Physico-chemical and biological characteristics of closed bioaugmented and conventional open intensive shrimp culture systems in controlled conditions

Valeriano L. Corre Jr., Rosy L. Janeo, Roman C. Sanares, Rhea J. Carton-Kawagoshi

Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Iloilo, Philippines. Corresponding author: V. L. Corre Jr., profcorre@yahoo.com

Abstract. The effects of the two culture systems, closed bioaugmented and open intensive system on the physico-chemical and biological conditions of the water were studied for 120 days in 1-m^3 concrete tanks with earthen bottom. The tanks were stocked with 25 *P. monodon* postlarvae/m^3. Physico-chemical and biological parameters of the tanks were analyzed weekly during the culture period. Results showed no significant difference on water quality parameters between treatments except for total alkalinity and water pH being significantly lower in all open system of water management while reactive phosphorous was lowest in the bioaugmented open system. Throughout the culture period chlorophyll *a* level remained stable in the closed bioaugmented system. Phytoplankton quality was dominated by *Nannochloropsis sp*. No significant difference was further observed on the total bacteria and presumptive *Vibrio* counts while the luminous bacteria count was significantly higher in the non-bioaugmented open system. Average body weight at harvest, survival rate and total production was highest in the closed bioaugmented system and lowest in the non-bioaugmented open system. These results imply that bioaugmentation and water management practice play a vital role in the maintenance of good water quality and attaining good shrimp production.

Key Words: water quality, bioaugmentation, closed system, conventional open system.

Introduction. The use of bacterial bioaugmentation agents (probiotics) in aquaculture is promising but needs further research efforts to determine their effectiveness in augmenting production (Gatesoupe 1999; Shariff et al 2001). Bioaugmentation is the concept of reducing the accumulation of organic wastes to environmentally safe levels through the use of micro- or macroorganisms (Thomas et al 1992). In shrimp culture, the first successful studies were reported by Maeda & Liao (1992) in the hatchery and Moriarty (1998) in ponds. However, there were also reports that the use of microbial products in the field was not as effective as in the laboratory (Chiayvareesajja & Boyd 1993). On the other hand, the use of probiotics (bioaugmentation) that is now widely used in conventional shrimp culture has been proven to inhibit the growth of pathogenic bacteria such as *Vibrio* sp. in ponds (Walker & Clymo 1996; Corre et al 2000).

In a poorly prepared shrimp pond in Malaysia, Shariff et al (2001) observed that survival rate, FCR and production of shrimps in bioaugmented ponds were relatively better than in the control ponds although no significant difference was obtained on water quality. In another study, Devaraja et al (2002) reported that the use of microbial products in shrimp farms could be of substantial benefit if used in larger areas.

The outbreak of white spot syndrome virus (WSSV) in shrimp culture in the Philippines triggered the shift from the open conventional bioaugmented system to closed bioaugmented system. The potential use of a closed zero-exchange system has been demonstrated for tilapia in Israel and in the USA and in high-density shrimp culture at Belize Aquaculture Ltd. in Central America (Funge-Smith & Phillips 2001; Burford et al

2003). This system creates a substrate of organic materials where heterotrophic microbial communities could thrive. This shift from an autotrophic to an intensively aerated heterotrophic (bacterial-controlled) system has been found to generate good results on the shrimp performance through the premise that aerobic microbes metabolize organic materials thereby contributing to its degradation, nitrification and the production of microbial protein ("flocs") (Avnimelech 2003; Burford et al 2003). Whereas uptake of inorganic nitrogen in an autotrophic system is limited by primary production, a heterotrophic system was found to have practically unlimited capacity and can tolerate intensification as the C:N ratio can be increased through the addition of carbonaceous materials (Avnimelech 2003; Burford et al 2003).

Recognizing the relative stability and environmental viability of this system, there is a need to establish baseline data on the potential application of this culture system at the local setting and its performance vis-à-vis the conventional open intensive system. This paper compares the production parameters of two culture systems, closed bioaugmented system and open intensive system and reports the effects of the two culture systems on the physico-chemical, biological and microbiological conditions of the water in tanks.

Material and Method

Culture facility. Twelve concrete tanks (1x1x1 m) with earthen bottom were prepared following the procedures described by Corre et al (2000). The tanks were filled with brackish water from the reservoir tank to a volume of 1.0 m^3. Water culture was done for three weeks, until the desired plankton count of more than 400,000 cells mL^{-1} was attained. Each tank was stocked with 25 shrimp juveniles/m^3. The shrimps were fed with commercially available shrimp pellets a day after stocking. Continuous aeration was provided using a ½ HP ring blower.

Experimental treatments. Four treatments were tested as follows: bioaugmented conventional open system (BOS); bioaugmented closed system (BCS); non-bioaugmented conventional open system (NBOS) and non-bioaugmented closed system (NBCS). Tanks for treatments 1 and 2 were applied with microbial bioaugmentation agent at 1.0 ppb for 1 to 30 days of culture, 1.5 ppb (DOC 31-60), 2.0 ppb (DOC 61-90) and 3 ppb (DOC 91 –120). Rice bran ("tiki-tiki") at 20% of the total daily feed ration was given at 9:00-10:00 AM to maximize life span of the microbial agents. No bioaugmentation agents were applied in treatments 3 and 4. Water change (25%) was done weekly in treatments 1 and 3 while treatments 2 and 4 had no or zero water change. The water volume in tanks for treatments 2 and 4 was maintained by daily addition of water from the reservoir.

Sampling. Water samples for physico-chemical and biological parameters were analyzed weekly. Total alkalinity, ammonia and particulate organic matter were analyzed following the procedures described by Strickland & Parsons (1972). Dissolved oxygen and temperature were monitored using a YSI Model 57 dissolved oxygen meter; water pH with Mettler Toledo pH meter and salinity with an Atago refractometer. Bacterial loads were quantified using the 10-fold dilution technique. Representative dilutions were be plated in duplicates onto nutrient agar with 1.5% sodium chloride for the total plate and luminous bacteria counts and thiosulfate citrate bile salt sucrose agar (TCBS) for the presumptive Vibrio count. The agar plates for bacterial count were incubated at room temperature for 20-24 hours.

Algal samples were counted in haemacytometer following the procedure described by Stafford (1999). Stock sampling of shrimps was done every 15 days interval until 120 days of culture.

Statistical analyses. Analysis of variance (ANOVA) was done using the SigmaStat software V. 7.5 (SPSS 1999) computer program to compare the effects of the different treatments.

Results and Discussion

Physico-chemical parameters of the water. The mean values of the water quality indicators monitored in this study remained within the recommended range for optimal growth and survival for *P. monodon* (Corre et al 2000; Chien 1992). No significant difference between treatments was observed on dissolved oxygen, salinity, water temperature, total ammonia-N and nitrite-N concentrations (P>0.05) (Table 1).

Table 1
Mean values of physico-chemical parameters of the water in the experimental tanks during the study period

Physico-chemical parameter	*Bioaugmented open system*	*Closed bioaugmented system*	*Non-bioaugmented open system*	*Non-bioaugmented closed system*
Unionized ammonia (mg/L)	0.19±0.02[a]	0.15±0.02[a]	0.16±0.02[a]	0.19±0.02[a]
Nitrite-N (ppm)	0.10±0.02[a]	0.10±0.02[a]	0.11±0.02[a]	0.11±0.02[a]
Reactive phosphorous (ppm)	0.08±0.01[a]	0.07±0.01[a]	0.04±0.003[b]	0.06±0.01[a]
Particulate organic matter (ppm)	3.6±0.6[ab]	2.7±0.3[a]	3.1±0.3[ab]	4.7±0.9[b]
Water pH	7.9±0.02[a]	8.0±0.02[b]	7.9±0.02[a]	7.8±0.03[c]
Total alkalinity (mg/L $CaCO_{3)}$	178±8.22[ab]	189±7.19[b]	159±6.61[bc]	156±6.72[c]
Dissolved oxygen (ppm)	5.2±0.11[a]	5.3±0.11[a]	5.4±0.11[a]	5.3±0.11[a]
Temperature (°C)	27.3±0.3[a]	27.3±0.3[a]	27.4±0.3[a]	27.5±0.4[a]
Salinity (ppt)	23±0.7[a]	21±0.9[a]	23±0.8[a]	21±0.9[a]
Chlorophyll *a* (ppm)	38±5.7[ab]	31±4.7[a]	35±4.9[a]	53±7.6[b]

Mean and standard errors for three replicates are shown. Values followed by different letters indicate significant difference between treatments (P<0.05), horizontal comparison only.

On the other hand, total alkalinity and water pH were significantly lower in all treatments with open system of water management while reactive phosphorous was lowest in the bioaugmented open system. It is noticeable though that despite the regular addition of hydrated lime, water pH gradually decreases from 8.2 at the start of the culture to 7.5 towards the end of the culture period (Figure 1). The same trend was observed on total alkalinity. This could be attributed to the increasing biomass of shrimps and bacteria in the culture system. Shrimps need calcium to harden their exoskeleton after molting while calcium is needed to neutralize the water since acid is produced by bacteria during decomposition of organic matter which could be the reason for the reduction in the level of water pH and total alkalinity. Likewise, it is but natural that the ammonia and phosphorous concentration of the water increases as the culture period increases (Figure 1). This is due to the accumulation of organic substances like uneaten feeds, plankton die-off and shrimp fecal matter. An erratic change in ammonia levels were observed from week 7 until the study was terminated, however, it could be noted that the change was gradual and the level more stable in the bioaugmented treatments.

Among treatments, the non-bioaugmented closed system had the highest chlorophyll *a* level (Table 1). However, the difference is not significant with the bioaugmented open system (P>0.05). Further, a stable chlorophyll *a* level was observed in the bioaugmented closed system throughout the culture period (Figure 2) which is in agreement with the report of Janeo et al (2009).

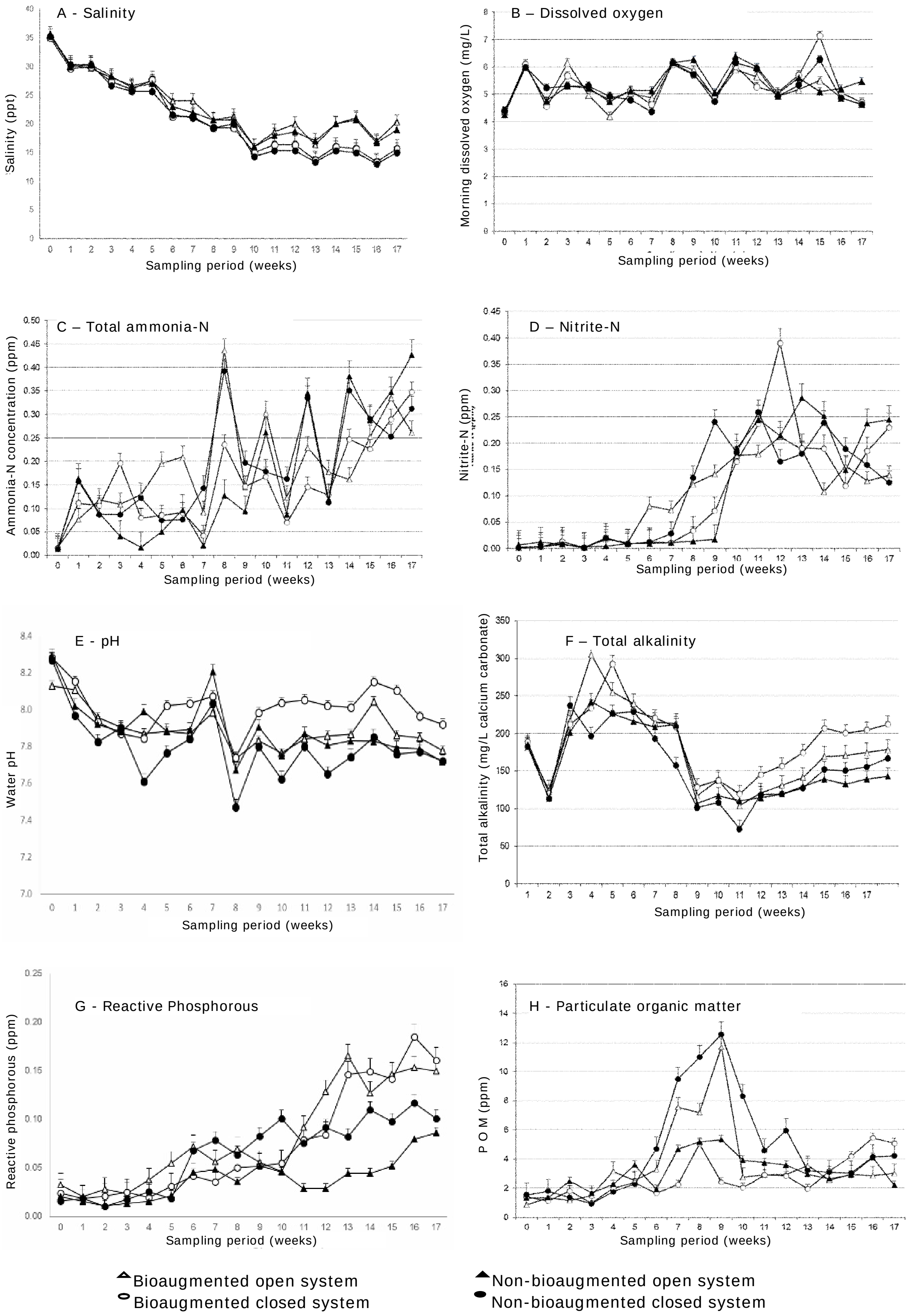

Figure 1. Profile of physico-chemical parameters of the water.

The phytoplankton count specifically the beneficial plankton was dominated by *Nannochloropsis sp.* It was noticed that in week ten, all the treatment tanks got a very low *Chlorella* count of about 8–28% only, the non-bioaugmented system having the lowest. Other beneficial phytoplankton consistently present was *Chlorella, Navicula* and *Nitzchia.* While the non-beneficial phytoplankton observed were *Merosmopedia*, *Oscillatoria, Pseudoanabaena* and *Anacytis.* Presence of zooplankton was seldom observed and was limited to nauplius which includes *Lycaea* and *Brachionus*. According to Boyd (1995), other benefits that can be derived from the use of probiotics include the reduction of blue–green algal populations that cause off-flavor, nitrate, nitrite, ammonia and phosphate levels, increased oxygen concentrations and promotion of organic matter decomposition. Ammonia level in closed system with bioaugmentation showed lower ammonia-N and these could be one of the positive effects of the use of these beneficial bacteria.

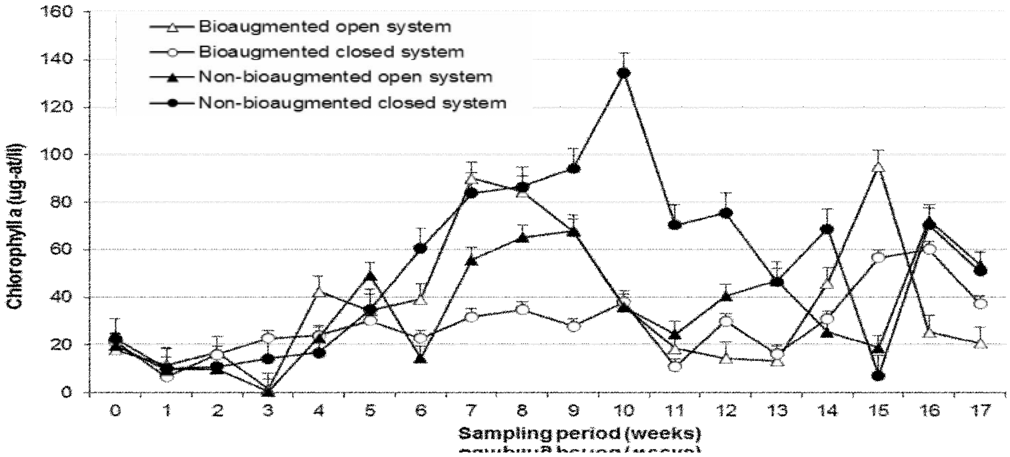

Figure 2. Chlorophyll *a* content of the water.

On the bacterial profile, no significant difference among treatments was observed on the total bacteria and presumptive *Vibrio* counts. Higher counts were observed from the 6th to the 12th week of culture (Figures 3 and 4).

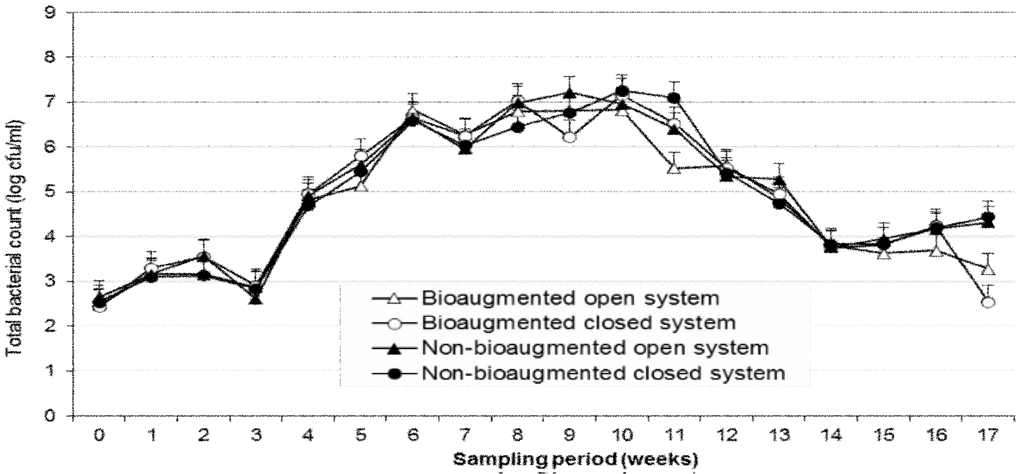

Figure 3. Total bacterial count of the water during the study period.

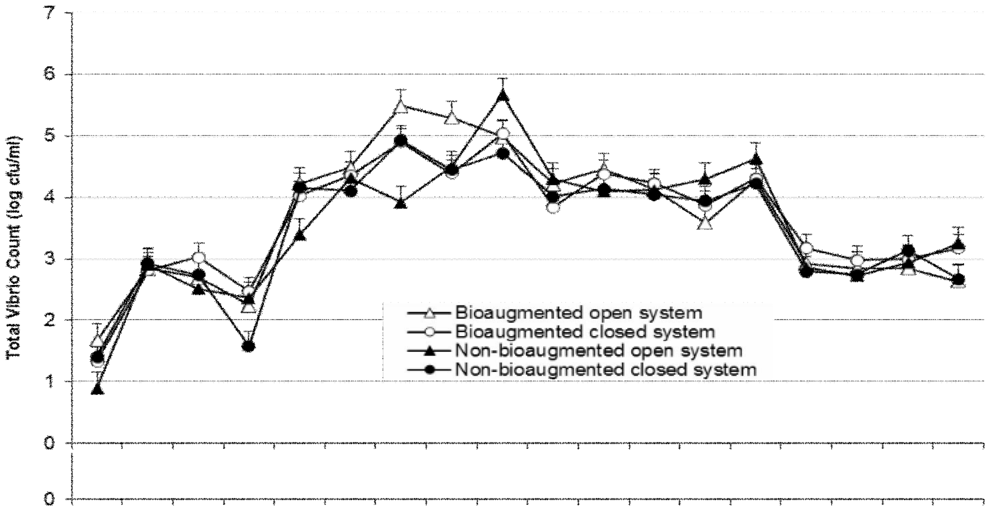

Figure 4. Presumptive *Vibrio* count of the water during the study period.

The luminous bacteria count in the NBOS was significantly higher among the treatments tested (Figure 5).

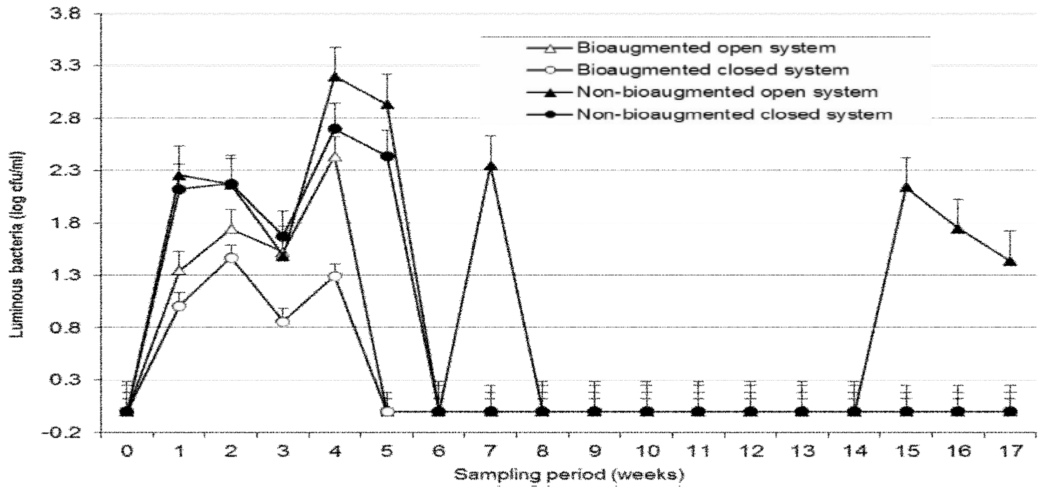

Figure 5. Luminous bacteria count of the water during the study period.

The count ranged from 0-8.3 x 10^2, 0-5.9 x 10^1, 0-1.8 x 10^3 and 0-8.3 x 10^2 in the BOS, BCS, NBOS and NBCS, respectively. Luminous *Vibrios* are natural fauna of sea and brackish waters that may have entered the culture tanks during water management. In their study, Janeo et al (2009) reported that regular application of bioaugmentation products in open system brackish water ponds significantly reduces the luminous bacterial count of the water. The same result was also observed in BOS tanks. According to Moriarty (1998) the luminous *Vibrio* species present in pond water were displaced when a select *Bacillus* species were added. This could best explain the low levels of luminous *Vibrios* in all bioaugmented treatments.

Average body weight, survival rate and production of shrimp. After 120-days of culture, the bioaugmented closed system (BCS) gave the significantly highest average body weight (ABW), survival rate and production among the treatments tested while the non-bioaugmented open system (NBOS) had the significantly lowest percent survival and shrimp biomass (Table 2). The mechanism of action of probiotic bacteria includes competitively excluding pathogenic bacteria or produce substances that can inhibit the growth of the pathogenic bacteria (Moriarty 1998); provide essential digestive enzymes to enhance the digestion process of the cultured animals (Bomba et al 2002); improve survival (Kumar et al 2006) and probiotic bacteria directly uptake or decompose the organic matter or toxic material in water thereby improving water quality (Ehrlich et al (1988) which gave significantly good result to BCS.

Table 2

Average body weight, mean survival and production of shrimp at harvest in tanks for a 120-day culture period

Parameters	*Average body weight at harvest (g)*	*Survival (%)*	*Biomass (g)*
Bioaugmented open system	25.9 ± 0.1 a	97.9 ± 0.4 a	634 ± 1.0 a
Bioaugmented closed system	26.7 ± 0.3 b	98.2 ± 0.2 a	655 ± 7.2 b
Non-bioaugmented open system	25.8 ± 0.1 a	87.8 ± 0.2 b	567 ± 1.2 c
Non-bioaugmented closed system	25.8 ± 0.1 a	97.7 ± 0.6 a	630 ± 2.0 a

Mean and standard errors for three replicates are shown. Values followed by different letters indicate significant difference between treatments ($P<0.05$), vertical comparison only.

The result of this experiment further validates recent data on the usefulness and the effectiveness of using probiotic bacteria in aquaculture system. Several researchers have done studies on the probiotic bacteria to improve the shrimp culture water, and achieved remarkable results (Gatesoupe 1999). When photosynthetic bacteria were added into the water, it could eliminate the ammonia-nitrogen, hydrogen sulfide and organic acids, and other harmful materials rapidly, improve the water quality and balance the pH. The heterotrophic bacteria may have chemical actions such as oxidation, ammonification, nitrification, denitrification, sulphurification and nitrogen fixation. When these bacteria were added into the water, they could decompose the excreta of fish or shrimp, remaining food materials, remains of plankton and other organic materials to carbon dioxide, nitrate and phosphate. These inorganic salts provide the nutrition for the growth of microalgae, while the bacteria grow rapidly and become the dominant group in the water, inhibiting the growth of pathogenic microorganisms. The photosynthesis of microalgae provides dissolved oxygen for oxidation and decomposition of the organic materials and for the respiration of the microbes and cultured animals. This kind of cycle may improve the nutrient cycle, and it can create a balance between bacteria and microalgae, and maintaining a good water quality environment for the cultured animals thus, a closed bioaugmented system of culture became possible.

Conclusions. This study elucidated the usefulness and effectiveness of using bacterial bioaugmentation in a closed or zero water exchange shrimp production system. The physico-chemical and biological parameters of the water remained stable throughout the culture period resulting to minimal stress on the cultured animals. In addition, the nitrifying actions of microbial community resulted to rapid removal of ammonia and nitrite in the culture water which contributed to the maintenance of optimum water quality for good growth and survival of shrimps making this approach valuable in zero water exchange systems. This scheme provides a biosecure and environment-friendly approach to prevent disease outbreaks and maintain good culture environment for the cultured animals.

Acknowledgements. We thank the UPV In-house Research Grants for funding this study. The CFOS-IA-Brackishwater Aquaculture Center for the use of facilities; Jovito Ambata, Edwin Batislaong and Engr. Reynold Jaspe for the tank maintenance.

References

Avnimelech Y., 2003 Control of microbial activity in aquaculture systems: active suspension ponds. World Aquaculture 34(4):19-21.

Bomba A., Nemcoa R., Gancarcikova S., Herich R., Guba P., Mudronova D., 2002 Improvement of the probiotic effect of microorganisms by their combination with maltodextrins, fructo-oligosaccharides and polyunsaturated fatty acids. British Journal of Nutrition 88(Suppl. 1):95–99.
Boyd C. E., 1995 Bottom soils, sediments and pond aquaculture. Chapman & Hall, New York, USA 348 pp.
Burford M. A., Thompson P. J., Bauman H., Pearson D. C., 2003 Microbial communities affect water quality, shrimp performance at Belize aquaculture. Global Aquaculture Advocate pp. 64-65.
Chiayvareesajja A., Boyd C. E., 1993 Effects of zeolite, formalin, bacterial suspension and aeration on total ammonia nitrogen concentration. Aquaculture 116:33-45.
Chien Y. W., 1992 Water quality requirements and management for marine shrimp culture. In: Proceedings of the Special Session on Shrimp Farming. Wyban J. (ed), pp. 144–156, World Aquaculture Society, Baton Rouge, LA, USA.
Corre Jr. V. L., Janeo R. L., Caipang C. M. A., Calpe A. T., 2000 Use of probiotics and reservoir with “green water” and other tips for a successful shrimp culture. Aquaculture Asia 5:34-38.
Devaraja T. N., Yusoff F. M., Shariff M., 2002 Changes in bacterial populations and shrimp production in ponds treated with commercial microbial products. Aquaculture 206:245-256.
Ehrlich K. E., Horsfall F. L., Cantin M. C., Turcotte A., 1988 Bioaugmentation technology to improve aquacultural production and to protect and to restore water bodies. In: Proceedings of Aquaculture International Congress, Vancouver, Canada, pp. 54–56.
Funge-Smith S., Phillips M. J., 2001 Aquaculture systems and species. In: Aquaculture in the Third Millenium. Technical Proceedings of the Conference on Aquaculture in the Third Millenium, Bangkok, Thailand, 20-25 February 2000. Subasinghe R. P., Bueno P., Phillips M. J., Hough C., McGladdery S. E., Arthur J. R. (eds), pp. 129-135, NACA, Bangkok and FAO, Rome.
Gatesoupe F. J., 1999 The use of probiotics in aquaculture. Aquaculture 180:147-165.
Janeo R. L., Corre V. L., Sakata T., 2009 Water quality and phytoplankton stability in response to application frequency of bioaugmentation agent in shrimp ponds. Aquacultural Engineering 40:120-125.
Kumar R., Mukherjee S. C., Prasad K. P., Pal A. K., 2006 Evaluation of *Bacillus subtilis* as a probiotic to Indian major carp *Labeo rohita* (Ham.). Aquaculture Research 37:1215–1221.
Maeda M., Liao I. C., 1992 Effect of bacterial population on the growth of prawn larva, *Penaeus monodon*. Bulletin of National Research Institute of Aquaculture 21:25-29.
Moriarty D. J. W., 1998 Control of luminous *Vibrio* species in penaeid aquaculture ponds. Aquaculture 164:351-358.
Shariff M., Yusoff F. M., Devaraja T. N., Srinivasa Rao P. S., 2001 The effectiveness of a commercial microbial product in poorly prepared tiger shrimp, *Penaeus monodon* (Fabricius) ponds. Aquaculture Research 32:181-187.
SPSS, 1999 SigmaStat Statistical Software. SPSS Marketing Department, Chicago, IL, USA.
Stafford C., 1999 A guide to phytoplankton of aquaculture ponds. Collection analysis and identification. Department of Primary Industries, Queensland, p. 59.
Strickland J. D., Parsons T. R., 1972 A practical handbook of seawater analysis. 2^{nd} ed., Fisheries Research Board of Canada, Ottawa, Canada, 310 pp.
Thomas G. M., Ward C. H., Raymond R. L., Wilson J. T., Loehr B. C., 1992 Bioremediation. In: Encyclopedia of microbiology. Leperberg J. (ed), pp. 369-385, Academic Press London.
Walker T., Clymo P., 1996 Fighting prawn diseases with bacterial inoculation in ponds. Austasia Aquaculture 10:45-47.

Received: 14 February 2016. Accepted: 16 March 2016. Published online: 30 April 2016.
Authors:
Valeriano Lopez Corre Jr., University of the Philippines Visayas, College of Fisheries and Ocean Sciences, Institute of Aquaculture, Philippines, Iloilo, Miag-ao 5023, e-mail: profcorre@yahoo.com
Rosy Lumajen Janeo, University of the Philippines Visayas, College of Fisheries and Ocean Sciences, Institute of Aquaculture, Philippines, Iloilo, Miag-ao 5023, e-mail: rosyjaneo@yahoo.com
Roman de la Cruz Sanares, University of the Philippines Visayas, College of Fisheries and Ocean Sciences, Institute of Aquaculture, Philippines, Iloilo, Miag-ao 5023, e-mail: rcsanares2002@yahoo.com
Rhea Joy Carton-Kawagoshi, University of the Philippines Visayas, College of Fisheries and Ocean Sciences, Institute of Aquaculture, Philippines, Iloilo, Miag-ao 5023, e-mail: pitsel@yahoo.com

How to cite this article:
Corre Jr. V. L., Janeo R. L., Sanares R. C., Carton-Kawagoshi R. J., 2016 Physico-chemical and biological characteristics of closed bioaugmented and conventional open intensive shrimp culture systems in controlled conditions. AACL Bioflux 9(2):429-437.