

Filter Feeding of Freshwater Mussel, *Ensidens ingallsianusingallsianus*, for Water Quality Control in Tilapia Closed Culture System

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ABSTRACT

A two-month experiment growing juvenile tilapia with and without freshwater mussel, *Ensidens ingallsianusingallsianus*, in an outdoor closed culture system without water exchange was conducted to evaluate the possibility of using the freshwater mussel to improve water quality. Average values of turbidity, chlorophyll *a*, total particulate matter, particulate organic matter, particulate nitrogen, particulate phosphorus and total ammonia nitrogen were 8.5 NTU, 100.8 µg/L, 14.2 mg/L, 11.5 mg/L, 2.139 mg/L, 0.120 mg/L, and 0.286 mg/L in tilapia with freshwater mussel treatment, compared to average values of 22.0 NTU, 324.1 µg/L, 42.5 mg/L, 34.1 mg/L, 5.291 mg/L, 0.305 mg/L, and 0.599 mg/L in tilapia only treatment. Average values of turbidity, chlorophyll *a*, total particulate matter, particulate organic matter, particulate nitrogen, and particulate phosphorus in tilapia with freshwater mussel treatment were significantly lower ($P \leq 0.05$) than those in tilapia only treatment. However, there was no significant difference ($P \geq 0.05$) in the average values of total ammonia nitrogen between the two treatments. Results of this experiment showed that *E. ingallsianusingallsianus* was effective in removing particulate matter, particulate nitrogen, phytoplankton and particulate phosphorus from water in tilapia culture tanks, resulting in decreased rates of 61.4% of turbidity, 66.6% of total particulate matter, 66.3% of particulate organic matter, 68.9% of chlorophyll *a*, 59.6% of particulate nitrogen and 60.7% particulate phosphorus. There was no significant difference ($P \leq 0.05$) between production rate, survival rate and growth rate of tilapia in both treatments.

Keywords: tilapia, freshwater mussel, *Ensidens ingallsianusingallsianus*, closed culture system

INTRODUCTION

In intensive fish culture, uneaten feed and metabolic wastes from fish are the major causes of water quality deterioration in culture pond sand bodies of water receiving

pond effluents. According to Chowdhury *et al.* (2013), total estimated solid wastes output of tilapia was 331-364 kg tonne⁻¹ of feed consumed or 423-496 kg tonne⁻¹ of fish produced. Solid nitrogen waste output was 7.6-8.3 kg tonne⁻¹ of fish produced and solid

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phosphorus waste output was 5.6-5.9 kg tonne⁻¹ of fish produced. Dissolved N waste output was 40.9-46.2 kg tonne⁻¹ of fish produced and dissolved phosphorus waste output was 4.2-5.0 kg tonne⁻¹ of fish produced. Part of these solid wastes suspend in water while another part would settle to the pond bottom. In intensive culture of tilapia, disturbance by fish generate resuspension of bottom sediments. Microalgae growth stimulated by nutrients released from fish wastes and uneaten feed also contribute to the particulate matter concentration in water in the form of phytoplankton. Because of their potential negative effects, removal of these solids is commonly practiced as a means to manage water quality in recirculating fish culture systems.

Bivalves are suspension-feeders that can highly efficiently clear seston particles greater than 3- μ diameter from the water column (Bayne and Newell, 1983; Bayne and Hawkins, 1992). Filtered particles are sorted prior to ingestion. Less nutritious and excess particles are immediately rejected as pseudofeces while ingested material is digested and the remains are excreted as feces. Feces and pseudofeces form biodeposits which settle to the bottom. Filtration by freshwater bivalves such as the Asiatic clam (*Corbicula fluminea*) and zebra mussel (*Dreissena polymorpha*) reduce water turbidity, phytoplankton concentration, and improved water quality in the river estuary and lake (Phelps, 1994; Leach, 1993). Polyculture of green mussels (*Perna viridis*), brown mussel (*Perna Indica*), or oyster (*Crassostrea* sp.) with shrimp can be used to control shrimp disease caused by luminous bacteria (Tendencia, 2007). Particulate organic

waste from aquaculture farms can be utilized as feed for the bivalves. Blue mussels, *Mytilus edulis* have the capability of capturing and absorbing excess particulate fish food released from the salmon farm (MacDonald, *et al.*, 2011). Greater increases in shell height and monthly instantaneous growth rates of oysters suspended at the salmon farm were also observed (Jones and Iwama, 1991). However, integrated Tasmanian blue mussels (*Mytilus planulatus*) – Atlantic salmon (*Salmo salar*) culture by growing mussels 70 and 100 m from the fish cages did not enhance growth of the mussels because phytoplankton production within the farm was not enhanced and mussels were cultured too distant to intercept settling particulate wastes emanating from the fish cage (Cheshuk, *et al.*, 2003)

Freshwater mussel (*Ensis ingallsianusingallsianus*) is a bivalve commonly found in standing and running waters in Thailand. In this study, we investigated the possibility of using *E. ingallsianusingallsianus* to improve water quality of tilapia intensive closed culture systems by studying the effect of co-culturing freshwater mussel and tilapia on particulate matter, particulate organic matter particulate nitrogen, particulate phosphorus, chlorophyll *a* concentration and other related water quality parameters, including production and growth of tilapia.

MATERIALS AND METHODS

The experiment was conducted in 75x155x60 cm (WxLxH) fiberglass tanks using water recirculation system without water

exchange. Cement board was used to partition fiberglass tank into two compartments for tilapia and bivalve. Bivalve compartment was 75x40x60 cm (WxLxH) and fish compartment was 75x115x60 cm (WxLxH). In the fish compartment, a 90 cm long baffle was placed perpendicular to the tank partition to direct water flow. The tanks were filled with 50 cm water. Water from the lower part of the bivalve section was airlifted into the fish section through two 0.5 inch PVC tubes, flowed around center baffle back to the bivalve section through a plastic screen on a rectangular hole (15x30 cm) on another side of the partition. The hole was cut at 10 cm above tank bottom level. Recirculation rate of water through airlift system was 4 L/min. Two plastic trays (35x55x15 cm (WxLxH) were placed on a PVC stand in the bivalve section. The first tray was placed 10 cm above tank bottom and the second tray was 10 cm above the first tray. Two air stones were placed on the tank bottom under the trays. Stocking rate of the bivalve was 3.0 kg/tank (1.5 kg/tray). Average weight and average number of the bivalves stocked per tank were 12.36 g and 243, respectively. Two air stones were placed in the fish compartment on both sides of the center baffle. Water used in this experiment was stored tap water mixed with water from a tilapia pond. Total volume of water in each experimental tank was 0.58 m³. Tilapia were stocked at the rate of 10 fish/tank. Initial weight of fish was between 70 and 90 g. Volume of water in the fish compartment was 0.43 m³ while that in the bivalve compartment was 0.15m³. Initial stocking rate was 0.76-0.81 kg /0.58 m³ or 1.3-1.4 kg/ m³ of total water volume for tilapia, and 3.0 kg/ 0.58 m³ or 5.17 kg/ m³ of total water volume for the bivalve.

The experiment consisted of three replications. Three similar tanks were also set up for control with the same stocking rate of tilapia without bivalve in plastic baskets. Fish were fed twice daily to satiation with 30% protein commercial pelleted feed. Experimental period was 8 weeks. Water samples were collected once a week from the fish compartment to analyze for dissolved oxygen, pH, turbidity, total particulate matter, particulate organic matter, particulate nitrogen, particulate phosphorus, chlorophyll *a*, total ammonia nitrogen, pH and dissolved oxygen. Experimental animals were counted and weighted at the beginning and at the end of the experiment to calculate survival rate and growth rate.

Total particulate matter was analyzed by filtering the samples through G/FC glass fiber filter, dried in an oven at 103-105^oC overnight and weighed (APHA *et al.*, 2005). Then the samples were transferred to a muffle furnace and ignited at 450^oC for 4 h, cooled in a dessicator and weighed to obtain particulate organic matter fraction which was equal to the weight lost after 4 h ignition in the muffle furnace. Particulate nitrogen was analyzed using Kjeltac 1035 analyzer unit, Tecator:Digestion block, Foss, Model 2520. Particulate phosphorus was analyzed spectrophotometrically after percholic acid digestion. Chlorophyll *a* content was analyzed using spectrophotometric determination method after extracting with acetone (APHA *et al.*, 2005). Turbidity was measured by Turbidimeter HACH 2100Q. Dissolved oxygen was measured by YSI Dissolved oxygen meter. pH of water was measured by YSI pH meter. Total ammonia nitrogen was measured by Phenate Method (APHA

et al., 2005). Data were analyzed for mean and standard deviation and compared by t-test.

RESULTS AND DISCUSSION

Results from this experiment indicated that introduction of freshwater mussel (*Ensis ingallsianusingallsianus*) into closed culture system of tilapia could improve water quality by reducing water turbidity, phytoplankton, total particulate matter, particulate organic matter, particulate nitrogen, and particulate phosphorus (Table 1, Figure 1). Statistical analysis indicated that average values of turbidity, chlorophyll *a*, total particulate matter, particulate organic matter, particulate nitrogen, and particulate phosphorus in tanks with tilapia and freshwater mussel were significantly lower ($P \leq 0.05$) than those with tilapia only. Average values of turbidity, chlorophyll *a*, total particulate matter, particulate organic

matter, particulate nitrogen, and particulate phosphorus in the tilapia with freshwater mussel treatment were 8.5 NTU, 100.8 $\mu\text{g/L}$, 14.2 mg/L, 11.5 mg/L, 2.139 mg/L and 0.120 mg/L, respectively, compared to average values of 22.0 NTU, 324.1 $\mu\text{g/L}$, 42.5 mg/L, 34.1 mg/L, 5.291 mg/L and 0.305 mg/L of turbidity, chlorophyll *a*, total particulate matter, particulate organic matter, particulate nitrogen, and particulate phosphorus, respectively, in tilapia only treatment (Table 1).

In the tilapia only treatment, weekly average values of turbidity, chlorophyll *a*, total particulate matter, particulate organic matter, particulate nitrogen, and particulate phosphorus steadily increased from weeks 1 to 4, then continuously decreased until week 8 with the exception of particulate nitrogen which continued to increase until week 5 (Figure 2). Weekly average values were 5.2 and 31.0 NTU for turbidity, 42.1 and 658.6 $\mu\text{g/L}$ for chlorophyll *a*, 8.1 and 75.8 mg/L

Table 1. Comparison of average values of turbidity, total particulate matter (TPM), particulate organic matter (POM), chlorophyll *a*, total ammonia nitrogen (TAN), particulate nitrogen (PN), particulate phosphorus (PP), dissolved oxygen (DO) and pH of water in tanks culturing tilapia and tilapia + freshwater mussel (mean \pm S.D.)

water quality parameter	initial	tilapia	tilapia + bivalve	% decrease
Turbidity (NTU)	3.1	22.0 \pm 11.6 ^a	8.5 \pm 6.1 ^b	61.4
TPM (mg/L)	5.6	42.5 \pm 26.2 ^a	14.2 \pm 11.3 ^b	66.6
POM (mg/L)	5.1	34.1 \pm 21.1 ^a	11.5 \pm 9.5 ^b	66.3
Chlorophyll <i>a</i> ($\mu\text{g/L}$)	11.8	324.1 \pm 222.2 ^a	100.8 \pm 88.3 ^b	68.9
TAN (mg/L)	0.022	0.599 \pm 0.752 ^a	0.286 \pm 0.132 ^a	-
PN (mg/L)	0.199	5.291 \pm 5.634 ^a	2.139 \pm 1.565 ^b	59.6
PP (mg/L)	0.011	0.305 \pm 0.189 ^a	0.120 \pm 0.082 ^b	59.2
DO (mg/L)	8.0	7.1 \pm 0.64 ^a	7.1 \pm 0.59 ^a	-
pH	8.2	8.3 \pm 0.30 ^a	7.9 \pm 0.14 ^b	-

Average values denoted by different superscript in each parameter are statistically significant different ($P \leq 0.05$)

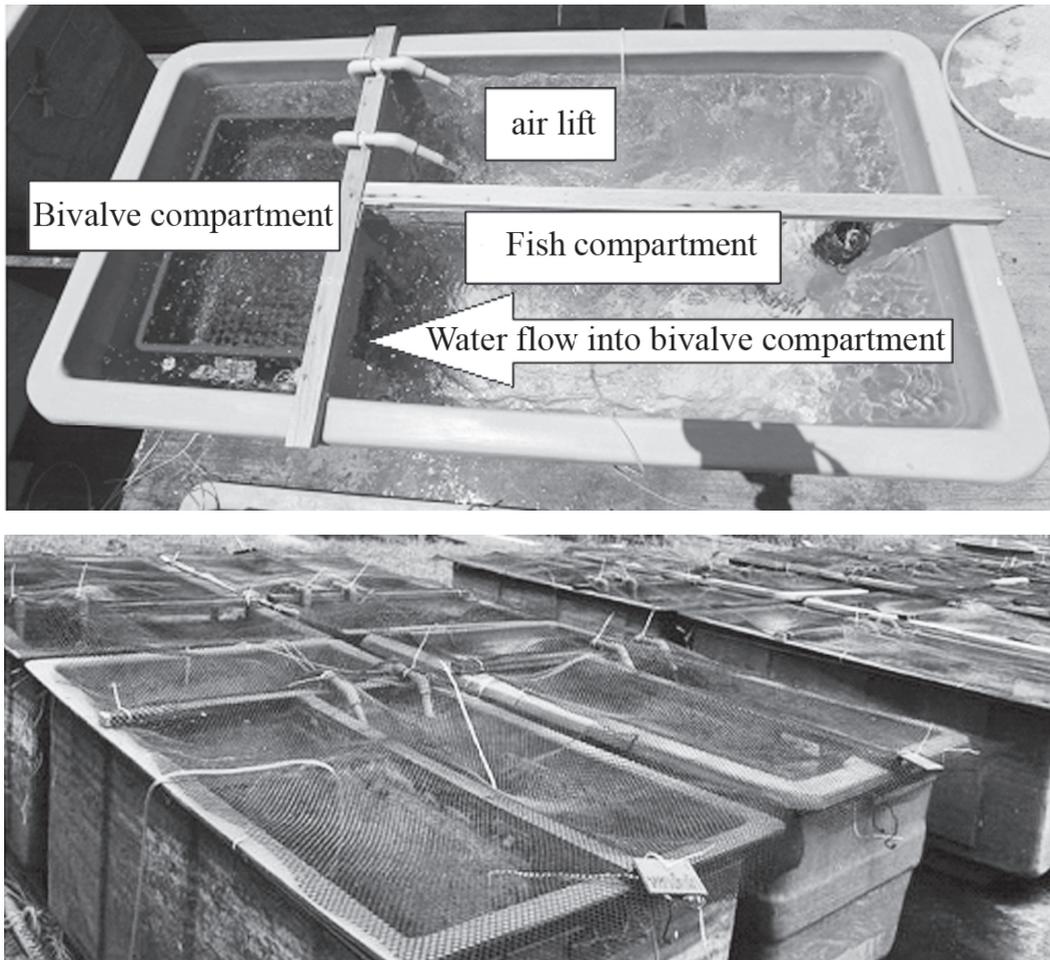


Figure 1. Experimental tanks.

for total particulate matter, 8.1 and 60.1 mg/L for particulate organic matter, 0.223 and 18.445 mg/L for particulate nitrogen and 0.049 and 0.631 mg/L for particulate phosphorus (Figure 2).

In the tilapia with freshwater mussel treatment, weekly average values of these water quality parameters except particulate nitrogen exhibited an increasing trend from weeks 1 to 6, then continuously decreased until to week 8. Weekly average values were

between 1.3 and 17.0 NTU for turbidity, 7.5 and 232.3 $\mu\text{g/L}$ for chlorophyll *a*, 1.8 and 35.4 mg/L for total particulate matter, 1.5 and 29.3 mg/L for particulate organic matter, 0.025 and mg/L for particulate nitrogen and 0.005 and 0.245 mg/L for particulate phosphorus (Figure 2). Decreasing trend of turbidity, particulate matter, particulate organic matter, chlorophyll *a*, particulate nitrogen and particulate phosphorus in both treatments in the last two weeks were likely caused by heavy rain during that period.

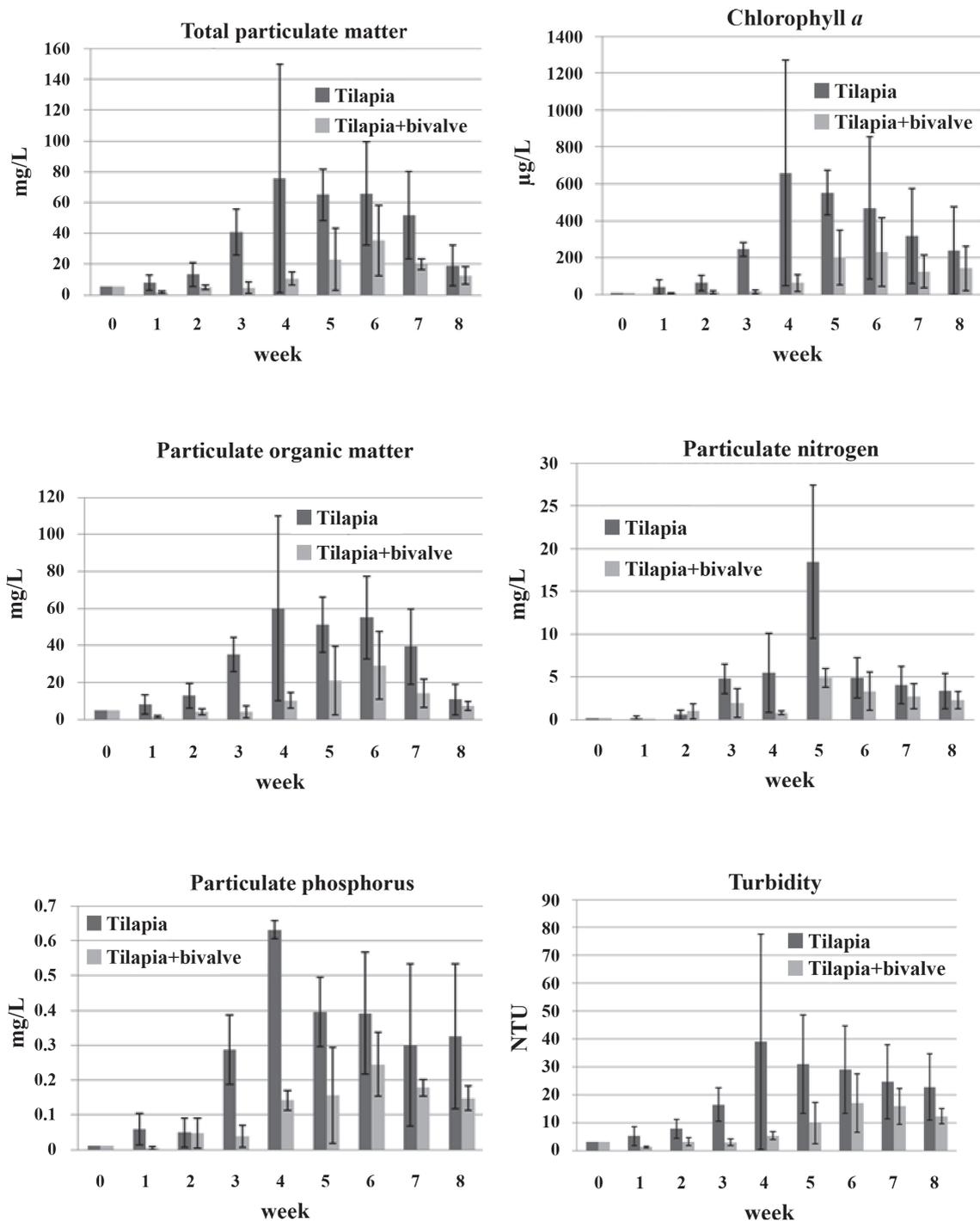


Figure 2. Average values of total particulate matter, chlorophyll *a*, particulate organic matter, particulate nitrogen, particulate phosphorus and turbidity of water in tanks culturing tilapia and tilapia + freshwater mussel.

The decrease in total particulate matter, particulate organic matter, particulate nitrogen and particulate phosphorus in water in tilapia with freshwater mussel treatment indicated the possibility of using freshwater mussel to improve water quality in intensive culture system of tilapia by reducing particulate organic matter and particulate nutrients from water column. Based on the results of this study, freshwater mussel could reduce turbidity, particulate matter, particulate organic matter, chlorophyll *a*, particulate nitrogen and particulate phosphorus in water in intensive culture system of tilapia by 61.4, 66.6, 66.3, 68.9, 59.6 and 60.7%, respectively. These results are similar to the results of Sterling and Okumus (1995) and Neori, *et al.* (2004) cited by Gifford, *et al.* (2005) which reported that culturing oyster or mussel with salmon could reduce nutrient concentrations in the effluent. Soto and Mena (1999) conducted an experiment growing juvenile salmon in closed culture systems with and without freshwater mussel (*Diplodon chilensis*) in outdoor tanks. They reported that within 18 days, *D. chilensis* reduced chlorophyll *a* concentration in tanks with mussel by two orders of magnitude (from ~ 300 to 3 µg/L) compared to tanks without the bivalve. Concentration of total phosphorus was also reduced by about one order of magnitude after 18 days until day 39. Cordova and Martinez-Porchas (2006) also reported that polyculture of Pacific white shrimp, *Litopenaeus vannamei*, giant oyster, *Crassostrea gigas*, and black clam, *Chione fluctifraga* in earthen ponds resulted in significantly decrease of chlorophyll *a*.

There were no statistically significant difference ($P \geq 0.05$) in the average values of

total ammonia nitrogen and dissolved oxygen between the two treatments. Average values of total ammonia and dissolved oxygen were 0.599 mg/L and 7.1 mg/L in tilapia only treatment and 0.120 and 7.1 mg/L in tilapia with freshwater mussel treatment (Table 1). Weekly average values from weeks 1 to 8 were between 0.031 and 1.918 mg/L for of total ammonia nitrogen in the tilapia only treatment and between 0.221 and 0.551 mg/L in tilapia with freshwater mussel treatment (Figure 3). This finding is different from those reported by Martinez-Cordova and Martinez-Porchas (2006) who reported that polyculture of Pacific white shrimp, *Litopenaeus vannamei*, giant oyster, *Crassostrea gigas*, and black clam, *Chione fluctifraga* in earthen ponds resulted in significantly decreasing total ammonium nitrogen.

Weekly average values from weeks 1 to 8 of dissolved oxygen were between 6.4 and 8.1 mg/L for tilapia only treatment and between 6.4 and 7.9 mg/L in tilapia with freshwater mussel treatment. Average value of water pH in tilapia only treatment was 8.3, which was significantly higher ($P \leq 0.05$) than the average value of pH 7.9 in tilapia with freshwater mussel treatment (Table 1). Weekly average values from weeks 1 to 8 of water pH were between 7.8 and 8.7 in tilapia only treatment and between 7.7 and 8.2 in tilapia with freshwater mussel treatment (Figure 3).

Soto and Mena (1999) reported that freshwater mussel, *D. chilensis*, reduced the concentration of ammonia by about one order of magnitude after 18 days until day 39 in closed culture system of salmon in outdoor

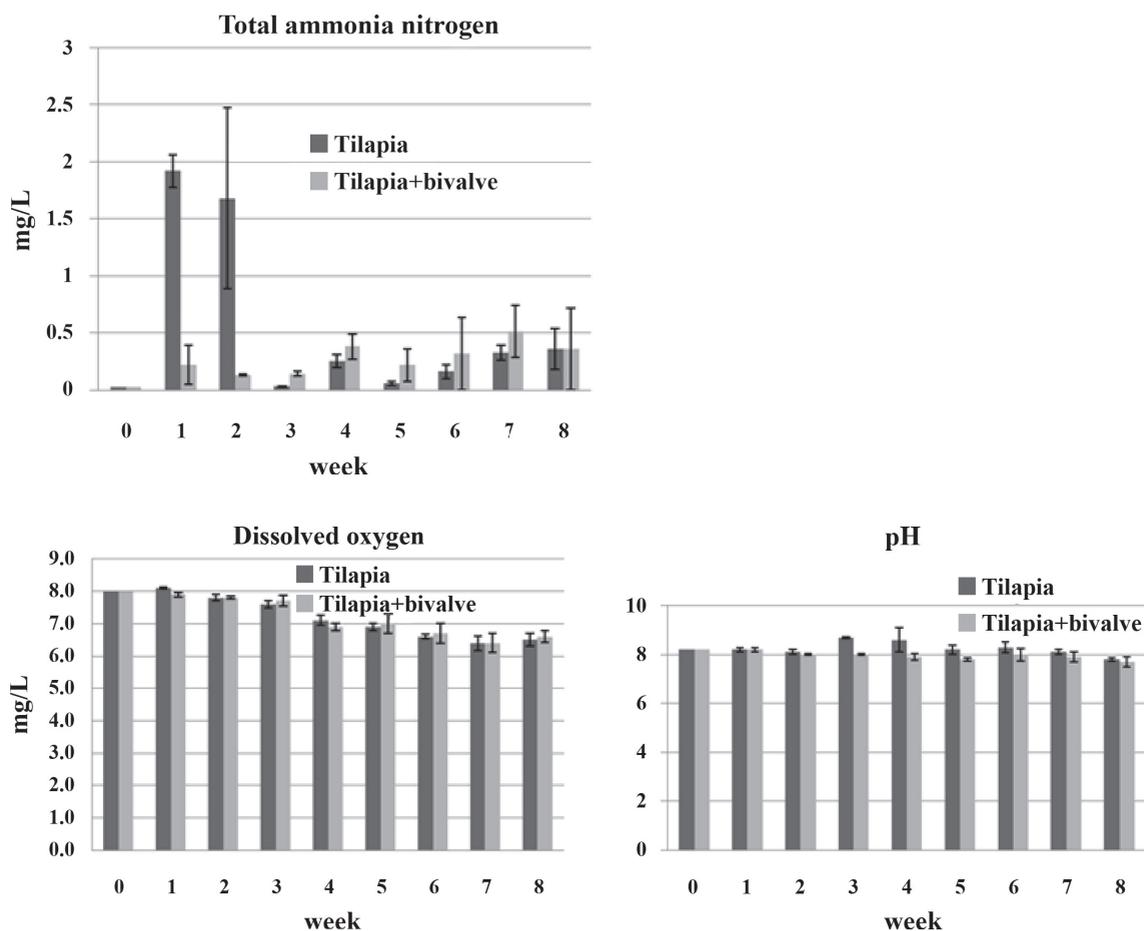


Figure 3. Average values of total ammonia nitrogen, dissolved oxygen and pH of water in tanks culturing tilapia and tilapia + freshwater mussel.

tank experiments. According to the results of this study, the introduction of freshwater mussel, *E. ingallsianusingallsianus*, into tilapia closed culture system did not reduce total ammonia nitrogen concentration in water. This is in contrast with the finding of Soto and Mena (1999). However, in the first two weeks, average values of total ammonia nitrogen in tilapia with freshwater mussel treatment were significantly lower ($P \leq 0.05$) than those of tilapia only treatment (Figure 3). Steep increase in total ammonia concentration in tilapia treatment in the first

week likely resulted from the combines effect of the accumulation of particulate organic matter which decomposed and released ammonia and the density of phytoplankton which took up the ammonia. Average concentration of particulate organic matter at the end of first week was 8.1 mg/L in tilapia treatment comparing to average concentration of 1.5 mg/L in tilapia and freshwater mussel treatment (Figure 2). Average concentration chlorophyll *a* at the end of first week in tilapia treatment was 42.1 $\mu\text{g/L}$. Dense algal bloom beginning from third week to the end of the

experiment in which chlorophyll *a* content rose to concentration level of 240.7-658.6 µg/L resulting in steeply decreased of total ammonia nitrogen to the level of 0.031-0.328 mg/L (Figure 2 and 3).

In tilapia and freshwater mussel treatment, filtration of particulate matter by the bivalve kept chlorophyll *a* content below 233 µg/L and kept total ammonia concentration below 0.516 mg/l throughout the experimental period (Figure 2 and 3).

Filtration of particulate matter by freshwater mussel removed large portion of phytoplankton and other particulate matter (uneaten feed, fish feces and dead algal cells) from water column resulting in the reduction of chlorophyll *a*, particulate organic matter, particulate nitrogen, particulate phosphorus and turbidity of water. Part of filtered particulate matter was ingested and assimilated by the bivalves while another part including feces and pseudo feces settled down to the bottom. Most of settleable particulate matter settled down to the bottom of the bivalve compartment. In tilapia treatment in which no freshwater mussel was put into the bivalve baskets, most of settleable particulate matter was also settled down in the bivalve compartment. Small amount of sediment were found in fish compartments of both treatments. Bottom sediment disturbance by tilapia generated resuspension of settleable particulate matter which was carried out from fish compartment with water that flow into bivalve compartment.

There were no significant differences ($P \geq 0.05$) between the average total weight at harvest, size at harvest, the increase in

bodyweight, survival rate and feed conversion ratio of fish in the two treatments. In tilapia only treatment, the average total weight of fish at harvest was 2726.7 g per tank which was equivalent to the production rate of 2.73 kg/m³. Average body weight of fish at harvest was 272.7 g. Average increase in body weight was 196.5 g/fish/8 weeks and average feed conversion ratio was 1.28. In tilapia with freshwater mussel treatment, the average total weight of fish at harvest was 2820.0 g per tank which was equivalent to a production rate of 2.82 kg/m³. Average size of fish at harvest was 282.0 g. Average increase of body weight was 202.7 g/fish/8 weeks and average feed conversion ratio was 1.25 (Table 2). Survival rate of the experimental fish were 100% in both treatments.

Production rate, survival rate and growth of tilapia in both treatments were not significantly different which indicated that closed culture system without water exchange could give an average production rate of at least 2.73 kg/m³. However, it has to be noted that part of the culture tank was partitioned and acted as settling area for settleable particulate matter. It also may be possible that higher production rate of tilapia can be obtained from co-culture of freshwater bivalve with tilapia using higher stocking rate of fish. However, more study is needed to obtain proper stocking densities of bivalve and fish in order to obtain optimum production.

Average survival rate of freshwater mussel was 70.8% and average growth rate was 0.14 g/individual/8 weeks (Table 3). In this experiment there was neither water exchange nor sediment removal. A thick

Table 2. Survival rate, growth rate, final body weight and feed conversion ratio of tilapia cultured with and without freshwater mussel (mean±S.D.)

Treatment	tilapia	Tilapia + bivalve
Initial number	10	10
Initial total weight (g)	761.7±53.9	793.3±94.5
Average initial weight (g/fish)	76.2±5.4	79.3±9.5
Survival rate (%)	100	100
Final total weight (g)	2726.7±64.3 ^a	2820.0±485.0 ^a
Final average weight (g)	272.7±6.4 ^a	282.0±48.5 ^a
Average growth rate (g/fish/8 week)	196.5±7.4 ^a	202.7±50.1 ^a
Average growth rate (%)	259.1±25.4 ^a	259.2±81.6 ^a
Feed conversion ratio	1.28±0.04 ^a	1.25±0.06 ^a

Average values denoted by different superscript in each parameter are statistically significant different (<0.05)

Table 3. Survival rate, growth rate and final body weight of freshwater mussel cultured with tilapia (mean±S.D.)

Initial number	243±8.5
Initial total weight (g)	3000
Average initial weight (g/individual)	12.36±0.44
Survival rate (%)	70.8±13.8
Final total weight (g)	2146.7±404.6
Final average weight (g)	12.49±0.37
Average growth rate (g/individual/8weeks)	0.14±0.11
Average growth rate (%)	1.1±0.9

layer of sediment was found at the bottom of the bivalve compartment after harvest. Toxic metabolites generated from the decomposition of organic sediment were likely to be the cause of slow growth rate and high mortality of the freshwater mussel. Periodical removal of sediment from the bivalve compartment should be an effective means to improve environmental conditions. High stocking density of the bivalve might also result in negative effects on the bivalve themselves.

Even though higher production of fish was not obtained from co-culture of freshwater mussel with tilapia, the result of this experiment indicated that freshwater mussel-tilapia co-culture can be used as a means to reduce particulate nutrients and particulate organic matter including phytoplankton in intensive culture system of tilapia which will result in better environmental condition in culture tank and less pollutant (organic matter, phytoplankton and nutrients) loading in fish culture effluents.

CONCLUSION

Results from this investigation indicated that freshwater mussel, *E. ingallsianusingallsianus*, can be used to improve water quality in tilapia intensive culture systems by reducing total particulate matter, particulate organic matter, phytoplankton, particulate nitrogen and particulate phosphorus. This will directly result in the reduction of pollutants loading in fish farm effluents and better environmental conditions in fish culture systems. However, more studies are needed to obtain proper stocking densities of bivalve and fish to obtain optimum production. Farm scale investigation and the development of hatchery techniques to produce juvenile freshwater mussel is necessary.

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