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Abstract

The aim was to assess the efficacy of prophylactic health products (PHPs) on shrimp (*Penaeus monodon*) post larvae (PL) during the nursery phase. This included five treatments: in-feed probiotic (F_PRO), water probiotic (W_PRO), a combination of water and in-feed probiotic (FW_SYN), biofloc, a combination of biofloc and in-feed probiotic (F_PRO& biofloc), and a no treatment control, five replicates each. Each tank was filled with 450 liter water and stocked with 700 PL15, weight 0.008±0.00 g at a density of 1.56 PL/L, reared for 27 days. There were no significant differences in water temperature, dissolved oxygen, alkalinity, and total ammonia nitrogen among treatments and control. pH and nitrite were lower in biofloc and F_PRO&biofloc compared to other treatments. The final body weight, weight gain, specific growth rate and food coversion ratio were significantly higher ($p \leq 0.05$) in F_PRO& biofloc compared to other treatments and control. A salinity stress test showed a siginificantly higher survival rate in F_PRO& biofloc followed by biofloc than other treatments and control. Our study indicated that rearing shrimp in biofloc alone or combined with in-feed probiotic might increase growth and resistance to environmental stressors. Further on-farm trials are required to confirm the efficacy of the PHPs.

Key words: Probiotics, Biofloc, *Penaeus monodon*, Nursing, Bangladesh

1. Introduction

Declining capture fisheries and growing domestic and international demand for seafood is driving intensification of smallholder aquaculture throughout much of Asia and Africa. A need to optimise returns from small production units makes small-scale aquaculturists particularly vulnerable to changes in input costs and efficiencies and a receptive audience for independent advice. Most critically, this intensification brings serious health management challenges for smallholders with limited diagnostic capacity. Consequently, they resort to prophylactic treatments, including probiotics, prebiotics and immunomodulators, of uncertain provenance and efficacy also raising environmental, animal and consumer health and safety concerns (Rico et al., 2012; Rico et al., 2013). Here, these are referred to as prophylactic health products (PHPs).

Bangladesh is one of the most suitable countries for aquaculture production because of its favorable biophysical resources and agro-climatic conditions (Ahmed, 2013). In which the costal aquaculture is dominated by black tiger shrimp (*Penaeus monodon*) farming. Rapidly growing domestic and export market demand for seafood products are driving intensification of aquaculture, led by smallholder farmers (Henriksson et al., 2018; Little et al., 2018). This intensification brings serious health management challenges, particularly for smallholders (Ali et al., 2016; Ali et al., 2018) and restrictions on antibiotic use are being imposed (Bondad-Reantaso et al., 2005). Over the last few years, farmers in Bangladesh like other places have become heavily dependent on a proliferating range of PHPs.

Probiotics are the most widely used PHPs in aquaculture for disease prevention, particularly bacterial diseases via competitive exclusion and immunomodulation (Hai, 2015; Hoseinifar et al., 2018). Probiotics are also considered an environmentally safe alternative compared to the prophylactic use of antibiotics. Previous studies showed that PHPs could play an important role in maintaining water quality, soil quality and health as well as increasing the growth and

74 survival rate of shrimp (Decamp et al., 2008; Wang et al., 2008). However, the provenance and
75 efficacy of PHPs is often uncertain and the economic burden of unjustified claims is likely to
76 fall most heavily on smallholders (Ali et al., 2016). Therefore, the effectiveness of such
77 products should be assessed in order to guide the farmers on which PHPs to be used and at
78 what stage of the production cycle. Furthermore, there are no regulatory frameworks, and
79 consequently no quality assurance, for the production and marketing of these products. The
80 lack of knowledge about the effectivness of such products in aquaculture particularly by
81 smallholder farmers is a limiting factor for the appropriate use (Ali et al., 2018).
82 The biofloc system is a revolution in aquaculture; it exploits the proliferation of beneficial
83 microorganisms to maintain water quality and provide better nutrition to cultured organisms
84 (Emerenciano et al., 2017). Biofloc stimulates heterotrophic microbial growth that assimilates
85 nitrogenous waste that can be utilized as a feed for the cultured aquatic organisms (De Schryver
86 et al., 2008). It also keeps nitrogenous waste below toxic levels and improves the feed nutrient
87 utilization efficiency (Crab et al., 2009), and provides extra essential nutrients (Xu et al., 2012)
88 and exogenous digestive enzymes (Xu and Pan, 2012). The application of biofloc has
89 significantly increased the survival and growth of pink shrimp post larvae production
90 (Emerenciano et al., 2011). Microorganisms developed in biofloc system provide protection
91 against infection via competition with pathogenic organisms (Crab et al., 2010; Emerenciano
92 et al., 2017).
93 A systematic surveys of shrimp grow-out farms, hatcheries and aqua shops found over 200
94 PHPs in Bangladesh (IMAQulate project, unpublished data). Although some proprietary and
95 academic studies have been conducted to evaluate the effectiveness of PHPs on the culture of
96 aquatic organisms, the efficacy of their application at shrimp juvenile stage is not fully
97 understood. In this study, the juvenile production stage was identified as a key intervention
98 point because: i) there is a high mortality rate at the juvenile production stage, ii) poor quality

juveniles are likely to compromise grow-out performance, iii) the cost of application of PHPs at juvenile production stage is lower at the juvenile production stage compared to grow-out phases, and iv) there is a growing trend of extended nursing of shrimp juveniles from 3 to 4 weeks in more biosecure lined/covered ponds (IMAQulate project, unpublished data). Therefore, the aim of the present study was to assess the effectiveness of the individual and a combined use of probiotics against 'no-treatment' control and a biofloc system during an extended nursing period.

2. Materials and methods

2.1 Study site and experimental animal

The experiment was conductecd at Radiant Hatchery and Culture Ltd., Noabeki, Shayamnagar, Satkhira, Bangladesh (22°21'17.0"N, 89°12'13.2"E). Specific pathogen free (SPF) post larvae (PL15), with an average weight and length of 0.0077±0.0031 g and 1.30±0.14 cm, respectively, were collected from MKA hatchery, Cox's Bazar district (21°16'50.4"N, 92°02'58.0"E). PLs were acclimated to a salinity of 10 in tanks for 24 hours prior to transfer to the experimental tanks.

2.2 Experimental design

PLs were randomly allocated into a negative control group with no addition of PHPs and five treatment groups namely in-feed probiotic (F_PRO), water probiotic (W_PRO), a combination of in-feed & water probiotic (FW_SYN), biofloc and a combination of in-feed probiotic and biofloc (F_PRO & biofloc), respectively (Table 1). Treatments were unknown (blind) for the nursery technician and lab technician to avoid bias. Thirty fiberglass tanks of 600 L capacity were used as experimental units, with 5 replicates per treatment. Each tank was filled with 450 L water and stocked with 700 PLs at a density of 1.56 PL/L then reared for 27 days. Growth,

survival, feed conversation ratio (FCR), final biomass, final weight and length were recorded at the end of an extended nursing phase.

2.3 Tanks preparations

Fiberglass tanks were cleaned, disinfected and filled with 450 L water of 10 salinity. Around 10% volume of water was exchanged with fresh water weekly in all treatments for 27 days except BFT and F_PRO&biofloc treatments (zero water exchange). Tanks were randomly allocated for F_PRO, W_PRO, FW_SYN, BFT, F_PRO& biofloc and control after stocking with PLs.

2.4 PHPs and biofloc

For this trial, PHPs were selected based on previously developed tool 'pedigree analysis tool-IMAQUlate project', in which PHPs were assessed according to the following factors: use-prevalence, declared manufacturing quality assurance certification, declared mode of action, active ingredient composition, and concentration . The latter two factors were assessed by through laboratory analysis and compared against manufacturer declarations on the product packaging. The PHPs were selected from 'high-scoring' products that were assessed using the pedigree analysis tool. The dose, frequency and mode of application of probiotics (Table 1) were administered according to the manufacturer's instructions. In-feed probiotic was added as 5g/kg feed every meal for F_PRO, FW_SYN and F_PRO&biofloc treatments. In-feed probiotic powder, a mixture of *Bacillus subtilis, Bacillus licheniformis, Bacillus pumilus, was mixed to the feed 30 minutes before every meal.* Water probiotic, *Bacillus subtilis, Bacillus licheniformis*, was added as 1 g/1000 L of water once a day for W_PRO and FW_SYN treatments. The water probiotic powder was first added to one litter of tank water and kept under aeration, one hour before adding to the tanks according to the manufacturer instructions. In biofloc and F_PRO&biofloc treatments, sucrose was added 3 times per day as an external

source of carbon (C) to adjust the Carbon (C): Nitrogen (N) ratio at 16:1. The amount of C and N in feed and the consequent amount of sucrose were calculated according to Emerenciano et al. (2012). The amount of sucrose was calculated every day based on the amount of feed and the adjusted C:N ratio.

2.5 Feed and feeding management

For the first three days of the experiment, PLs were fed on *Artemia* and commercial feed "FRIPPAK Raceway 500", 3 times/day. From the 4^{th} to 10^{th} days, PLs were fed FRIPPAK Raceway 500 feed only and then commercial feed FRIPPAK Raceway 700 was used until end of the experiment. The approximate analysis of ''FRIPPAK Raceway 500 and FRIPPAK Raceway 700'' was; protein 46%, lipid 7%, fiber 3% and moisture 9%. From the 4^{th} day, feeding regime was according to the manufacturer's instructions, 6 times/day at 02:00, 06:00, 10:00, 14:00, 18:00 and 22:00 h, starting with 1 g/day/tank then a daily increase of 0.3 g.

2.6 Water quality parameters

Water temperature, dissolved oxygen (DO) and pH were measured twice a day using a portable temperature-DO meter (Lutron PDO 519) and HANNA pH meter (HI98107), respectively. The total ammonia nitrogen (TAN) and nitrite (NO_2-N) were measured daily using colorimetric test kits: Advance Pharma Co Ltd., Thailand and Marine Leader Co., Ltd., Thailand, respectively. Alkalinity was measured daily by titration-based chemical test kits (Hanna Instrument - HI3811). Salinity was measured daily using a refractometer (ATAGO-Master refractometer, Tokyo, Japan). Total suspended solids (TSS) were measured daily using a Imhoff Cone (1000 mL, DIN 12672, VITLAB) in BFT and F_PRO&BFT treatments only.

2.7 Growth parameters

The initial body weight and length of the PL15 (n=60) was measured at the day of stocking of the nursery tanks. At the end of the experiment, 30 juveniles were randomly collected from each tank to measure the final body weight and length. Samples used for these measures were then discarded. The observed body weight and food intake data were used to calculate the following growth indices:

$$Weight\ gain\ = Final\ weight(g) - Initial\ weight\ (g)$$

$$Specific\ growth\ rate\ (SGR)\ (\%) = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} x100$$

Where In is a logarithmic number and W_1 and W_2 are the weights at times t_1 and t_2, respectively, with t_1 and t_2 being the first and final day of the experiment, respectively.

$$Length\ gain\ = Final\ length\ (cm) - Initial\ length\ (cm)$$

$$Survival\ (\%) = \frac{Shrimp\ number\ at\ the\ end\ of\ the\ experiment}{Shrimp\ number\ at\ the\ beginning\ of\ the\ experiment} x100$$

$$Food\ conversion\ ratio\ (FCR) = \frac{Total\ feed\ given(g)}{Wet\ weight\ gain(g)}$$

The weight length relationship (condition factor, k) was calculated according to Ali et al. (2008) using the equation:

$$Condition\ factor\ (K) = \frac{W}{L^3} x100$$

Where W is the weight of shrimps (g) and L is total length of shrimp (cm)

2.8 Microbiological analyses

Water and shrimp samples were analysed for the total bacterial count (TBC) and total *Vibrio* count (TVC) at the end of the experiment. Nutrient agar (Allen et al., 2004) and thiosulfate citrate bile salt-sucrose (TCBS) agar media were used for the TBC and TVC (Jorgensen et al., 2015), respectively. Five juveniles were randomly collected from each replicate. The whole animal was processed for preparation of stock solution and weighed by an electric balance aseptically (HR-200). The weighed samples were then taken in a sterile vortex mixer with 5 ml alkaline saline peptone water for proper mixing. The mixed samples were taken in sterile eppendorf tubes for centrifugation at 3,000 rpm for 4 minutes (Centrifuge (5415 D). After centrifugation the supernatant was collected in a sterile falcon tube, followed by serial dilutions. One ml of diluted solution was poured on solid media aseptically by sterile micropipette and was spread out properly with a sterilized L-shaped glass rod. All plates were incubated at 30ºC for 24 hours after spreading. After incubation the bacterial colonies grown in the plates were counted, considering only the plates containing between 10 and 300 colonies.

2.9 Salinity stress test

At the end of the experiment, 30 animals randomly selected from each tank were placed in 5L aerated plastic container with 5 salinity with no feeding (Chen et al., 2016). The salinity was reduced from 5 to 1 after 24 h, and further reduced from 1 to 0 after another 24 h. Dead animals were collected and counted every 4 h.

2.10 Statistical Analyses

All data were entered into MS Excel (Microsoft Corporation) and Statistical Package for Social Science, SPSS 16.0 (SPSS, Chicago, IL, USA). A one-way analysis of variance (ANOVA) followed by the Duncan Multiple Range Test was used to determine the significance of

differences between treatments. For the challenge test, survival in each treatment and the control was plotted using Kaplan-Meier survival estimates and Mantel-Cox log-rank tests (two-way) were performed to determine whether significant differences existed in survival between treatments. A p value of ≤ 0.05 was considered to indicate statistical significance.

2.11. Ethics statement

The experimental animals and protocols used in this study were approved by the Animal Welfare and Ethical Review Body (AWERB) at the University of Sterling.

3. Results and discussions

3.1 Water quality

All water quality parameters (Table 2) were within the optimal range for growth of shrimp (Kannupandi et al., 2002; Krishnaprakash, 2007; Uddin et al., 2013). There were no significant difference in the mean values of temperature, DO, alkalinity, and TAN between treatments. Nitrite (NO_3-N) was significantly lower ($P \leq 0.05$) in biofloc and F_PRO&biofloc compared to other treatments. These results implied that the addition of sucrose as a carbon source had a role in inorganic nitrogen reduction through stimulating the growth of heterotrophic bacteria (Emerenciano et al., 2012; Luo et al., 2014). The pH was also significantly lower ($P \leq 0.05$) in biofloc treatments (BFT and F_PRO&biofloc) than only probiotic treatments and control. These low values of the water pH may be due to the high level of inorganic carbon caused by the decomposition of bacterial organic matter (Panjaitan, 2010). The TSS levels (Table 2) were increased gradually over the experimental period. However, the mean values of TSS in the present experiment were lower than the range recommended for shrimp farming in biofloc system (Samocha et al., 2007).

239 **3.2 Growth performance**

240 The final body weight and weight gain of juveniles were significantly higher ($P \leq 0.05$) in
241 F_PRO&biofloc compared to other treatments and control, with biofloc at an intermediate level
242 (Table 3). Most of the studies on biofloc systems reported a significant increase in the growth
243 performance of the cultured animals (Azim and Little, 2008; Xu and Pan, 2012; Luo et al.,
244 2014). In the present study, shrimp were observed to occasionally graze on the bioflocs that
245 were suspended in the water in the biofloc and F_PRO&biofloc. Hence, the bioflocs act as an
246 important and additional natural food source that enhance growth rates of the shrimp
247 (Wasielesky et al., 2006; Arnold et al., 2009). Bioflocs could provide more proteins, lipids,
248 minerals and vitamins necessary for growth (Thompson et al., 2002; Moss et al., 2006). A
249 significantly higher ($P \leq 0.05$) SGR was found in F_PRO&biofloc than other treatments and
250 CTRL, with no significant differences ($p \geq 0.05$) betweeen biofloc and F_PRO& biofloc (Table
251 3). The results in the present study indicated that juveniles treated in the biofloc systems (BFT
252 and F_PRO& biofloc) performed better growth than only probiotic and control. This result is
253 in agreement with the findings of Xu et al. (2012). At the end of experiment, juvenile survival
254 rates were above 75%, with no significant differences ($p \geq 0.05$) between treatments and control
255 (Table 3). The high survival rate indicated the acceptable culture condition in tanks and diets
256 provided. Similar survival rates were reported by Zhou et al. (2009) and Xu et al. (2013) for
257 shrimp treated with probiotics and biofloc system, respectively. The FCR varied from 0.74 to
258 0.90 (Table 3) and it was significnalty higher ($p \leq 0.05$) in treatment with biofloc &F_PRO
259 than other treatments and CTRL; however, FCR did not vary significnatly ($p \geq 0.05$) between
260 biofloc and biofloc &F_PRO. A similar trend of results reproted in previous studies for shrimp
261 produced in biofloc system (Ray and Lotz, 2014; Effendy et al., 2016).

262

3.3 Microbiological parameters

The average TBC in water samples taken from W_PRO was higher ($3.2\pm0.73 \times 10^6$ CFU ml^{-1}) ($p \geq 0.05$) than other treatments and control (Table 4). The average TBC in shrimp samples varied from 3.1 ± 1.1 to $6.0\pm1.9 \times 10^6$ CFU g^{-1} with no significent differences ($p \geq 0.05$) among treatments. The TBC was higher in tanks that received a combination treatment of in-feed probiotic and biofloc compared to control. A similar tendency was also reported by Nimrat et al. (2012) and Kumar et al. (2017) for shrimp treated with probiotics and molasses, respectively. Our results showed that the use of biofloc as a culture system contributed to an increase in the bacterial abundance in the gut of shrimp, with the addition of the probiotic mixture. Hu et al. (2017) reported that the use of probiotic and molasses promoted the formation and development of a beneficial microbial community in intensive shrimp culture. These results corroborate the findings of the present study in which a combination of sucrose and probiotic increased the total bacterial count in the F_PRO& biofloc. The TVC in the water and shrimp samples was found to be higher ($p \geq 0.05$) in the control compared to treatments with the use of probiotics and sucrose (Table 4). In the present study, the application of probiotics and sucrose in treatments containing *Bacillus spp* within the probiotic may have been responsible for the lower TVC in the water and shrimp compared to those in the control. This is in line with Li et al. (2009) and Villaseñor et al. (2013) reported the addition of a commercial probiotic mixture containing *Bacillus* was able to modify the gut bacterial community in shrimp included decreasing the number of *Vibrio*. In many cases, *Vibrio spp.* are opportunists, causing disease and mortality in animals only in a physiologically stressed condition (Alderman and Hastings 1998).

3.4 Salinity stress test

The survival rate in the salinity stress test varied among different treatments (Fig. 1). A highly significant ($P \leq 0.05$) survival rate was observed in juveniles treated with F_PRO&BFT followed by biofloc than other treatments and control. The present study indicated that the application of biofloc technology could improve juveniles quality and performance and consequently the tolerance rate to stressful conditions. This result is consistent with a previous study conducted by Ekasari et al. (2015). Stress tests are commonly used methods to assess the quality of cultured species (MacNiven and Little, 2001). Salinity stress test has been used to assess the osmotic capability as an indicator of the general robustness of the animal (MacNiven and Little, 2001 and Salze et al., 2008). *Penaeus monodon* exhibits hyper-osmotic regulation at low salinity levels, and hypo-osmotic regulation at high salinity levels (Cheng and Liao 1986).

4. Conclusion

This study found a higher growth performance of shrimp reared in biofloc alone or in combination with in-feed probiotic than only probiotic treatments and control. The survival rate was significantly higher in animals that received a combined treatment of in-feed probiotic and biofloc followed by biofloc compared to other treatments and control over the period of salinity stress test. The study also showed that pH and nitrite concentrations were significnalty lower in the biofloc treatment compared to other treatments and the control. However, these results were based on an experiment under controlled commercial hatchery conditions, and 'on farm' environmental conditions may be different. Further on-farm trials would be useful to furtehr explore the effficacy of micro-biofloc tank systems alone or combined with in-feed probiotic to smallholder farmers.

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Conflict of interest statement

Authors are declaring that they do not have any conflict of interest.

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Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

List of Figures

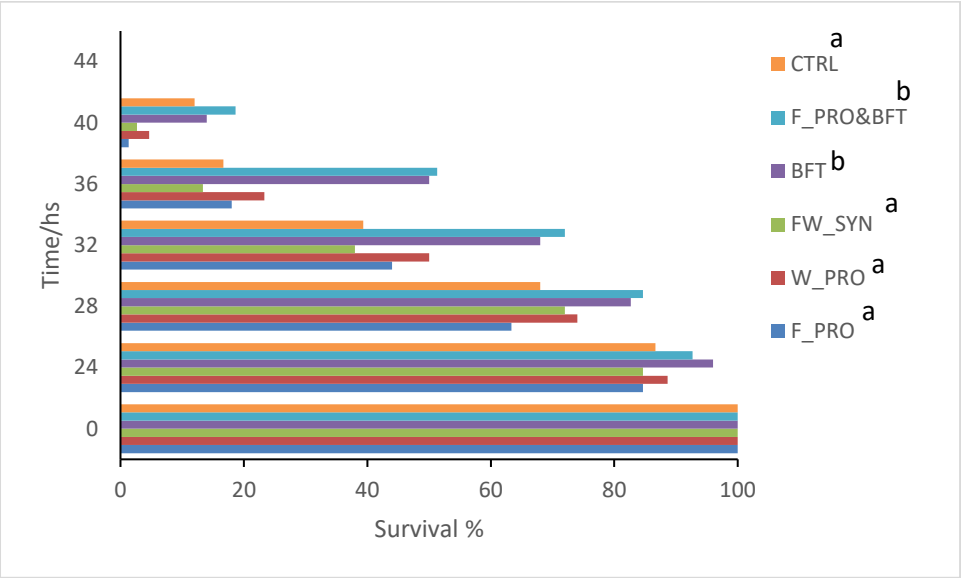

Figure 1: Cumulative survival of shrimp juveniles exposed to salinity stress test at the end of an extended nursery phase using Kaplan-Meier (Log-rank Mantel Cox) . Groups that do not share letters are significantly different ($p < 0.05$).

495 **List of Tables**

496

497 **Table 1:** Experimental design and treatments of *Penaeus monodon* at the extended nursing

498 phase.

Code	Description	PHPs active ingredient/s
F_PRO	In-feed probiotic (a)	*Bacillus subtilis, Bacillus licheniformis, Bacillus pumilus (a)*
W_PRO	Water probiotic (b)	*Bacillus subtilis, Bacillus licheniformis (b)*
FW_SYN	In-feed and water probiotic (c)	a&b
Biofloc	Biofloc (d)	Sucrose (d)
F_PRO&biofloc	In-feed probiotic and biofloc (e)	a&d
Control	Control	No additives

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507 Table 2: Mean (± SE) values of water quality parameters during the extended nursery phase

Parameters	Treatments					
	F_PRO	**W_PRO**	**FW_SYN**	**biofloc**	**F_PRO&biofloc**	**Control**
Temperature (ºC)	29 ± 0.05	29 ± 0.03	29 ± 0.05	29 ± 0.10	29 ± 0.05	29 ± 0.06
DO (mg/l)	7.2 ± 0.01	7.2 ± 0.01	7.2 ± 0.01	7.2 ± 0.03	7.2 ± 0.02	7.2 ± 0.02
pH	7.93 ± 0.01^{a}	7.91 ± 0.00^{a}	7.91 ± 0.00^{a}	7.86 ± 0.01^{b}	7.87 ± 0.01^{b}	7.93 ± 0.01^{a}
TAN (mg/l)	0.40 ± 0.02	0.54 ± 0.06	0.47 ± 0.05	0.50 ± 0.04	0.57 ± 0.04	0.41 ± 0.03
Nitrite (mg/l)	1.0 ± 0.09^{a}	1.2 ± 0.06^{b}	1.1 ± 0.05^{ab}	0.34 ± 0.06^{c}	0.36 ± 0.03^{c}	1.1 ± 0.06^{ab}
Alkalinity (mg/l)	170 ± 2.4	172 ± 3.0	166 ± 3.4	159 ± 3.4	170 ± 5.1	169 ± 2.3
TSS (mg/l)	--	--	--	1.92±0.02	1.96±0.02	--

508 Different subscripts within rows indicate significent differences ($P \leq 0.05$)

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525 Table 3: Growth parameters (mean ± SE) at the end of the extended nursery phase

	Treatments					
Parameters	**F_PRO**	**W_PRO**	**FW_SYN**	**Biofloc**	**F_PRO&biofloc**	**Control**
Weight gain (g)	0.24 ± 0.01^{a}	0.25 ± 0.01^{a}	0.25 ± 0.01^{a}	0.28 ± 0.01^{ab}	0.30 ± 0.02^{b}	0.24 ± 0.01^{a}
SGR (%)	5.6 ± 0.09^{a}	5.6 ± 0.07^{a}	5.6 ± 0.06^{a}	5.8 ± 0.07^{ab}	5.9 ± 0.09^{b}	5.6 ± 0.06^{a}
Survival rate (%)	79 ± 1.2	79 ± 1.3	75 ± 1.6	79 ± 1.8	78 ± 1.6	79 ± 2.1
Length gain (cm)	1.9 ± 0.07^{a}	2.1 ± 0.04^{b}	2.1 ± 0.07^{b}	2.1 ± 0.06^{ab}	2.1 ± 0.04^{b}	2.0 ± 0.10^{ab}
Condition factor	0.63 ± 0.02^{a}	0.62 ± 0.02^{a}	0.63 ± 0.02^{a}	0.64 ± 0.01^{ab}	0.69 ± 0.02^{b}	0.62 ± 0.02^{a}
FCR	0.90 ± 0.06^{a}	0.86 ± 0.05^{ab}	0.90 ± 0.04^{a}	0.78 ± 0.05^{ab}	0.74 ± 0.05^{b}	0.90 ± 0.03^{a}

526 Different subscripts within rows indicate significent differences ($P \leq 0.05$)

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543 Table 4: Total bacterial count (TBC) and total vibrio count (TVC) of water and shrimp

544 samples at the end of the extended nursery phase

Treatments	**TBC**		**TVC**	
	Water (10^6 CFU ml^{-1})	Shrimp (10^6 CFU g^{-1})	Water (10^3 CFU ml^{-1})	Shrimp (10^3 CFU g^{-1})
F_PRO	1.8±0.53	4.8±1.1	5.8±3.6	2.9±2.1
W_PRO	3.2±0.73	5.6±1.4	10±3.3	9.9±5.2
FW_SYN	1.4±0.22	5.9±1.5	7.7±2.8	6.3±5.1
Biofloc	2.3±1.1	3.9±0.58	3.8±2.4	7.0±4.2
F_PRO&biofloc	2.9±1.1	6.0±1.9	1.2±0.86	6.1±1.4
Control	1.4±1.1	3.1±1.1	12±6.1	10±6.2

545 Different subscripts within colluman indicate significent differences ($P \leq 0.05$)