



Natural productivity of pacific white shrimp *Litopenaeus vannamei* during the nursery stage

Josol Marjon, Dion Neil Colantro, Charity Asutilla, Florito Guarin,
Jemelyn Castanares, Jomel Baobao, Maria Danesa S. Rabia*

Bohol Island State University, Calape, Bohol, Philippines

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Abstract

Natural productivity of pacific white shrimp by using inorganic and organic fertilizer was investigated. Nursery culture was carried out for 15 days in 2 m³ indoor concrete tanks with stocking density of one postlarvae (PL) liter⁻¹ using PL16 shrimp. During the first week of the experiment there was no significant difference in the phytoplankton densities and microbial flocs in the two treatments. A day after the 3rd fertilization, the algae bloomed in the tanks applied with urea which resulted to mass mortality of all stocks. The results demonstrated that weekly periodic application of urea at a rate of 50 kg ha⁻¹ in a zero-water exchange set up during the nursery of *L. vannamei* is not viable. Periodic fertilization using chicken manure at a rate of 625 kg ha⁻¹ improved feed conversion rate as well as shrimp survival and production. On the other hand, fertilization using chicken manure improved feed conversion rate as well as shrimp survival and production. Shrimp survival was 92.68%, specific growth rate (% day⁻¹) was 14.05 in terms of weight and 18.17 in terms of length and yield was 128.37 g m⁻³.

* **Corresponding Author:** Maria Danesa ✉ mariadanesa.rabia@bisu.edu.ph

Introduction

World aquaculture has grown dramatically in the last 50 years, from a production of less than a million tonnes in the early 1950s, production in 2006 have risen to 51.7 million tonnes excluding aquatic plants (FAO, 2008). In terms of value, shrimp is by far the largest commodity representing 17% of the total value of international-traded fishery products in 2006 (FAO, 2009). From 2000 to 2005, its global market has expanded from less than \$1 billion to \$5.8 billion (FAO, 2008). The increase in shrimp production is brought about by so many factors. One of these is the intensification of culture systems. Advancements in aquaculture engineering and biotechnology among others have triggered the transformation from extensive to intensive culture of shrimps. Introduction of new aquaculture species in certain regions is another major factor. Example is the introduction of the Pacific white shrimp *Litopenaeus vannamei* to Asia. In 1996, *L. vannamei* was introduced on a commercial scale in mainland China and Taiwan and subsequently spread to the Philippines and other neighbouring countries (FAO, 2004).

However, the intensification of shrimp aquaculture is coupled with pollution, environmental degradation and occurrence of diseases caused by heavy effluent discharge and pathogen contamination of water supply. Thus, reducing waste outputs of shrimp aquaculture operations is essential to ensure long-term sustainability. According to McIntosh *et al.* (2001) this could be achieved by improving feed and water management, application of water treatment to wastewater and adoption of zero or minimal water exchange. The latter is the soundest possible option because it does not only control effluent discharge but also reduces introduction of water borne pathogens, thus increasing biosecurity of the cultured organism (Gomez-Jimenez, 2005). The bioflocs technology is an important discovery in the zero-water exchange or closed system of shrimp farming. Bioflocs technology (BFT) is a system wherein heterotrophic bacteria, algae and other microorganisms are grown in flocs-controlled

conditions within the culture period (Jorand *et al.*, 1995). The microbial biomass or bioflocs utilize dissolved and particulate nitrogenous compounds in the water coming from the waste of the cultured organism; the assimilation of these compounds is stimulated by addition of carbon sources to the system (Avnimelech, 1999). The presence of bioflocs does not only improve water quality but also serves as additional food for the cultured organism. The BFT represents a sustainable way of producing food by the production of new biomass grown on the nutrient waste and is used as an alternative food source (Crab *et al.*, 2007). Studies on zero water exchange during the nursery culture of *L. vannamei* showed several benefits. Baloi *et al.* (2010) reported higher survival of *L. vannamei* post larvae cultured using reclaimed water from a super intensive zero-water exchange pond in tanks without water exchange for seven days. Widanarni *et al.* (2010) demonstrated that the optimum carbon-nitrogen ratio using the biofloc technology in *L. vannamei* nursery production obtained the best shrimp growth, yield and feed efficiency.

Another strategy to improve sustainability while increasing shrimp production is to promote natural productivity in the culture system. Natural food production in an aquatic system is enhanced by fertilization. Inorganic and organic fertilizers supplement nutrients needed to increase primary productivity that supports the maintenance of other communities occupying higher trophic levels. Stahl (1979) reported that natural food alone consisting primarily of a mixture of organic detritus and soil produce growth rates in freshwater prawn *Macrobrachium rosenbergii* postlarvae better than commercial production ponds. Anderson *et al.* (1987) demonstrated that 53–77% of the growth of juvenile *L. vannamei* raised in earthen ponds was due to the assimilation of in situ natural pond biota. Porchas-Cornejo *et al.* (2011) reported that promotion of natural feed had a positive effect on weight gain, survival and final biomass of *L. vannamei*. Martinez-Cordova *et al.* (1998) found out that the growth and biomass of *L.*

vannamei were higher in fertilized ponds despite lower feeding rate. *L. vannamei* grown extensively (without supplemental feeding) in fertilized ponds resulted to moderate to high survival rates (Jaspe *et al.*, 2010). However, research on the use of different fertilizers in a zero-water exchange set up during the nursery of *L. vannamei* is limited.

This study determined the effects of periodic fertilization on natural productivity, growth and survival in the nursery of *L. vannamei* under a zero-water exchange set up.

Materials and methods

Experimental design and set up

The experiment utilized a complete randomized design with two treatments: inorganic fertilizer (urea) and organic fertilizer (chicken manure). Rearing of *L. vanammei* postlarvae lasted for 15 days (from PL 16 to PL 30). It was conducted in the facilities of the Central Bangus Hatchery located in Pangangan Island, Calape, Bohol. The experiment used six (2 m³) indoor concrete tanks assigned to the two treatments. The tanks were chlorinated and washed prior to use. The tanks were provided with 7-10 cm mud substrate. Tea seed was added at 100 ppm to eradicate naturally occurring predators in the mud and lime was applied at 100 ppm subsequently. Chicken manure and urea were applied initially at 1250 kg ha⁻¹ and 100 kg ha⁻¹ a week before stocking and added weekly at a rate of 625 kg ha⁻¹ and 50 kg ha⁻¹ respectively to maintain green water condition. Tanks were filled with seawater with a salinity range of 30-35 ppt. Pond green water was inoculated in the tanks to provide initial algal population. Each tank was equipped with aeration line to provide sufficient dissolved oxygen level.

Stocking and feeding

Pacific white shrimp *L. vannamei* postlarvae (PL 14) were purchased from a private hatchery (Dobe Export International). The shrimps were acclimatized in a ten-tonner rectangular tank with same salinity and temperature of the hatchery

water and were fed on satiation twice a day. Stocking in the experimental tanks was done early morning on the third day after obtaining the fry at a rate of 1 individual liter⁻¹. Initial weight and length of the shrimps were 17.85 mg and 1.49 cm. First feeding was done a day after stocking based on 2.5% body weight. Subsequent feeding was adjusted based on the actual body weight. Feeding ration was administered four times a day at 8 am, 10 am, 1 pm and 4 pm.

Water quality and natural productivity monitoring

Phytoplankton and microbial-floc population in the rearing water were monitored weekly and physico-chemical parameters such as temperature, salinity, dissolved oxygen and pH were checked daily. Temperature and dissolved oxygen was measured using portable DO meter, pH with portable pH meter and salinity with a refractometer. Dominant phytoplankton, zooplanktons and microbial flocs were counted weekly using a standard haemocytometer under a compound microscope.

Growth and survival monitoring

Weight of the shrimps was monitored weekly to assess growth and adjustment of the feed ration. The shrimps were harvested after 15 days and counted individually. One hundred (100) representative samples from each tank were weighed using 1.0 g precision electronic balance and length measured using Vernier caliper to the nearest 0.01 cm. To estimate the specific growth rate (SGR), food consumption rate (FCR) and survival rate (SR), the following formulas were used: $SGR = [(\ln \text{ final weight} - \ln \text{ initial weight}) / \text{days}] \times 100$. Where: ln = natural logarithm of final and initial weight. $FCR = \text{weight of feeds consumed} / \text{weight gained}$ and $SR = (\text{recovered stocks} / \text{total stocks}) \times 100$.

Statistical analysis

To determine significant differences in the water quality parameters, microbial floc and phytoplankton counts, growth, survival, yield and FCR between treatments, T-test for independent sample was used.

Results and discussion

Water quality

Good water quality is essential in the success of shrimp nursery operation. Table 1 shows the minimum, maximum and mean levels of these parameters all throughout the duration of the culture period. The mean daily values recorded during the experiment fall under the optimum range for culture of *L. vannamei*. Moreover, the results of all the water quality parameters showed no significant difference between the two treatments. The mean salinities of the two treatments were at 37.1 ppt and 36.7 ppt respectively. Ponce-Palafox *et al.* (1997) found out that the optimum range for best survival and growth

of *L. vannamei* is between 33-40 ppt. Dissolved oxygen is one of the most important water quality parameters, minimum DO standard for good growth of shrimps is at 3 ppm (Fast and Boyd, 1992). The mean dissolved oxygen concentrations of the two treatments were at 5.0 ppm and 5.1 ppm respectively. The temperature readings were relatively stable all throughout the culture period in all the experimental tanks which fall under the optimum temperature for shrimps at 26°C to 33°C. Mean pH in the two treatments were at 7.6 and 7.5 with very minimal fluctuation until the end of the experiment. The recorded pH fall under the optimum pH in aquaculture system at 7.5 to 8.5.

Table 1. Mean, minimum and maximum value of DO, pH, temperature and salinity of water in Pacific white shrimp (*L. vannamei*) nursery culture with different fertilizers

Treatment/Parameters	Salinity (ppt)			Dissolved Oxygen			Temperature (°C)			pH		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Inorganic Fertilizer (Urea)	33.7	40.0	37.1 ^a	4.8	5.2	5.0 ^a	26.7	29.0	27.6 ^a	7.6	10.2	8.1 ^a
Organic Fertilizer (Chicken Manure)	32.0	39.0	36.7 ^a	4.5	5.7	5.1 ^a	26.4	29.0	27.6 ^a	7.5	10.2	7.6 ^a

Means with different letters indicate significant differences between treatments ($P < 0.05$).

Phytoplankton and microbial flocs

Phytoplanktons play a significant role in stabilizing the whole pond ecosystem and in minimizing the fluctuations of water quality. A suitable phytoplankton population enriches the system with oxygen through photosynthesis during day light hours and lowers the levels of CO₂, NH₃, NO₂ and H₂S. During the first week of the experiment there was no significant difference in the phytoplankton densities between the two treatments (Table 2). Common phytoplanktons observed were diatoms and *Chaetoceros* species. A day after the 3rd fertilization, the algae bloomed manifested by dark green coloration of the water in the tanks applied with urea. Mean phytoplankton count in these tanks increased from $372,500 \pm 2,500$ cells ml⁻¹ to $513,333 \pm 11,273$ cells ml⁻¹ and was significantly higher compared to the tanks fertilized with chicken manure. Generally, the phytoplankton densities all throughout the experiment were relatively lower compared to the healthy phytoplankton abundance reported by Cordova *et al.* (2008) in ponds applied with organic and inorganic fertilizer at 530,000 – 980,000 cells m⁻¹.

The denseness could be attributed to the use filtered seawater. Pond green water was only added in the tanks to provide initial algal population. Phytoplanktons aggregate with microorganisms in the aquaculture system to form microbial flocs. Bio-flocs or the microbial flocs are mixture of microorganisms (bacteria, phytoplanktons and zooplanktons), algae and other particles (Jorand *et al.*, 1995). Typical flocs are irregular by shape, have a broad distribution of particle sizes, are fine, easily compressible, highly porous and are permeable to fluids (Chu and Lee, 2004). Microbial floc or flocculated particles were observed to be increasing as shown in Table 2. The build-up rates of the floc on the 2nd week followed the standard community succession principle wherein from a clear water condition, algal bloom follows and bacterial communities are established when the water turns to brown. Build-up of flocculated particles in two-week time was relatively low.

The microbial flocs observed in the experiment were visibly large and distinct under the microscope. Optimum water quality conditions are

important in maintaining healthy population of microbial flocs and these were met during the experiment. High dissolved oxygen in the system is not only essential for the shrimps but also for the metabolic activity of the cells and structure of the microbial flocs. A trend towards larger and more compact flocs at higher dissolved oxygen concentrations was noted by Wilen and Balmer (1999). Temperature is also important in microbial floc morphology.

Krishna and Van Loosdrecht (1999) observed that higher temperatures (30-35°C) resulted in bulking of the sludge due to the excessive production of extracellular polysaccharide, thus, intermediate water temperature of 20-25°C would be best to obtain stable microbial flocs. Similarly, pH has an effect on microbial floc, according to Mikkelsen *et al.* (1996) changes in pH determine the stability of bio-flocs present in the system.

Table 2. Mean phytoplankton and microbial floc count in the water of Pacific white shrimp (*L.vannamei*) nursery culture with different fertilizers

Treatment	Phytoplanktons (cell density ml ⁻¹)		Microbial Flocs (cell density ml ⁻¹)	
	Initial	Day 7	Initial	Day 7
Inorganic Fertilizer (Urea)	372,500 ± 2,500 ^a	513,333 ± 11,273 ^a	723 ± 13 ^a	797 ± 17 ^a
Organic Fertilizer (Chicken Manure)	365,833 ± 3,819 ^a	423,333 ± 3819 ^b	732 ± 38 ^a	836 ± 15 ^a

Means with different letters indicate significant differences between treatments (P<0.05).

Growth and survival

Growth, survival and yield are summarized in Table 2. A day after the 3rd fertilization, the algae bloomed in the tanks applied with urea which resulted to mass mortality of all stocks. However, the fatality could not be attributed to the increase in phytoplankton density because it was relatively lower compared to a healthy phytoplankton bloom (Cordova *et al.*, 2008). Furthermore, dissolved oxygen remained at its optimum level and apparently not the cause of mortality. One possible reasons of the fatality could be the increase of NH₃ in the system triggered by the addition of urea. In the experiment conducted by Das *et al.* (2005) application of organic and inorganic fertilizer increased inorganic nutrients including ammonia. Application of inorganic fertilizer gave peak values of nitrogen species such as ammonia, nitrite and nitrate earlier during first to second week. Although natural food production was enhanced by application of organic and inorganic fertilizers, the results showed that weekly application of urea at 50 kg ha⁻¹ significantly affects survival, causing mass mortality of stocks. On the other hand, survival of shrimps in tanks fertilized with chicken manure was 92.68% with specific growth rate (% day⁻¹) of 14.05 in terms of weight and 18.17 in terms of length and

total yield of 128.37 g m⁻¹. Lara-Anguiano *et al.* (2013) found out that *L.vannamei* culture in tanks with zero water exchange using organic fertilizer (molasses) had higher survival and production rates and reduced ammonia concentrations toward the end of the experiment while the use of inorganic fertilizer caused increased nitrogen and phosphorus.

Conclusion

Periodic fertilization using chicken manure at a rate of 625 kg ha⁻¹ improved feed conversion rate as well as shrimp survival and production. Moreover, the results indicate that weekly periodic application of urea at a rate of 50 kg ha⁻¹ without any water replacement in tanks during the nursery of *L. vannamei* is not viable.

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