Thesis for the Degree of Master of Fisheries Science

Effect of Biofloc Feeding on Growth and Survival Rate of *Marphysa sanguinea* (Polychaeta: Eunicidae) Juveniles

By

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Department of Fisheries Biology

The Graduate School

Pukyong National University

August 2017

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바위털갯지렁이 (*Marphysa sanguinea*: Eunicidae) 치충의 성장 및 생존율에 미치는 바이오플락 급이 효과

Advisor: Prof. Chang-Hoon Kim

By

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A thesis submitted in a partial fulfillment of the requirement
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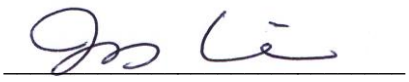

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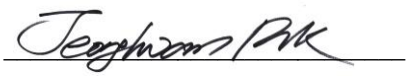

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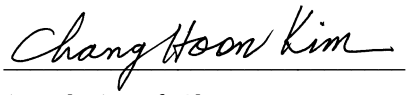

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Abstract

The rockworm *Marphysa sanguinea* (Montagu, 1815) **(**Polychaeta: Eunicidae), one of the economically valuable species, has been increasing in demand not only for fishing bait but also for living food resource of fin fish and shrimp. Rockworm farms have recently started developing artificial seed production techniques. The aim of this study is to demonstrate the suitability of biofloc feeding for *M. sanguinea* juveniles. Two experiments have been conducted for three months on biofloc feeding for *M. sanguinea* juveniles at Fisheries Science and Technology Center (FSTC), Pukyong National University.

The first experiment of biofloc feeding was carried out on five feeding rates (3%, 6%, 9%, 12% and 15% of total body weight) with four replicates. Biofloc was fed everyday with shrimp feed powder 50% of crude protein (DongA One Corporation) and glucose in 1.55 of C/N ratio. The stocking density was 300 inds/bottle of 20 L capacity. In total, 20 bottles were used with worms at a mean initial weight of 0.057 ± 0.009 g. Worms were fed at the rate of 3% of body weight in the beginning. After 3 months of rearing, worms showed mean final weight of 0.084 ± 0.02 g, specific growth rate of 0.21% and survival rate of 81.9 % at 15% feeding rate. However, 100% survival rate was found at 9% feeding rate with specific growth rate of 0.052% and mean final weight of 0.046 g, which was lower than those of the initial weight. That is, out of five different feeding rates, 15% feeding rate resulted in the highest growth rate, but 3% and 9% feeding rates resulted in the highest survival rates. The second experiment was conducted on the effects of stocking density on the growth and survival rate of *M sanguinea* in 16 bottles; each bottle of 20-L capacity. The experiment was conducted on a feed supply system of 16 bottles connected to a 400-L acrylic tank of biofloc feed mixed with shrimp feed powder and glucose. Juvenile worms (average weight 0.179 ± 0.017 g) were stocked into 16 bottles with different densities (100, 200, 400, 800 inds/bottle) with four replicate bottles per treatment. Feeding rates were 0.05 g, 0.07 g, 0.08 g and 0.16 g according to 3% of body weight in each treatment, respectively. Water was exchanged at the rate of about 0.55%, 1.11%, 2.22% and 4.44% every day. Specific growth rate (%/day) was checked during 12 weeks in all treatments, and harvested biomass increased proportionally to stocking density (0.613%, 0.019%, 0.099% and 0.601%/day increasing stocking density, respectively). The results demonstrated that the growth and survival rate of *M. sanguinea* juveniles were significantly affected by different stocking densities ($p < 0.05$).

1. Introduction

World Aquaculture has been growing at an annual rate of 8.9–9.1% since the 1970s. This high growth rate is due to solving the shortage of protein food supplies, which is particularly suffered in the developing countries (Subasinghe, 2005; Gutierrez-Wing and Malone, 2006; Matos *et al.*, 2006). The global shrimp market has expanded from less than $1 billion to $5.8 billion (US) from 2000 to 2005 (FAO, 2008). To meet the growing demand, the shrimp industry is shifting from extensive rearing systems to more intensive rearing systems. However, environmental (i.e. discharge of farm effluents) and economical limitations (higher prices of feed ingredients, especially fishmeal) could hamper the growth of the shrimp industry. The expansion of the aquaculture production is restricted by the pressure it can cause on the environment, that is, the discharge of waste products in the water bodies and its dependence on fish oil and fishmeal (De Schryver *et al.*, 2008). In order to be completely successful, the aquaculture industry needs to develop technology that will support economic and environmental sustainability (Kuhn *et al.*, 2010). The technology is to implement cheaper alternative ingredients to fishmeal and to effectively reduce the costs of feed as feed costs can account for 50% of operational expense (Van Wyk *et al.*, 1999), while reducing the pressure on overexploited natural fisheries (Tacon *et al.*, 2006; Naylor *et al.*, 2009). Thus, it is important to determine if alternative ingredients derived from biologically treating fish waste, bioflocs (microbial flocs), could be a suitable replacement ingredient in marine shrimp diets. If implemented successfully, this option would offer sustainability to fishmeal. According to Kuhn *et al.* (2010), initial cost estimates for biofloc production were approximately $400 to $1000 per ton of dry ingredients, which was projected to be less than the ingredients such as fishmeal and soybean meal. Over the period of January 2008 to May 2009, the global fishmeal market varied from a low mean of about $900 to a high mean of $1250 per metric ton. During the same time frame, soybean meal varied approximately from a low mean of $375 to a high mean of $550 (FAO, 2009). Thus, this study suggests that biofloc can be a viable and more sustainable feed alternative, considering its cost, generating method, and potential possibility of easing the pressure on wild fisheries by reducing the demand for fishmeal to some extent.

Aquaculture produces large quantities of wastes that contain solids (e.g. feces and uneaten feed) and nutrients (e.g. nitrogen and phosphorus) which can be detrimental to the environment, if managed improperly. These solids and nutrients originate from uneaten feed, feces, and animal urea/ammonia (Maillard *et al.*, 2005; Sharrer *et al.*, 2007). If released

directly to the environment, these solids and nutrients can be pollutants resulting in environmental issues such as eutrophication (Wetzel, 2001) or can be toxic directly to aquatic fauna (Timmons *et al*., 2002; Boardman *et al*., 2004). The most common method for dealing with this pollution has been a continuous replacement of the pond water with new clean water from the water source (Gutierrez-Wing and Malone, 2006). For instance, Penaeid shrimp requires about 20 m^3 fresh water per kg shrimp producded (Wang, 2003).

An average farm with a production of 1000 kg shrimp/ha/yr and with total pond surface of 5 ha means a water use of ca. 270 m^3/day. A relatively new alternative to previous approaches is the biofloc technology (BFT) aquaculture (Avnimelech, 2006). In the system, a co-culture of heterotrophic bacteria and algae is grown in flocs under controlled conditions within the culture pond. The system is based on the knowledge of conventional domestic wastewater treatment systems and is applied to aquaculture environments. Microbial biomass grows on fish excreta, resulting in a removal of unwanted components from the water. The major driving force is the intensive growth of heterotrophic bacteria. They consume organic carbon; 1.0 g of carbohydrate-C yields about 0.4 g of bacterial cell dry weight-C. Thereby, they immobilize mineral nitrogen depending on the bacterial C/N-ratio. As such, Avnimelech (1999) calculated a carbohydrate need of 20 g to immobilize 1.0 g of N, based on a microbial C/N-ratio of 4 and a 50% C in dry carbohydrate. The study aimed to reduce inorganic nitrogen accumulation in an extensive shrimp culture system by (1) increasing the C/N ratio of the feed through reducing its protein content and by (2) reducing the use of fresh water per kg shrimp produced (Wang, 2003).

The rockworm, *Marphysa sanguinea* (Montagu, 1815) (Polycheata: Eunicidae), is a large, often conspicuous, and dioecious benthic polychaete. Based on the conceptual knowledge of sexual maturation, spawning cycle and physiological characteristics of *M. sanguniea*, this study tried to develop artificial seed production techniques under the laboratory conditions. The rock worm has multiple stages in the reproductive biology such as multiplying, growing, maturing and spawning stages, and also resting and degenerative stages during gonadal development processes. In the natural seawater flow-through system, gonads of female and male matured from January to April. Spawning and spermatogenic spurt were observed from May to August. Then, resting stage was observed from September to November. Finally, gonadal development was observed in the following year. Investigation on the yearly egg production provided basic information for artificial seed production and reproductive parameters (i.e. weight, the number of segments, absolute fecundity and egg size) (El Barhoumi, 2013).

Marphysa sanguinea can be found in a variety of benthic environments, including soft muddy sediments around oyster reefs, sandy or muddy tidal flats among other fouling organisms on dock pilings and buoys, and inside holes of calcareous rocks (Ruppert and Fox, 1988). When inhabiting sediments, these worms use their muscular bodies to make burrows in the sand or mud. The rockworm is a large polychaete, reaching lengths of up to 40 cm with a lifespan of ca. 90 days. As with other organisms, growth and lifespan may vary with food availability, water temperature, salinity, predator abundance, and additional environmental factors. While reports are scarce on populations of *M. sanguinea*, rockworms from the Venice Lagoon in Italy were documented to spawn throughout April and May (Prevedelli *et al.*, 2007).

Marphysa sanguinea, one of the most economically valuable species, is in great demand for the feed in fish aquaculture. It is an iteroparous species and has no difference between males and females, spawning without epitokal metamorphosis (El Barhoumi *et al*., 2013). The most abundant inhabitation is in soft muddy sediments around oyster reefs, sandy or muddy tidal (Rupert and Fox, 1988). It burrows in sediment and can reach a maximum length of 40 cm (Lardicci and Castelli, 1986). In addition, *M. sanguinea* is known as an euryhaline polychaete with a wide distribution. At present, this species has been recorded in many regions around the world, including the south coast of England, Eastern Scheldt in South-Western Netherlands, and the Italians coast (Prevedelli *et al.*, 2007; Hutchings *et al.*, 2011), Portugal (Garcês and Pereira, 2011), Tunisia (El Barhoumi *et al.*, 2013) and Khnifiss lagoon in South of Morocco (Ouassas *et al.*, 2015). Until now, there are actually not many researches about *M. sanguinea* life cycle. Prevedelli *et al.* (2007) described the life cycle of *M. sanguinea* in the Venice Lagoon-Italia in detail with many traits such as sediment characteristics, reproductive traits, gametogenic cycle, larval development (trochophore from 0 - 1 day, matatrochophore from 1 - 7 days, nectochaete from 7 - 14 days) and juvenile from 14 - 60 days and adult more than 60 days. The reduction in the dispersal phase, together with the development of a strong anterior musculature, brought to an early acquisition of the benthic habitat, which was attained with the construction of the mucous tube inside of which the larvae lived.

Marphysa sanguinea is a large-sized gonochoric species with annual iteroparous strategy and synchronous spawning at population level. The gonadal activity was at a maximum during summer period in both sexes. From May to September, the greater number of immature oocytes could be observed in females; starting in November, immature oocytes decreased, and those with a wider diameter increased progressively in number. April and May

are the main spawning seasons with a 1:1 sex ratio of male to female (Prevedelli *et al.*, 2007; El Barhoumi *et al.*, 2013; Ouassas *et al.*, 2015).

1.1 Problem statement

Polychaetes are among the most frequent and abundant marine metazoans in benthic environments. A variety of beneficial features can be ascribed to biofloc technology, from water quality control to in situ feed production and some possible extra features. Biofloc technology offers aquaculture a sustainable tool to simultaneously address its environmental, social and economic issues concurrent with its growth. Researchers challenge further development of this technique, and farmers implement it in their future aquaculture systems. The basics of the technology is not so improved, but its fine-tuning and implementation will need further research and development from the present and future generation of researchers and farmers in order to make this technique a keystone of future sustainable aquaculture.

This study attempts to summarize current information about the feeding biology of the worms. As worms can survive well in the biofloc feeding system and management of the successful artificial seed production, it is possible that much higher levels could be achieved with improved management of biofloc feeding.

1.2 Hypothesis

According to Nahar and Siddik (2015) successfully cultured and genetically improved farmed tilapia (GIFT) with biofloc technology and culture of mono sex tilapia using periphyton is more economic and beneficial than wheat bran and even commercial tilapia feed for the culture of mono sex tilapia in the farming system of Bangladesh. Hence, biofloc technology provides a sustainable tool to simultaneously address the environmental, social and economic issues that are related to the growth of this particular aquaculture sector. It is a challenge to researchers and tilapia farmers to further develop and refine this technique as well as to implement this technology in future aquaculture systems (Choo and Caipang, 2015).

Based on these findings in using of biofloc technology in tilapia culture, this study hypothesized that biofloc feeding in different quantities and different stocking densities could easily make an impact and effect on the growth and survival rate of rockworm *Marphysa sanguinea* juveniles.

1.3 Objectives of the study

This study aims to show that a variety of beneficial features can be ascribed to biofloc technology, from water quality control to in situ feed production and some possible extra features. Biofloc technology offers aquaculture a sustainable tool to simultaneously address its environmental, social and economic issues concurrent with its growth. Different feeding rates were an important factor affecting the *M. sanguinea*. Thus, it is important to the success of experiment to determine the optimal feeding rate. In different stocking densities, the high level of the density means more survival and growth. This study is to reveal the most appropriate stocking densities for worms in artificial rearing culture, and also to investigate the effects of stocking densities on growth and survival of worms.

1.4 Justification of the study

According to FAO (2016), the aquaculture industry has been increasing rapidly at a rate of 9% per year since the 1970s. But land costs and strong dependence on fishmeal and fish oil are the main impediment to the expansion of aquaculture. As with commercial feed for aquaculture, such ingredients are among the prime constituents. About 50% expenditure for aquaculture production derived from feed costs, which is predominant due to the cost of protein component in commercial diets. The environmental congenial and cost effective aquaculture system called ‘‘Biofloc Technology (BFT)’’ is considered an efficient alternative system since nutrients could be continuously recycled and reused. The technology is supportive for sustainability of aquaculture, cost effective and environmentally sustainable.

1.5 Significance of the study

Primarily, this research can help understand scientific findings and sustainable analyses of the culture of *Marphysa sanguinea* from the environmental, economic and social perspective. Secondly, results from this experiments can help develop rearing techniques, which enhance survival and growth rate at the juvenile stage, consequently boosting commercial production of the species for the numerous aquaculture purpose.

2. Materials and methods

2.1 Design of experiments

Different feeding rates experiment design

- Feeding system once per day
- Three sediment types (mixture of oyster shells, coarse sand, sand about 7.5 cm height) used together as culture sediment
- Initial weight of juvenile : 0.06 ± 0.01 g
- Five feeding rates (3%, 6%, 9%, 12%, 15% of total body weight)
- Four replicates of each feeding rate
- Three months of experimental period

Different stocking densities experiment design

- Feeding system once per day
- Three sediment types (mixture of oyster shells, coarse sand, sand about 15 cm height) used together as culture sediment
- Initial weight of juvenile : 0.179 ± 0.016 g
- Four different stocking densities (100 inds, 200 inds, 400 inds, 800 inds)
- Four replicates of each stocking density
- Three months of experimental period

2.1.1 Source of juvenile worm

This experiment was conducted at Fishery Science and Technology Center (FSTC), Pukyong National University, Goseong, South Korea. The FSTC is situated about 110 km southwest of Busan. The center occupies a global position of 34˚ 58´ 34.7160" N and 128˚ 19´ 24.9960" E an altitude of 14 m.

Experimental juvenile *Marphysa sanguinea* were obtained by artificial seed production in breeding season from May, 2016 to July, 2016. The rockworm is a gonochoric species, with individuals being either male or female (Prevedelli *et al.*, 2007). Populations reproduce sexually and are capable of producing multiple egg clutches throughout their lifetime in a process called iteroparity. Scientists speculate that copulation between male and female occurs within the burrow, where eggs remain until they reach the free-swimming

larval stage. At this time, larvae are released from burrow into the water column, and then taken out and cultured in bottles until the juvenile stage. Individuals from the same population are known to have synchronous spawning events. During this collection, the sediment was scooped bit by bit into plastic trays and spread evenly on the trays to expose the worms. Worms were then siphoned out with a pipette. For the first experiment, 6000 juvenile worms were collected and placed in 20 separate bowls (300 individuals per bowl). For the second experiment, 6000 juvenile worms were collected and placed in 16 separate bowls (100, 200, 400 and 800 inds/bowl), and each stocking density was four replicates.

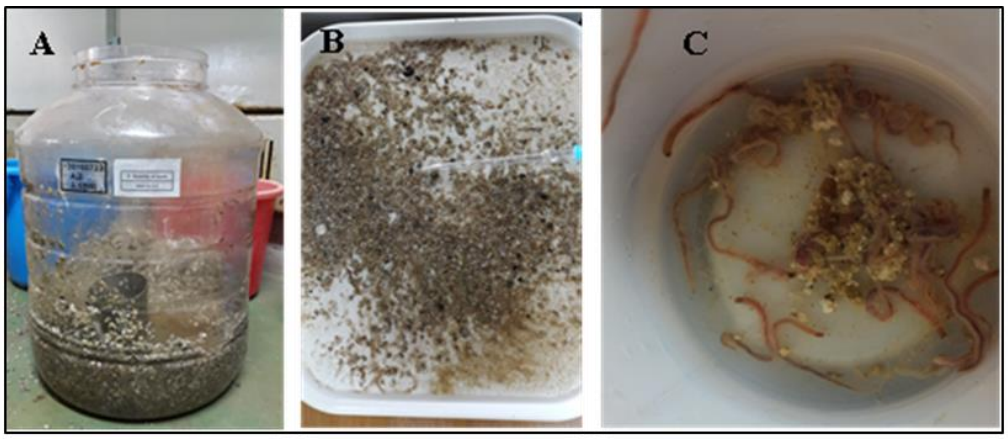

Fig. 1. Specimen collection for the experiment. (A) Scooping out the rearing substrate where juvenile worms burrow in 20 L experiment bottle, (B) collection of worms, and (C) the collected juvenile worms.

2.1.2 Weighing of juvenile worms

At the termination of the experiment, worms in each bowl were counted and 300 worms from each bowl were measured for total growth weight, and survival rate were monitored and recorded. Weight was determined by Mettler Toledo analytical balance as shown in Fig. 2 below. Individuals were placed on tissue paper to blot any water before weighing.

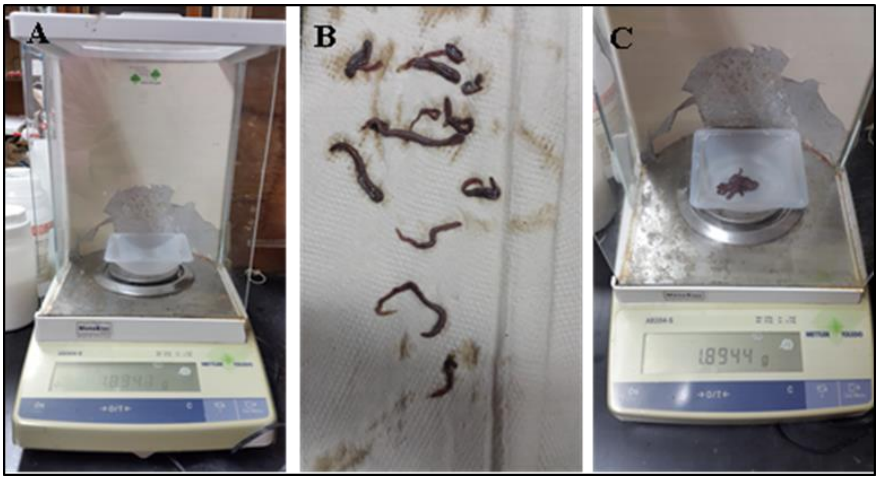

Fig. 2. Weighing of juvenile worms. (A) Mettler Toledo Analytical balance used to weigh, (B) blotting of worms before weighing, and (C) weighing of individuals.

2.1.3 Preparing experiment bottles

Experimental culture unit consisted of plastic bottles of 20 L capacity (Fig. 3) 37.5 x 28 cm in length and width. All bottles were washed, and substrates were filled with the mixture of oyster shells, coarse sand and sand about 7.5 cm high including water outlet. In the second experiment each bottle was put with 15 cm high of substrates. The outlet pipes were covered with spongy 6 x 4 cm size to prevent worms from escaping. Meanwhile, the overflow pipes were out since the worms lived in the bottom layer of the sediment.

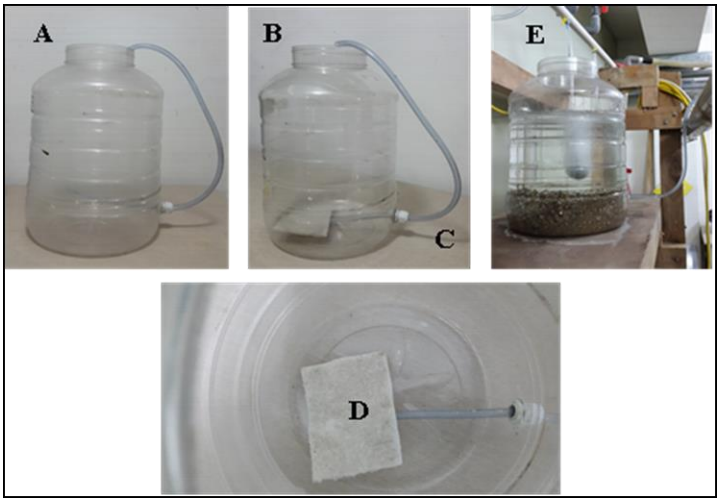

Fig. 3. Procedure of rearing bottle preparation for the experiment. (A) a transparent polyethylene 20 L bottle with outlet tube hose, (B-C) a bottle showing the spongy-type covering filter (D) upper bottom outlet, and (E) the last setting of bottle filled with substrate and aeration.

2.1.4 Sediment collection

Three types of sediments were used: oyster shells, coarse sand and sand, which were collected from the research center and oyster farm near the center, respectively. Oyster shells, coarse sand and sand were filtered through 2 and 5 mm sieves, respectively. They were then treated by thoroughly washing with freshwater and sun dried thereafter.

2.1.5 System set-up

Each bottle was filled with sediment (about 7.5 cm height of oyster shells, coarse sand, and sand). In the experiment room, for the first experiment, bottles were arranged in 3 feet height of the 1st floor with 10 bottles facing each other; in total, 20 bottles were placed at the 1st floor (Figs. 4-6). In the middle of two sides, outlet pipes from bottles were directed into their holes (50 x 4 m) using PVC pipes separately. These PVC pipes were dropped to 400 L capacity of acrylic tank for water outlet. The tank was prepared with biofloc sea water by providing shrimp feed powder and glucose every day. It was connected to experiment bottles, which were supplied with each amount of biofloc one time a day for different feeding rates experiment. Each bottle was then stocked with 300 juvenile worms of initial weight 0.06 ± 0.01 g. They were allowed to acclimate, burrow into the sediment bottom and make their tubes for a period of 48 hours. All replicates received a constant aeration. Dissolved oxygen (DO) averaged 5.19 ± 0.07 mg/L and pH 7.74 ± 0.18, according to C:N 20, and glucose and shrimp feed powder were mixed and prepared for feed in 400 L of acrylic tank.

For the second experiment, bottles were arranged in 3 feet height of 2nd floor, facing each other; in total, 16 bottles were placed. In the middle of two sides, outlet pipes from bottles were directed into their holes (Ø 50 mm x 4 m) using PVC pipe separately. These PVC pipes were dropped to 400 L capacity of acrylic tank for water outlet connected with biofloc sea water. Four types of different stocking densities were 100, 200, 400 and 800 juvenile worms with the initial weight of 0.156 ± 0.011 g, 0.131 ± 0.003 g, 0.215 ± 0.041 g and 0.214 ± 0.024 g, respectively. They were allowed to acclimate, burrow into the sediment bottom and make their tubes for a period of 48 hrs. All replicates received a constant aeration. Dissolved oxygen (DO) averaged 5.18 ± 0.04 mg/L and pH 7.64 ± 0.14. According to C:N 20, glucose and shrimp feed powder were mixed and prepared for feed in 400 L of acrylic tank.

Fig. 4. The first experiment set-up. (A) arrangement of rearing bottles and an acrylic tank for biofloc preparation in the experiment room, (B) biofloc tank, and (C) PVC pipe line (Ø 50 mm) with holes for adjusting water level by connecting with drainage outlets.

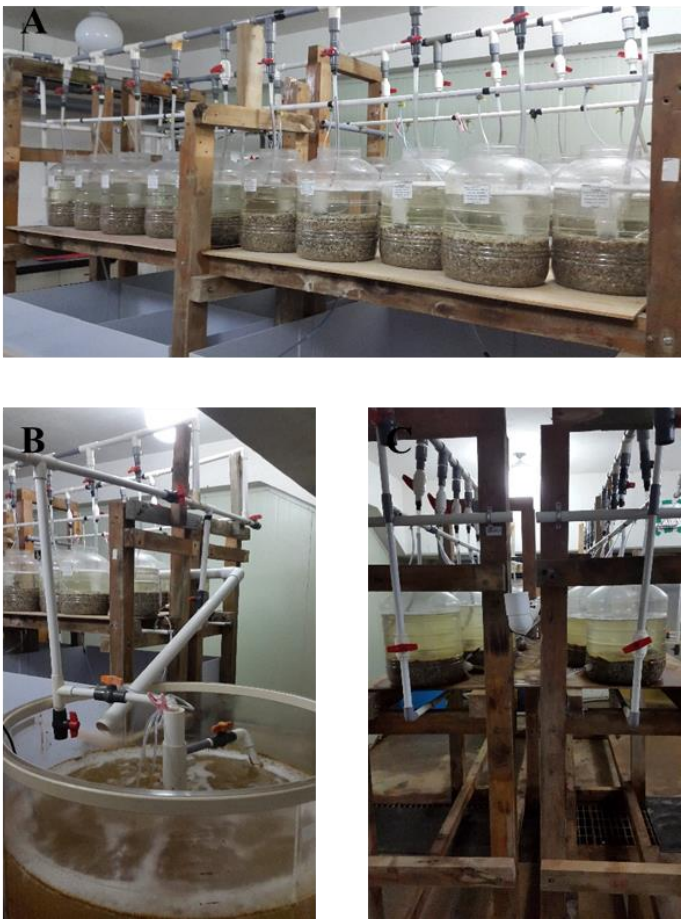

Fig. 5. The second experiment set-up. (A) arrangement of rearing bottles in the experiment room, (B) acrylic tank for biofloc preparation, and (C) outlet connected with Ø 50 mm PVC pipe lines.

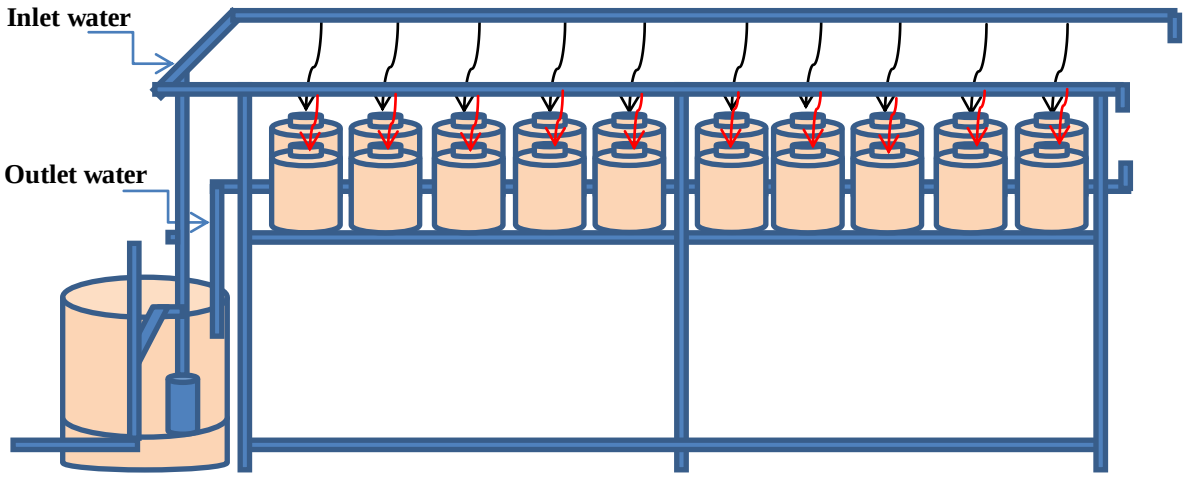

Fig. 6. A schematic diagram of the experiment set-up.

2.1.6 Feeding of worms

The worms were fed on shrimp feed mixed with biofloc water of different quantities of feed everyday throughout the experiment period. Five different feeding quantities (shrimp feed powder + biofloc) were assigned to 20 L bottles with 0.05 g, 0.1 g, 0.2 g, 0.3 g and 0.4 g for the first experiment. Then, four different stocking densities were assigned to 20 L bottles with 0.05 g, 0.07 g, 0.08 g and 0.16 g for the second experiment, respectively. Each different quantity experiment has four replicates.

Fig. 7. Shrimp feed powder and glucose feed for biofloc preparation in the experiment.

2.1.7 Experimental water preparation

Seawater (31.9 psu) was pumped from the Jaran Bay adjacent to the Research Center and tap water was the source of water used in the experiment. About one week was spent to gradually vary the experimental water salinity and to decrease it by about 23-25 psu of seawater. Varying salinity involved appropriate portions mixed with freshwater and seawater, both of which were stored in two separate tanks (1000 L and 2000 L capacity each) in the experiment room (Fig. 8). These tanks received a constant aeration. After one week, pumps were turned on to distribute water into all experimental bottles about 18 L.

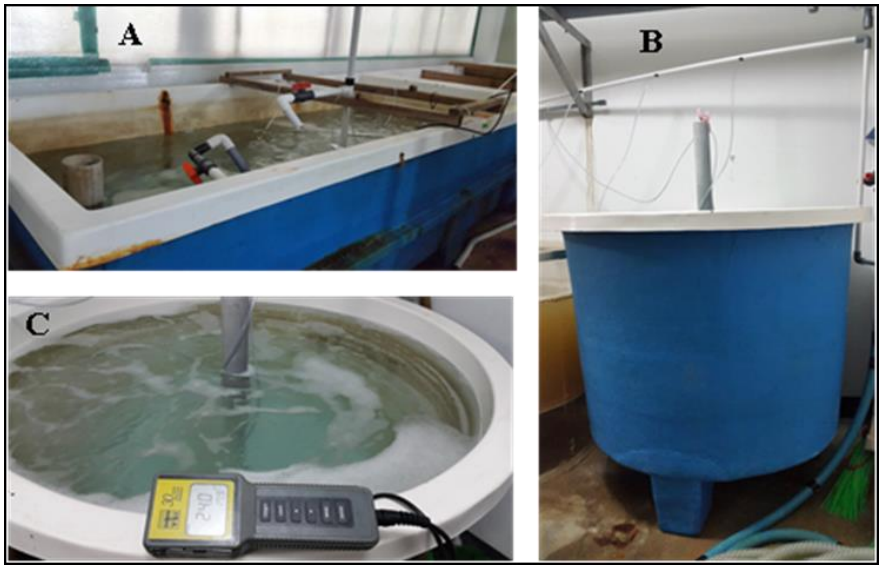

Fig. 8. Experimental seawater preparation. (A) 2 ton tank and (B) 1 ton tank for a stock of sterilized seawater to supplement or to exchange the recirculation water and (C) salinity check.

2.2 Water quality analysis

Temperature, DO, pH and salinity were measured daily by Hydrolab Minisonde 5 (Made in Korea) together with Hydrotech Archer (Made in USA). Other water parameters analyzed by specific methods include: NO_3-N, tested by cadmium reduction method, NO_2-N, tested by diazotization method, and NH_3-N, tested by indophenols methods.

2.3 Data collection

Data were collected after experimental period (3 months) basically on survival and growth rates of worms. During the collection process, identified individual worms were siphoned out by pipette, as described in previous chapter 2.1.1. Worms from each replicate were placed in separate bowls. Thirty individuals of worms were selected by random sampling from each bottle in all replicates to check for the survival. Growth of juveniles was measured by weighing all samples by a Mettler Toledo analytical balance, as described in 2.1.2.

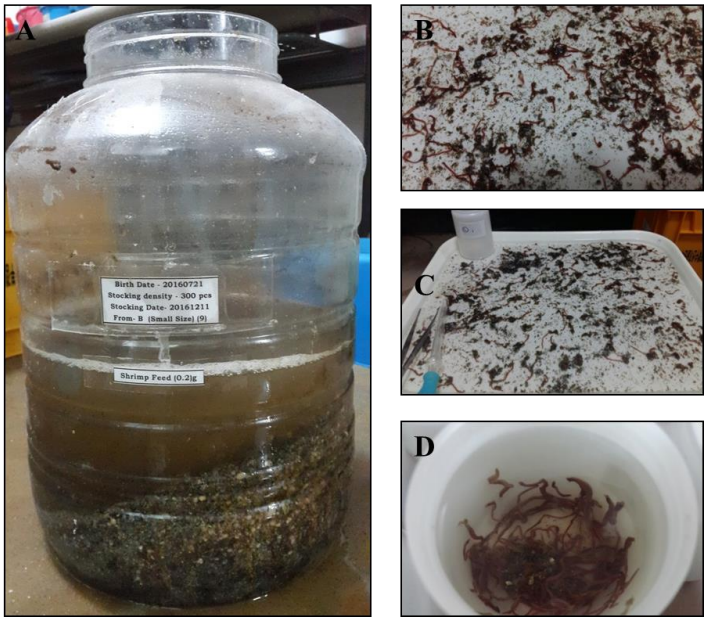

Fig. 9. Collection of juvenile worms at the end of experiment. (A) a rearing bottle for scooping sediment bit by bit, (B) sediment spread on tray, (C) siphoning out identified worm, and (D) collected worms kept in a bowl.

The equations below show how survival rate, weight gain and specific growth rate were calculated:

$$Sr = \frac{Nf}{Ni} \text{ x } 100 \qquad (1)$$

Where *Sr* denotes survival rate, *Nf* ; number of survivors and *Ni* ; initial number of stocked worms.

$$WG = \frac{Wf - Wi}{Wi} \text{ x } 100 \qquad (2)$$

Where *WG* denotes weight gain, *Wf* ; final body weight and *Wi* ; initial body weight.

$$SGR = \frac{lnWf - lnWi}{day} \text{ x } 100 \qquad (3)$$

Where *SGR* denotes specific growth rate, *lnWf* ; natural log of final body weight, *lnWi* ; log of initial body weight; *day* represents days of feeding.

2.4 Statistical analysis

The values were recorded as mean ± standard deviation. The statistical significance of difference in the mean and standard deviation ($p<0.05$) was analyzed by one-way ANOVA test on the comparison of the test and the control groups by the SPSS 15. Duncan's multiple range was used to compare differences among individual means (Zar, 1984). Differences were considered significant at P levels<0.05.

3. Results

3.1 Effect of different feeding rates on survival rate (%) of worms

There was a statistically significant difference between groups as determined by one way ANOVA (P=0.006). Survival remained relatively low at all different rates of feeding for 3 months of culture. Unexpectedly high mortality occurred in the feeding rate 15% body weight per day, and the final survival rate was 81.9% after 3 months. 3% and 9% body weight per day were shown to have the highest survival rate, 100%, at the end of the experiment. There was no statistically significant difference P<0.05 as described by Duncan's homogeneity of variance (Fig. 10, Table 1).

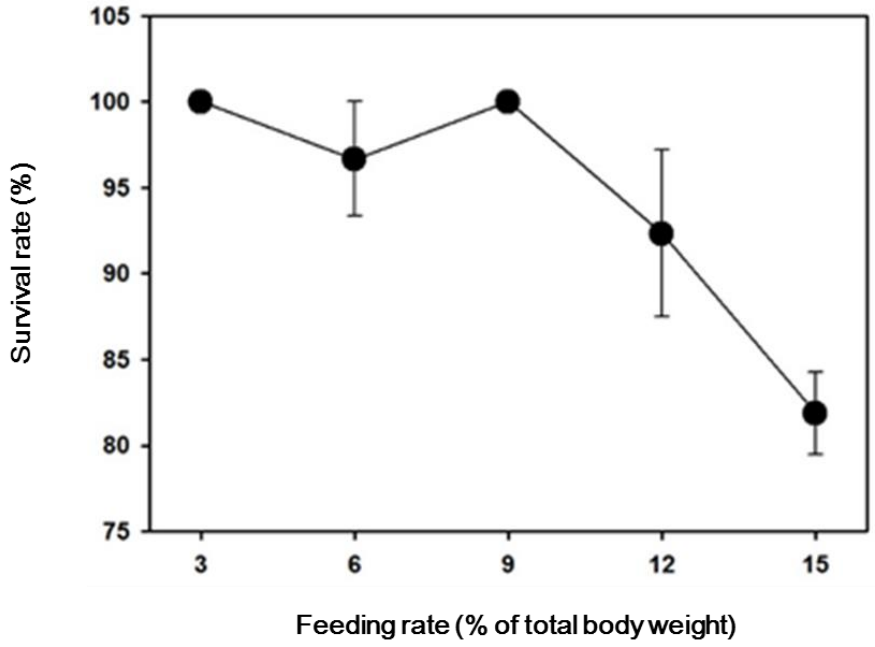

Fig. 10. Survival rate of *Marphysa sanguinea* juveniles at different feeding rates for 3 months.

3.2 Effect of different feeding rates on specific growth rate (%/day) of worms

Specific growth rate (%/day) was also significantly affected by five different feeding rates as determined by one-way ANOVA (P= 0.566). A significant difference was found at specific growth rate (%/day) among juvenile worms at feeding rate of 15% with other four different 3, 6, 9 and 12% of total body weight. The maximum of specific growth rate was observed at 15% feeding rate (0.344 ± 0.068), which had a significant difference from that of other groups (P<0.05), followed by 3% (0.302 ± 0.283), 6% (0.216 ± 0.090), 12% (0.190 ± 0.007). The lowest of specific growth rate was observed at 9% (0.046 ± 0.076), which was significantly different from that of other treatments (P<0.05).

Contrary to survival rate, the highest final weight was recorded at 15% (0.344 ± 0.068 g). There was no statistical significant difference from feeding rate of 9% body weight per day as described by Duncan's homogeneity of variance test. The lowest final weight of 0.046 ± 0.076 g was recorded in the control group. See Fig.11 and Table 1 below.

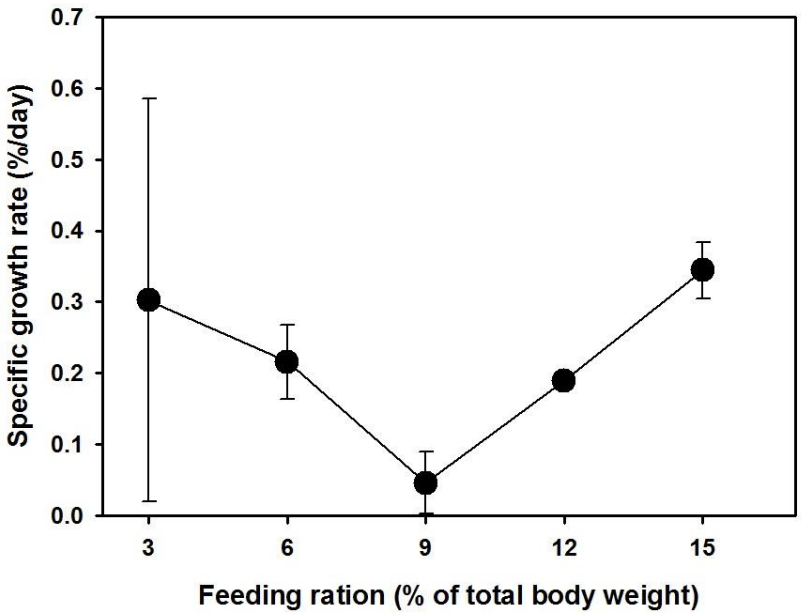

Fig. 11. Specific growth rate (%/day) of *Marphysa sanguinea* juveniles at different feeding rates for 3 months.

Table 1. Specific growth rate (%/day) and survival rate (%) of *Marphysa sanguinea* juveniles at different feeding rates for 3 months

Different feeding rates (%)	Initial weight (g)	Final weight (g)	SGR (%/day)	SR (%)
3	0.040 ± 0.016	0.057 ± 0.02	0.302	100
6	0.062 ± 0.004	0.081 ± 0.008	0.216	96.67
9	0.077 ± 0.006	0.082 ± 0.014	0.046	100
12	0.053 ± 0.005	0.067 ± 0.007	0.190	92.33
15	0.055 ± 0.012	0.084 ± 0.021	0.344	81.89

Table 2. One way ANOVA test on the effect of different feeding rates on specific growth rate (%/day) of *Marphysa sanguinea* juveniles

Feeding rates (% of total body weight)					P
3	6	9	12	15	
0.302±0.283	0.216±0.090	0.046±0.076	0.190±0.007	0.344±0.068	0.566

Table 3. One way ANOVA test on effect of different feeding rates on survival rate (%) of *Marphysa sanguinea* juveniles

Feeding rates (% of total body weight)					P
3	6	9	12	15	
100.0±0.0	96.7±5.8	100.0±0.0	92.3±8.4	81.9±4.2	0.006

3.3 Effect of different stocking densities on survival rate (%) of worms

There was a statistically significant difference between groups as determined by one way ANOVA (P=0.001). Survival remained relatively low at all different stocking densities for 3 months of culture. An unexpected high mortality was found at different stocking densities, as 800 inds. group that showed a final survival rate of 48.4%, after 3 months (Fig. 12, Table 4).

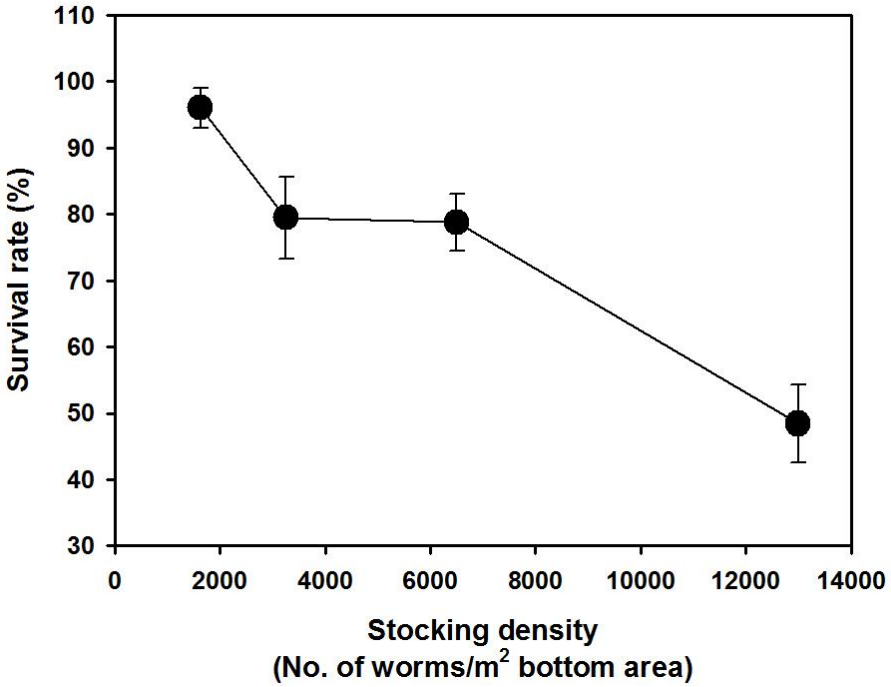

Fig. 12. Survival rate (%) of *Marphysa sanguinea* juveniles at different stocking densities for 3 months.

3.4 Effect of different stocking densities on specific growth rate (%/day) of worms

Specific growth rate (%/day) was also significantly affected by different stocking densities as determined by one-way ANOVA (P= 0.002). Contrary to survival rate, the highest final weight was recorded at 100 inds/bottle (0.613 ± 0.07 g) and 800 inds/bottle (0.601 ± 0.277). There was no statistical significant difference between 200 and 400 inds/bottle as described by Duncan's homogeneity of variance test. The lowest final weight of 0.019 ± 0.038 g was recorded at 200 inds/bottle (Fig. 13, Table 1).

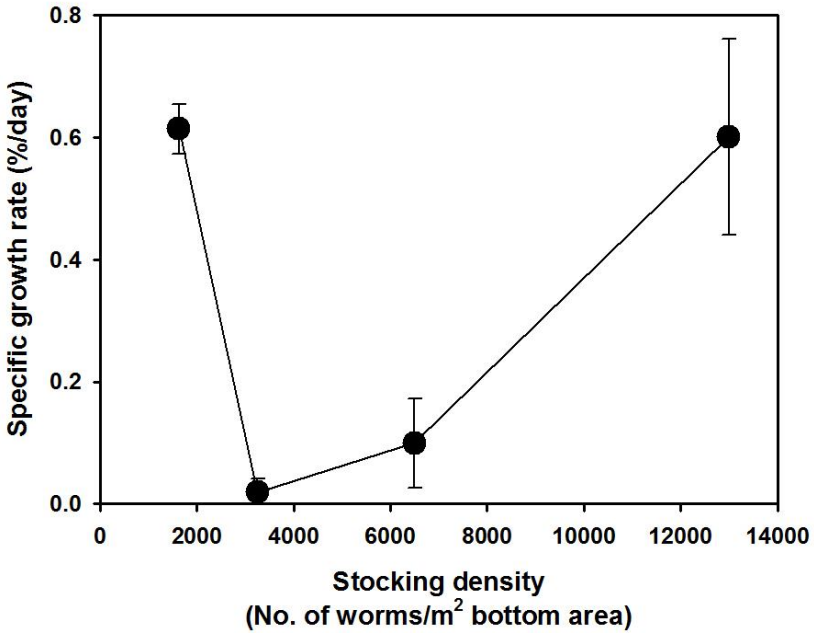

Fig. 13. Specific growth rate (%/day) of *Marphysa sanguinea* juveniles at different stocking densities for 3 months.

Table 4. Specific growth rate (%/day) and survival rate (%) of *Marphysa sanguinea* juveniles culture at different stocking densities for 3 months

Different stocking densities (inds)	Initial weight (g)	Final weight (g)	SGR (%/day)	SR (%)
100	0.154 ± 0.013	0.321 ± 0.031	0.613	96.00
200	0.129 ± 0.001	0.132 ± 0.005	0.019	79.50
400	0.198 ± 0.031	0.221 ± 0.007	0.099	78.75
800	0.206 ± 0.026	0.429 ± 0.097	0.601	48.42

Table 5. One way ANOVA test on the effect of different stocking densities on specific growth rate (%/day) of *Marphysa sanguinea* juveniles

	Stocking density				P
Worm number	100	200	400	800	-
Stocking Density (individuals/m^2)	1,630	3,260	6,520	13,040	-
Specific growth rate (%/day)	0.613 ± 0.070^a	0.019 ± 0.038^b	0.099 ± 0.126^b	0.601 ± 0.277^a	0.002

Table 6. One way ANOVA test on the effect of different stocking densities on survival rate (%) of *Marphysa sanguinea* juveniles

	Stocking density				P
Worm number	100	200	400	800	-
Stocking Density (individuals/m^2)	1,630	3,260	6,520	13,040	-
Survival rate (%)	96.0 ± 5.3^a	79.5 ± 10.7^b	78.8 ± 7.4^b	48.4 ± 10.1^c	0.001

3. 5 Water quality analysis for different feeding rates

Trends of various factors of water quality tested in the biofloc tank and experimental bottles for different feeding rates are shown in Figs. 14 to 21 and Figs. 22 to 29, respectively.

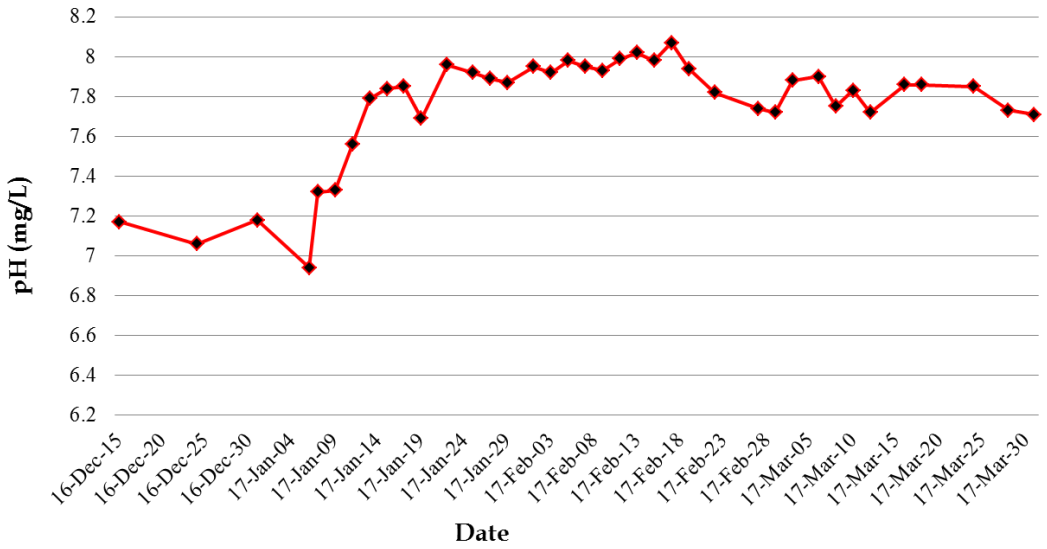

Fig. 14. pH variation in a biofloc tank for different feeding rates from December 2016 to March 2017.

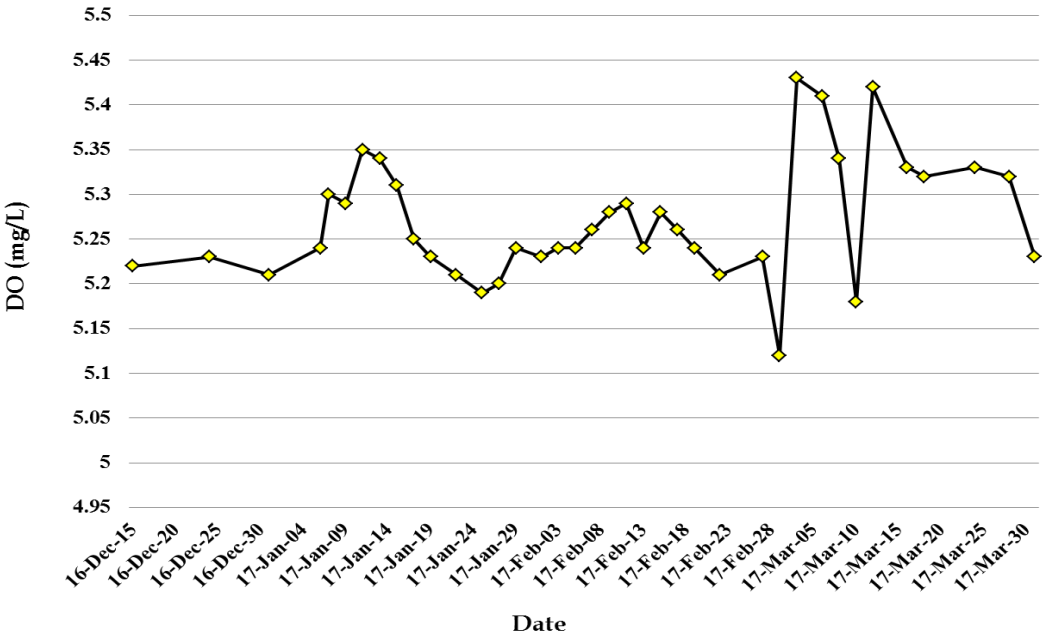

Fig. 15. Dissolved oxygen (DO) variation in a biofloc tank for different feeding rates from December 2016 to March 2017.

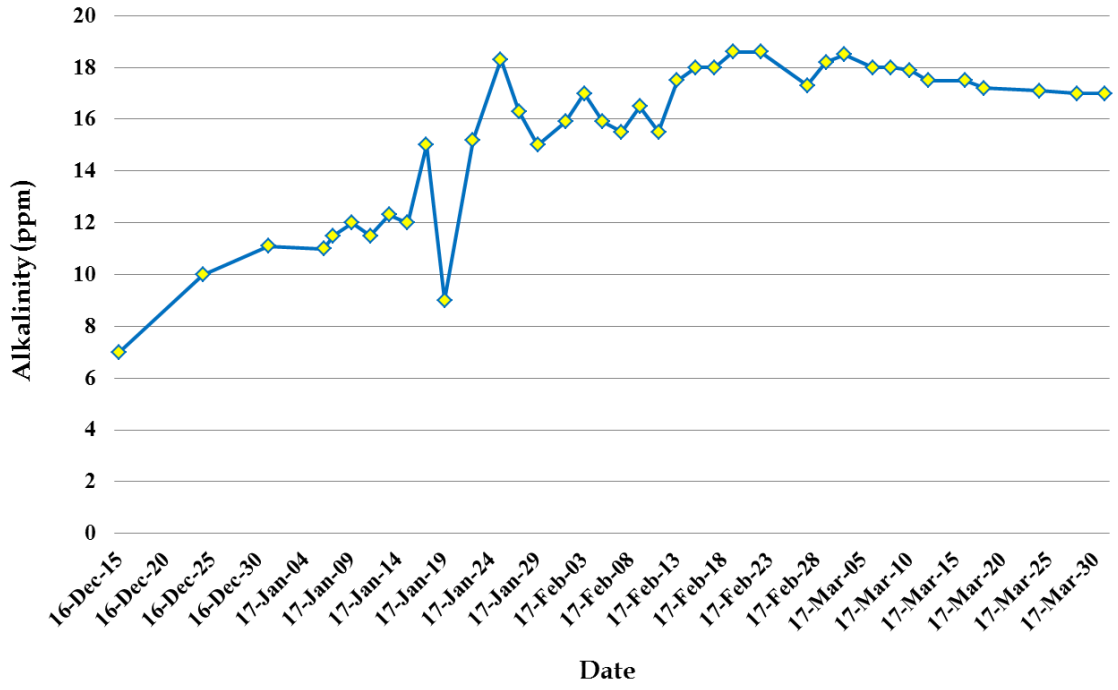

Fig. 16. Alkalinity variation in a biofloc tank for different feeding rates from December 2016 to March 2017.

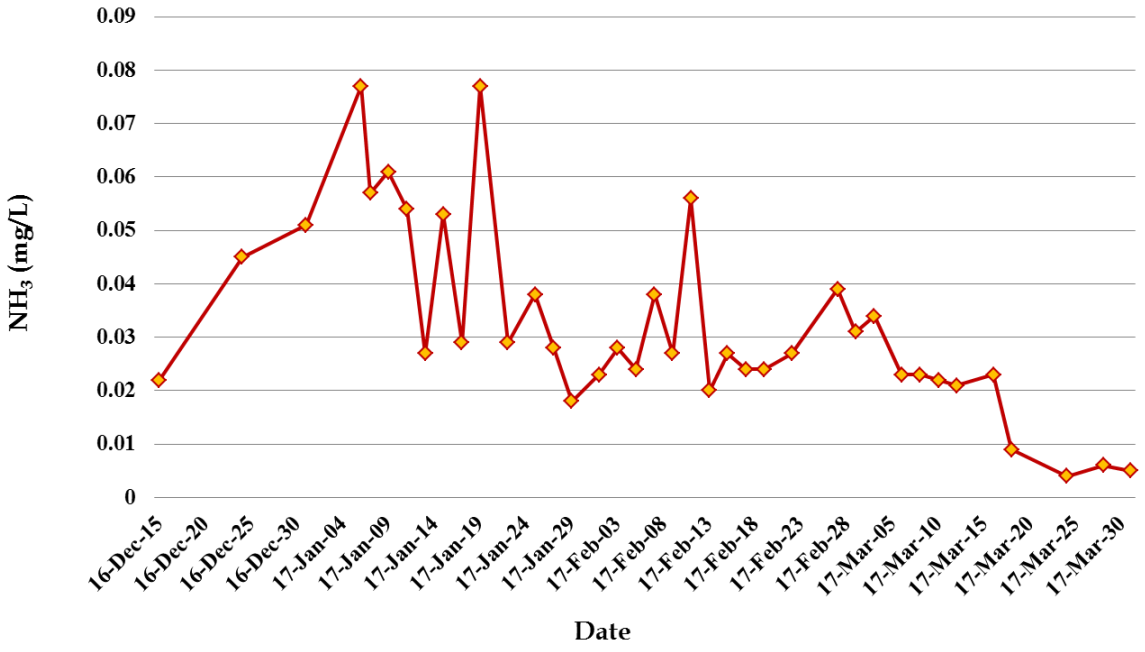

Fig. 17. Ammonia variation in a biofloc tank for different feeding rates from December 2016 to March 2017.

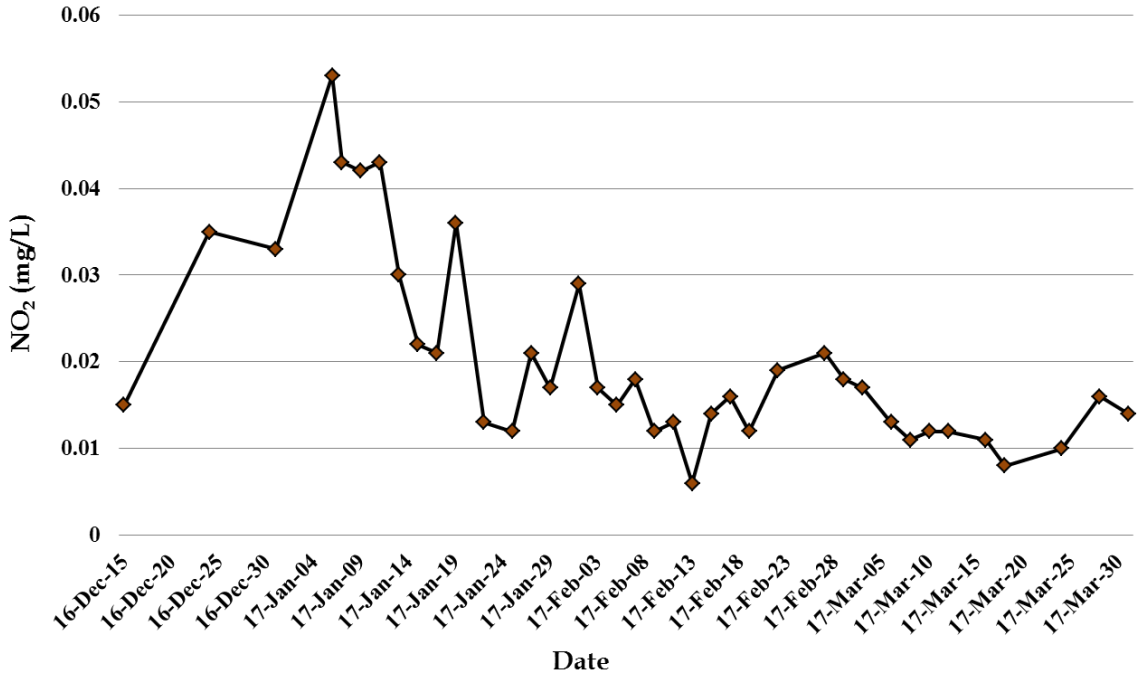

Fig. 18. Nitrite variation in a biofloc tank for different feeding rates from December 2016 to March 2017.

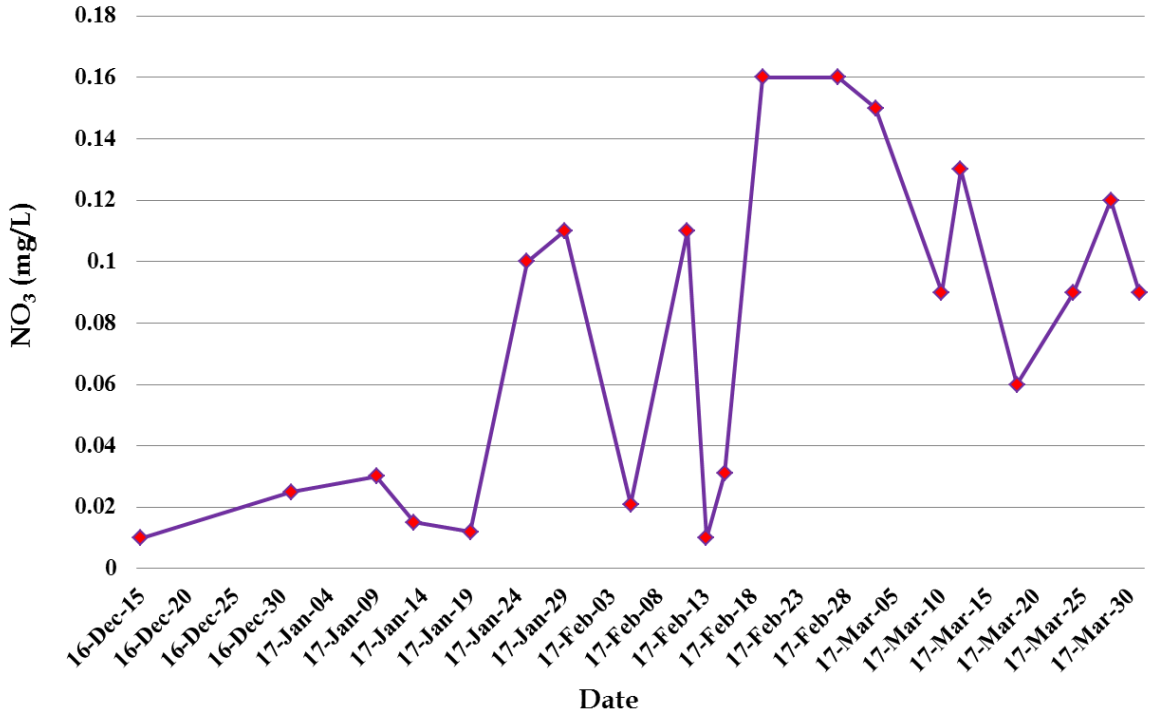

Fig. 19. Nitrate variation in a biofloc tank for different feeding rates from December 2016 to March 2017.

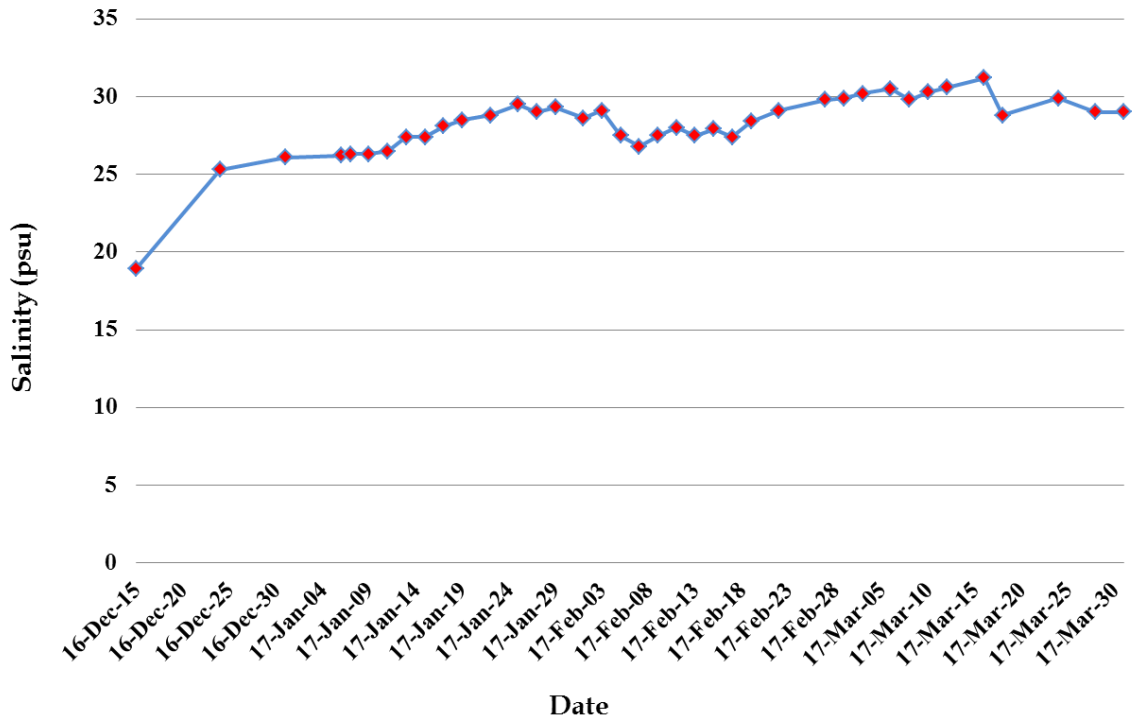

Fig. 20. Salinity variation in a biofloc tank for different feeding rates from December 2016 to March 2017.

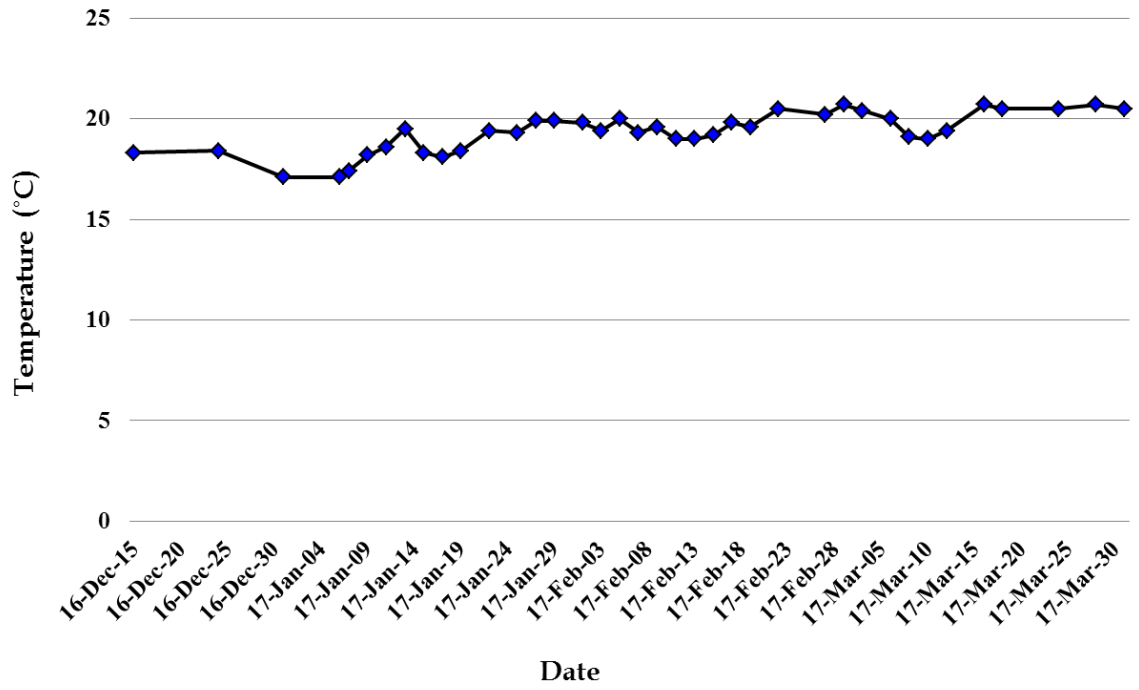

Fig. 21. Temperature variation in a biofloc tank for different feeding rates from December 2016 to March 2017.

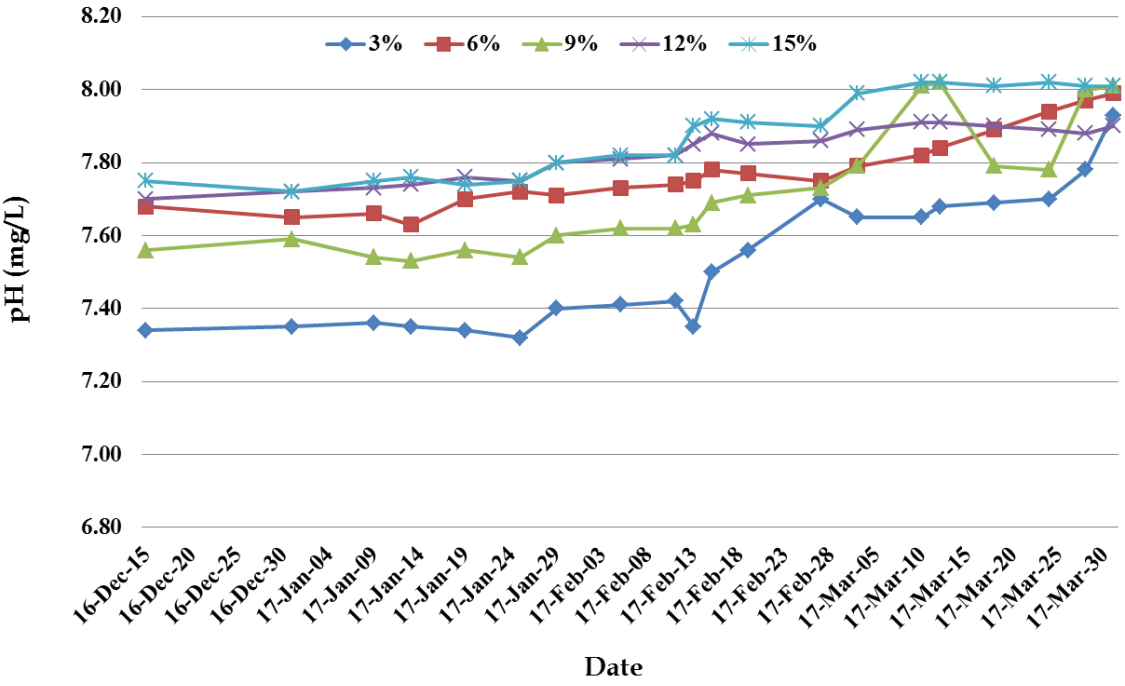

Fig. 22. pH variation in experimental bottles for different feeding rates from December 2016 to March 2017.

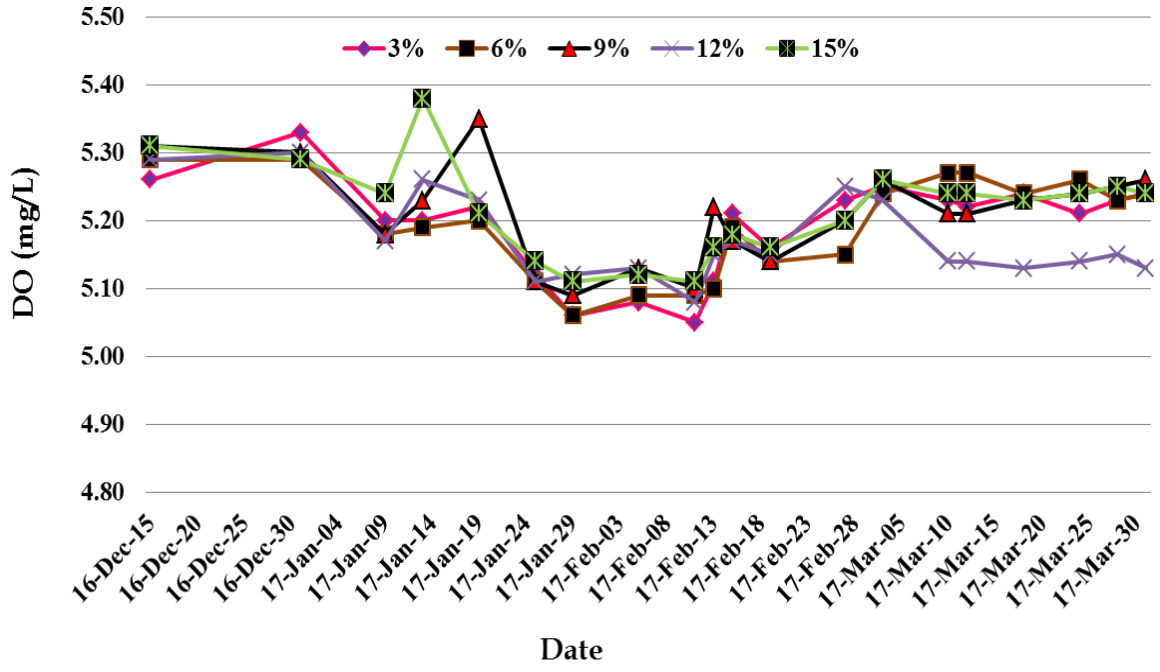

Fig. 23. Dissolved oxygen variation in experimental bottles for different feeding rates from December 2016 to March 2017.

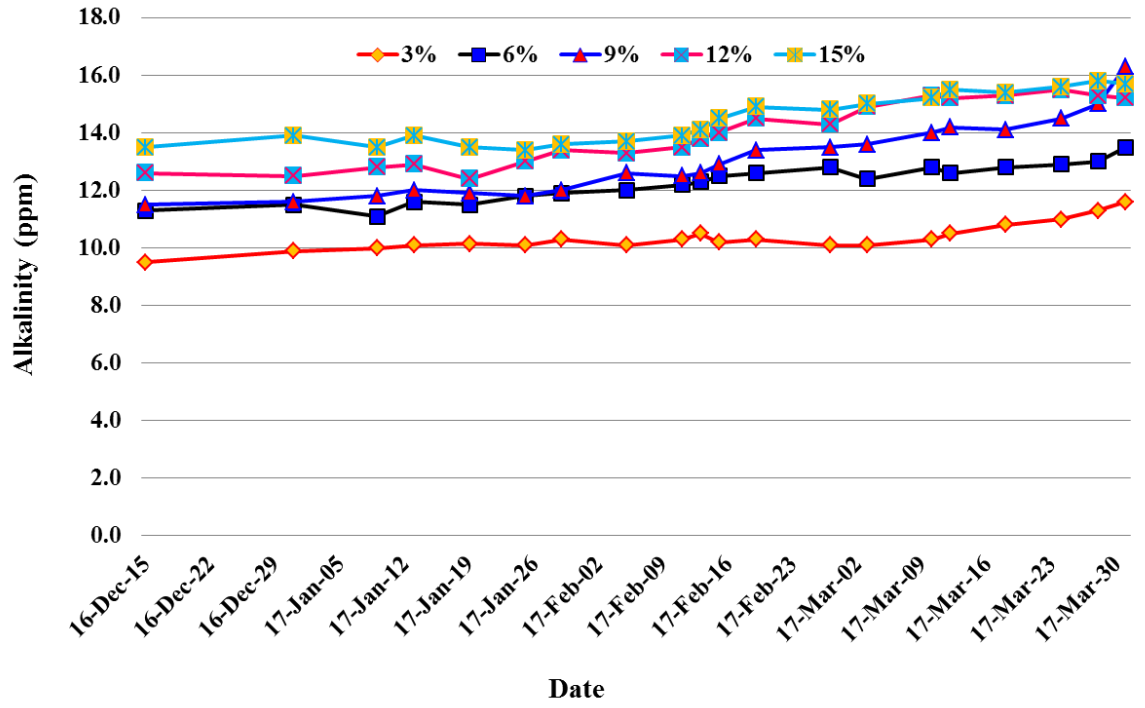

Fig. 24. Alkalinity variation in experimental bottles for different feeding rates from December 2016 to March 2017.

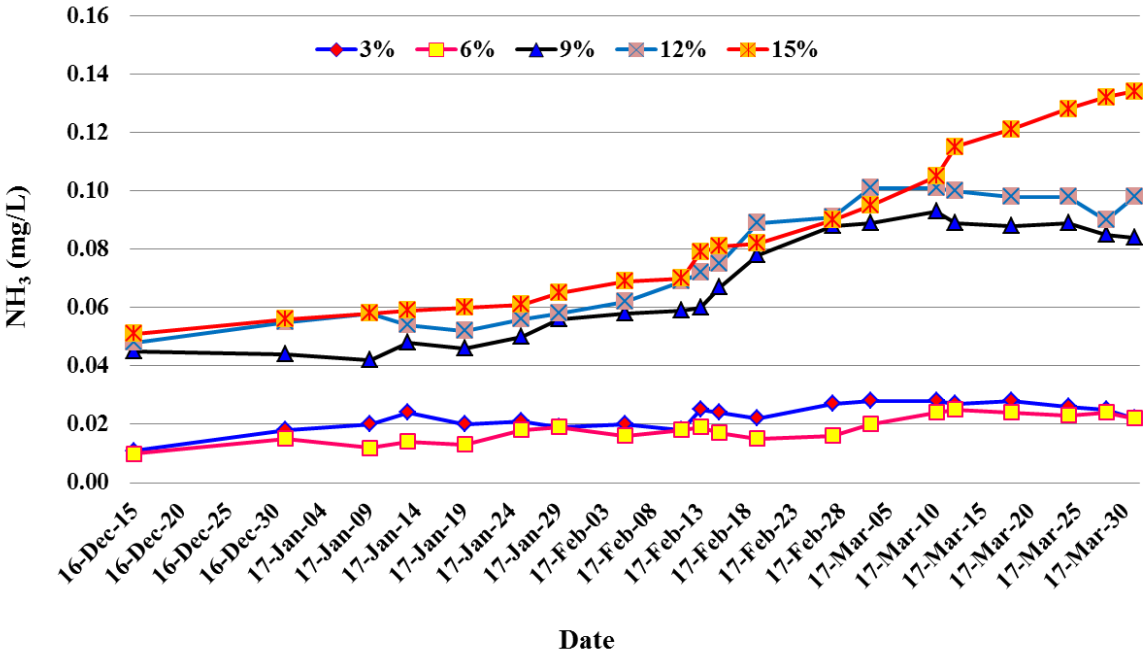

Fig. 25. Ammonia variation in experimental bottles for different feeding rates from December 2016 to March 2017.

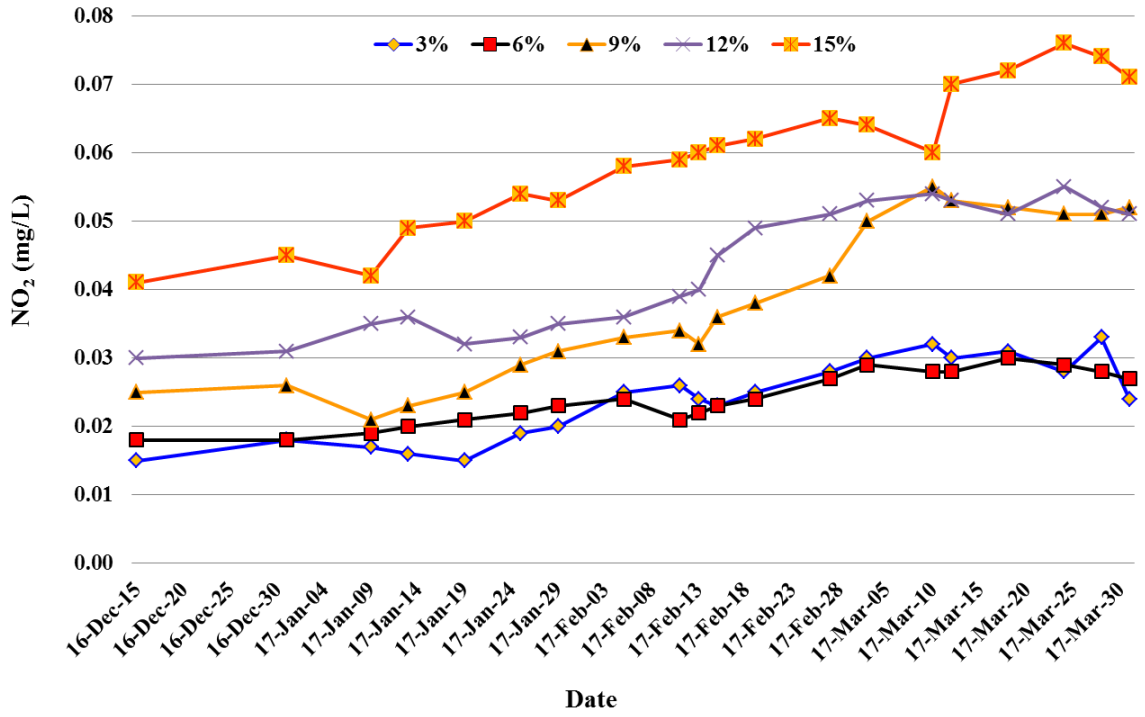

Fig. 26. Nitrite variation in experimental bottles for different feeding rates from December 2016 to March 2017.

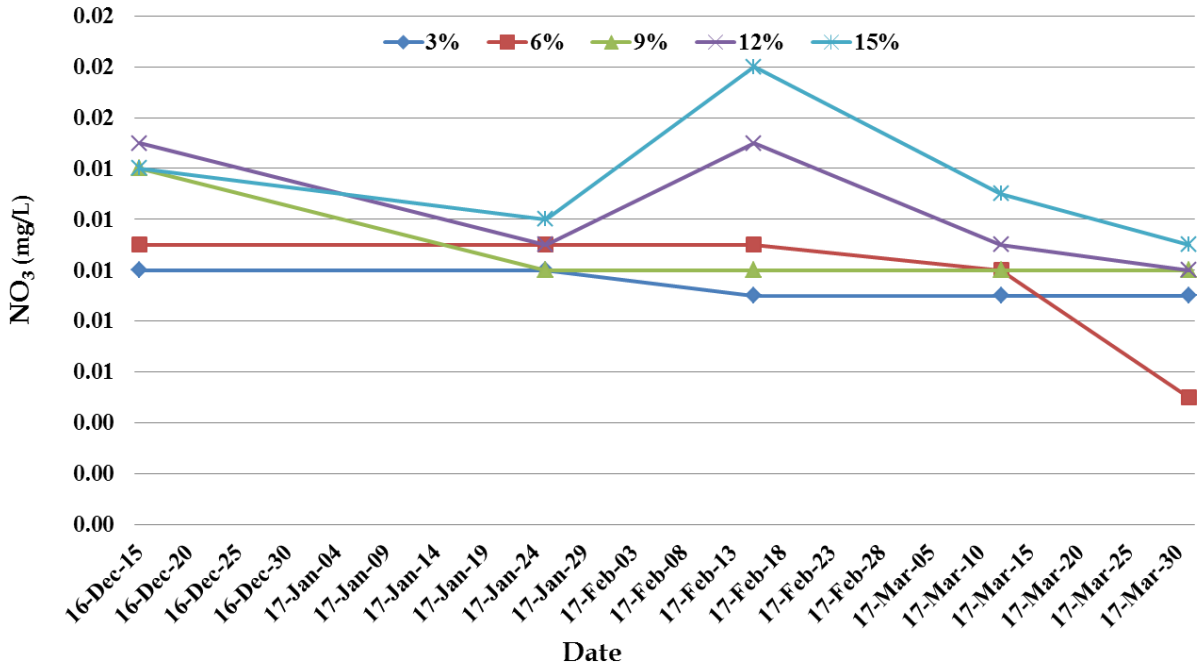

Fig. 27. Nitrate variation in experimental bottles for different feeding rates from December 2016 to March 2017.

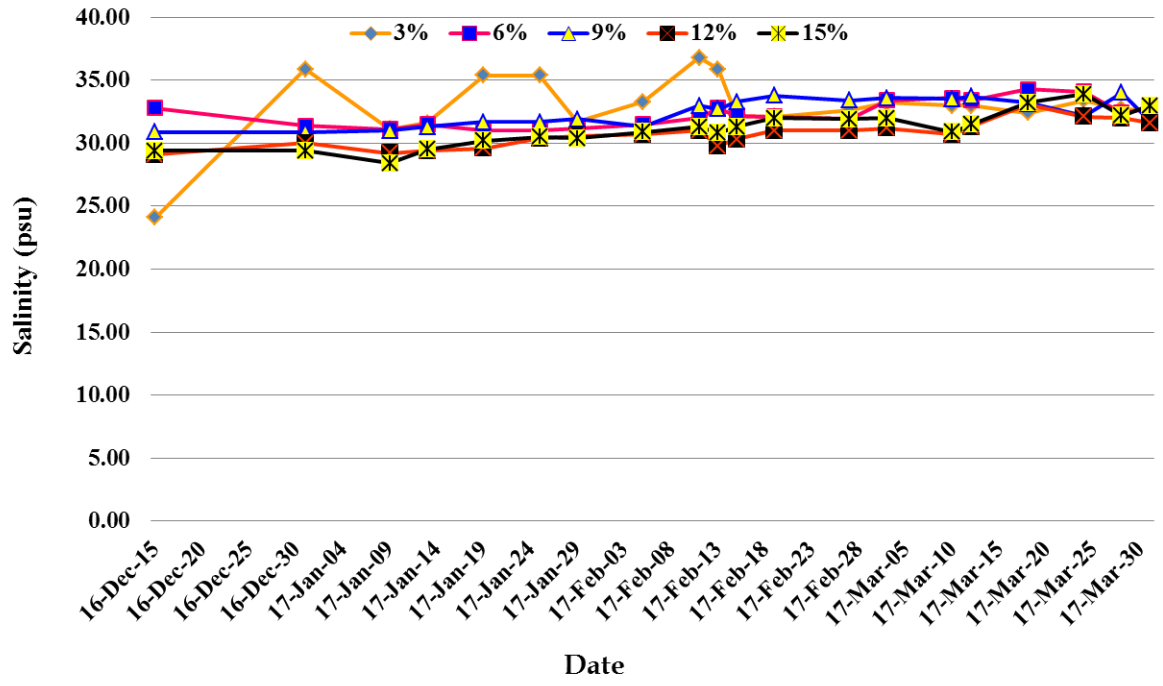

Fig. 28. Salinity variation in experimental bottles for different feeding rates from December 2016 to March 2017.

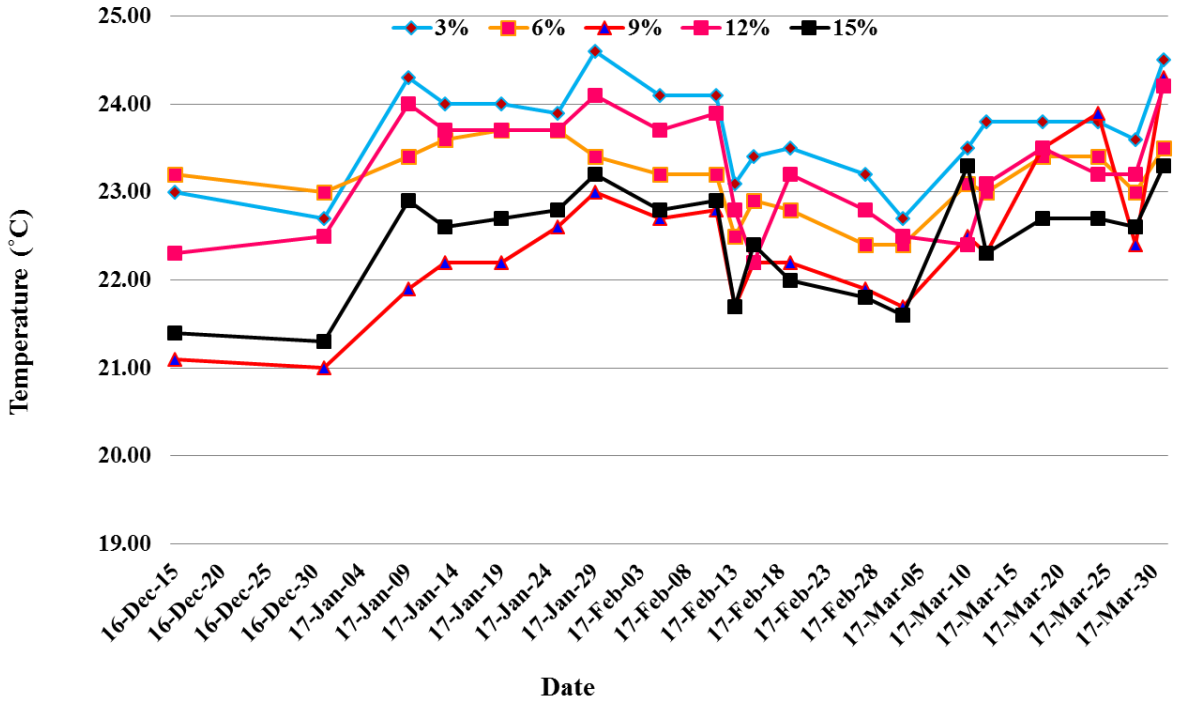

Fig. 29. Water temperature variation in experimental bottles for different feeding rates from December 2016 to March 2017.

3.6 Water quality analysis for different stocking densities

Trends of various factors of water quality tested in a biofloc tank and experimental bottles for different stocking densities are shown in Figs. 30 to 37 and Figs. 38 to 45, respectively.

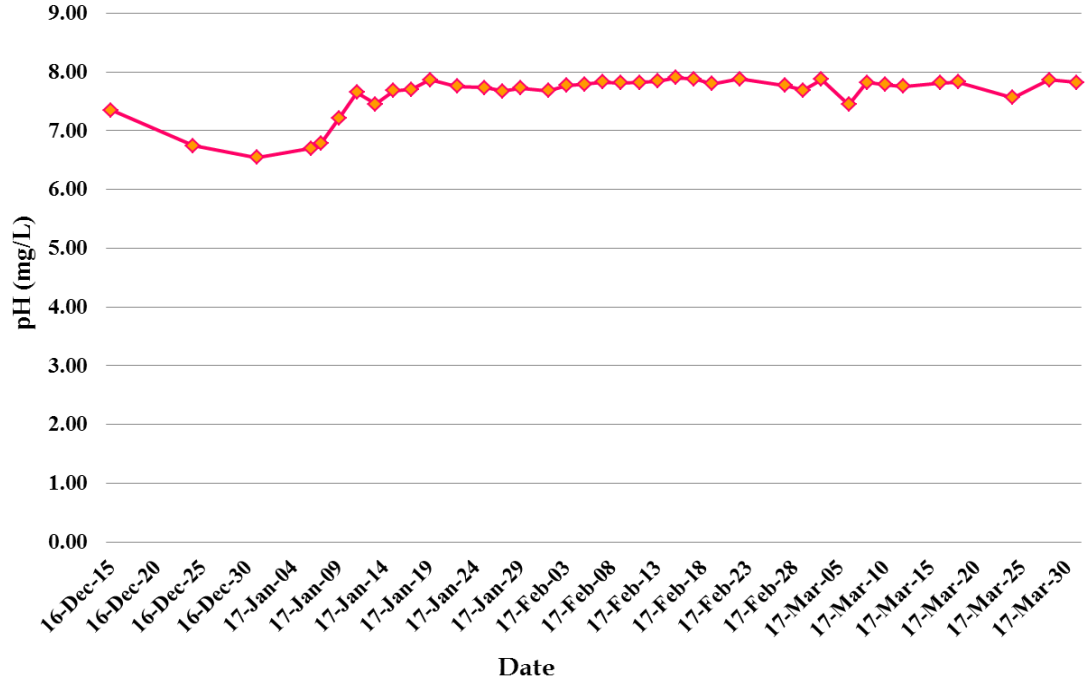

Fig. 30. pH variation in a biofloc tank for different stocking densities from December 2016 to March 2017.

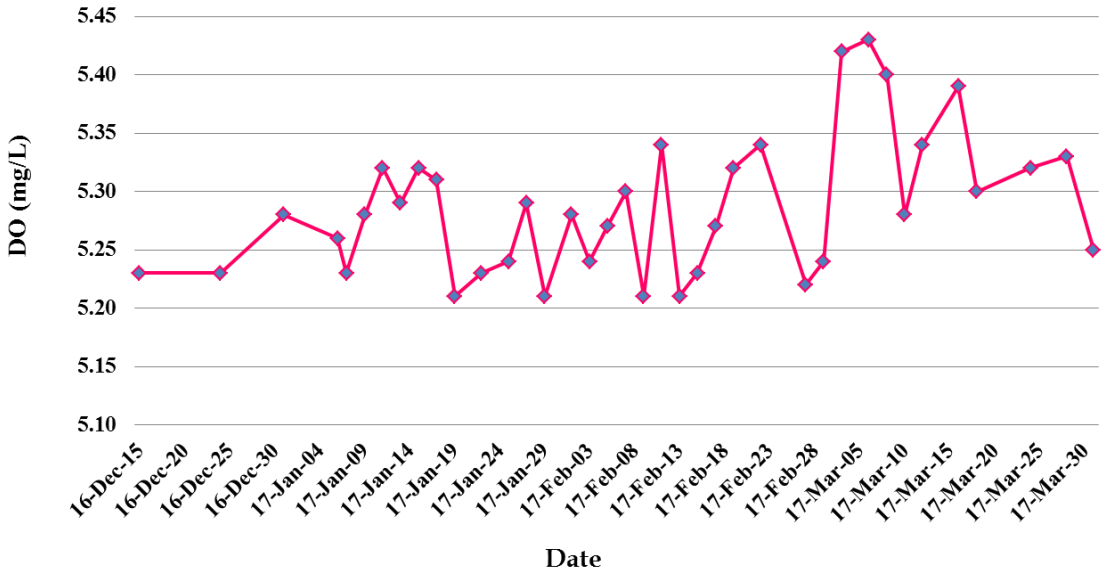

Fig. 31. Dissolved oxygen variation in a biofloc tank for different stocking densities from December 2016 to March 2017.

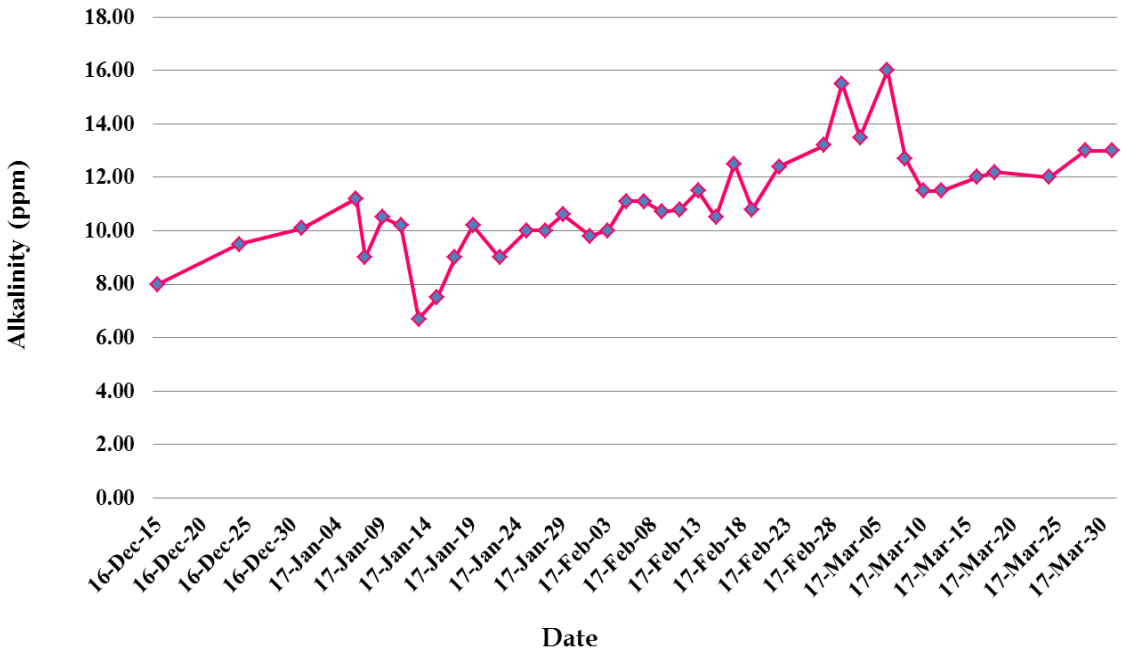

Fig. 32. Alkalinity variation in a biofloc tank for different stocking densities from December 2016 to March 2017.

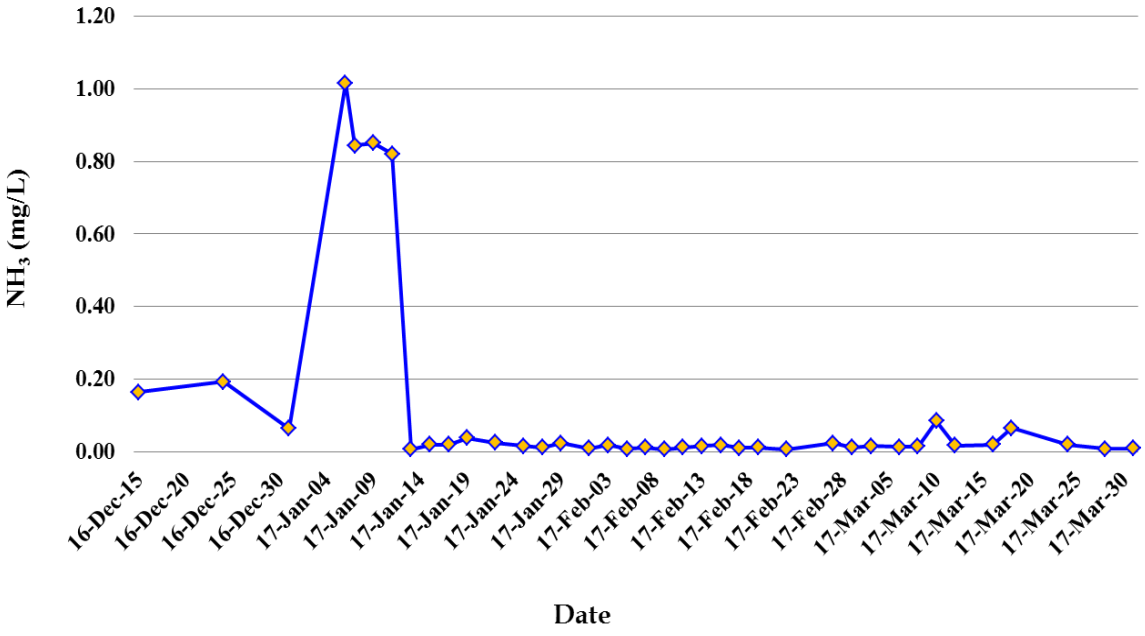

Fig. 33. Ammonia variation in a biofloc tank for different stocking densities from December 2016 to March 2017.

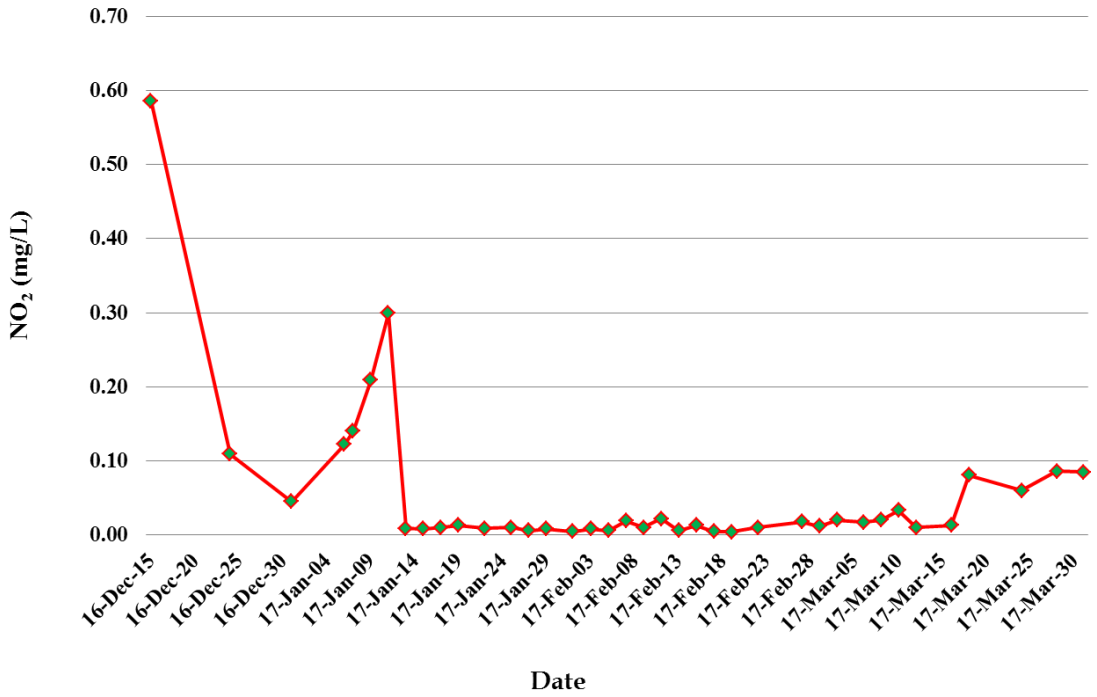

Fig. 34. Nitrite variation in a biofloc tank for different stocking densities from December 2016 to March 2017.

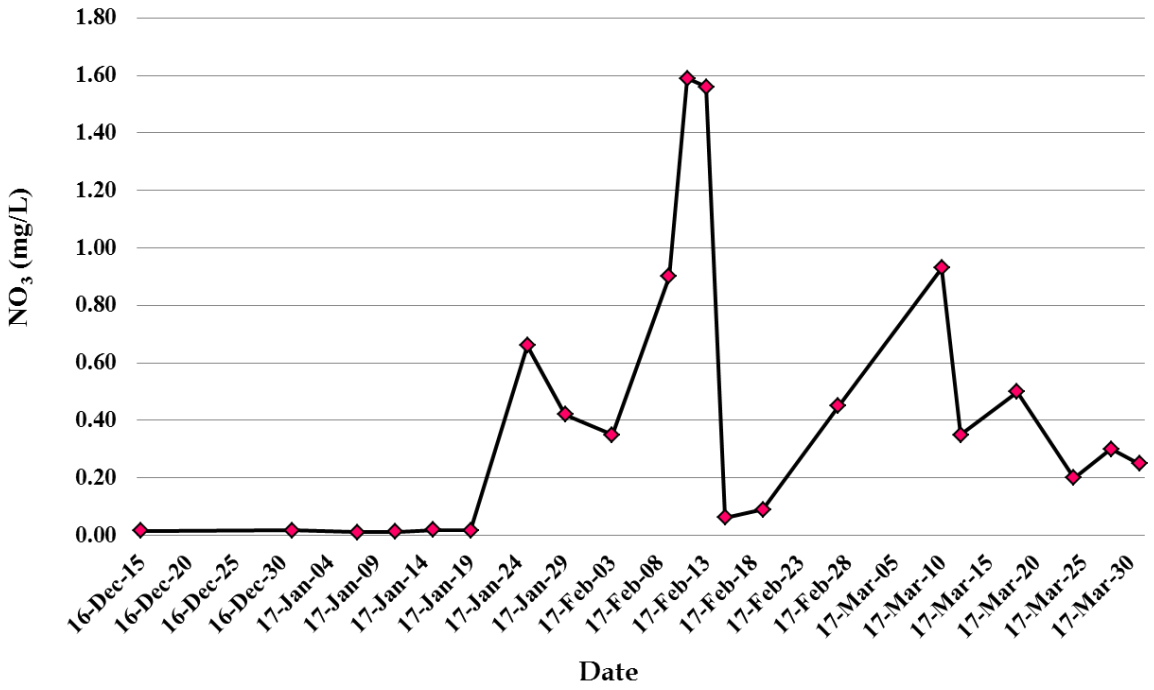

Fig. 35. Nitrate variation in a biofloc tank for different stocking densities from December 2016 to March 2017.

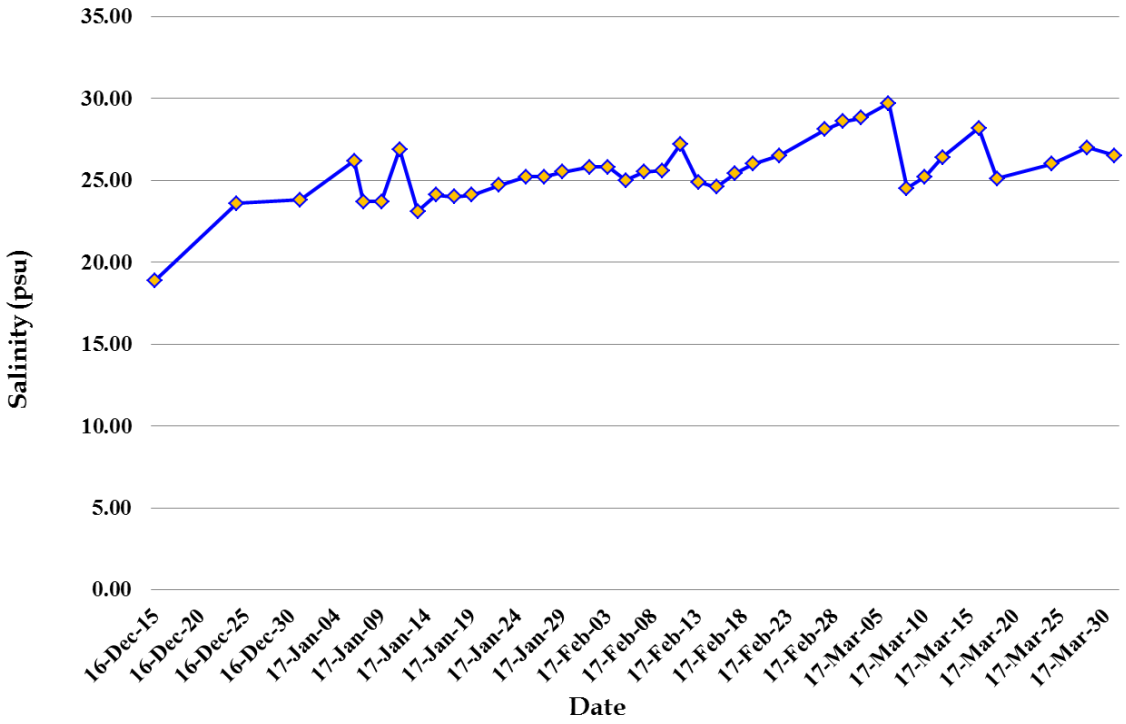

Fig. 36. Salinity variation in a biofloc tank for different stocking densities from December 2016 to March 2017.

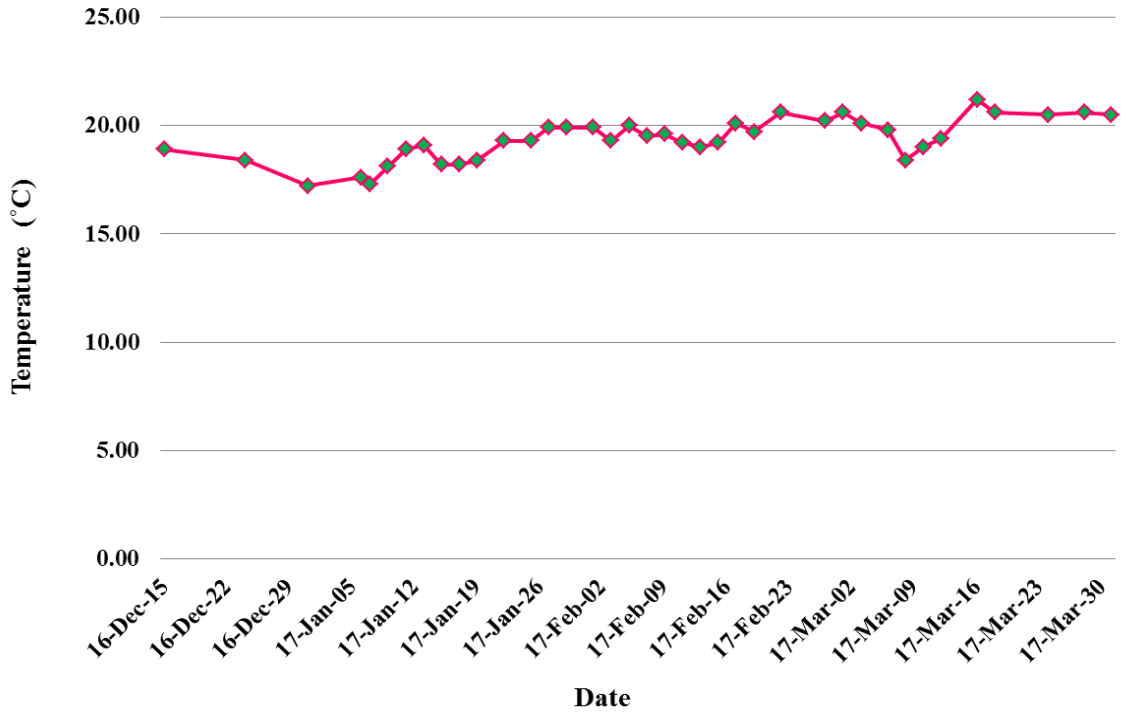

Fig. 37. Temperature variation in a biofloc tank for different stocking densities from December 2016 to March 2017.

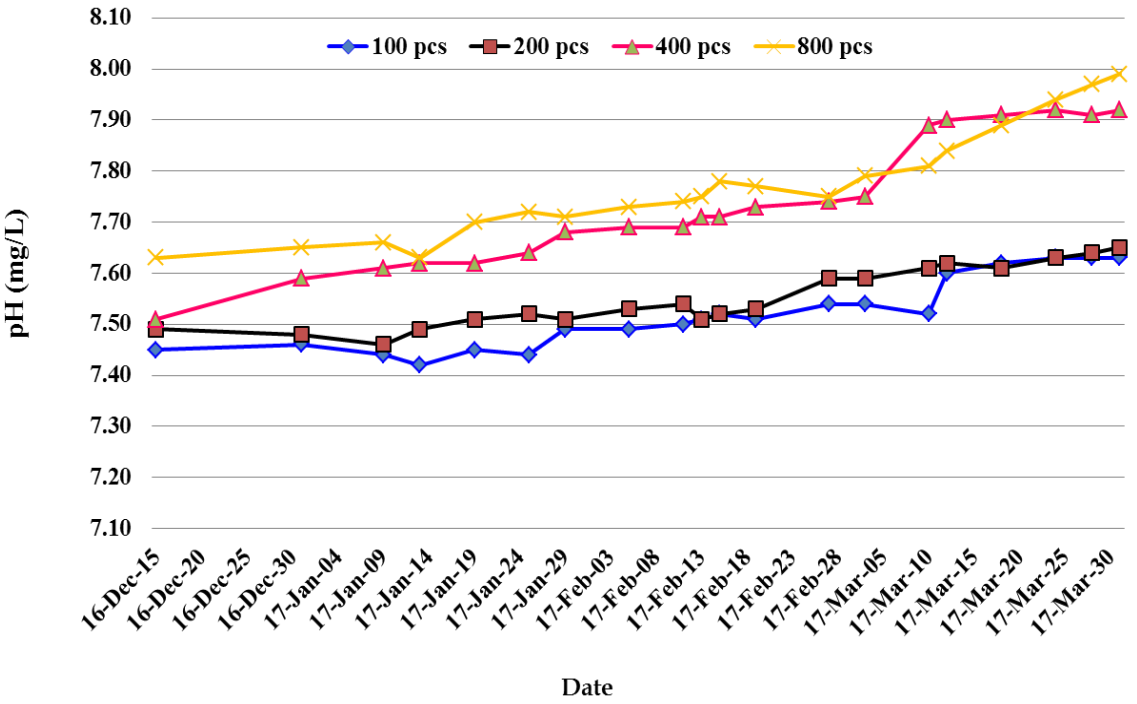

Fig. 38. pH variation in experimental bottles at different stocking densities from December 2016 to March 2017.

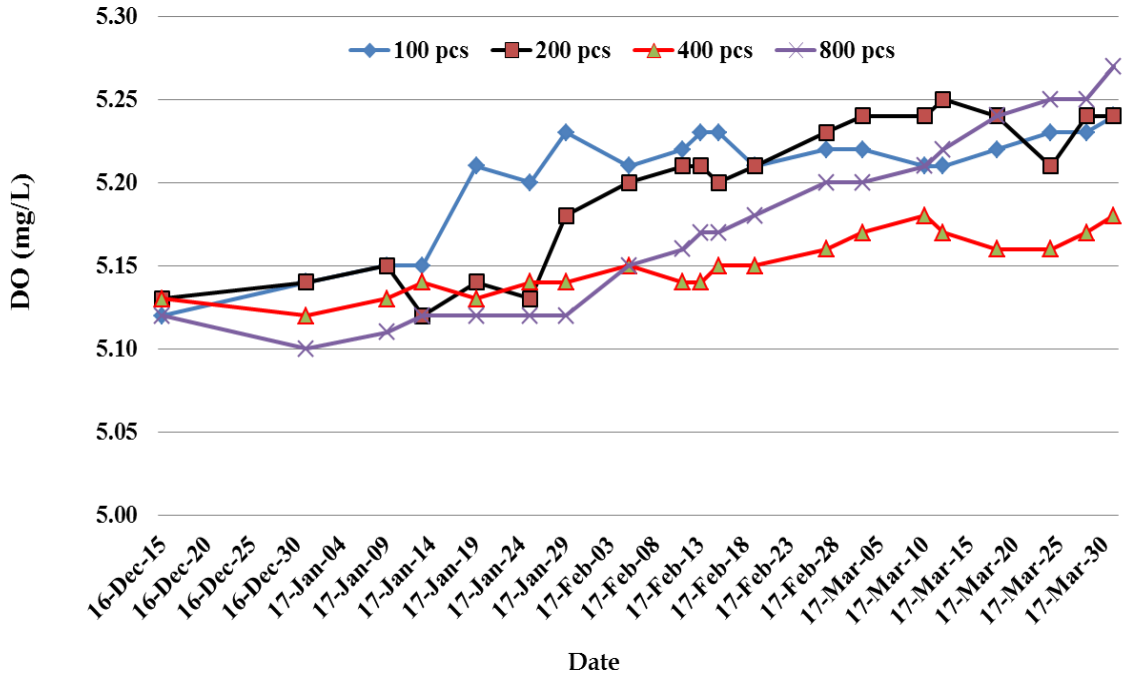

Fig. 39. Dissolved oxygen variation in experimental bottles at different stocking densities from December 2016 to March 2017.

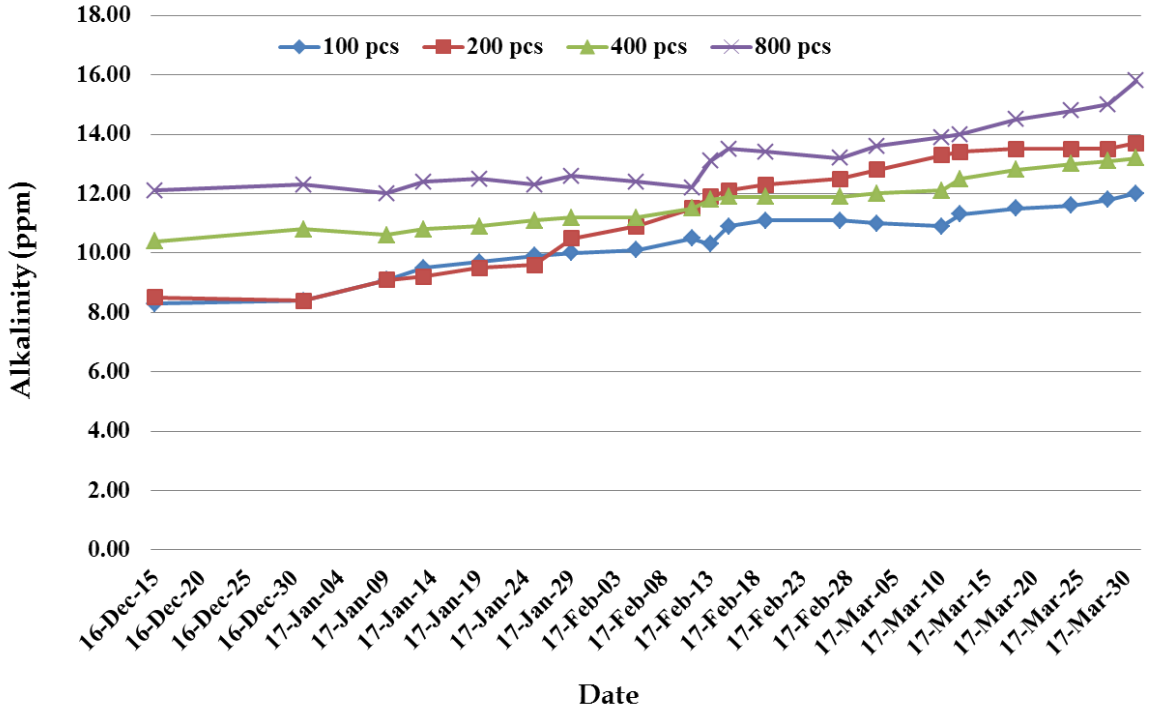

Fig. 40. Alkalinity variation in experimental bottles at different stocking densities from December 2016 to March 2017.

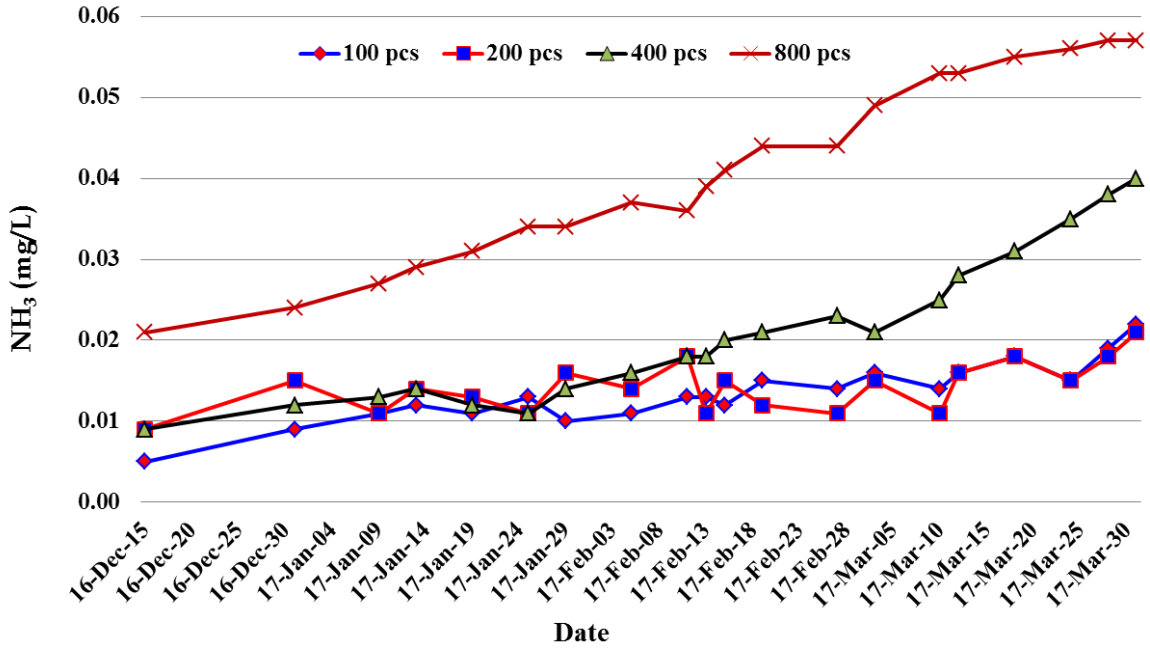

Fig. 41. Ammonia variation in experimental bottles at different stocking densities from December 2016 to March 2017.

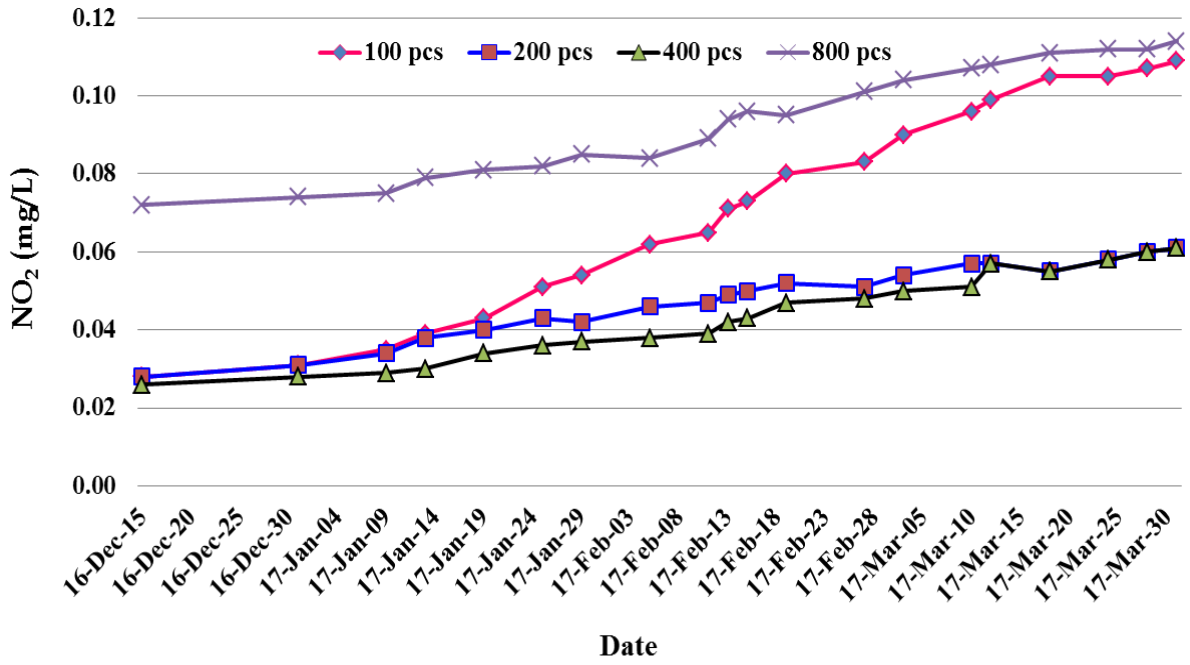

Fig. 42. Nitrite variation in experimental bottles at different stocking densities from December 2016 to March 2017.

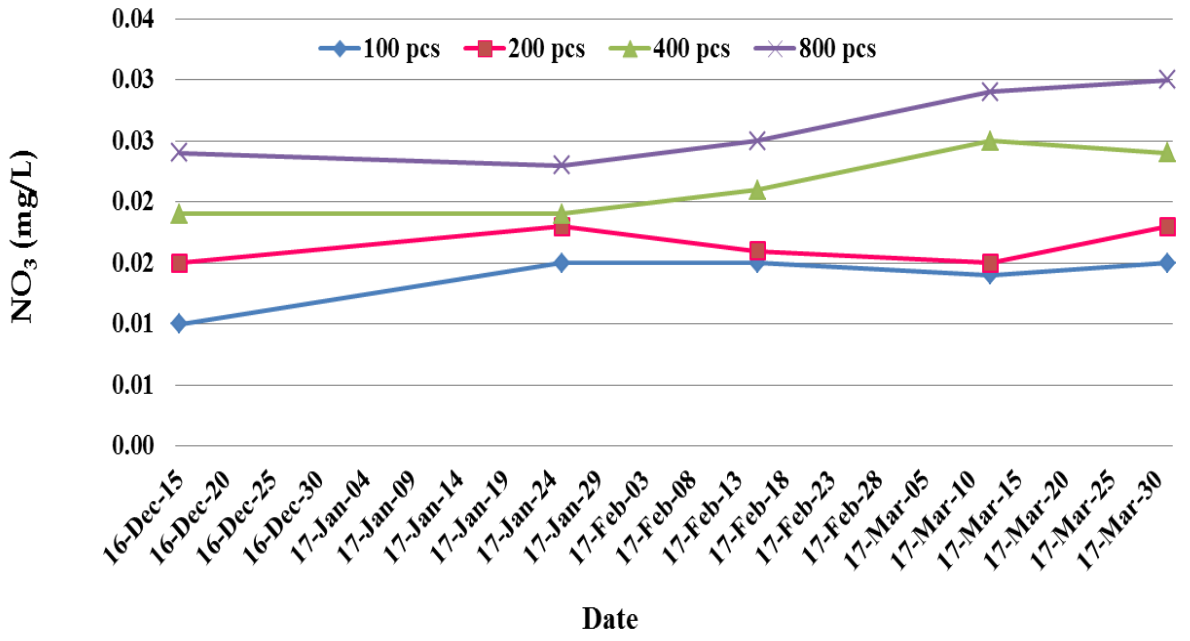

Fig. 43. Nitrate variation in experimental bottles at different stocking densities from December 2016 to March 2017.

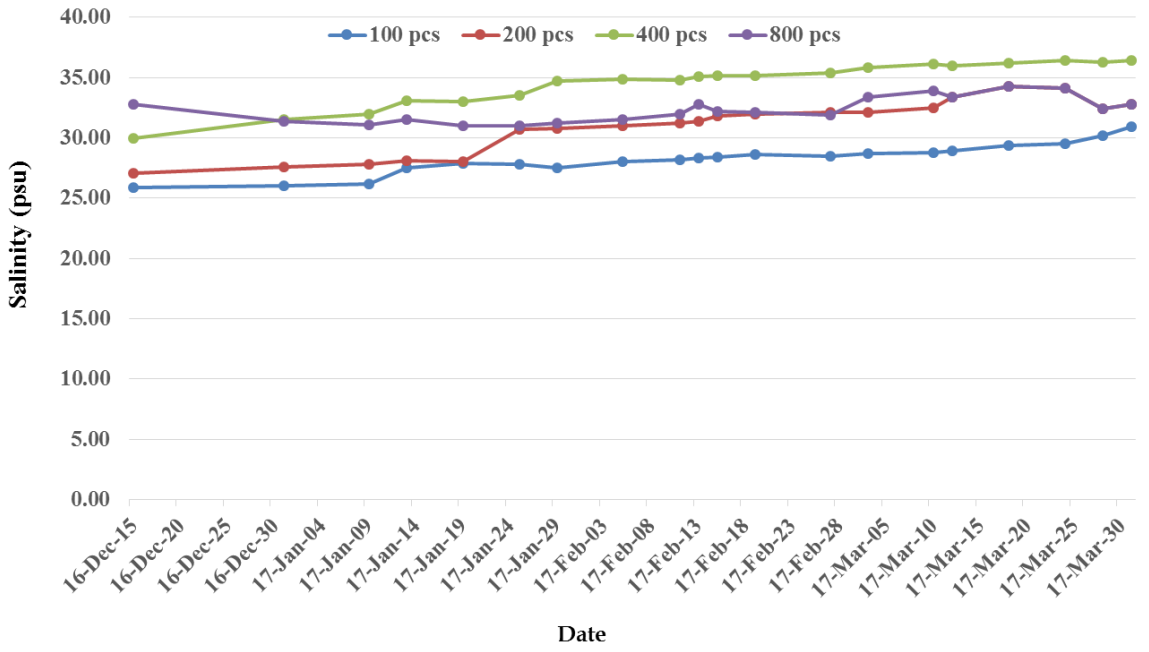

Fig. 44. Salinity variation in experimental bottles at different stocking densities from December 2016 to March 2017.

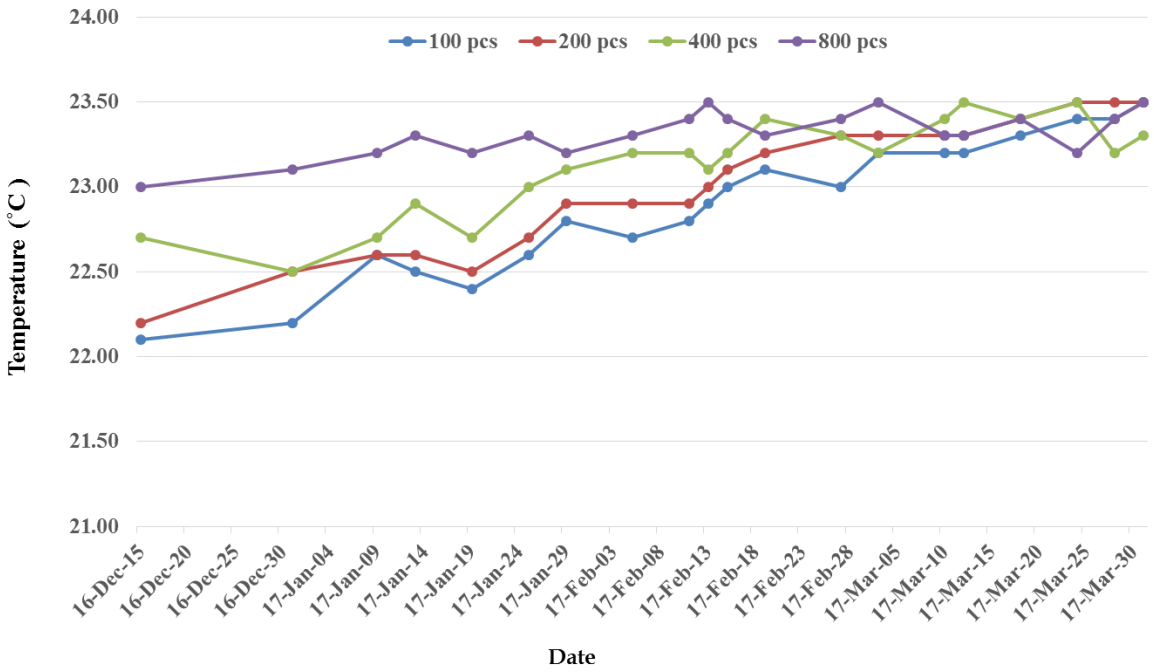

Fig. 45. Water temperature variation in experimental bottles at different stocking densities from December 2016 to March 2017.

4. Discussion

Results from the present study support the hypothesis that different feeding rates and different stocking densities were significantly affected on the growth and survival rate of *M. sanguinea*. Feeding frequency was one of the most important considerations in aquaculture practice that could affect overall growth, survival as well as habitat of aquaculture. Again, the optimization of feeding frequency was considered a significant factor, as profit was the main motivating reason in aquaculture (Ferdous, 2014). Proximate composition of the formulated diet was analyzed to verify the accuracy of the formulation. Biofloc consisted of crude protein 35-50%, crude lipid 0.6-12% and high ash 21-32% (Emerenciano *et al.,* 2012). Shrimp feed powder contained crude protein 50.0%, crude fat 7.0%, crude fiber 4.0% and crude ash 17.0% (Dong A One Corporation), and the optimum protein requirement for growth of polychaete worm was 17.33% (Pharmacopeia, 2007). Although the amount of feed application differed according to treatments, the same formulated diet was provided throughout the experimental period. Because the main purpose of this experiment was to evaluate the effectiveness of different feeding quantities.

In different feeding rates experiment: The values of water quality parameters such as water temperature, dissolved oxygen, pH, ammonia, nitrite, nitrate and alkalinity in different treatments were studied. The mean values of water temperature in different treatments were 23.68 ± 0.545, 23.14 ± 0.397, 22.405 ± 0.828, 23.235 ± 0.639 and 22.45 ± 0.618˚C in different feeding rates of 3, 6, 9, 12 and 15% of total body weight, respectively. The highest 24.6˚C and the lowest 21.0˚C of water temperature in the present study might be due to winter season. The amount of dissolved oxygen in the water was directly influenced by temperature and also affected metabolism in both microbial community and the cultured species, which determined worm growth aspects. In BFT setup, an intermediate water temperature of 20-25˚C could be the best to obtain stable flocs (Craig and Helfrich, 2002). Very low temperature was not good for worms, which did not survive at temperature below 10˚C for more than a few days (Nahar, 2015).

In the present study, daytime temperature did not fall below 18.3˚C during the experiment period. Dissolved oxygen varied from 5.05 to 5.38 mg/L with mean values of 5.192 ± 0.073, 5.191 ± 0.073, 5.209 ± 0.071, 5.173 ± 0.063 and 5.215 ± 0.070 mg/L in different feeding rates of 3, 6, 9, 12 and 15% of total body weight, respectively. 5 to 7 mg/L of dissolved oxygen content of water was considered to be fair or good in respect of productivity, and water having dissolved oxygen below 5 mg/L to be unproductive (Nahar *at*

al., 2015). Even slightly higher dissolved oxygen was found in the present study, but this low dissolved oxygen level did not give any negative effect because worms had a high tolerance to low dissolved oxygen levels. The pH values of pond water under different treatments were found to be alkaline, ranging from 7.34 to 8.02 with mean values of 7.52 ± 0.18, 7.78 ± 0.10, 7.72 ± 0.17, 7.83 ± 0.07 and 7.88 ± 0.11 in 3, 6, 9, 12 and 15% of total body weight, respectively. Notably, changes in pH directly influence the stability of both bioflocs present and cultured worm (Swingle, 1969). Swingle stated that pH range from 6.5 to 9.0 was suitable for aquaculture, while pH more than 9 was unsuitable. The pH was an environmental stressor which had leading effects on physiological functioning of some worms. It was very difficult to control pH in any given biofloc system probably due to different chemical and biological processes in BFT units. The effectiveness of BFT was also investigated for maintaining good water quality in over-wintering for the culture of worms. BFT was a more sustainable and environmentally friendly aquaculture system, which has been tried both at laboratory and commercial scale for various aquaculture (Emerenciano *et al.,* 2013). Therefore, information on biofloc parameters and their influencing factors could be important in the development of BFT and aquaculture at large (Nahar *et al.,* 2015).

Ammonia, nitrite and nitrate also had effects in this experiment. The highest level of NH_3 was 0.134 mg/L and the lowest was 0.01 mg/L. The highest level of NO_2 was 0.076 mg/L and the lowest was 0.015 mg/L. The highest level of NO_3 was 0.018 mg/L and the lowest was 0.009 mg/L. All these levels became high during experiment period. Ammonia was a toxic compound that could adversely affect worm health. The nature and degree of toxicity depended on many factors, including the chemical form of ammonia, the pH and temperature of the water, the length of exposure, and the life stage of the exposed worm (Rahman, 1992). Ammonia was also contained to a lesser extent in drilling substrates (used in exploration drilling). Issues associated with toxicity testing and the consequences of ammonia exposure were also discussed. Acute toxicity studies for ammonia followed standard guidelines that included exposure of worms to ammonia under static conditions using starved, resting, unstressed animals (Stephen *et al.,* 1985; Randall, 2002). However, toxicity tests might underestimate the toxicity of ammonia in natural environments because they were conducted in artificial conditions that minimize stress and internal ammonia levels (Ip *et al.,* 2001; Brinkman *et al.,* 2009). Among other biological changes, stress in worms increased ammonia production and ammonia toxicity (Randall *et al.,* 2002).

The maximum body weight gain (BWG) was observed in 15% of feed rate (0.028 ± 0.008) and the minimum BWG was observed in 9% of feed rate (0.010 ± 0.001). The specific

growth rate (SGR) was improved significantly ($P<0.05$) when the feeding quantity was increased. The growth data clearly indicated that SGR was observed in 15% of total body weight (0.081 ± 0.017). A significant difference was found at specific growth rate (%/day) among juvenile worms at feeding rate of 15% with other four different 3, 6, 9 and 12% of total body weight. The maximum of specific growth rate was observed at 15% feeding rate (0.344 ± 0.068), which had a significant difference from that of other groups ($P<0.05$), followed by 3% (0.302 ± 0.283), 6% (0.216 ± 0.090), 12% (0.190 ± 0.007). The lowest of specific growth rate was observed at 9% (0.046 ± 0.076), which was significantly different from that of other treatments ($P<0.05$).

The growth parameters were significantly affected by different feeding rates ($P<0.05$). The effect of different feeding rates on survival rate showed significant differences among treatments, and feeding rates had a positive effect on survival rate. The maximum of survival rate (100%) was obtained at 3 and 9% of total body weight. However, these treatments had no significant differences. The lowest survival rate was observed at 15% of feeding rate (81.9 ± 4.2) and was significantly different from other treatments ($P<0.05$). Although 15% of feeding rate had maximum growth weight gain, but its survival rate of 86.42% was less than 3, 6, 9 and 12% of total body weight. Effect of feeding frequency on survival rate showed significant differences among treatments, and feeding frequency had a positive effect on survival rate. No effects of feeding frequency was also reported on survival rate. Feeding frequencies did not significantly affect survival rates on sea bream (*Sparus aurata)* and on juvenile black sea turbot (*Psetta maxima*) (Nekoubin, 2012).

In this condition, the survival rate and specific growth rate, and the effect of high feeding rate on worm survival rate might be dependent upon the biological characteristics. Such as tolerance to environmental change, life stage, social interaction and behavior might be explainable by their competition for territories, as the high feeding had an effect on growth and production (Palomino, 2001).

Internal ammonia levels increased when acute toxicity tests were carried out on Environmental Protection Agency (EPA) guidelines (Randall *et al.*, 2002; Stephen *et al.*, 1985). Therefore, unfed worms may be more sensitive to external ammonia than fed worms (Randall *et al.*, 2002). The toxicity of ammonia may be affected by combinations of biotic factors (e.g., quiescent water in exposure tanks, feeding of test organisms, and other stressors) (Brinkman *et al.*, 2009).

But the results also indicated that more study was needed to determine the influences of other factors (such as quiescent exposure waters and combinations of other stressors) along

with ammonia toxicity to worms in the bottles (Brinkman *et al.,* 2009). In response to concentrations of ammonia, some worms have been shown to suffer impaired performance of the startle-escape response. In this study, the only two performance variables that were not affected by ammonia were responsiveness (an indicator of acoustic or visual sensitivity and motivation to escape) and directionality (an indicator of overall sensory performance) (McKenzie *et al.,* 2008).

Nitrite entered a worm culture system after feed was digested by worms and the excess nitrogen was converted into ammonia, which was then excreted as waste into the water. Total ammonia nitrogen (TAN; NH3 and NH4+) was then converted to nitrite (NO_2) which, under normal conditions, was quickly converted to non-toxic nitrate (NO_3) by naturally occurring bacteria. Uneaten (wasted) feed and other organic material also broke down into ammonia, nitrite, and nitrate in a similar manner. Nitrite problems were typically more likely to arise in closed, intensive culture systems due to insufficient, inefficient, or malfunctioning filtration systems. High nitrite concentrations in ponds occurred more frequently in the fall and spring, when temperatures were fluctuating, resulting in the breakdown of the nitrogen cycle due to decreased plankton and/or bacterial activity. A reduction in plankton activity in ponds (because of lower temperatures, nutrient depletion, cloudy weather, herbicide treatments, etc.) can result in less ammonia assimilated by the algae, thus increasing the load on the nitrifying bacteria.

In this experiment, poor survival of polychaete worm was observed at 15% of feeding rate, and the highest survival rate was found at 3% and 9%. But the rate of specific growth rate was not so much high compared to other feeding rates. Generally, this feeding frequency was optimal for the condition of survival rate and feed utilization was the most efficient at this rate of feeding (Jamabo, 2015). According this result, it was pertinent to consider the prevailing physio-chemical characteristics (water temperature, pH, Dissolved Oxygen) that caused worms not to eat well. The minimum level of specific growth rate was at 3% and 9% of feeding rates.

Results from the current study indicated that feed treatments influenced some water parameters such as ammonia, nitrite and nitrate, whereas only biofloc feed in the condition was not enough to improve the growth rate and percent survival of the rockworm polychaete *Marphysa sanguinea* juvenile. Hence, it is suggested that a mixture with artificial feed is a good tool for well growth and will maintain the percent survival and development of juvenile worm in artificial conditions, for the benefit of the culture management of this juvenile worm.

The second experiment: Juvenile worms were stocked at four densities with 0.55%, 1.11%,

2.22% and 4.44% rates, and water exchange depended on the feeding amount for 3 months experiment. In the beginning of the experiment, mean weight was not significantly different among the densities at stocking rate ($P<0.05$). The overall averages of juveniles were 0.154 ± 0.013 g, 0.129 ± 0.001 g, 0.198 ± 0.031 g and 0.206 ± 0.026 g in weight. At the end of the experiment, the specific growth rate ranged between 0.613 ± 0.070 g at the lowest stocking density (100 inds/bottle) and 0.601 ± 0.277 g at highest stocking density (800 inds/bottle). The overall data of specific growth rate (%/day) and survival of juvenile worm reared at four stocking densities and water exchange rate for a period of three months in 20 L bottles are presented in Table 4. It can be concluded from this table, specific growth rate (%/day) and survival were the best ($P<0.05$) at the lowest stocking density. The results of the analyses of variance of survival rates were showed highly significantly ($P<0.05$) affected by stocking densities and water change rates.

The number of surviving worm was the most important consideration in BFT culture system of polychaete juvenile. The mean survival of worm in different stocking varied from 48.43% to 91.5%. There was no significant ($P<0.05$) difference in survival of worms among different treatments.

The values of water quality parameter such as water temperature, dissolved oxygen, pH in different treatments were checked every 2 days. The mean values of different water temperature were 22.8˚C, 23.01˚C, 23.12˚C, 23.31˚C in 100 inds, 200 inds, 400 inds and 800 inds, respectively. The highest 23.31˚C and the lowest 22.8˚C water temperature in the present study might be due to the winter season. The amount of dissolved oxygen in the water was directly influenced by temperature and also affected metabolism in both microbial community and the cultured species, which determined worm growth aspects. In BFT setups, an intermediate water temperature of 20-25˚C could be the best to obtain stable flocs proposed (Craig and Helfrich, 2002).

Dissolved oxygen (DO) varied from 5.15 to 5.20 mg/L with mean values of 5.20 ± 0.034, 5.19 ± 0.04, 5.15 ± 0.017, 5.17 ± 0.053 in 100 inds, 200 inds, 400 inds and 800 inds, respectively. Dissolved oxygen (DO) of 5.15 to 5.20 mg/L of dissolved oxygen content of water was considered to be fair or good in respect of productivity, and water having dissolved oxygen below 5 mg/L to be unproductive. The pH values of pond water under different treatments were found to be alkaline, ranging from 7.51 mg/L to 7.77 mg/L with mean values of 7.64 mg/L in 100 inds, 200 inds, 400 inds and 800 inds, respectively. Notably, scientists concurred that changes in pH directly influenced the stability of both bioflocs present and experimental worm culture in the bottles. The pH was an environmental stressor which had a

leading effect on physiological functioning of worms. It was very difficult to control pH in any given biofloc system probably due to different chemical and biological processes in BFT units. The effectiveness of BFT was also investigated for maintaining good water quality in winter season for experiment (Nahar *et al.,* 2012).

Moreover, the growth performance of polychaete was significantly related to the stocking density of worms (Table 4). The results from this study showed that there was an inverse relationship between density and growth potential of worms when it was stocked at a very high density. But interestingly, at a very low density (100 inds), growth rate and survival rate were higher than at the stocking density of 200 inds and 400 inds. The maximum growth of worm was obtained at the stocking level of 800 inds/bottle, but survival rate was 48.44%.

It was observed that increased biomass of worms in bottles had a significant positive effect on the final mean body weight. Worms stocked in bottles at lower density showed better growth than at higher density. The lower growth performance of worms at higher stocking density could have been caused by voluntary appetite suppression, more expenditure of energy caused by intense antagonistic behavioral interaction, and competition for food and living space and increased stress. It was also reported that increasing stocking density of worms might have led to diminishing social dominance, resulting in lower individual growth rates (Gaber *et al.,* 2012). Furthermore, stress due to reduction in space availability was reported to be the primary factor for growth inhibition in other fish like summer flounder (*Paralichthys dentatus*) stocked at high densities (King *et al.,* 2000). It has been suggested that high stocking densities could lead to poor water quality (high ammonia, high nitrite, low oxygen) which in turn led to reduced growth performance (Brett, 1979; Pickeringn and Pottinger, 1987; Kebus *et al.,* 1992; Kindschi and Koby, 1994; Wagner *et al.,* 1995).

It was extremely unlikely that poor water quality was a factor in this study. First, because all treatments were associated with the same recirculating water and biological filter, water quality was probably identical in all treatments. Second, non-ionized ammonia never exceeded 0.05 ppm and dissolved oxygen never fell below saturation. Lastly, no loss of appetite was found in any of the treatments that could indicate poor water quality and/or stress. Thus, the recirculating system and biological filter were capable of maintaining ammonia below and dissolved oxygen above normally stressful levels. The systems in which the experiment eliminated two of the variables (high ammonia, low oxygen) were often associated with high stocking densities. Apart from water quality issues, food consumption and feeding behavior might also be affected by stocking density (Holm *et al.,* 1990; Martinez-Tapia and Fernandez-Pato, 1991). In such instances, high number of worms per unit

area could cause an increase in agonistic feeding behavior, which in turn increased stress and decreases growth (Gaber, 2012). On the other hand, low stocking densities, lack of competition for food and/or social hierarchy could lead to decreased feed utilization efficiency resulting in stunted growth. Here, the difficulty of tracing food particles may have led to the reduction of feed consumption, and to the flushing of uneaten food with the drainage water, causing the deterioration of feed utilization efficiency. Such wastage of feeding materials could increase the production cost to a great extent, and the gross yield at such low densities was too poor to compensate the cost (Suman, 2010).

Hence, an optimum density level in terms of economic viability of worm culture must be established. Thus, this study is to help postulate an optimum stocking density level of worms for maximum utilization of food and space with minimum stress and energy expenditure resulting in higher growth potential of the worms.

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