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An innovative biofloc technology for the nursery production of Pacific whiteleg shrimp, *Penaeus vannamei* in tanks

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Abstract

Nursery production of shrimp is usually done in small ponds; however, the use of small and circular tanks with plastic liners is gaining popularity. From an industry standpoint, there is still a need to assess how nursery systems can be of benefit to the shrimp production cycle. Hence, the use of small circular tanks coupled with the incorporation of biofloc technology was assessed in terms of its viability during the nursery production of the Pacific whiteleg shrimp, *Penaeus vannamei*. A 450m^2 plastic lined circular tank was installed and prepared for the stocking of *P. vannamei* postlarvae (PLs) at a density of 500 PLs per m^2. Biofloc was produced and maintained throughout the nursery phase using brown sugar as carbon source at a carbon to nitrogen (C:N) ratio of 10. Water quality was monitored daily, while presumptive Vibrios were enumerated weekly. Sampling for growth was done at the 14th day post-stocking and weekly until harvest on the 30th day. The different water quality parameters were within optimum levels required for shrimp growth. Presumptive Vibrios were dominated by the yellow colonies. At the end of the nursery phase, there was 100% survival and the shrimp attained an average body weight of 1.26 g and a feed conversion ratio (FCR) of 0.43. Our results indicate that the use of small circular tanks with biofloc during the nursery production phase of whiteleg shrimp is feasible and can be incorporated in the grow-out culture of this shrimp species.

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Introduction

Biofloc technology offers a viable approach towards high-density culture of shrimp because it can maintain good water quality with minimal or no water exchange through nutrient recycling (Avnimelech, 1999; Kuhn *et al.*, 2009; Fatimah *et al.*, 2019). In particular, nitrogenous wastes are converted into microbial biomass that can be used in situ by the cultured animals (Kumar *et al.*, 2020). The sustainability of this system relies heavily on the growth of microorganisms in the culture medium coupled with minimum or zero water exchange. The microorganisms that are present in a biofloc system has two important roles: (1) maintenance of water quality as a result of the uptake of nitrogenous waste materials, thereby producing microbial protein "in situ"; and (2) improved nutrition efficiency through reduction of feed conversion ratio and a decreased feed costs (Emerenciano *et al.*, 2013). Through addition of carbohydrate sources to the water and adjusting the carbon to nitrogen ratio (C/N), the heterotrophic bacteria are able to absorb nutrients and maintain the production of bioflocs (Khanjani *et al.*, 2017), which in turn facilitate the removal of ammonia-nitrogen and nitrite (NO_2-N) (Asaduzzaman *et al.* 2008; Gao *et al.* 2012). Moreover, as disease outbreaks and their impact on commercial shrimp farming operations during the past years have greatly impacted the operational management of shrimp farms, the use of biofloc technology is increasingly identified as one possible approach for disease prevention in shrimp culture (Hargreaves, 2013). Short- and long-term nursery trials in shrimp demonstrated the importance of bioflocs as a means of preventing the negative effects of ammonia in the culture system as well as a source of natural food for the shrimp post-larvae (Emerenciano *et al.*, 2011; Correia *et al.*, 2014; Mishra *et al.*, 2008; Samocha *et al.*, 2007; Suita *et al.*, 2016; Wasielesky *et al.*, 2013; Schveitzer *et al.*, 2017).

The nursery system is an intermediate step between the post-larval (PL) stage and the grow-out phase in shrimp culture (Mishra *et al.*, 2008). During this phase, shrimps PLs are reared at high densities for 15 - 60 days that involves precise technical management, feeding and water quality monitoring (Jory and Cabrera, 2012; Samocha, 2010; Schveitzer *et al.*, 2017). Here in the Philippines, traditional shrimp farmers carry out shrimp nursery activities in small ponds; however, with issues on disease outbreaks and biosecurity issues during the grow-out phase, the use of small and circular tanks with plastic liners is gaining popularity among shrimp growers. From an industry standpoint, there is still a need to assess how nursery systems can be of benefit to the shrimp production cycle. Hence, the use of small circular tanks coupled with the incorporation of biofloc technology was assessed in terms of its viability during the nursery production of the Pacific whiteleg shrimp, *Penaeus vannamei*.

Materials and methods

Preparation of nursery tank

Round-shaped tank with an area of 450 m^2, framed with steel, padded with plastic liner, and surrounded by oxygen diffusion system at 3 horsepower (hp) capacity and paddlewheel aerators (2 units with a total capacity of 2 hp) was used for the nursery production of whiteleg shrimp (Fig. 1). Farming super-intensive white leg shrimp in steel frame round tank with plastic liner offers the following advantages: ability to control environmental problems and stabilizes environmental parameters to reduce environment pollution. Moreover, the use of small circular tanks facilitates rapid installation and removal of the structures and at the same time these tanks are able to maximize space for culture of the organisms. The tank has a central drain or a shrimp tank toilet that facilitates efficient removal of sludge as well as shrimp wastes and uneaten feeds that settled at the central portion of the tank as a result of water circulation.

Water culture and biofloc production

A short water cultivation phase was done prior to stocking and this involved the addition of a commercially available probiotics following the manufacturer's instructions, brown sugar at a C:N ratio of 6:1 to support heterotrophic bacteria and a commercially available pond water colorant to provide shade to the shrimp PLs. The addition of the colorant was followed using the protocol provided by the manufacturer. After 5 days of water cultivation, the tank was stocked with *L. vannamei* postlarvae (PLs) at a density of 500 PLs per m^2. Biofloc was produced and maintained throughout the 30-day nursery phase using brown sugar as carbon source and applied by completely dissolving in water at carbon to nitrogen (C:N) ratio of 10. The amount of brown sugar that was added to the nursery tank was calculated based on the amount of daily feeds given to the PLs using a biofloc calculator (Fig. 2). The biofloc calculator was specially prepared for the shrimp farmers by just providing details including amount of feed per day, the crude protein and moisture content of the feeds. The values of the crude protein and moisture content were obtained from the feed proximate data that are printed on the feeding bags. Once these details are provided, the amount of brown sugar that will be added for that day is immediately known. There was daily application of brown sugar for the first week after stocking, followed by application every 2 days from the 8th day of culture (DOC) until the 14th DOC. From 15th until 23rd DOC, the application was done every 3 days and finally, every 4 days from 24th DOC until the day before transfer to the grow-out ponds. Brown sugar was completely dissolved in pond water and broadcasted directly to the tank. The amount of brown sugar to be added daily is divided equally and is applied one hour after the morning feed and at the afternoon feeding. The application schedule of brown sugar to maintain biofloc production in a 450 m^2 nursery tank during the 30-day nursery production phase is shown in Table 1.

Monitoring of water quality and bacterial analysis

Water quality was monitored twice daily using commercially available kits. The parameters that were monitored include: dissolved oxygen, pH, salinity, ammonia-N, nitrite-N and nitrate-N. Presumptive Vibrios were enumerated weekly using thiosulfate-citrate-bile salts-sucrose (TCBS) agar and the ratio of the green (presumptive pathogenic) and yellow (presumptive non-pathogenic) colonies were obtained. The seeded agar media were placed in an incubator at 30°C. After a 24-h incubation period, the colony forming units per milliliter (CFU mL^{-1}) were counted. Sampling for growth was done at the 14th day post-stocking and weekly until harvest on the 30th day.

Shrimp sampling

During sampling, shrimp were checked for gut fullness, external appearance and the presence of any mortalities in the feeding trays. The average weight, survival and feed conversion (FCR) ratio of the shrimp during the nursery production phase were computed prior to transfer of the shrimp juveniles to the grow-out ponds.

Results and discussion

During the 30-day nursery phase, the water quality parameters were within the optimum levels required for shrimp farming even though there was minimal water exchange. Table 2 shows the range of values of the different water quality parameters that were assessed during the nursery phase. Of particular importance was the low level of nitrogenous wastes that were monitored in the nursery tank. Ferreira *et al.* (2020) stressed that the addition of sugar in a biofloc system was largely responsible in controlling the spikes in levels of nitrogenous compounds in the water. The accumulation of nitrite during the latter part of the nursery production phase indicates that the nitrification process is occurring in the nursery tank. A similar observation was also obtained by Ferreira *et al.* (2020) using different biofloc production systems in the nursery phase of whiteleg shrimp. The bacteria that convert ammonia into nitrite have faster growth rate than those that convert nitrite to nitrate, especially in saltwater (Madigan *et al.*, 2016); hence, there were more readings and higher levels of nitrite than nitrate during the 30-day nursery production phase. Moreover, the levels of these different water quality parameters were used as guide in facilitating water exchange in the nursery tank. In cases when water exchange was necessary, this was carried out by replacing at most 10% of the water in the nursery tank with water coming from the reservoir. The volume and frequency of water exchange were dependent on the water transparency in the nursery tank. This was to prevent excessive phytoplankton bloom that may result in oxygen depletion during night time. Reservoir water was allowed to stabilize for 3-5 days before being pumped to the nursery tank.

Table 1. Application schedule of brown sugar for biofloc production maintenance in the nursery tank during the 30-day rearing period of the shrimp post-larvae.

DOC	Feed Amount	Carbont Amount (Brown sugar)	Amount to be Applied (Kgs)	When to Apply (DOC)
1	0.6			
2	0.63	0.12	0.12	2
3	0.7	0.15	0.15	3
4	0.74	0.1	0.15	4
5	0.78	0.15	0.15	5
6	0.83	0.17	0.17	6
7	0.96	0.2	0.2	7
8	1.5	0.3	0.6	9
9	1.7	0.3		
10	2	0.4	0.9	11
11	2.5	0.5		
12	3	0.6	1.2	13
13	3.2	0.6		
14	3.8	0.75	2.65	15-16
15	4.5	0.9		
16	4.9	1		
17	5.5	1	3.5	18-19
18	b	1.2		
19	6.8	1.3		
20	7.7	1.5	4.6	21-22
21	7.8	1.5		
22	8.4	1.6		
23	9	1.7	7.5	24-2G
24	9.6	1.9		
25	10	1.9		
26	10.7	2		
27	11.4	2.3	10.3	27-29
28	12.6	2.5		
29	13.5	2.7		
30	14	2.8		

Top draining was used to replace water in the nursery tank after heavy rains to prevent sudden changes in the water quality parameters. On the other hand, bottom draining from the central drain or shrimp tank toilet was carried out to remove sludge, uneaten feeds, feces and dead shrimp that accumulated at the central portion of the tank. Wastewater from the nursery tank is directed towards the settlement ponds before being released back to the waterways. Total bacteria and presumptive Vibrios that were monitored weekly in the nursery tank are shown in Figure 3. Total bacteria in the water of nursery tank was at least 10 times higher than the population the presumptive Vibrios during the nursery production phase.

The *Vibrio* population in the nursery tank was dominated by yellow colonies (non-pathogenic); however, throughout the duration of the nursery production phase, green *Vibrio* colonies (pathogenic) were not detected. Luminous bacteria were also not detected in the water of the nursery tank.

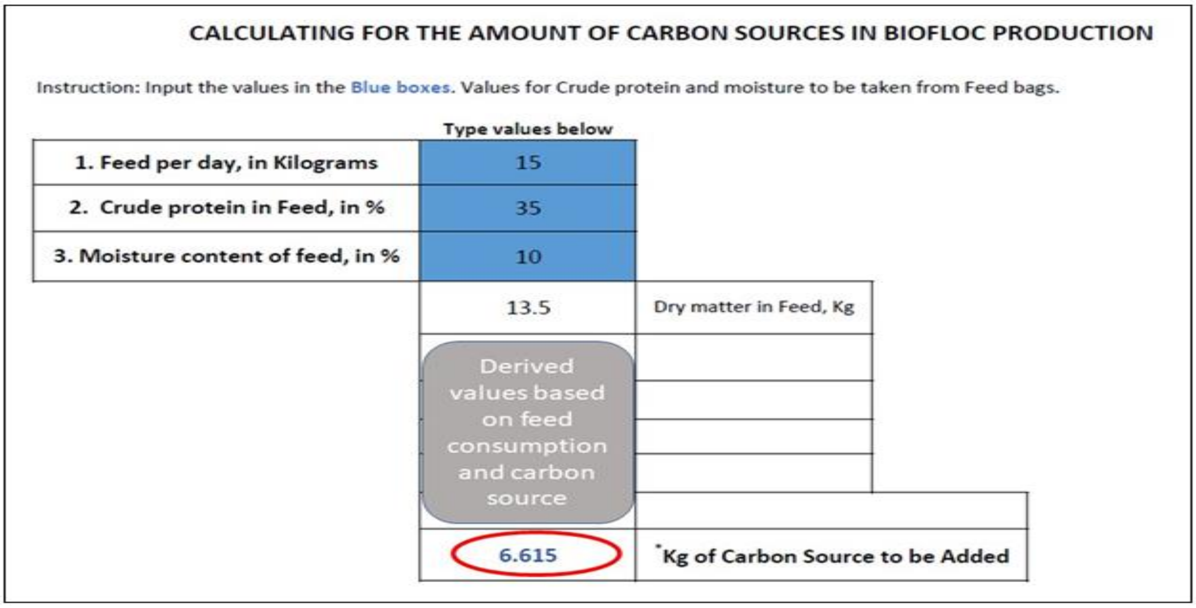
Table 2. Range of physico-chemical water quality parameters in the nursery tank over a 30-day rearing period of whiteleg shrimp post-larvae.

Parameter	Range
Water Temperature (°C)	27 - 32
Salinity (ppt)	18 - 24
pH	7.9 - 8.7
Dissolved Oxygen (ppm)	5.0 - 8.0
Anunonia-N (ppm)	0 - 1.0
Nitrite-N (ppm)	0 - 0.3
Nitrate-N (ppm)	Not detected

Though it is generally believed that the supply of feed and organic inputs in tanks increase organic matter content, which in turn favor the growth of Vibrios (Ferreira *et al.*, 2011), the results in our present study proved otherwise: the *Vibrio* population in the nursery tank was kept in check due to the presence of bioflocs. In shrimp culture, the *Vibrio* populations are regularly monitored because under certain conditions they can be harmful to shrimp, resulting in either excessive mortality or significant reduction in growth (Aguirre-Guzmán *et al.*, 2004). Earlier studies in shrimp biofloc systems showed that Vibrios are significantly reduced and disease resistance of the shrimp is enhanced in the presence of bioflocs (Crab *et al.*, 2010; Aguilera-Rivera *et al.*, 2019; Sajali *et al.*, 2019) resulting in lower incidences of vibriosis and mortality (Cardona *et al.*, 2015; Anand *et al.*, 2017; Lee *et al.*, 2017).

Fig. 1. A 450 m^2 circular tank with plastic liner installed for the nursery production of whiteleg shrimp. Photo by Michelle Sarupan.

In fact, Kumar *et al.* (2020) demonstrated that the reduction of Vibrios, particularly the strain of *V. parahaemolyticus* that causes acute hepatopancreatic necrosis disease (AHPND), was possibly due to the switch from free-living virulent planktonic phenotype to a non-virulent biofilm phenotype. This is turn lowered incidence of mortality as a consequence of AHPND.



CALCULATING FOR THE AMOUNT OF CARBON SOURCES IN BIOFLOC PRODUCTION

Instruction: Input the values in the Blue boxes. Values for Crude protein and moisture to be taken from Feed bags.

	Type values below	
1. Feed per day, in Kilograms	15	
2. Crude protein in Feed, in %	35	
3. Moisture content of feed, in %	10	
	13.5	Dry matter in Feed, Kg
	Derived values based on feed consumption and carbon source	
	6.615	*Kg of Carbon Source to be Added

Fig. 2. Calculation of the amount of carbon source using a biofloc calculator. This farmer-friendly guide enables the farmer to compute for the amount of carbon that will be added to the nursery tank by inputting the desired C;N ratio, amount of feed per day, the crude protein and moisture contents of the feeds.

It is worthy to note that in spite of the beneficial effects of bioflocs in shrimp culture, one cannot have complete control over the dynamics and composition of microbial communities that take place within the production system of shrimp larvae during the nursery phase (Ferreira *et al.*, 2020). This is due to the complexity of interactions that occur in the system, and this warrants additional studies particularly on the metagenomics aspects of biofloc technology in the nursery phase of shrimp culture. After a period of 30 days in the nursery tank, the shrimp attained an average body weight of 1.26 g and a feed conversion ratio (FCR) of 0.43. Survival rate of the shrimp stock was 100%.

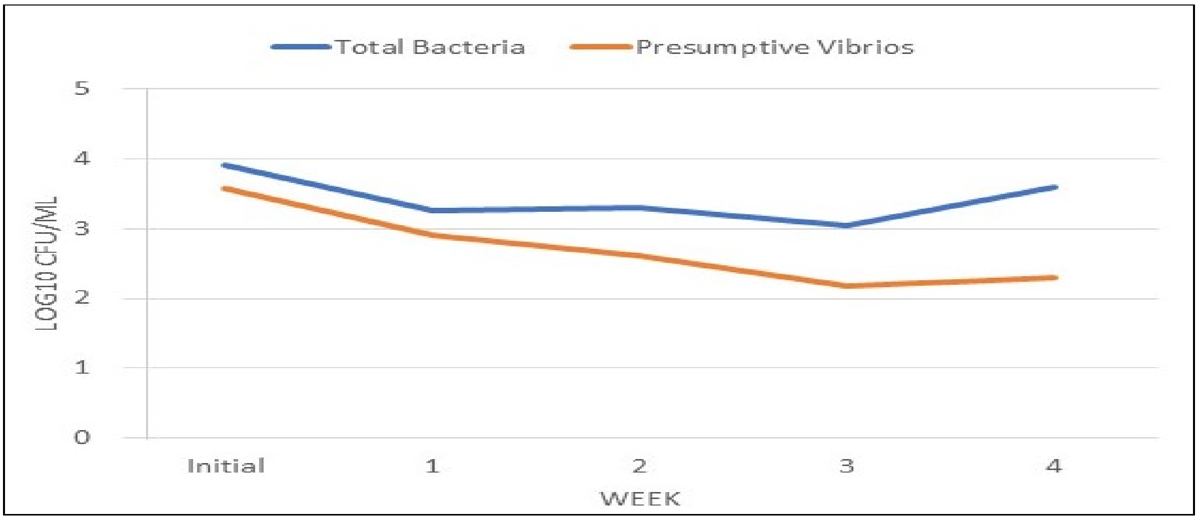


Fig. 3. Profile of total bacteria and presumptive Vibrios in the nursery tank over a 30-day rearing period of whiteleg shrimp post-larvae.

Our results were consistent with earlier studies on the nursery production of whiteleg shrimp in biofloc system, wherein there is a generally high survival rate and low feed conversion ratio (Mishra *et al.*, 2008; Serra *et al.*, 2015; Khanjani *et al.*, 2017). According to Moss (2002), the manipulation of the microbial community in a biofloc culture system can provide additional source of food for the shrimp; thereby increasing growth rate and reducing FCR, which were observed in this study. Monitoring of the shrimp in the grow-out pond (with an area of 3,000 m^2 with a stocking density of 40 shrimp per m^2) after transfer from the nursery tank showed that after 46 days, the shrimp attained an average weight of 30 g. At harvest, there was 100% survival rate with a productivity of 11.2 tons per hectare and FCR of 0.94. The present study has practical implications for shrimp culture. There is reduction in the amount of water that is required during the nursery phase of shrimp culture. This means reducing the costs related to pumping, refilling, disinfection of water, as well as in decreasing environmental impacts and improving biosecurity within the shrimp farm (Boyd 2003). Moreover, under normal conditions, the grow-out phase is reduced because culture of shrimp in the grow-out ponds can be simultaneously done with the nursery phase; thus, after harvest, the grow-out ponds can be cleaned and immediately stocked with bigger-sized shrimp juveniles. Using this scheme, productivity is increased by at least 50% and ensures almost year-round production of shrimp. With the further incorporation of biofloc technology in the nursery production systems, there is improvement in growth, survival and disease resistance in shrimp in addition to improvement in water quality and microbial population in the rearing water of the shrimp larvae.

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