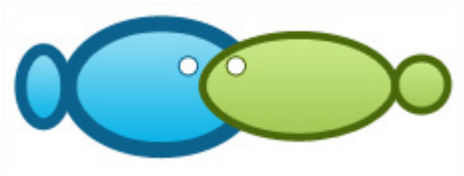

# Potential of *Gracilariopsis bailiniae* and *Oreochromis mossambicus* in improving water quality in intensive *Litopenaeus vannamei* tank culture

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**Abstract**. Shrimp production has grown rapidly but it has been responsible for ecosystems deterioration because of its nutrient rich effluents. This study was conducted to investigate the potential of seaweed (*Gracilariopsis bailiniae*) and tilapia *(Oreochromis mossambicus)* in improving water quality in intensive shrimp tank culture. Shrimps were cultured outdoors in plastic tanks at a stocking rate of 400 shrimp $m^{-3}$ triplicated for 60 days. The water was recirculated in a low water exchange management regime at an average rate of 6 L $hr^{-1}$. Unionized ammonia, bacterial counts and presumptive *Vibrio* counts were measured once weekly. Chlorophyll *a* was measured weekly while phytoplankton density was measured thrice for the whole culture period. The results showed that both *G. bailiniae* and *O. mossambicus* have the potential to control the growth of pathogenic *Vibrio*. Both inhibited the growth of pathogenic green *Vibrio* by promoting the growth of beneficial yellow *Vibrio*. Both also encouraged the stable growth of beneficial phytoplankton (*Nannochloropsis* and *Chlorella*) which control blooms and collapse of phytoplankton in the culture system. Further, unionized ammonia was reduced in shrimps integrated with *G. bailiniae*. This investigation presented the potential of seaweed (*G. bailiniae*) and tilapia (*O. mossambicus*) in improving water quality in an intensive shrimp tank culture.
**Key Words**: bioremediation, effluents, integrated aquaculture, microalgae, pathogenic *Vibrio*.

**Introduction**. Aquaculture system has been responsible for the deterioration of ecosystems due to the large amounts of nutrients into the marine environment through excretory products and excess feed. The major challenge in aquaculture industries is how to minimize the negative effects of nutrients in the pond water and aquaculture effluents (Marinho-Soriano et al 2002; Rabiei et al 2014).

Cost effective technologies for high shrimp yield with minimal impact are necessary for the sustainable expansion of the shrimp industry (Samocha et al 2015). One efficient and inexpensive way of treating culture effluents is through integrated multi-trophic aquaculture (IMTA) (Carton-Kawagoshi et al 2014). In the Philippines, the concept of integrated aquaculture is not new and has been applied for decades but was usually done in pond systems (Largo et al 2016). IMTA systems uses marine species that were commercially viable and environmentally sustainable based on the concept that the wastes (uneaten feeds, feces and metabolic excretion) of one species are useful input for another species working in natural self-cleansing mechanism (Lavania-Baloo et al 2014). The genus *Gracilaria* (Rhodophyta) have high bioremediation efficiency and market value such as agar-agar, feed, and food for human (Huo et al 2012) and species *Gracilariopsis bailiniae* is a good IMTA biofilter. *G. bailiniae* has the capacity to utilize both $NH_4^+$ and $NO_3^-$ levels (Carton et al 2011). Stocking of finfishes like tilapia with other species is also beneficial. It stabilizes plankton population and density that promotes the production of "green water" which reduces the growth of luminous bacteria, contains higher percentage of beneficial phytoplankton, and stabilizes water pH (Corre et al 2015).

Seaweed is a suitable candidate to reduce the amount of dissolved nutrients being released from the shrimp effluents while tilapia can prevent multiplication of luminous

*Vibrio* in shrimp culture. Studies on tilapia (*O. mossambicus)* in 'green water' technology and the biofiltering efficiency of seaweed (*Gracilaria* spp.*)* are well documented but little is known on *O. mossambicus and G. bailiniae*'s effects on the water quality of intensive shrimp (*Litopenaeus vannamei*) tank culture. Therefore, this study is necessary to investigate the potential of seaweed and tilapia in improving water quality of shrimps in an intensive shrimp (*L. vannamei*) tank culture.

## Material and Method

***Experimental treatments and culture conditions.*** The study was conducted at Brackishwater Aquaculture Center of the University of the Philippines Visayas - College of Fisheries and Ocean Sciences. The treatments used in this study were (a) *L. vannamei* only (control group); (b) *L. vannamei* integrated with seaweed (*G. bailiniae)* or shrimp-seaweed; (c) *L. vannamei* integrated with tilapia (*O. mossambicus*) or shrimp-tilapia; and (d) *L. vannamei* integrated with seaweed (*G. bailiniae)* and tilapia (*O. mossambicus)* or shrimp-seaweed-tilapia. Shrimps of about 3.04–4.17 g were stocked at 400 shrimp $m^{-3}$ in 175 L capacity plastic tanks for 60 days. Prior to stocking of *L. vannamei*, reservoir and grow-out tanks were filled with seawater to 120 L. Effluents of *L. vannamei* were recirculated from the shrimp boxes to the corresponding reservoir in a low water exchange management regime with an average flow rate of 6 L $h^{-1}$ using an aquarium pump. Seaweed (*G. bailiniae)* stocked in this experiment was obtained from a *Gracilaria* farm in Leganes, Iloilo, Philippines. *G. bailiniae* was washed with running seawater, cleaned of epiphytes and acclimated in effluent-free seawater for one week. *G. bailiniae* was stocked at 2 kg $m^{-2}$ (Carton-Kawagoshi et al 2014) and tilapia (*O. mossambicus*) at 350g $m^{-3}$. *L. vannamei* was stocked in the shrimp tanks while *G. bailiniae* and *O. mossambicus* were stocked in the corresponding reservoir tanks of the treatments. *L. vannamei* was fed four times daily with 42% protein commercial *L. vannamei* feeds while *O. mossambicus* was fed with 27% protein commercial tilapia feeds twice daily. Feed ration was adjusted weekly based on average body weight and survival. Uneaten feeds and faeces were siphoned weekly every after stock sampling. Connecting pipes, pumps and waterlines of the recirculating system were also checked and monitored to ensure consistent water flow rate during the culture period. These ensure sufficient water flow for optimal biofiltration. There was no water change during the culture period. Water loss due to evaporation, water sampling and siphoning of uneaten feeds and faeces during stock sampling was compensated by addition of seawater. This was done to maintain the desired water level. The culture was done outdoor with the set up shaded with transparent roofs to avoid rainfall effect on the experiment so that light and temperature conditions would be similar to that in the field.

***Water quality sampling***. Unionized ammonia was analyzed once a week following the procedure of Strickland & Parsons (1972). Water samples for chlorophyll *a* were filtered using GF/C filter paper and then extracted in 90% acetone solution and were done following the method of Strickland & Parsons (1972). Quantitative analysis of phytoplankton was done weekly using a haemacytometer and a compound microscope following the procedure of Martinez et al (1975). Phytoplankton was identified using keys and illustrations by Prescott (1962) and other phycological taxonomic references available online. Total bacterial count and total *vibrio* count were determined weekly. Bacterial counts using the spread plate method was done on nutrient agar (NA) supplemented with NaCl, thiosulfate citrate bile salts (TCBS) agar following the method of Reilly (1982) as cited in Janeo et al (2009). The agar plates were incubated for 24 hours at room temperature. Yellow and green colonies (*Vibrio*) were then counted after 24 hours.

***Statistical analysis***. Data analyses were performed using the software SPSS 20.0 version. One-way ANOVA and Duncan Multiple Range Test (DMRT) were used to find out statistical differences among the different treatments. Significant level was set at $p<0.05$. Bacterial counts were log 10 transformed.

**Results**. Mean unionized ammonia was significantly highest in shrimp-tilapia (1.55 ppm) but this did not differ to the level obtained in water of shrimp-seaweed-tilapia (1.49 ppm). The control group had the lowest level of unionized ammonia of 1.06 ppm but this did not differ with the unionized ammonia level of water in shrimp-seaweed (1.22 ppm) as shown in Table 1.

Table 1

Mean values and standard errors of the water and growth parameters in the experimental units during the 60-day culture period

| *Treatment* | *Control* | *With seaweed* | *With Tilapia* | *With seaweed and Tilapia* |
|---|---|---|---|---|
| Unionized ammonia (ppm) | $1.06 \pm 0.05^{a}$ | $1.22 \pm 0.05^{ab}$ | $1.55 \pm 0.14^{c}$ | $1.49 \pm 0.11^{bc}$ |
| Chlorophyll *a* ($\mu g$ liter$^{-1}$) | $5.86 \pm 0.82^{b}$ | $2.89 \pm 0.20^{a}$ | $2.85 \pm 0.19^{a}$ | $4.23 \pm 0.36^{a}$ |
| Phytoplankton (cells ml$^{-1}$ x 10,000) | $35.2 \pm 2.94^{c}$ | $20.6 \pm 3.17^{ab}$ | $15.66 \pm 1.20^{a}$ | $26.8 \pm 2.67^{bc}$ |
| Green colony- forming *Vibrio* (%) | $16 \pm 3.06^{c}$ | $10 \pm 0.05^{b}$ | $3 \pm 0.88^{a}$ | $4 \pm 0.00^{a}$ |
| Yellow colony- forming *Vibrio* (%) | $84 \pm 3.06^{a}$ | $90 \pm 2.85^{b}$ | $97 \pm 0.58^{b}$ | $96 \pm 1.20^{b}$ |
| Survival of *L. vannamei* (%) | $49 \pm 1.76$ | $67 \pm 8.00$ | $61 \pm 4.35$ | $58 \pm 3.06$ |

Values in the same column with different superscript differ significantly ($p<0.05$).

Figure 1 presented the weekly mean unionized level of the water samples in the experimental units of *L. vannamei* for 60 days. The unionized ammonia started to increase significantly in the shrimp-seaweed-tilapia and significantly lowest in shrimp-seaweed but the values did not differ in unionized ammonia level of water samples in shrimp-tilapia and the control group in Week 1. However, for Week 2 unionized level of water in shrimp-seaweed significantly decreased from the water of the control, shrimp-tilapia, and shrimp-seaweed-tilapia.

Mean concentration of chlorophyll *a* (Table 1) was significantly highest in water of the control (5.86 $\mu g$ $L^{-1}$) followed by shrimp-seaweed-tilapia, shrimp-seaweed, and shrimp-tilapia respectively (4.23 $\mu g$ $L^{-1}$; 2.89 $\mu g$ $L^{-1}$; and 2.85 $\mu g$ $L^{-1}$). Weekly trends of chlorophyll *a* concentrations presented in Figure 2 were fluctuating. Chlorophyll *a* level was significantly highest in water in the control starting at Week 4 up to the end of the culture period. The concentrations dropped off at Week 4 and increased at Week 5 to Week 7 and dropped again at Week 8.

Mean phytoplankton density seen in Table 1 showed that water in the control had the highest density (35.2x10, 000 cells $mL^{-1}$) followed by the water in shrimp-seaweed-tilapia (26.8x10, 000 cells $mL^{-1}$), and shrimp-seaweed (20.6x10, 000 cells $mL^{-1}$). Mean total cell count was significantly lowest in water of shrimp-tilapia (15.5x10, 000 cells $mL^{-1}$). The abundance of phytoplankton in all treatments was dominated by *Nannochloropsis* and *Chlorella* throughout the 60-day culture period (90%-97%). The remaining percentage was composed by cyanophytes (*Oscillatoria, Chroococcus, and Chlorococcum*) and the diatoms (*Melosira, Navicula, Nitzschia, and Coscinodiscus*).

Figure 3 showing the phytoplankton density for three samplings. It was observed that the phytoplankton density for the control group was significantly highest for Week 3 and Week 6. However, there was an abrupt decreased in the phytoplankton abundance at Week 9 in the water of the control. It was further noticed that the phytoplankton cell count in water in shrimp-seaweed, shrimp-tilapia, and shrimp-seaweed-tilapia were stable throughout the culture period.

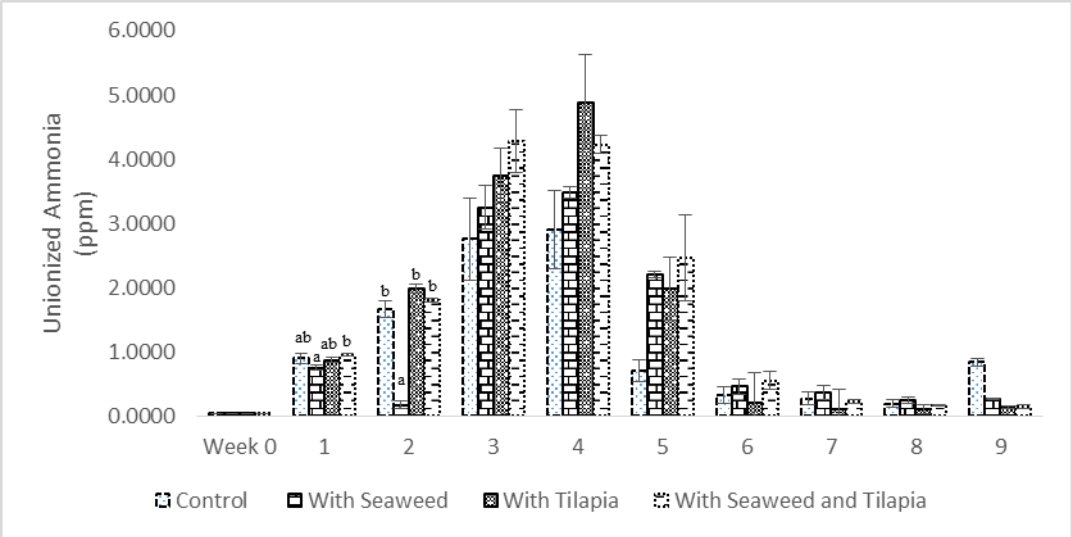


Figure 1. Mean weekly unionized ammonia level (n=3, mean±SE) in water in experimental units during the 60 day culture period. Values with different labels are significantly different ($p<0.05$).

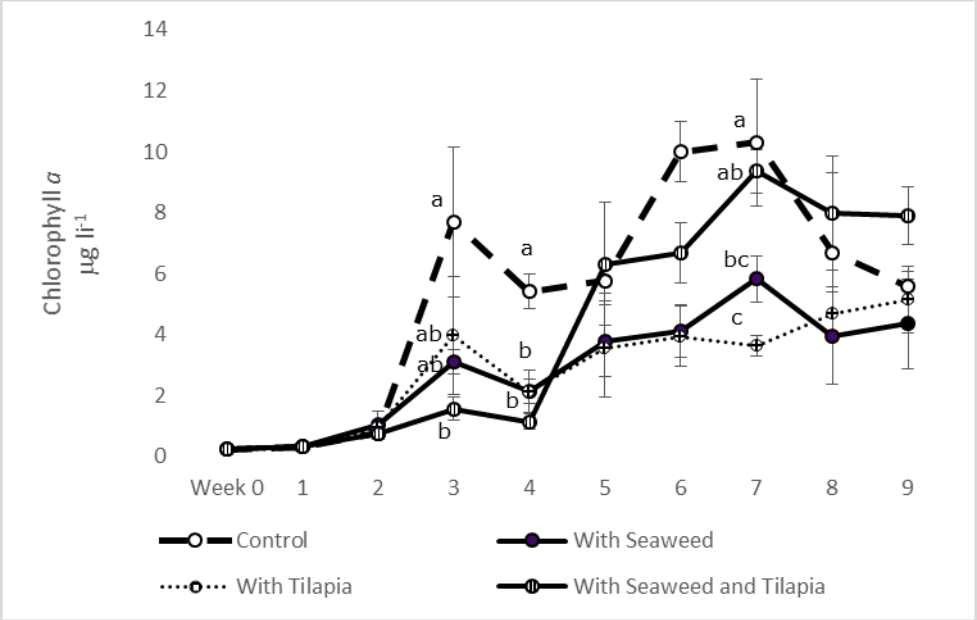


Figure 2. Mean weekly chlorophyll *a* level in water in experimental units during the 60-day culture period. Values with different labels are significantly different ($p<0.05$).

Bacterial counts on nutrient agar plates and *Vibrio* counts on TCBS plates did not differ significantly among treatments. Total plate counts of bacteria ranged from $2.66x10^4$ cfu $mL^{-1}$ (in shrimp-tilapia) to $1.10x10^5$ cfu $mL^{-1}$ (in shrimp-seaweed-tilapia). Further, *Vibrio* counts ranged from $9.30x10^2$ cfu $mL^{-1}$ (shrimp-seaweed) to $1.70x10^3$ cfu $mL^{-1}$ (shrimp-tilapia). The same result was achieved in the yellow- forming *Vibrio*. Water in the shrimp-seaweed had the lowest yellow colony *Vibrio* count ($9.00x10^2$ cfu $mL^{-1}$) and was highest in shrimp-tilapia ($1.67x10^3$ cfu $mL^{-1}$). However, for green- forming colony *Vibrio* in water in the control had the highest count followed by the shrimp-seaweed, shrimp-seaweed-tilapia, and was lowest in shrimp-tilapia respectively ($9.51x10^1$ cfu $mL^{-1}$; $3.46x10^1$ cfu $mL^{-1}$; $3.36x10^1$ cfu $mL^{-1}$ and $2.85x10^1$ cfu $mL^{-1}$).

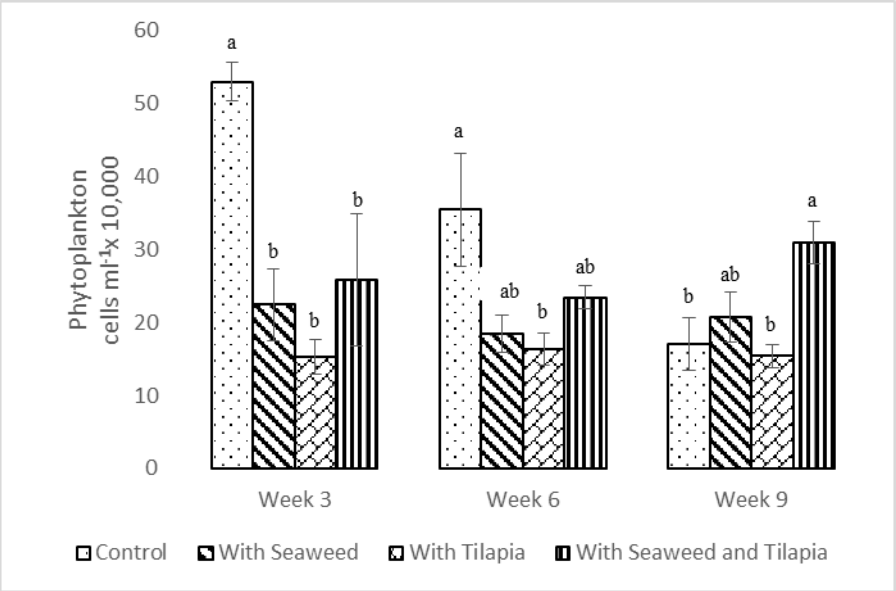


Figure 3. Mean phytoplankton count in water in experimental units during the 60-day culture period. Values with different labels are significantly different ($p<0.05$).

The trends for weekly total bacterial counts and total *Vibrio* counts are presented in Figure 4 and Figure 5.

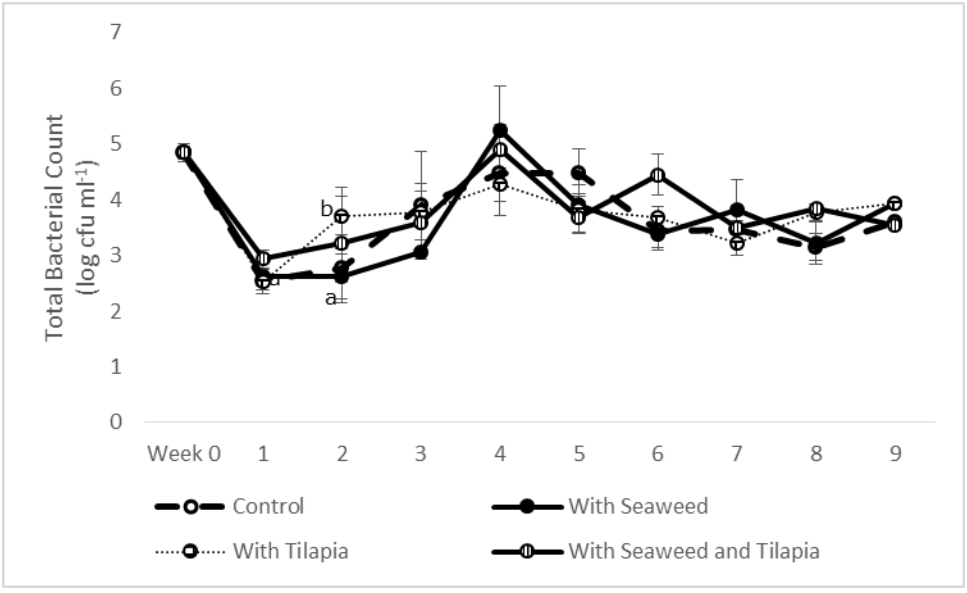


Figure 4. Weekly total bacterial count in water in experimental units during the 60-day culture period. Values with different labels are significantly different ($p<0.05$).

Weekly counts of the bacteria and *Vibrio* were observed to vary from the start up to the end of the experiment. However, it was noted that during the culture period, green-forming colony *Vibrio* in the water of shrimp-tilapia was lowest (Figure 6) and was even reduced up to 0 count at Week 6. Yellow-forming colony *Vibrio* was highest in water of shrimp-tilapia (Figure 7) among other treatments.

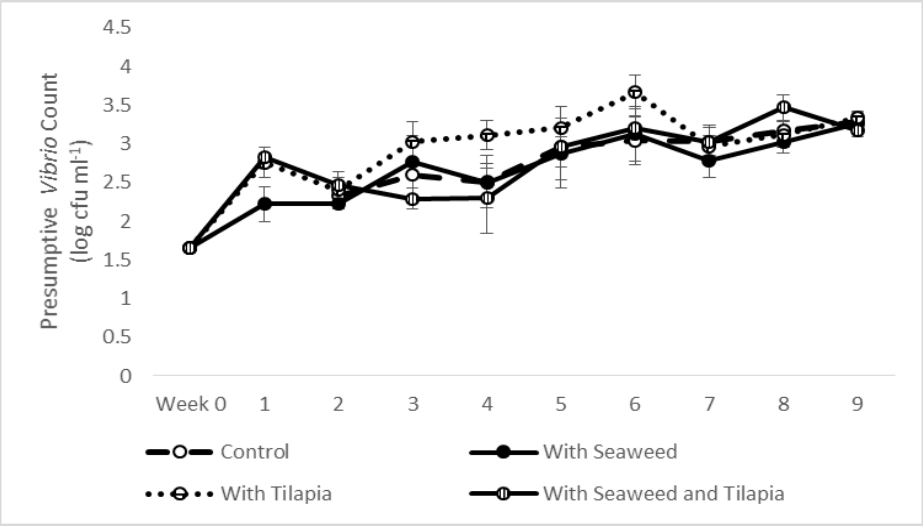


Figure 5. Weekly presumptive *Vibrio* count in water in experimental units during the 60-day culture period.

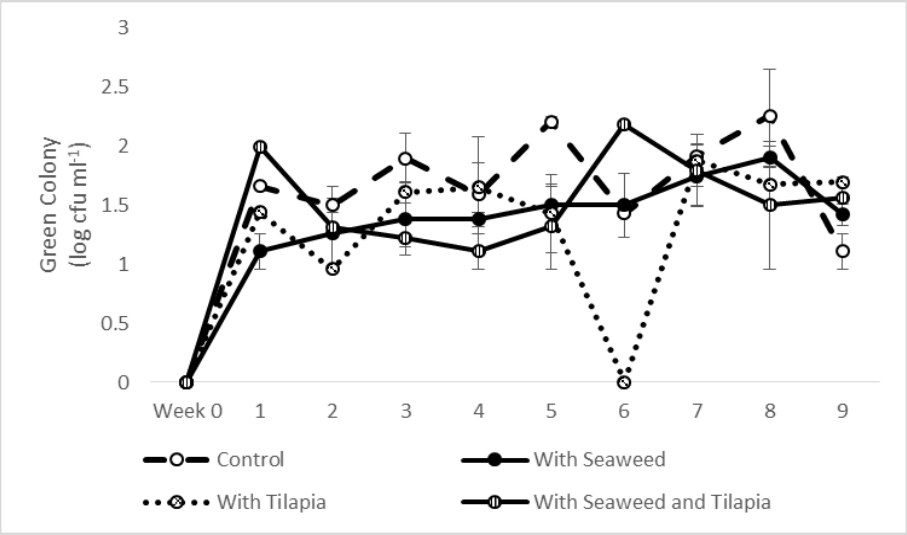


Figure 6. Weekly green-colony forming *Vibrio* in water in experimental units during the 60-day culture period.

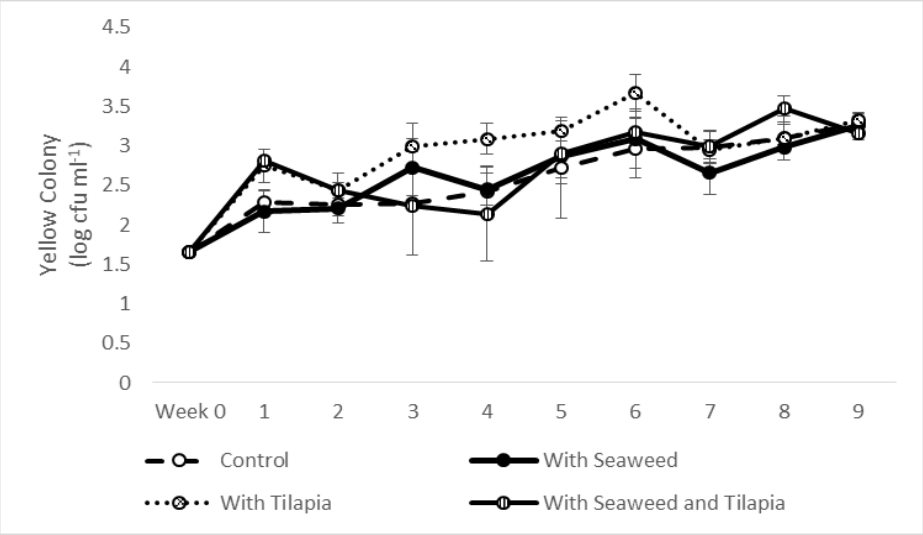


Figure 7. Weekly yellow-colony forming *Vibrio* count in water samples during the 60-day culture period.

The percentage compositions of green colony and yellow colony of the different treatments as seen in Table 1 showed that the mean percentage composition of green-forming colony *Vibrio* for the water in the control was significantly highest (16%). Green-forming colony *Vibrio* in water in shrimp-seaweed-tilapia (4%) and shrimp-tilapia (3%) were statistically lowest. Mean percentage composition of yellow-colony forming *Vibrio* on the other hand was significantly lowest in the water of the control (84%) and was significantly highest in shrimp-tilapia (97%), shrimp-seaweed-tilapia (96%) and the shrimp-seaweed (90%).

**Discussion**. The main objective of the present study was to find out the potential of *G. bailiniae* and *O. mossambicus* in improving water quality and it was shown by the results that shrimp-seaweed had comparably low level of unionized ammonia with that of the control. *G. bailiniae* is reported to utilize nitrogen in the form of ammonium and optimized uptake rate in a low water exchange. Unionized ammonia in shrimp-seaweed was higher than the control although they did not differ significantly. Survival of the shrimps in shrimp-seaweed was highest (67%) than the control (49%). In effect, feed ration of shrimps in shrimp-seaweed was also higher which was the source of nutrients in the system making level of unionized ammonia higher. Thus, it is certain that *G. bailiniae* utilized ammonium from the shrimp effluents because of its comparable results with the control despite its high level of nutrients out of its daily feed ration. Additional feeds given to the tilapia in the reservoir aside from the shrimp feeds contributed to the increase of nutrients because there was continues supply of nutrients in the system. According to Burford & Lorenzen (2004) feed inputs in shrimp ponds resulted to the bulk of N inputs and most enters as TAN (Total-Ammonia N). It was found out in their study that TAN levels increased with increasing stocking densities and decreasing water exchange rates. It must be noted that the present study is an intensive culture system and cultured in low water exchange. Higher stocking density also requires higher feed ration. Further, integration of tilapia also requires feeding for sustainability. Average body weight of the shrimps and tilapia increased through time thus requiring more feeds for growth.

Phytoplankton biomass is estimated by measuring the amount of cholorophyll *a.* Shrimp culture ponds exhibit higher level of chlorophyll *a* (Cardozo et al 2011). Chlorophyll *a* levels in this experiment was significantly highest in the water of shrimps without integration from the rest of the treatments. The level of chlorophyll *a* level in this study was affected by the low water movement, amount of feeds given, and survival. Increasing stocking densities increased chlorophyll concentrations and increased water exchange rates decreased chlorophyll concentrations (Burford & Lorenzen 2004; Cardozo et al 2011). The increased levels of chlorophyll *a* are due to the large amount of nitrogen and phosphorous from formulated food used to stimulate phytoplankton growth (Casé et al 2008; Cardozo et al 2011). Cremen et al (2007) stated that microalgae require nitrogen and phosphorous for growth and reproduction. This is where the importance of seaweed and tilapia integration in terms of bioremediation comes in. *G. bailiniae* aside from its nutrient removal capacity has the ability to control phytoplankton blooms at higher stocking densities making it an efficient and stable component in closed culture systems (Carton-Kawagoshi et al 2014). Phytoplankton in the control bloomed at the early stage of the culture period but collapsed at the latter part. However, treatments integrated with both seaweed and tilapia and each of both had relatively stable phytoplankton abundance throughout the culture period. Similar trend was also observed by Jaspe et al (2011a) where there was a decreasing trend in phytoplankton population on the shrimp monoculture system whereas in white shrimp-milkfish polyculture ponds, the growth of the phytoplankton was more or less stable (Jaspe et al 2011b). Microalgae blooms are considered stressful and undesirable in shrimp ponds (Janeo et al 2009) because of its big possibility of frequent phytoplankton crashes in the system. According to Janeo et al (2009) blooms will cause a significant increase in ammonia, decrease in dissolved oxygen, and rise in organic material, and stressed shrimp that are more susceptible to disease. Casé et al (2008) also stated that phytoplankton make excellent indicator of environmental conditions and aquatic health because they are sensitive to

changes in water quality, respond to low dissolved oxygen levels, high nutrient levels, toxic contaminants, poor food quality, and predation.

In this study, *Nannochloropsis* species dominated throughout the culture period in all experimental treatments. The results of the phytoplankton abundance and composition of the present study were comparable to the results of Cremen et al (2007) and Janeo et al (2009). Diatoms and green algae are considered beneficial algae as they form food items for most of the aquatic invertebrates. As stated by Tendencia et al (2015), Chlorophyceae, i.e. algae, enhanced nutrient uptake in effluent streams resulting in improved water quality in *Penaeus monodon* Fabricius culture ponds.

Higher presumptive *Vibrio* counts in shrimps with tilapia in this study were comparable to the results of Tendencia et al (2004). Total bacteria and presumptive *Vibrio* counts of the water in shrimp-seaweed were comparable to the counts of the water in shrimp-tilapia and luminous bacterial count in water samples of shrimps integrated with seaweed and/or tilapia were almost negligible. The presence of tilapia has specific antibacterial activity against *V. harveyi* (Tendencia et al 2004). Maftuch et al (2016) investigated the effect of extracted seaweed *Gracilaria verrucosa* on fish pathogenic bacteria. The active antibacterial fraction of *G. verrucosa* indicated fractions containing antibacterial compounds such as alkaloid, flavonoid, tannin, and phenolic compounds. This had weak antibacterial activity against *Vibrio harveyi* and *Vibrio algynoliticus* bacteria. Tendencia & de la Peña (2010) also found out that *Gracilaria heteroclada* has the potential to control the growth of luminous bacteria.

The low percentage of green *Vibrio* spp. and high percentage of yellow *Vibrio* spp. in the water samples of the shrimp-tilapia, and shrimp-seaweed, and shrimp-seaweed-tilapia verify the results of Tendencia et al (2004), Huervana et al (2006) and Cadiz et al (2016). As cited in Cadiz et al (2016), most *Vibrio* spp. which are pathogenic to shrimp form green colonies when grown in TCBS agar while yellow colonies were reported to have beneficial effects. Tilapia has an effective control in potentially pathogenic *Vibrios* in the water and shrimps without integration had the highest percentage of green-forming colonies of *Vibrio.* Luminous *Vibrio* was detected in some weeks of monitoring although the count was almost negligible in the control and undetectable on the remaining weeks. *Vibrio harveyi* has the adaptation to switch off bioluminescence or enter into a non-culturable state when conditions are not favourable to their optimum growth (Huervana et al 2006). In this study, although it is a recirculating system and where there was nutrients inflow, other factors such as amounts of nutrients, water flow, and microflora density have to be considered.

**Conclusions**. This study shows the potential of *G. bailiniae* and *O. mossambicus* in improving water quality in intensive low water exchange shrimp tank culture. *G. bailiniae* and *O. mossambicus,* both have the potential to control the growth of pathogenic *Vibrio* and encourage the stable growth of beneficial phytoplankton which control blooms and collapse of phytoplankton. In addition, unionized ammonia was reduced in shrimps integrated with *G. bailiniae*. This investigation presented the potential of seaweed (*G. bailiniae*) and tilapia (*T. mossambicus*) in improving water quality in intensive shrimp tank culture. This is necessary because it could serve as basis for larger scale and even commercial scale culture.

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