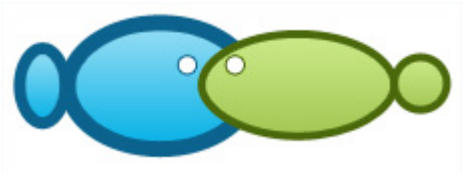

Comparative efficacies of tilapia green water and biofloc technology (BFT) in suppressing population growth of green Vibrios and *Vibrio parahaemolyticus* in the intensive tank culture of *Penaeus vannamei*

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Abstract. The use of tilapia green water (TGW) and biofloc technology (BFT) to control abundance of green colony forming *Vibrio* species and *Vibrio parahaemolyticus* in the intensive tank culture of *Penaeus vannamei* was evaluated. The study was performed over a 60-d outdoor culture of *P. vannamei* in 1000-L concrete tank with three replicates each culture system. The densities of *Vibrio* spp. in the rearing water and attached on surfaces were monitored at 6-d intervals. *V. parahemolyticus* was enumerated using a chromogenic bacterial medium. The BFT culture system promoted higher densities of total culturable *Vibrio* species in both water and surface samples while TGW has consistently lower densities of attached total culturable Vibrios. Percentage of green colony Vibrios in the total culturable *Vibrio* count in the water were generally lower in TGW and BFT. Furthermore, the densities of *V. parahaemolyticus* in the water samples as well as on surfaces were generally lower in the TGW than in the other culture system. The present findings demonstrated that the practice of TGW technology is an effective ecological method of controlling growth of potentially pathogenic bacterial species such as the green colony-forming Vibrios and *V. parahaemolyticus* in the intensive tank culture of *P. vannamei.*
Key Words: shrimp culture system, bacterial diseases, *Vibrio* species, white shrimp.

Introduction. Bacterial diseases have historically been among the most important problem facing aquaculturists and *Vibrio* spp. may well be the longest known pathogen in aquaculture (Hawke 2000). *Vibrio* species are highly abundant in saline water environments and some strains are considered opportunistic pathogens of immunocompromised cultured shrimp under unfavourable environmental conditions. *Vibrio parahaemolyticus* for instance, one of the dominant *Vibrio* spp. frequently isolated in estuaries and aquaculture farms (Thakur et al 2004; Gopal et al 2005; Alagappan et al 2013), has been associated with shrimp diseases such as the red disease and tail necrosis (Jayasree et al 2006). Recently, a strain of this *Vibrio* sp. was identified as the causative agent of acute hepatopancreatic necrosis disease (AHPND) in penaeid shrimp. The bacterium was isolated from an AHPND-positive shrimp in Vietnam and the pure culture of the isolate was able to induce 100% mortality to experimentally infected healthy shrimp (Tran et al 2013). AHPND emerged in 2009 and has since then affected major shrimp-producing countries in Asia. The disease causes high and rapid mortality in 20 to 30 days after stocking in grow out ponds (Joshi et al 2014). Outbreaks of the disease in different areas of Asia resulted in production losses, loss of income and profit for shrimp producers, and impacts on trade. Viet Nam reported an estimated production loss of US$0.1 billion in 2011 while 39,000 Ha of shrimp farm were affected in Malaysia (FAO 2014).

The devastating impacts of outbreaks of diseases with bacterial etiology such as luminous vibriosis in the shrimp industry have led to development of innovative culture

techniques to control growth of important bacterial pathogens. Among these innovative techniques, are culture of shrimp using either biofloc technology (BFT) or tilapia green water (TGW). The TGW technique involves the use of the rearing water of Tilapia in shrimp culture (Corre et al 1999). On the other hand, BFT implements pond management utilizing minimal water exchange and promoting development of dense microbial population by increasing carbon: nitrogen ratio in the water (Avnimelec 1999; Ebeling et al 2006). According to Tendencia et al (2004), the presence of tilapia in the rearing water of shrimp can directly inhibit the growth of *V. harveyi* which could be attributed to the inherent property of tilapia such as mucus and other metabolites that have antagonistic effect against *Vibrio* spp. Also, the green water is characterized by the abundance of micro algae such as *Chlorella* and *Nannochloropsis* which are known to be highly beneficial for farmed aquatic animals. The addition of *Nannochloropsis* sp. was reported to enhance the survival of mud crab *Scylla serrata* larvae despite exposure to pathogenic concentration of *V. harveyi* which is due to the microalgae's role in bacterial growth inhibition, improvement of water quality, and enrichment of live food (Toledo et al 2005). In addition, extracts of the micro alga in methanol, *n*-hexane, and ethyl acetate exhibited vibriostatic activity when bioassayed with *V. harveyi* (Seraspe et al 2005). On the other hand, the practice of growing shrimp using BFT has been found to be advantageous than the conventional culture practices. The utilization of limited water exchange in BFT can minimize the introduction of pathogens with the incoming water. The improved biosecurity in this management practice can reduce crop losses due to outbreaks of infectious diseases. Moreover, it was documented that bioflocs grown on glycerol were able to protect gnotobiotic brine shrimp (*Artemia franciscana*) against pathogenic *V. harveyi* (Crab et al 2012).

The potential of both TGW and BFT in controlling growth of *Vibrio harveyi* has been reported. However, the effectiveness of both systems in suppressing growth of other potential pathogens such as *Vibrio parahaemolyticus* in the intensive culture of shrimp still needs to be proven. Therefore, the present study aims to evaluate the posible effects of using TGW and BFT on the population growth of total culturable *Vibrio* species, particularly *V. parahaemolyticus*, in the intensive tank-based culture of *Penaeus vannamei*.

Material and Method

Culture conditions and experimental animals. The study was conducted at the facilities of the Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas from July 3 to September 3, 2015. The experiment was carried out in nine concrete tanks with 1000L working volume and provided with uniform aeration. The tanks were filled with disinfected seawater adjusted to 25 ppt with freshwater. Prior to stocking, growth of phytoplankton was stimulated by adding of 10 ppm commercial inorganic fertilizer urea (46-0-0).

The experiment evaluated two innovative culture systems (TGW and BFT) in shrimp grow out production and a control treatment where shrimp was reared in a conventional culture practice. A regular water exchange using unfiltered seawater pumped directly from the source was observed in the control tanks. Initial water change was done 15 days after stocking and weekly thereafter. The green water culture (TGW) system followed the protocol presented by Corre et al (1999). High-saline tolerant *Tilapia hornorum* were stocked in a tank with mature water at a stocking rate of 30 fry per m^3. The green water produced in the tilapia tanks was used to replace the water lost during water exchange in shrimp tanks. Frequency of water change in the control was also practiced in the TGW. The treatment tanks for the BFT utilized a limited water exchange system with molasses as carbon source. The supplementation of carbon was based on the addition of 15.17 unit carbohydrates to every one unit of ammonia (Ebeling et al 2006). Supplementation was adjusted whenever floc level (measured by the Imhoff cone) exceeds 15 mL L^{-1}. Juvenile *P. vannamei* (ABW = 2.21g) were stocked at a density of 150 shrimp per m^3 after two weeks of water culture. Shrimp were fed twice daily at 07:00 and 17:00 h with commercial vannamei feeds. Adjustment in feeding rate and feed

type was done based on the shrimp consumption and growth performance obtained during biweekly sampling.

Water temperature ranged between 27–31°C, salinity was within 15–25 ppt, dissolved oxygen between 3.5–6.90 mg L^{-1} and pH between 6.7–8.00.

Sample collection. Samples for bacterial enumeration were collected at six-day intervals between 9:00–10:00 AM. Water samples were collected in sterile plastic sampling bottles taking necessary precautions to avoid cross contaminations. For the enumeration of *Vibrio* species colonizing surfaces, a polystyrene plate was submerged in each tank at the start of the experiment. During sample collection, the plate was rinsed with sterile saline solution to remove unattached bacteria. Samples were collected by swabbing one known area with a sterile cotton swab (the area previously sampled were not anymore used for the succeeding sample collections). The swab was then immediately soaked in a sterile saline solution and vortex mixed to release attached microorganisms to the diluent.

All samples were subjected to a 10-fold serial dilution using sterile (120°C for 15 minutes) seawater. Representative dilutions were plated onto thiosulphate citrate bile sucrose agar (TCBS) in duplicates for the enumeration total culturable *Vibrio* count. Morphology and number of colonies on TCBS were recorded after 18–24 hours of incubation at 30°C.

Green colonies observed in the TCBS were replica plated onto the chromogenic media (HiCrome^{TM} Vibrio Agar) for the enumeration of *V. parahaemolyticus.* The colonies that developed bluish green coloration within 18 to 24 hours were recorded as *V. parahaemolyticus* count. Counts of the rearing water were expressed as colony forming unit (cfu) per mL while that of attached *Vibrio* sp. were expressed as cfu per cm^2.

Statistical analyses. Data were analyzed by one-way analysis of variance (ANOVA) for each sampling period. If the effects are significant, the means were subjected to post hoc analysis using Duncan Multiple Range Test (DMRT). All statistical analyses were performed using SPSS ver. 16.0 with significance level set at 0.05. Bacterial counts were log 10 transformed before analysis but untransformed data are presented in figures.

Results. The density of total culturable *Vibrio* in the water samples were comparable among culture systems. Significant differences were observed only starting day of culture (DOC) 48 onwards with significantly higher counts observed in the BFT tanks. The counts ranged from 10^2–10^4 cfu/mL in control and TGW; and 10^2–10^5 cfu/mL in BFT (Figure 1). On the other hand, density of *Vibrio* spp. colonizing surfaces were found to be generally lower in the TGW tanks (10^1-10^3 cfu/cm^2) with significant differences observed at DOC 12 to 30. The counts in both control (10^1–10^5 cfu/cm^2) and BFT (10^1–10^4 cfu/cm^2) culture systems were comparable all through out the culture period (Figure 2).

Table 1 presents the percentage composition of green-colony Vibrios in the total culturable *Vibrio* count in the water and surface samples. Lower percentage of green Vibrios were observed in both TGW (0–29.8%) and BFT (0–63.7%) than in the control (0–67.3%). However, green *Vibrio* spp. were found to dominate the total population of Vibrios attached on surfaces of all culture systems.

Figure 3 shows the *Vibrio parahaemolyticus* count in the rearing water of the three culture systems. Although increasing as the culture period progressed, the density of *V. parahaemolyticus* in water samples were observed to be consistently lower in the TGW (0–10^3 cfu/mL) tanks than in the other two culture systems (0–10^4 cfu/mL). Similar occurrence was observed in the surface samples (Figure 4).

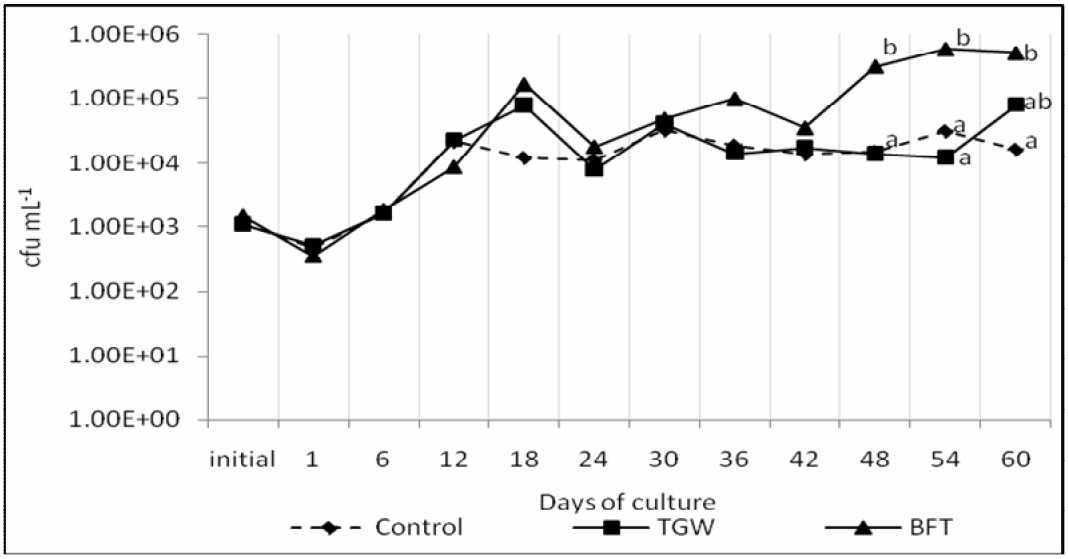

Figure 1. Total culturable *Vibrio* count in the water samples at different days of culture (DOC) of *P. vannamei* in different culture systems. Values during the same DOC with different labels are significantly different ($p < 0.05$).

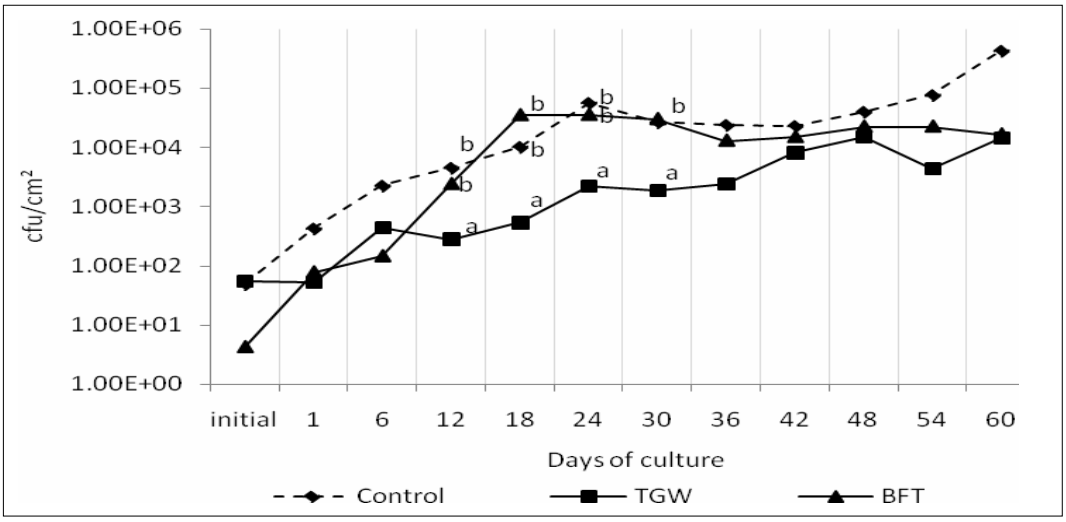

Figure 2. Total culturable *Vibrio* count in the surface samples at different days of culture (DOC) of *P. vannamei* in different culture systems. Values during the same DOC with different labels are significantly different ($p < 0.05$).

Table 1

Percentage composition of green-colony forming Vibrios in the total culturable *Vibrio* count at different days of culture of *P. vannamei* in different culture systems

Culture system	*Days of culture (DOC)*											
	initial	*1*	*6*	*12*	*18*	*24*	*30*	*36*	*42*	*48*	*54*	*60*
	% Green Vibrios in water samples											
Control	0.0	3.2	0.0	33.6	59.6	67.3	92.8	26.2	36.2	58.3	89.2	93.4
TGW	0.0	0.0	0.0	24.1	0.3	15.6	8.5	5.3	0.0	29.8	9.9	13.2
BFT	0.0	0.0	0.0	30.3	59.2	56.2	63.7	32.9	4.0	3.3	7.9	1.5
	% Green Vibrios in surface samples											
Control	0.0	12.3	31.9	96.1	95.4	46.4	95.7	96.9	84.7	64.8	42.1	14.8
TGW	0.0	0.0	0.0	53.1	91.5	53.7	45.3	25.0	4.8	67.1	50.0	64.9
BFT	0.0	0.0	0.0	98.7	99.8	93.6	99.2	73.1	82.2	48.6	35.3	51.1

TGW - tilapia green water; BFT - Biofloc technology.

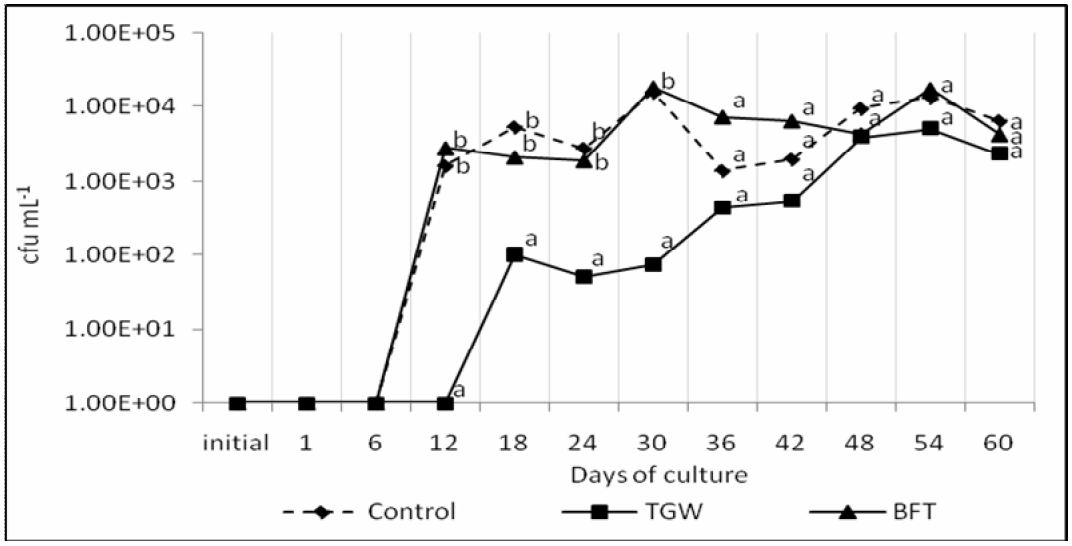

Figure 3. *Vibrio parahaemolyticus* count in the water samples at different days of culture (DOC) of *P. vannamei* in different culture systems. Values during the same DOC with different labels are significantly different ($p < 0.05$).

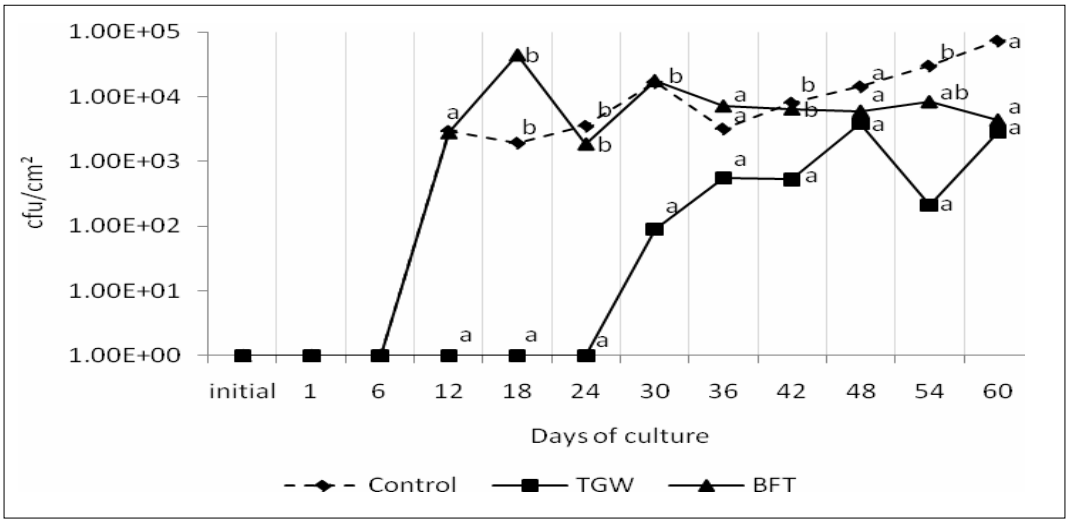

Figure 4. *Vibrio parahaemolyticus* count in the surface samples at different days of culture (DOC) of *P. vannamei* in different culture systems. Values during the same DOC with different labels are significantly different ($p < 0.05$).

Discussion. The present study evaluated the effects of different culture systems practiced in shrimp grow out production on the population growth of Vibrios in the tank-based culture of *P. vannamei*. The findings revealed that biofloc technology (BFT) promoted higher densities of total culturable Vibrios in the shrimp culture environment. The practice of increasing carbón:nitrogen ratio enhanced growth of heterotrophic bacterial species present in the system. *Vibrio* sp. have long served as models for heterotrophic bacterial processes and are known to efficiently utilized wide spectrum of carbohydrates present in the water (Thompson et al 2004; Takemura et al 2014). An increase in the *Vibrio* count as C:N ratio increases was also previously reported (Michaud et al 2006). The counts observed in the water samples of shrimp culture using the control was comparable with that reported by Gopal et al (2005) and Tendencia et al (2015). *Vibrio* count observed in the tilapia green water (TGW) culture in the present study was similar with the findings of Corre et al (2005).

Both control and BFT harboured higher densities of attached Vibrios than TGW. The green water is characterized by the high abundance of microalgae such as *Chlorella* which are known to be highly beneficial for farmed aquatic animals (Tendencia & de la Peña 2003; Corre et al 2005). The utilization of microalgae has been reported to have

improved production in hatchery operations of penaeid shrimps due to the antagonistic effects of these microalgae against virulent bacterial strains (Ronquillo et al 1998). It was also demonstrated that some species of microalgae may produce N-acyl-homoserine lactone (AHL) mimics which prevents the swarming behaviour and virulence of pathogenic bacterial species (Misciattelli et al 1998). Pathogenic *Vibrios* such as *V. harveyi* and *V. parahaemolyticus* utilises the AHL signalling system in order to transform to swarming phase and attached on surfaces at the same time express virulence. It was suggested that it is possible to block the AHL pathway by using AHL mimics which could bind to the bacterial AHL receptors and prevent swarming and expression of virulence (Misciattelli et al 1998; McCarter 1999).

Most *Vibrio* spp. pathogenic to shrimp such as *V. harveyi* and *V. parahaemolyticus* form green colonies when grown in TCBS agar (Musa et al 2008; Felix et al 2011) while those that form yellow colonies were reported to have beneficial effects (Thompson et al 2010; Tendencia et al 2011). The low percentage of green *Vibrio* spp. in the water samples of both TGW and BFT (Table 1) indicates that these culture systems promotes the dominance of yellow Vibrios in the rearing water of shrimp. Addition of molasses could have promoted the proliferation of yellow Vibrios in BFT since these *Vibrio* spp. utilizes sucrose while the green ones do not (Rahman et al 2010). Also, a decreasing trend in the percentage of green Vibrios in the BFT tanks was observed in the present study which suggests that there might be a shift in the *Vibrio* species abundance within the system to favour the dominance of other species that may have beneficial roles in the shrimp culture environment. The lower percentage of green Vibrios in the TGW suggests that there is an effective control of potentially pathogenic Vibrios in the water as demonstrated in the findings of Tendencia & de la Peña (2003), Tendencia et al (2004), Corre et al (2005), and Lio-Po et al (2005). Similarly, Huervana et al (2006) reported low percentage of green-colony Vibrios in the "green water" obtained from the broodstock tank of *Oreochromis mossambicus.*

The data generated on the occurence of *Vibrio parahaemolyticus* in the present study revealed the presence of this *Vibrio* species in all culture systems although at varying levels. The mere presence of this *Vibrio* sp. will not cause any harm to the cultured animal unless the host (the shrimp) is immunologically compromised. Generally lower *V. parahaemolyticus* counts were documented in the TGW tanks than in the control and BFT. A previous study reported that Vibrionaceae dominates the bacterial flora of both BFT and non BFT culture systems and some of the isolates are possibly pathogenic (Luis-Villaseñor et al 2015). The lower counts of *V. parahaemolyticus* in the TGW tanks documented in the present study again proves the effectivity of TGW technique in controlling the growth of potentially pathogenic Vibrios in shrimp culture. Several authors reported the positive effect of using "green water" in preventing important bacterial disease in shrimp such as luminous vibriosis. It was documented that stocking all male tilapia (*Tilapia hornorum*) at a biomass not lower than 300 g/m^3 can efficiently inhibit the growth of luminous bacteria in shrimp culture which could be attributed to the inherent property of tilapia such as mucus and other metabolites as well as with the microflora associated with tilapia culture that have the anti- luminous *Vibrio* ability (Tendencia et al 2004). Leaño et al (2005) was able to isolate filamentous fungi in the gut and mucus of tilapia which are known to be antimicrobial producers. In addition, Lio-Po et al (2005) investigated the ability of "green water" in inhibiting the growth of *Vibrio harveyi* in the grow out culture of *P. monodon* by screening associated isolates of bacteria, fungi, phytoplankton, and skin mucus of tilapia. The results of the study revealed that "green water" produced by tilapia is effective in preventing outbreaks of luminous vibriosis due to the anti-luminous *Vibrio* factors in the bacterial, fungal, phytoplankton, and fish skin mucus component of the "green water". Probably, these factors are also associated with the effectivity of tilapia green water in controlling proliferation of *V. parahaemolyticus* in shrimp grow out culture. On the other hand, findings of Tendencia et al (2006) revealed that the increase in shrimp biomass reduces the efficiency of the presence of tilapia in controlling the growth of luminous bacteria in the water. This could be the reason why there is an increasing trend in the densities of *V. parahaemolyticus* in TGW. Higher shrimp biomass requires larger amounts of organic inputs which resulted to the increased

carbon load of the water that might have enhanced microbial growth. Probably, the anti-*Vibrio* factors present in the TGW were not sufficient to control such bacterial loads during the later culture periods. Nevertheless, it is still evident in the present findings that the use of TGW in shrimp culture could result to a lower *V. parahaemolyticus* population in the culture environment compared to other culture systems.

Conclusions. The present findings provides evidence that the use of TGW in shrimp culture could promote lesser incidence of surface colonizing *Vibrio* species, lower percentage of green Vibrios in the rearing water as well as control proliferaton of *V. parahaemolyticus* during the earlier period of shrimp culture. This study could serve as basis for possible ecological methods of preventing outbreaks of diseases due to opportunistic pathogens such as *Vibrio* sp.

Acknowledgements. The authors gratefully acknowledge the financial support and assistance of the following: Department of Science and Technology - Accelerated Science and Technology Human Resource Development Program (DOST-ASTHRDP); University of the Philippines Visayas Office of the Vice Chancellor for Research and Extension (UPV-OVCRE); and UPV-DOST Shrimp Program.

References

Alagappan K. M., Deivasigamani B., Balamurugan S., 2013 Occurrences of pathogenic *Vibrio parahaemolyticus* from Vellar estuary and shrimp ponds. African Journal of Biotechnology 12:1624-1629.

Avnimelech Y., 1999 Carbon/nitrogen ratio as a control element in aquaculture systems. Aquaculture 176:227-235.

Corre V. L. Jr., Janeo R. L., Caipang C. M. A., Calpe A. T., 1999 Sustainable shrimp culture techniques: use of probiotics and reservoirs with “green water”. Philippine Council for Aquatic and Marine Research and Development, Los Baños, Laguna and University of the Philippines in the Visayas, Miag-ao, Iloilo, 32 pp.

Corre V. L. Jr., Janeo R., Ronquillo J. O., Kurokura H., 2005 Use of green water technology as biocontrol of luminous bacteria in intensive shrimp (*Penaeus monodon*) grow-out culture. UPV Journal of Natural Sciences 10:51-60.

Crab R., Defoirdt T., Bossier P., Verstraete W., 2012 Biofloc technology in aquaculture: beneficial effects and future challenges. Aquaculture 356-357:351-356.

Ebeling J. M., Timmons M. B., Bisogni J. J., 2006 Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. Aquaculture 257:346-358.

FAO, 2014 The state of the world fisheries and aquaculture. Food and Agriculture Organization. Rome, Italy, pp. 18-27, 213.

Felix F., Nugroho T., Silalahi S., Octavia Y., 2011 Molecular characteristics of *Vibrio* sp. causing black tiger prawn (*Penaeus monodon*) disease in Sumatra and Java shrimp ponds by 16S rDNA sequencing. Journal of Agricultural Technology 7:679-694.

Gopal S., Otta S. K., Kumar S., Karunasagar I., Nishibuchi M., Karunasagar I., 2005 Occurrence of *Vibrio* species in tropical shrimp culture environments; implication for food safety. International Journal of Food Microbiology 102:151-159.

Hawke J. P., 2000 Bacterial disease agents. In: Encyclopedia of aquaculture. Stickney J. (ed), John Wiley & Sons, Inc., Canada, pp 69-96.

Huervana F. H., De la Cruz J. J. Y., Caipang C. M. A., 2006 Inhibition of luminous *Vibrio harveyi* by “green water” obtained from tank culture of tilapia, *Oreochromis mossambicus*. Acta Ichthyologica et Piscatoria 36:17-23.

Jayasree L., Janakiram P., Madhavi R., 2006 Characterization of *Vibrio* spp. associated with diseased shrimp from culture ponds of Andhra Pradesh (India). Journal of the World Aquaculture Society 37:523-532.

Joshi J., Srisala J., Sakaew W., Prachumwat A., Sritunyalucksana K., Flegel T., Thitamadee S., 2014 Identification of bacterial agent(s) for acute hepatopancreatic necrosis syndrome, a new emerging shrimp disease. Suranaree Journal of Science and Technology 21:315-320.

Leaño E. M., Lio-Po G. D., Nadong L. A., Tirado A. C., Sadaba R. B., Guanzon N. G. Jr., 2005 Mycoflora of the 'green water' culture system of tiger shrimp *Penaeus monodon* Fabricius. Aquaculture Research 36:1581-1587.

Lio-Po G. D., Leaño E. M., Penaranda M. M. D., Villa-Franco A. U., Sombito C. D., Guanzon Jr. N. G., 2005 Anti-luminous *Vibrio* factors associated with the 'green water' growout culture of the tiger shrimp *Penaeus monodon*. Aquaculture 250:1-7.

Luis-Villaseñor I. E., Voltolina D., Audelo-Naranjo J. M., Pacheco-Marges M. R., Herrera-Espericueta V. E., Romero-Beltran E., 2015 Effects of biofloc promotion on water quality, growth, and biomass yield and heterotrophic community in *Litopenaeus vannamei* (Boone, 1931) experimental intensive culture. Italian Journal of Animal Science 14:3726.

McCarter L., 1999 The multiple identities of *Vibrio parahaemolyticus*. Journal of Molecular Microbiology and Biotechnology 1:51-57.

Michaud L., Blancheton J. P., Bruni V., Piedrahita R., 2006 Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. Aquacultural Engineering 34:224-233.

Misciattelli N., Jones D. A., Simoes N., Latchford J. W., Bridson P., 1998 Manipulation of bacterial populations in shrimp larval cultures fed artificial diets. In: Advances in shrimp biotechnology. Flegel T. W. (ed), National Center for Genetic Engineering and Biotechnology, Bangkok, pp. 109-116.

Musa N., Wei L. S., Wee W., 2008 Phenotypic and genotypic characteristics of *Vibrio harveyi* isolated from black tiger shrimp (*Penaeus monodon*). World Applied Sciences Journal 3:885-902.

Rahman S., Khan S. N., Naser M. N., Karim M. M., 2010 Isolation of *Vibrio* spp. from penaeid shrimp hatcheries and coastal waters of Cox's Bazar, Bangladesh. Asian Journal of Experimental Biological Science 1(2):288-293.

Ronquillo J. D., Saisho T., Yamasaki S., 1998 Application of phytoplankters as live diet and biocontrol in improving the hatchery production of commercially important penaeid shrimp in Asia (Abstract). In: Advances in shrimp biotechnology. Flegel T. W. (ed), National Center for Genetic Engineering and Biotechnology, Bangkok, pp. 107.

Seraspe E. B., Valenzuela J. B., Maeda H., Kamei Y., 2005 Bioactivity screening of extracts of *Nannochloropsis* sp. against *Vibrio harveyi* causative agent of luminous vibriosis in penaeid shrimps. UPV Journal of Natural Sciences 10:27-36.

Takemura A. F., Chien D. M., Polz M. F., 2014 Associations and dynamics of Vibrionaceae in the environment, from the genus to the population level. Frontiers in Microbiology 5:1-26.

Tendencia E. A., de la Peña M., 2003 Investigation of some components of the greenwater system which makes it effective in the initial control of luminous bacteria. Aquaculture 218:115-119.

Tendencia E. A., de la Peña M. R., Fermin A. C., Lio-Po G. D., Choresca Jr. C. H., Inui Y., 2004 Antibacterial activity of *Tilapia hornorum* against *Vibrio harveyi*. Aquaculture 232:145-152.

Tendencia E. A., de la Peña M. R., Choresca Jr. C. H., 2006 Effect of shrimp biomass and feeding on the anti-*Vibrio harveyi* activity of *Tilapia* sp. in a simulated shrimp-tilapia polyculture system. Aquaculture 253:154-162.

Tendencia E. A., Bosma R. H., Verreth J. A. J., 2011 White spot syndrome virus (WSSV) risk factors associated with shrimp farming practices in polyculture and monoculture farms in the Philippines. Aquaculture 311:87-93.

Tendencia E. A., Bosma R. H., Verdegem M. C. J., Verreth J. A. J., 2015 The potential effect of greenwater technology on water quality in the pond culture of *Penaeus monodon* Fabricus. Aquaculture Research 46:1-13.

Thakur A. B., Vaidya R. B., Suryawanshi S. A., 2004 *Vibrio* abundance in modified extensive culture ponds of *Penaeus monodon*. Indian Journal of Fisheries 51:147-152.
Thompson F. L., Iida T., Swings J., 2004 Biodiversity of Vibrios. Microbiology and Molecular Biology Reviews 68:403-431.
Thompson J., Gregory S., Plummer S., Shields R. J., Rowley A. F., 2010 An in vitro and in vivo assessment of the potential of *Vibrio* spp. as probiotics for the Pacific white shrimp, *Litopenaeus vannamei*. Journal of Applied Microbiology 109:1177-1187.
Toledo N., Dureza L., Ronquillo J., Itami T., Sakata T., Suarnaba V., 2005 In vivo assay of live microalgae on *Vibrio harveyi* co-cultured with *Scylla serrata* larvae. UPV Journal of Natural Sciences 10:91-102.
Tran L., Nunan L., Redman R. M., Mohney L. L., Pantoja C. R., Fitzsimmons K., Lightner D. V., 2013 Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. Diseases of Aquatic Organisms 105:45-55.

Received: 29 January 2016. Accepted: 28 February 2016. Published online: 09 March 2016.
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How to cite this article:
Cadiz R. E., Traifalgar R. F. M., Sanares R. C., Andrino-Felarca K. G. S., Corre Jr. V. L., 2016 Comparative efficacies of tilapia green water and biofloc technology (BFT) in suppressing population growth of green Vibrios and *Vibrio parahaemolyticus* in the intensive tank culture of *Penaeus vannamei*. AACL Bioflux 9(2):195-203.