Production performance of white shrimp (*Litopenaeus vannamei*) under sea floating net cages with biofloc and periphyton juvenile

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Abstract. To the post larvae (PL), intermediate rearing should apply before sea floating net cage situation(s). This understanding formed the basis of this current study, which was performed specifically to compare the production performance of biofloc (BFT) and periphyton (PPT) juvenile of white shrimp (*Litopenaeus vannamei*) to reach marketable size in floating net cage. The study consists of two steps, that is, intermediate rearing and grow-out. In intermediate rearing phase, post larvae were respectively stocked in triplicate at the density of 2666 PL m^{-3} for BFT and 1333 PL m^{-3} for PPT. PL was fed five times daily with the blind feeding methods and reared for 21 days prior grow-out. In grow-out phase, shrimp juvenile were then stocked in triplicate 3x3x3 m cages at the density of 550 m^{-2} and reared for 120 days. Juvenile were fed commercial pellet six times daily with restricted feeding method refer to feeding level of vannamei. Following periodic sampling with subsequent analysis, it was found that, intermediate rearing by using BFT shows better growth rate, final weight and length, survival rate, feed conversion and biomass production performance compared to PPT ($P<0.05$). In grow-out phase, BTF juvenile reach marketable size and also shows better growth performance, final weight, survival, feed conversion, and biomass production compared to the rest. Plausibly BFT performs better than PPT in terms of intermediate rearing to produce better seed quality.

Key Words: intermediate rearing, grow out, growth rate, survival rate.

Introduction. Whilst the global demand for shrimp is expected to reach 5 million ton/year, such production could only provide 3.6 million ton year^{-1}, out of which 2.37 million ton are belivedto be *Litopenaeus vannamei* cultured of brackish water ponds (FAO GLOBEFISH 2015). Disease outbreaks also have become a major problem in pond shrimp production (Leano & Mohan 2012; Lightner et al 2012; Velmurugan et al 2014; Karthikeyan et al 2015). It is also primarily caused by poor water quality and degraded carrying capacity (Primavera 1994; Avnimelech & Ritvo 2003; Herbeck et al 2013; Wu et al 2014; Bournazel et al 2015; Rekha et al 2015). Such conditions have however resulted in low productivity (Kumar et al 2012) and high production cost (Paterson & Miller 2014). An alternative to shrimp production system are needed, among others for example sea floating net cage which is associated with several advantages when compared to brackish water pond, i.e. good water circulation and high oxygen solubility (Paquotte et al 1998; Zarain-Herzberg et al 2006, 2010; FAO 2007) and no accumulation of solid waste near the cages (Alongi et al 2003). Likewise, no energy is required for aeration or water exchange (Paquotte et al 1998). In addition, the production yields are higher than those obtained in extensive and semi intensive ponds (Walford & Lam 1987; Paquotte et al 1998; Zarain-Herzberg et al 2006), and there is a high possibility for intensification due to high carrying capacity of sea water body (Zarain-Herzberg et al 2010), which enables the produced shrimp meat with better taste, texture, color and smell (Liang et al 2008; Maicá et al 2014).

Marine environment is different relative to inland aquaculture (brackish water pond) in terms of wave, current, light, transparency, plankton, suspended solid of water and other parameters. Thus, the shrimp seed should be prepared to be adapted with such prevailing conditions. The intermediate rearing or nursery should be done to the shrimp post larvae (PL) prior reared in the grow-out system in the sea (Zarain-Herzberg et al 2006, 2010). For improving production performance, Zarain-Herzberg et al (2006) attempted to install polystyrene substrates in floating net cage for shrimp shelter to grow periphyton as additional feed and shelter for the shrimp PL. For emphasis, periphyton refers to the entire complex of microalgae, heterotrophic bacterium, benthic organisms and detritus developed over submerged substrate and forms an additional food in aquatic production systems (Azim et al 2005; van Dam et al 2002; Anand et al 2013). Periphyton could increase nutrient utilization efficiency and improve shrimp immune-system (Bratvold & Browdy 2001; Zarain-Herzberg et al 2006; Sharma et al 2010; Zhang et al 2010; Promya & Chitmanat 2011; Kumar et al 2015). It also can significantly increase the growth of vannamei shrimp (Audelo-Naranjo et al 2011; Moss & Moss 2004), tiger shrimp (Kumar et al 2015; Khatoon et al 2009) and brown tiger prawn (*Penaeus esculentus*) (Burford et al 2004a).

Another technology that could be implemented for improving the intermediate rearing of shrimp PL is biofloc, which is a conglomeration of microbes, alga, protozoa and other microorganisms together with detritus (particles from death organisms) that formed unique ecosystem in form of floc of suspended particle, porous characteristic, very light, and has a diameter of 0.1 to several mm (Avnimelech 2012). Such microbes in biofloc as heterotroph bacteria could decompose organic materials and shrimp metabolites. In addition, their proliferation would depend on the floc substrates, oxygen levels, pH, C:N ratio, temperatures and other parameters (Avnimelech et al 1994; Avnimelech 2012; Ferreira et al 2015). Not only for water quality improvement, biofloc also provides food supplements with high proteins, polyunsaturated fatty acid (PUFA), and high lipids for shrimp (McIntosh et al 2000; Burford et al 2004a; Buford et al 2004b; Avnimelech 2006; Wasielesky et al 2006). In addition, it can reduce the use of commercial feed. Other authors have considered biofloc able to improve shrimp immune system and reduce pathogen bacteria proliferation by food and space competition (Crab et al 2007; Ferreira et al 2015), and could increase seed quality (De Schryver et al 2008; Avnimelech 2012).

Relevant information about the performance comparison between biofloc and periphyton of white shrimp juvenile in FNC grow-out system in the sea seem unvailable. To supplement existent literature, the present work was performed to compare the production performance of biofloc (BFT) and periphyton (PPT) juvenile of white shrimp (*Litopenaeus vannamei*) with target of marketable size in sea FNC. Production performance parameters evaluated include growth rate, final weight and length, survival rate, feed conversion and biomass production.

Material and Method. This study was conducted in two steps: shrimp PL intermediate rearing and grow-out. The intermediate rearing was conducted in indoor hatchery tanks for BFT at Karang Congkak Island and in FNC for PPT at Semak Daun Island waters, at Thousand Islands Jakarta, Indonesia. The grow-out rearing was conducted in FNC at Semak Daun Island waters. The location is a part of Sea Farming Station, Center for Coastal and Marine Resources Studies, Bogor Agricultural University. Semak Daun Island waters has a water depth of 0.5-12.1 m, salinty of 31-32 ppt, dissolved oxygen of 7.4-9.4 mg L^{-1}, temperature of 28.6-30.4ºC and current water of 1.2-552 cm sec^{-1}. The research was conducted on June-August in calmed West Seasson.

Intermediate rearing. Intermediate rearing of shrimp PL with BFT was done in 2.5x1.2x1.1 m of fiber glass tanks. The tank was filled with 1.5 m^3 (0.5 m depth) of sea water and added with 10% of biofloc inoculant, and the water were aerated during rearing period and no water exchange. After 14 days when the culture media become brownish-red in color and good smells, PL10 shrimp (specific pathogen free, SPF-certified) were stocked in triplicate tanks with density of 2,666 shrimp per m^3. The shrimp PL supplied by a reputable (private) hatchery was fed commercial pellet-powder with 40% protein. The feeding rate was 16% of the total biomass and the feeding frequency was five times daily. After 10 days of rearing, half portion of daily feed were changed to molasses and rice brans (with ration of 1:1) as carbon source to maintain C:N ratio in culture media around 1:12 to 1:15. Dolomites lime was added into the culture media between 10-15% of total feed when any drop in pH appeared. Intermediate rearing with PPT was conducted in triplicate of 3x3x1.5 m polyethelene cages with mesh size of 1 mm, equipped with 4 units of periphyton substrate made from 5 sheet of 50x50 cm PE net. Substrate were hanged 50 cm under surface sea water two weeks before stocking of shrimp PL. The cages were covered with net to illuminate the light. PL10 were stocked in cage with density of 1333 shrimp m^{-2} and fed commercial pellet-powder with 40% protein, about six times daily. The feeding rate of PL was referred to standard of white shrimp culture (blind feeding method) which is about 16% daily. In both system, shrimp PL were reared for 21 days and at the end of rearing shrimp juvenile were counted and measured for length and weight.

Grow-out rearing. Six polyethylene floating net cages with dimension of 3x3x3 m were used for shrimp grow out. The mesh size of net cages was adjusted to shrimp size, i.e. 1 mm for shrimp juvenile of 3-5 cm, 5 mm for 6-10 cm and 0.5 inch for 11-13 cm. The bottom of cages was layered by additional net with mesh size of one mm to protect the shrimp from predators and to prevent feed loss. Each cage equipped with one 1x1 m feeding tray made from wood frame and net with mesh size of 1 mm. The feeding tray were hanged horizontally at about 1 meter below the water surface. Biofloc juvenile (BFT) and periphyton juvenile (PPT) with size of 3-4 cm in length were reared in triplicate of cages with stocking density of 550 shrimp m^{-2}. Treatments were randomly assigned to cages. Shrimp juvenile were fed commercial pellet with 36-38% protein. The feeding rate during the first month of rearing was 6% of the total biomass. It was reduced to 5, 4 and 3% during the second, third and fourth month, respectively. The feeding frequency was six times daily. During rearing period (120 days) a part of wall surface of the cage nets were cleaned periodically from biofouling using plastic brush to ensure free water flow through the cage. In addition the remained biofouling has been maintained as natural food for the shrimp juvenile (Zarain-Herzberg et al 2006).

Test parameters. Test parameters for intermediate and grow-out rearing include resultant weight and length, daily growth, survival rate, feed conversion ratio (FCR) and biomass production. Weight and body length of shrimp in grow-out rearing were periodically measured using 30-40 shrimp samples every 10 days. Daily growth was calculated according to Ricker (1979), and FCR by Zonneveld et al (1991). At the end of the experiment, the survival rate was calculated by counting the total number of shrimp in each cage. Water quality consist of temperature, dissolved oxygen (DO), salinity, ammonia and pH were measured daily in the morning.

Data analysis. All data were presented as the mean ± standard deviation (SD) of replicated measurements (n = 3). Growth rate, final weight and length, survival rate, feed conversion ratio and biomass production were analyzed by analysis of variance (ANOVA) using the statistical software SPSS version 16 (paired t-test). Significance of differences was defined at $p<0.05$.

Results and Discussion. In the intermediate rearing, the final weight and length, daily growth, survival rate, FCR and biomass production of vannamei juvenile in BFT showed better performance compared to PPT ($p<0.05$) as presented in Table 1. In the grow-out rearing, the weight of BFT juvenile remained higher compared to PPT during 120 days of rearing as depicted by the growth curve in Figure 1. Final weight and length, daily growth, survival rate, FCR and biomass production of BFT juvenile showed better outcomes compared to PPT, as in the intermediate rearing ($P<0.05$) (Table 2).

Table 1
Production performance of white shrimp (*Litopenaeus vannamei*) in intermediate rearing using biofloc (BFT) and periphyton (PPT) technology

Production performance	*BFT*	*PPT*
Final weight (g)	0.37±0.03[a]	0.24±0.06[b]
Final length (g)	3.73±0.12[a]	3.23±0.23[b]
Daily growth (g day^{-1})	0.018±0.002[a]	0.011±0.003[b]
Survival rate (%)	83.96±2.96[a]	74.48±2.80[b]
Feed conversion ratio (FCR)	0.76±0.067[b]	3.43±0.03[a]
Biomass production (kg m^{-2})	0.80±0.07[a]	0.20±0.01[b]

Different letters indicate significant differences ($p<0.05$).

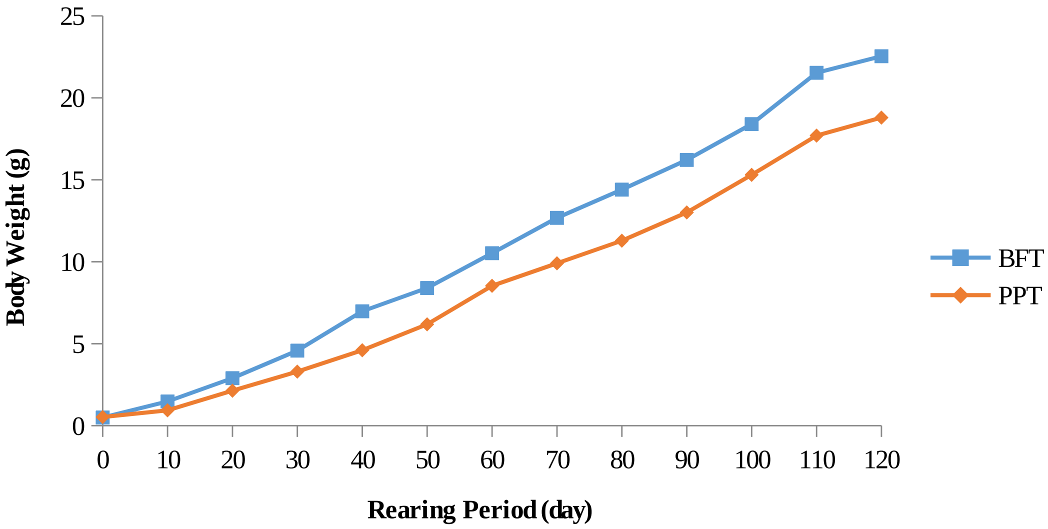

Figure 1. Growth curve of biofloc (BFT) and periphyton (PPT) juvenile of white shrimp (*Litopenaeus vannamei*) in sea net cages of grow-out rearing.

Table 2
Production performance of white shrimp (*Litopenaeus vannamei*) in sea net cages of grow-out rearing using biofloc (BFT) and periphyton (PPT) juvenile

Production performance	*BFT*	*PPT*
Final weight (g)	22.54±1.77[a]	18.79±1.44[b]
Daily growth (g day^{-1})	0.19±0.02[a]	0.15±0.01[b]
Survival rate (%)	58.93±1.67[a]	27.36±4.47[b]
Feed conversion ratio (FCR)	2.88±0.06[b]	2.925±0.06[a]
Biomass production (kg m^{-2})	6.45±1.63[a]	2.700±0.28[b]

Different letters indicate significant differences ($p<0.05$).

Water quality in grow-out cages appeared within tolerance range for white shrimp with some resemblances between treatments ($P>0.05$) (Tabel 3). Temperature ranged between 28.1-30.9^{o}C, DO 5.6-6.6 mg L^{-1}, salinity 31-33 ppt; ammonia 0.01-0.03 mg L^{-1} and pH 7.1-8.2. Data of water quality resembled both inside and outside of the cage ($P>0.05$).

Table 3
Water quality parameters inside and outside the floating net cage in grow-out rearing of white shrimp (*Litopenaeus vannamei*)

Parameters	*Inside rearing cage*		*Outside rearing cage*
	BFT	*PPT*	
Temperature (^{o}C)	28.3-30.9	28.1-30.7	28.0-31.5
Dissolved oxygen (mg L^{-1})	5.0-6.1	5.9-6.6	6.1-7.2
Salinity (ppt)	31-33	31-33	31-32
Ammonia (mg L^{-1})	0.01-0.02	0.01-0.03	< 0.01
pH	7.1-8.2	7.4-8.2	7.8-8.8

Intermediate rearing with BFT produced improved production performance compared to PPT, given by data of final weight, final length, daily growth, survival rate, FCR and biomass production of shrimp PL ($P<0.05$) (Table 1). The bacteria are intentionally cultured to become bioremediator that convert nitrogen waste (metabolites and feed waste) into microbes protein in biofloc system (Crab et al 2007). BFT also provide food supplement with high proteins, PUFA, and high lipids for PL (McIntosh et al 2000; Buford et al 2004a; Buford et al 2004b; Avnimelech 2006; Wasielesky et al 2006). Biofloc contains approximately, 0.9-16% poly-β-hydroxybutyrate to fulfill shrimp needs but not more than 1% (De Schryver & Verstraete 2009; De Schryver et al 2010; Sarmin et al 2013). Poly-β-hydroxybutyrate is an intracellular polimer product that produced by several kind of microorganisms as energy source and carbon. With the nutrition quality mentioned above, biofloc not only gives a high PL growth (0.018 g day^{-1}) but also can help save farmer from the use of commercial feed. It can be seen on the value of FCR was relatively low on shrimp with BFT compared to PPT. In addition, half portion of expensive commercial pellet feed of intermediate rearing with BFT was replaced with cheaper carbohydrates source feed ingredients (rice bran and molasses).

Herein also, production performance of intermediate rearing with PPT remained below BFT, even though the process technology have been tried in shrimp culture (Moss & Moss 2004; Burford et al 2004a, 2004b; Khatoon et al 2009; Audelo-Naranjo et al 2011; Kumar et al 2015). Periphyton is a cheap food source for the PL and easy to produce. Phytoplankton colonies are considered able to provide food for shrimp. In PL stage, vannamei consumes diatom, then algal filament, seagrass, zooplankton, then small molusca, tiny shrimp, polychaeta, other invertebrate and detritus aggregate (Azim et al 2005). Herein, poor production performance of the intermediate rearing with PPT may be caused by environment of sea FNC which more dynamic than rearing tanks of BFT. The water currents (about 11.1-12.4 cm) in the net cages made the PL shrimp hard to consume the attached periphyton which might have made the substrate to swing consistent with currents/waves, amidst the absence of data on feed intake of shrimp PL.

Survival rate of PL in BFT intermediate rearing (83.96%) was higher than PPT (74.48%). The result might be owed to a combination of factors: intermediate rearing tanks environment and presence of decomposer (microbes). The environment of rearing tanks of BFT seemed more tolerable compared to PPT in sea FNC. This could make it more favorable for shrimp PL. The presence of decomposer in rearing tank probably inhibits the proliferation of pathogenic bacteria amidst food and space competition (Crab et al 2007; Ferreira et al 2015). Biofloc contains poly-β-hydroxybutyrate that not only could enhance cultured shrimp growth but also exerts prevention and treatment effects on *Vibro* infection (Defoirdt et al 2007; De Schryver et al 2008; Ekasari et al 2014). With the superiority of survival and growth causing intermediate rearing of PL with BFT (0.8 kg m^{-3}) there would be a higher productivity than PPT (0.2 kg m^{-3}).

Excellent shrimp production performance was obtained by BFT juvenile of grow-out rearing in sea FNC. The production performance of BFT juvenile seemed improved compared to PPT juvenile in term of daily growth, final weight and length, survival rate, FCR and production biomass (Table 2). Better nutrition intake of PL during intermediate rearing lead BFT juvenile has higher performance than PPT juvenile in sea FNC. The daily growth and final weight of BFT juvenile reached 0.19 g day^{-1} and 22.54 g $shrimp^{-1}$ (size 44, mean 44 shrimp in one kg) respectively, while PPT juvenile only 0.15 g day^{-1} and 18.79 g $shrimp^{-1}$ respectively. In addition, daily growth data herein seemed in good agreement with data reported by Zarain-Herzberg et al (2006, 2010), seemingly higher to data reported by Paquotte et al (1998). The daily growth of BFT juvenile seemed lower than standard growth for vannamei culture in intensive brackish water pond (Wyban & Sweeney 1991). With daily growth of 0.19 g day^{-1} of BFT juvenile, about 105 days was than required to reach export size of shrimp (size 50-60) while for PPT juvenile, an increased rearing period needed amounted to about 134 days. Biomass production of BFT juvenile of 6.45 kg m^{-2} or 64.5 ton ha^{-1} would equal to intensive white shrimp production standard in brackish water pond (Wyban & Sweeney 1991).

Herein also, the survival rates both BFT and PPT juveniles (27.36-58.93%) seemed lower compared with those of Zarain-Herzberg et al (2006, 2010) that reported range of between 77.4-95.0%. The low survival rate could be associated with the longer rearing period (120 days) compared to those of Zarain-Herzberg et al (2006, 2010) i.e. 58-63 days and the incidence of lost shrimp. Shrimp mortality was determined by counting live shrimps at the end of rearing. The reason of lost shrimp is still unknown. It can be that the died shrimp bodies might well have been consumed by wild fish outside the cage in the night. Shrimp lost is also possibly owed to predator at outside of cage consuming small shrimp just from between the mesh. Stressed and weak shrimp probably found after sampling and net changing, usually became an easy target for the predator. Muscle damage would be observed followed by necrosis in the body of died shrimp. Before death, the shrimps showed the following symptoms: jerky movement in circular shape and stretching their body. Increased mortality seemed apparent when the shrimp reached above 10 g $shrimp^{-1}$ in weight. When reached that size, the shrimp may stress due to high stocking density (550 shrimp m^{-2}). In intensive brackish water pond the stocking density could only reach 60-300 shrimp m^2 and 30-50 shrimp m^{-2} for semi-intensive system (Wyban & Sweeney1991).

The FCR obtained in our trials resembled those estimated by Paquotte et al 1998 and were higher than those found by Zarain-Herzberg et al (2006, 2010). In brackish water pond, white shrimp FCR can reach 1.3-1.6 (Wyban & Sweeney 1991), which was seemingly lower than our study in sea FNC plausibly attributable to water productivity. Especially in pond situations, aquatic systems may produce higher water productivity compared with the sea FNC, which may well also facilitate the use of natural feed resource for the shrimp in view to reduce the use of commercial pellet feed. Sea water of FNC can be classified as oligotrophic waters, so shrimp relying only on the commercial pellet feed.

Water quality parameters inside rearing cage were still within a tolerant range, according to biological standard for white shrimp culture in ponds and marine-organisms. Water quality parameters inside and outside the cages seemingly differed only in ammonia and DO levels. Ammonia levels inside the FNC seemed higher compared with those outside the FNC. It is probably caused by the un-eaten food that gathered at the bottom of the cage, which might have produced some food waste that contained protein. The food waste would then decompose, producing ammonia that probably contributed to facilitate the blockage of the current owed to the increased biofuoling. Biofouling also blocked water circulation in the bottom, supported by DO and pH levels measurement. DO and pH levels inside the cage were lower than outside cage.

Conclusions. Production performance of BFT overall seemed improved compared with those of PPT in terms of intermediate rearing as it produced better seed quality for sea FNC culture of *L. vannamei*. BFT juvenile are recommended as useful candidate for white shrimp culture in sea FNC. Further study should be directed towards the increase of survival rate to reduce the FCR of white shrimp culture in sea FNC. Further study to resolve the “lost-shrimp” may also be useful especially for improving cage engineering because it may well facilitate shrimp sampling and net changing which can suppress the mortality. The study about shrimp feeding management including application of improved periphyton technology in sea FNC may serve as a clue to suppress FCR.

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