Screening Three Finfish Species for their Potential in Removing Organic Matter from the Effluent of White Leg Shrimps (*Litopenaeus vannamei*) Farming

Lich Q. Nguyen[1*], N. Bolan[2] & M. Kumar[3]

Keywords: Organic matter- Finfish- White leg shrimp- Shrimp wastewater- Vietnam

Summary

*White leg shrimp (*Litopenaeus vannamei*) farming effluent contains pollutants that include high levels of organic matter (OM) nutrients and growth-promoting substances. This study investigated the effects of varied concentrations of white leg shrimp (*Litopenaeus vannamei*) farm wastewater 0, 50, 75 and 100%, on the survival rate (SR) of three finfish species: tilapia (*Oreochromis niloticus*), grey mullet (*Mugil cephalus*) and rabbit fish (*Siganus guttatus*) as part of screening their potential in removing organic matter from the effluent of white leg shrimp farming. The different initial levels of shrimp wastewater from 50% to 100% had no significant effect on the survival rate of tilapia and mullet; but the survival rate of* S. guttatus *significantly decreased with increasing shrimp wastewater (P<0.05). The results showed that the removal of BOD, COD and TSS occurred in the range of 66-83, 68-81 and 30-54%; respectively and the removal efficiency of OM by mullet was higher than Tilapia in all treatments. The study also indicated that the reduction highest removal of BOD, COD and TSS was achieved being 83.1%, 80.7and 53,7% respectively, at the medium stocking density (25 fish/m²) of mullet.*

Résumé

Comparaison de trois espèces de poisson pour leur potentiel à réduire la matière organique des effluents de l'aquaculture des crevettes blanches (*Litopenaeus vannamei*)

*Les effluents des bassins de production de crevettes (*L. vannamei*) contiennent des éléments polluants tels que la matière organique (OM), des nutriments et des substances stimulant la croissance. Cette étude analyse les effets de trois concentrations (0, 50, 75 et 100%) d'effluents de bassins de production de crevettes, sur la survie (SR) de trois espèces de poisson: le tilapia (*Oreochromis niloticus*), le mulet gris (*Mugil cephalus*) et le poisson-lapin-doré (*Siganus guttatus*) afin de connaître leur potentialité à réduire le taux de matière organique de cet effluent. Les différents niveaux initiaux de ces effluents (50, 75 et 100%) n'ont pas d'effets significatifs sur la survie des tilapias et du mulet, alors que la mortalité de* S. guttatus *augmente significativement avec la concentration d'effluent (P<0,05). Les taux de réduction de BOD, COD et TSS étaient entre 66-83, 68-81 and 30-54%, respectivement. Le taux de réduction d'OM par mulet était plus élevé que pour le tilapia dans tous les traitements. Les taux de réduction les plus élevés, avec une densité moyenne de 25 mullet/m², sont observés pour des BOD, COD et TSS de 83%, 81% et 54% respectivement.*

[1]Hue University, College of Agriculture and Forestry, Hue City, Vietnam

[2]University Drive, The University of Newcastle, Faculty of Science and Information Technology, Global Centre for Environmental Research Callaghan, Australia

[3]Flinders University of South Australia, School of Biological Sciences, Ottoway, Australia

*Corresponding author: EMail: quanglichckcn@huaf.edu.vn

Introduction

Organic matter (OM) is the main pollutant from the effluent of intensive shrimp farming. In wastewater, OM includes both dissolved and suspended particles comprising mainly carbon chains. OM is readily available to microbes and algae; an increase in OM concentration will affect the structure of the phytoplankton community, thereby leading to eutrophication in the pond (9).

Without proper treatment, the shrimp wastewater discharge containing high OM unfavourably affects aquatic life, water quality and the environmental system.

In the last few decades, several methods have been applied to control OM in aquaculture wastewater. These methods include wetland treatment, polyculture systems, trickling filters, biofilms, membrane filtration, electro-coagulation and chemical treatment (4). Boopathy (2) suggested that most of these methods may be used to reduce OM from wastewater, but biological methods are the most advantageous. Nowadays, alternative systems for removing OM involve aquatic animals and/or plants from aquaculture wastewater have been studied. Troell *et al.* (7) used a polyculture system which combined seaweed, fish and shrimp to treat shrimp farming effluent. Their study suggested that the polyculture system could be used to remove OM, as well as produce an aquatic organism as a food source. Tian *et al.* (6) investigated a closed-polyculture system including Chinese shrimp (*Penaeus chinensis*), Taiwanese tilapia (*O. mossabicus* x *O. Niloticus*) and constricted tagelus (*Sinonovacula constricta*). The study indicated that chemical oxygen demand (COD) in a polyculture system was lower than that in shrimp monoculture, while other parameters such as pH, dissolved oxygen (DO) and nutrient concentrations were not significantly different. Erler *et al.* (3) used two finfish species (*Mugil cephalus* and *Siganid nebulosus*) and vertical artificial substrates (VAS) to treat shrimp farm effluent.

The study concluded that VAS significantly improved the settlement of particulates, while *Mugil cephalus* potentially reduced the production of nitrate (NO_3-N) when VAS was absent. However, when *Siganid nebulosus* was added, the total finfish nitrogen (N) retention increased. Limited information is available on the applicability of tilapia, grey mullet and siganid in removing organic matter from shrimp farm effluent.

Fish can filter feed on things such as phytoplankton, organic particles and bacterial films in the upper water column (8).

This study investigated the potential of finfish species for assimilating OM and their optimal stocking density for maximizing the removal of OM from intensive shrimp farm effluent through two subsequent experiments, the the first assessed survival rates and second the removal rates.

Materials and methods

Wastewater was collected at the shrimp farm effluent canal in the third month of the shrimp production cycle and diluted with seawater to obtain 75% and 50% wastewater concentrations. Three water quality parameters of the diluted treatments: (i) DO (±0.5), (ii) T (±0.5) and (iii) pH (±0.5) were adjusted to levels similar to the 100% treatment. All experiments were conducted at the Dien Mon Enterprise Shrimp Farming, Truong Son Company, Thua Thien Hue province, Vietnam. The water quality parameters including pH, temperature (T), salinity (S) and dissolved oxygen (DO) parameters were measured once in every two days at 8:00AM in the field. Water samples were taken at 25 - 40 cm in depth at 5 positions inside the tank, including the 4 corners and the centre, and then mixed together for analysis.

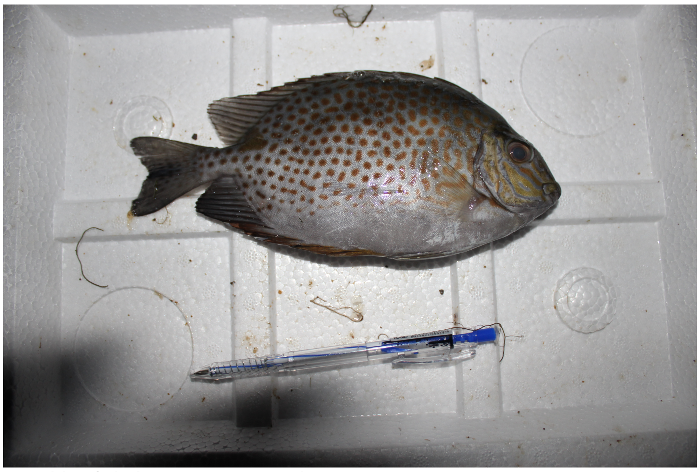
The concentration of TAN was measured by the Phenate method. TSS, BOD, COD, NO_3-N and PO_4-P were performed according to the standard method (1) in the laboratory.

Experiment 1

The survival rates of three local finfish species: tiliapia (*Oreochromis niloticus*) (Figure 1a), grey mullet (*Mugil cephalus*) (Figure 1b) and rabbit fish (*Siganus guttatus*) (Figure 1c), were tested at three different shrimp wastewater levels: 50%, 75% and 100% during 12 days of experiment period. At the beginning of the experiment, each tank contained 500 L of water composed of 100, 75 or 50% shrimp wastewater; the supplement was seawater.

a. Grey mullet (*Mugil cephalus).*

b. Tilapia (*Oreochromis niloticus*).



c. Rabbit fish (*Siganus guttatus*).

Figure 1: Three finish species were used in the experiments.

At days 4 and 8 of the experimental period, 250 L water of each tank was replaced with an identical mixture of seawater and shrimp wastewater collected from the water channel. Oxygen was supplied from the aerator system to maintain the DO level of the water above 4.0 mg/L during the experiment.

The experimental set-up had three parallel replicates at the same time. The average wet weight of the fish at the start of the experiment was 20.2 ±2.1 g for *O. niloticus*, 20.4 ±2.2 for *M. cephalus* and 20.5±3.1 for *S. guttatus*. Each tank contained 15 fish.

Experiment 2

Experiment 2 assessed the optimal stocking density for maximum biodegradation of the OM from shrimp wastewater by the fish species selected from experiment 1. The two species were tiliapia (*Oreochromis niloticus*) and grey mullet (*Mugil cephalus*). The mean wet weight of *M. cephalus*, 21.4 ±2.1g, was tested at three densities: 20 (M-20), 25 (M-25) and 30 (M-30) mullets/m^3, which approximated 400, 500 and 600 g/m^3, respectively. The mean wet weight of tilapia, 21.1 ±1.4g, was tested at the same densities; the treatments, referred to here, are: T-20, T-25 and T-30. Both species had a control of 0 fish/m^3 referred to here as CT and CM, respectively. The experiment was conducted over 8 days with three parallel replications at the same time.

Data analysis

The survival rate of the animals was calculated by Equation I.

$$SR = \frac{N_{en}}{N_{in}} \times 100 \qquad \text{(I)}$$

where: *SR* is the survival rate (%), N_{en} is the number of animals at the end of experiment (individuals) and N_{in} is the number of animals at the initiation of experiment (individuals).

The mean and the standard deviation (SD) values of the effluent water quality indicators of the three finfish species in three different shrimp wastewater levels were calculated by using descriptive statistics. The treatment efficiency was calculated by using the mean difference between initial and final values. The two experiments were analysed by using a one-way ANOVA followed by Tukey post hoc test and Tamhane's test (when equal variances were not assumed). SPSS® software Version 21 was used for the mentioned analysis.

The differences were considered significant at $P<0.05$. The time series was interpreted by using a straightforward analysis of the plotted graphs prepared in MS-Excel®.

Results and discussion

Screening the potential of finfish

Water quality

During the experiment, the mean values of Salinity (S), Temperature (T) and DO fluctuated ranging from 21.7 – 22.7%, 30.4 – 31.5 °C and 5.7 – 6.3 mg/L, respectively in the treatments.

At 100% shrimp wastewater level, the pH was slightly higher than that in the 75% and 50% levels for all the three fish species treatments, while the concentration of DO was usually lower than that in other two treatments of wastewater levels (50% and 75%) (Table 1).

At the initial stocking, the concentrations of both TAN and NO_2-N were not different among the three finfish species (Table 2).

Total ammonia Nitrogen (TAN) concentrations slightly increased with the increased concentration of shrimp wastewater (Figure 2).

The TAN increased from an initial level of 4.7 mg/L to 5.4, 5.1 and 5.5 mg/L for treatments T-100, M-110 and S-100, respectively. At treatments of 75% and 50% wastewater levels, TAN increased from 3.2 mg/L to 3.7 mg/L, and from 1.9 mg/L to 2.5 mg/L, respectively after 12 days of experimental period. At three different shrimp wastewater levels, the NO_2-N concentration increased in *O. niloticus* and *S. guttatus* treatments, while that in *M. cephalus* treatments slightly decreased (Figure 3). At the end of the experiment, the concentration of NO_2-N for each of the three treatments: M-100, M-75 and M-50 was reduced by 23%, 23.7% and 7.5%, respectively.

Table 1

The means ± SD, of salinity, T, pH, and DO in the different treatments for the 12 days experimental period.

Treatments	Salinity (‰)	Temperature (°C)	pH ($logH^{+}$)	DO (mg/L)
T-100	21.8 ±11.0	31.5 ±3.8	7.8 ±0.15	5.8 ±0.2
T-75	21.9 ±10.7	31.3 ±4.0	7.6 ±0.18	6.0 ±0.1
T-50	22.2 ±10.2	31.3 ±4.0	7.7 ±0.15	6.1 ±0.1
M-100	21.9 ±11.8	31.3 ±3.8	7.7 ±0.19	5.9 ±0.1
M-75	22.6 ±11.1	30.4 ±3.2	7.5 ±0.05	5.9 ±0.2
M-50	22.6 ±11.3	30.3 ±2.9	7.5 ±0.11	6.3 ±0.2
S-100	21.8 ±11.7	31.3 ±3.7	7.7 ±0.11	5.9 ±0.2
S-75	22.5 ±11.1	30.5 ±3.3	7.6 ±0.15	6.0 ±0.2
S-50	22.7 ±11.0	30.5 ±3.3	7.6 ±0.17	6.3 ±0.2

Note: T-100, T-75 and T-50, M-100, M-75 and M-50, and S-100, S-75 and S-50: *O. niloticus*, *M. cephalus* and *S. guttatus*, respectively, stocked in 100, 75 and 50% shrimp wastewater.

Table 2

The initial and average values for days 2, 4, 6, 8 and 12 of TAN and NO_2-N in the three different finfish species for the 12-day experiment (means ± SD).

	O. niloticus		*M. cephalus*		*S. guttatus*	
	Initial	Final	Initial	Final	Initial	Final
TAN (mg/L)	3.2 ±1.4	3.8 ±1.4^{a}	3.2 ±1.4	3.5 ±1.4^{a}	3.2 ±1.4	3.7 ±1.3^{a}
NO_2-N (mg/L)	2.8 ±1.2	3.1 ±1.0^{a}	2.8 ±1.2	2.6 ±0.8^{b}	2.8 ±1.2	3.2±1.0^{a}

Different superscripts for the final values in the same row indicate that the values are significantly different at p<0.05.

Table 3

The survival rate of three finfish species at different shrimp wastewater levels at day 12.

Fish species	Shrimp wastewater level (%)			Mean survival rate
	100	75	50	
O. niloticus	91 ±1.9^{a,i}	92 ±1.9^{a,i}	94 ±1.9^{a,i}	93 ±2.2^{a}
M. cephalus	82 ±1.9^{a,i}	84 ±1.9^{a,ij}	91 ±1.9^{a,j}	86 ±4.3^{b}
S. guttatus	37 ±6.7^{b}	49 ±5.1^{bij}	54 ±3.8^{b,j}	47 ±9.1^{c}

a,b,c Different superscripts in the same column indicate that the values are different (p<0.05).

i,j,k Different superscripts in the same row indicate that the values are different (p<0.05).

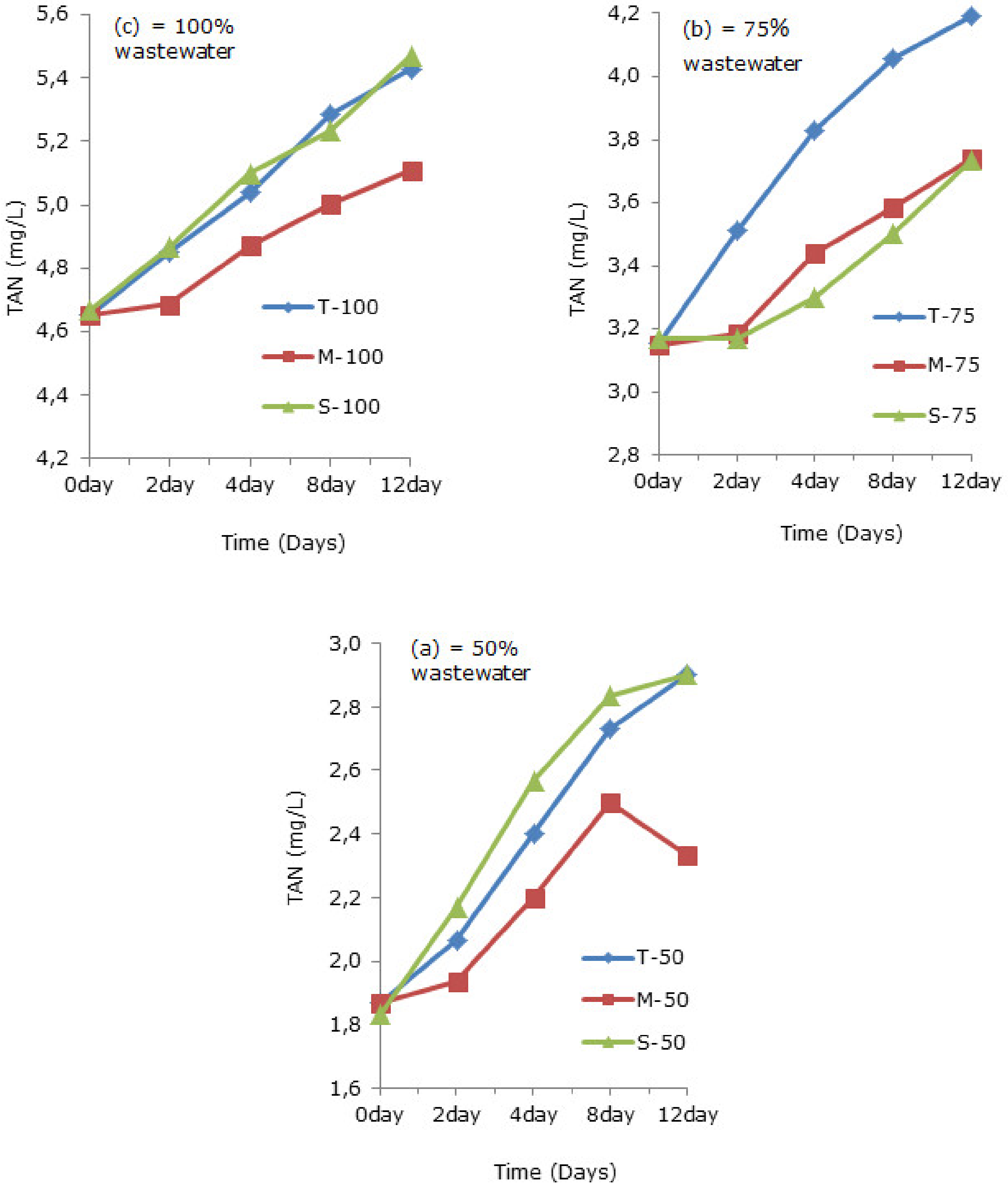


Figure 2: Effects of three selected finfish species (T= Tilipia ; M= Mullet ; S= Siganus) on TAN at three different shrimp wastewater levels.

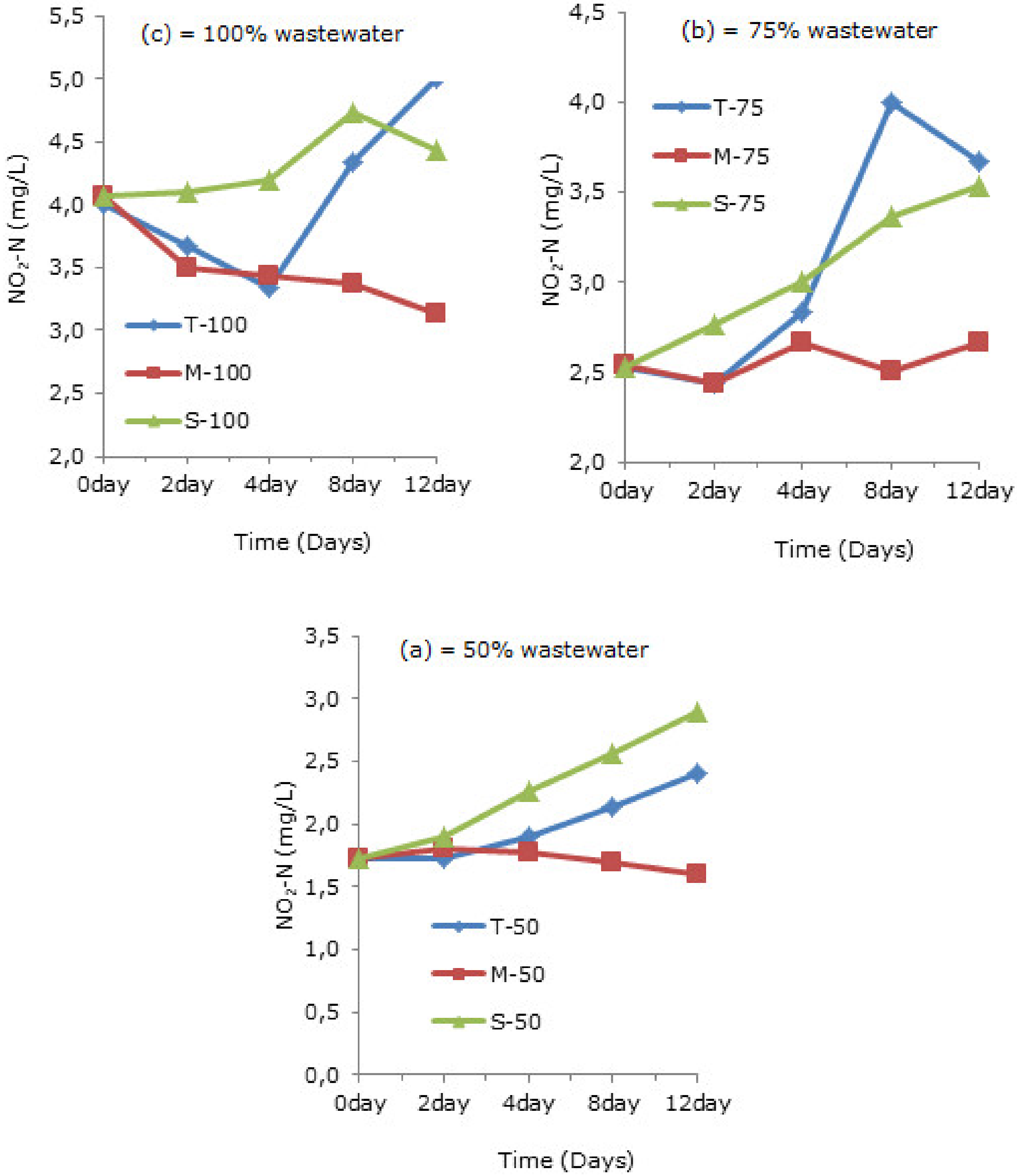


Figure 3: Effects of three finfish species on NO_2-N at three different shrimp wastewater levels.

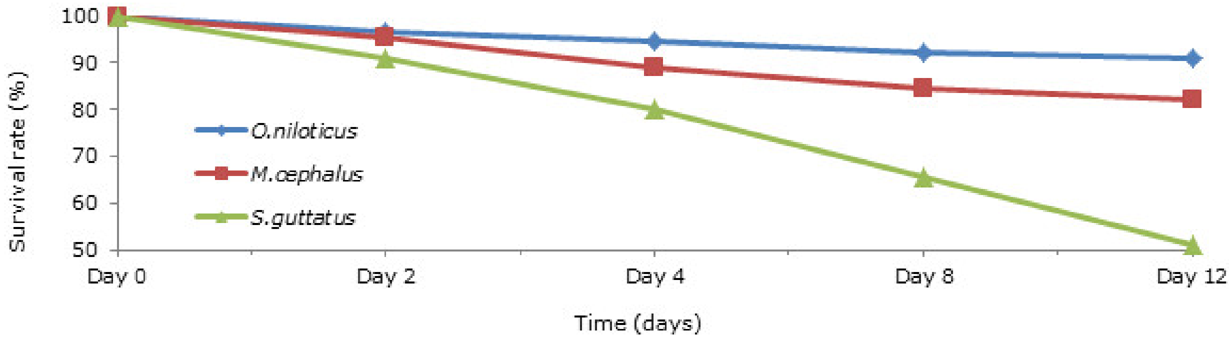


Figure 4: Comparison of the survival rate of the three selected finfish species.

The mean level of NO_2-N in *M. cephalus* treatment was significantly lower (P<0.05) compared with that of the other two species (Table 2). The survival rate (SR) of two species *O. Niloticus* and *M. cephalus* for the three shrimp wastewater levels was not different after the 12-day-experimental period (Table 3). However, the SR of *S. guttatus* decreased from 54% to 37% when shrimp wastewater level was increased from 50% to 100%. The mean SR of the three species was significantly different (P<0.05). At the end of the experiment, the mean SRs were 93%, 86% and 47% for *O. Niloticus*, *M. cephalus* and *S. Guttatus*, respectively (Table 3). Over the 12 day-period, the mortality rate of *O. Niloticus* and *M. cephalus* was steady, while the mortality among the *S. Guttatus* accelerated (Figure 4).

However, all three finfish species showed stress at 100% treatment which had 5 mg/L TAN. At the lower level of 0.1 mg/L NH3, the SRs of *O. niloticus* and *M. cephalus* were around 94 and 91%, but when the level of NH3 increased to 0.2 mg/L and 0.3 mg/L, the SRs decreased to about 92% and 91% for *O. niloticus* and 84% and 82% for *M. cephalus*, respectively. The survival rate of *S. guttatus* also significantly decreased from 54 to 37% when NH3 concentrations increased from 0.1 to 0.3 mg/L (P<0,05). The NH_3 and the NO_2-N toxicity levels in the water significantly influenced the fish biomass. Increasing the fish stocking density, likewise increased the concentrations of NH_3 and NO_2-N. This may be caused by different DO levels in the tanks due to different ecological niches these fish species were feeding on. Morris and North (5) reported that the oxygen consumption not only depended on the animal species, but also on their ecological niches, morphological changes at different life stages and biomass stocking densities. These may be the main reasons for the limited nitrification process in the tanks which increased NH_3 and NO_2-N concentrations in the water.

The results indicated that *S. guttatus* was less tolerant and had little capacity in removing OM from shrimp farm effluent compared with *O. niloticus* and *M. cephalus* which showed more capacity in biodegrading OM; these two species also had wider toxicity and salinity tolerance. The SR of *O. niloticus* and *M. cephalus* was not significantly affected with shrimp wastewater at 50% and 100%, but that of *S. guttatus* was reduced correspondingly with increased shrimp wastewater concentration.

Impact of fish stocking density

O. niloticus *densities and water quality*

The water quality parameters were similar among the treatments (P>0.05), except for DO (Table 4). The mean DO concentration decreased with increasing fish stocking density. The concentration of TAN, NO_3-N and PO_4-P increased with increasing *O. niloticus* stocking density from 20 to 30 tilapia/m^3, and these reached the highest level in the highest stocking density (T-30). The levels of TAN, NO_3-N and PO_4-P nearly doubled during the experiment, but the mean values did not differ between the treatments, except for DO. In the control, the mean value of NO_3-N was significantly lower than that in the three treatments, while the DO level was significantly higher (Table 4).

Table 4

Mean (± SD) values of T, pH, DO, TAN, NO_3-N and PO_4-P at three different *O. niloticus* densities.

	T (⁰C)	pH (logH⁺)	DO (mg/L)	TAN (mg/L)	NO_3-N (mg/L)	PO_4-P (mg/L)
T-20	33.0±0.4[a]	7.9±0.12[a]	5.9±0.22[a]	2.5±0.80[a]	4.6±0.60[a]	1.4±0.42[a]
T-25	32.9±0.3[a]	8.0±0.13[a]	5.6±0.19[a]	2.6±0.73[a]	4.8±0.64[a]	1.4±0.47[a]
T-30	33.0±0.3[a]	7.9±0.12[a]	5.2±0.36[b]	2.8±0.71[a]	4.8±0.69[a]	1.5±0.51[a]
CT	33.3±0.1[a]	8.2±0.22[a]	6.4±0.43[c]	2.5±0.52[a]	3.7±0.36[b]	1.5±0.42[a]

Different superscripts in the same column indicate that the values are different ($p<0.05$). T-20: 20 *O. niloticus* (400 g/m³); T-25: 25 *O. niloticus* (500 g/m³); T-30: 30 *O. niloticus* (600 g/m³); CT: is control (no fish).

Table 5

Initial, final and reduction values of BOD, COD and TSS by O. niloticus in three treatments (T-20, T-25 and T-30) and the control (CT)

	BOD			COD			TSS		
	Initial (mg/L)	Final (mg/L)	Removal (%)	Initial (mg/L)	Final (mg/L)	Removal (%)	Initial (mg/L)	Final (mg/L)	Removal (%)
T-20	58,6	20.1±0.8	66 ±1.3[a]	92,7	28.4±0.8	69 ±0.9[a]	342	204±10.6	40 ±3.1[a]
T-25	58,6	12.6±0.2	78 ±0.4[b]	92,7	21.8±0.7	77 ±0.8[a]	342	233±1.44	32 ±0.4[b]
T-30	58,6	16.4±1.4	72 ±2.3[ab]	92,7	21.2±0.6	77 ±0.6[a]	342	240±2.12	30 ±0.6[b]
CT	58,6	42.5±0.4	28 ±0.6[c]	92,7	64.6±1.1	30 ±1.3[b]	342	240±2.1	30 ±0.6[b]

Different superscripts in the same column indicate that the values are different ($p< 0.05$). T-20: 20 *O. niloticus* (400 g/m³); T-25: 25 *O. niloticus* (500 g/m³); T-30: 30 *O. niloticus* (600 g/m³); CT: is control (no fish).

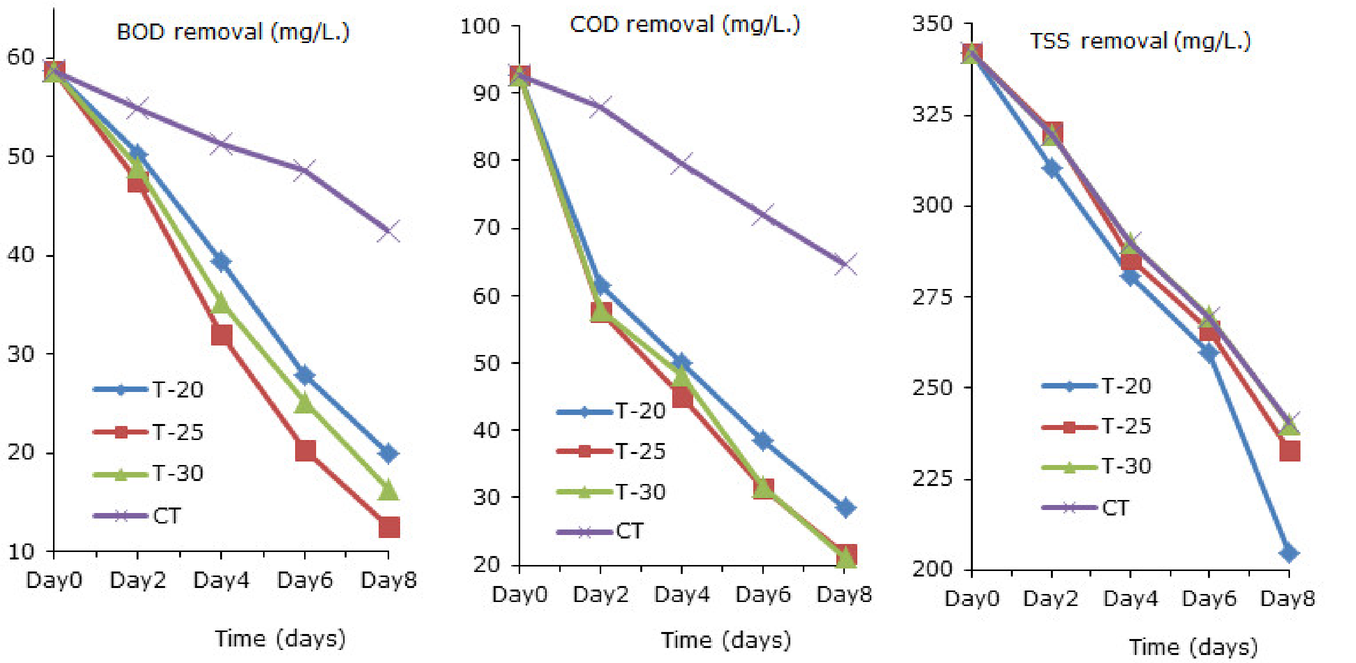


Figure 5: Reduction of BOD, COD and TSS by *O. niloticus* in three treatments (T-20, T-25 and T-30) and the control (CT).

Table 6

Mean (± SD) values of T, pH, DO, TAN, NO_3-N and PO_4-P at three different M. cephalus densities and the control.

	T (^{0}C)	pH (logH^+)	DO (mg/L)	TAN (mg/L)	NO_3-N (mg/L)	PO_4-P (mg/L)
M-20	31.8 ±0.7[a]	7.7 ±0.1[a]	5.5 ±0.24[a]	2.2 ±0.05[a]	5.4 ±0.04[a]	1.3 ±0.05[a]
M-25	31.8 ±0.7[a]	7.7 ±0.1[a]	5.4 ±0.22[a]	2.2 ±0.06[a]	5.1 ±0.03[a]	1.3 ±0.01[a]
M-30	31.8 ±0.7[a]	7.7 ±0.1[a]	5.4 ±0.15[a]	2.3 ±0.04[a]	4.8 ±0.03[a]	1.4 ±0.01[a]
CM	32.0 ±0.7[a]	7.8 ±0.1[a]	6.2 ±0.32[b]	3.3 ±0.18[b]	4.0 ±0.28[b]	1.3 ±0.02[a]

Different superscripts in the same column indicate that the values are different ($p<0.05$). M-20: 20 *M. cephalus* (400 g/m^3); M-25: 25 *M. cephalus* (500 g/m^3); M-30: 30 *M. cephalus* (600 g/m^3); CM: is control (no fish).

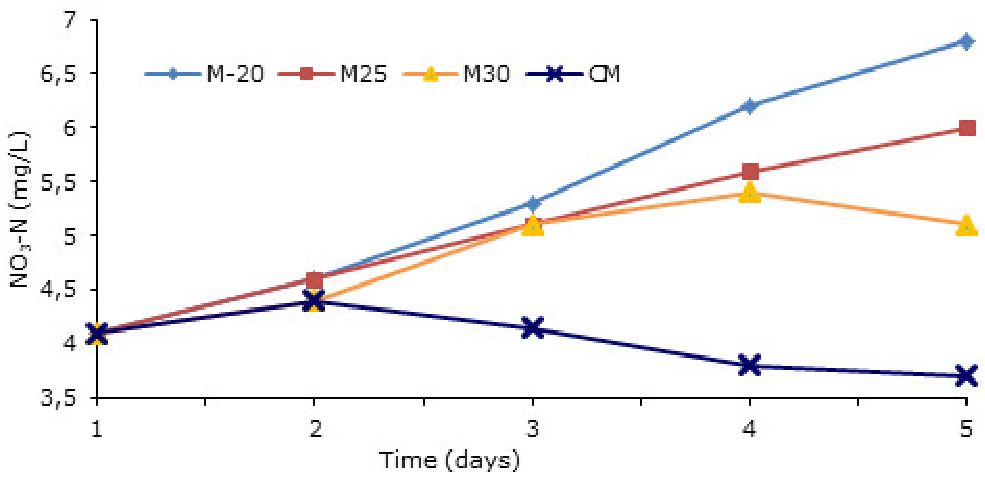


Figure 6: The NO_3-N level in three *M. Cephalus* treatment densities (M-20, M-25 and M-30) and the control (CM).

The BOD and COD concentrations were reduced during the experiment in all three treatments (Table 5). The reduction of BOD and COD was significantly stronger in all treatments compared with that in the control ($P<0.05$). In the three treatments, the removal rates were around 66 – 78% for BOD, 69 – 77% for COD, while in the control these rates were 28 and 30%, respectively (Figure 5). The removal rate of BOD was slightly better for the medium stocking density of *O. niloticus*; however, the difference between medium and high densities was not statistically significant. The latter might be related to the increased TAN concentration and the reduced DO level in the highest *O. niloticus* stocking density, which affected the survival and growth rate.

The reduction of TSS was significant at the low density (T-20), but not for the other densities and that of the control. At the end of the experiment, TSS was reduced by 40%, 32% and 30% in low, medium and high stocking densities; respectively, and that in the control, by 30% (from 342 mg/L to 240 mg/L). The low removal of TSS at higher stocking density may be explained by the higher production of waste compared to the low and medium stocking densities which resulted in increasing the internal TSS concentration.

M. cephalus *densities and water quality*

The T, pH, DO, TAN, NO_3-N and PO_4-P did not differ between the three stocking densities of *M. cephalus* (Table 6). The mean values of DO and TAN in the control were significantly higher than that in the treatments ($p<0.05$), while NO_3-N was lower in the control.

The concentration of NO_3-N significantly ($P<0.05$) increased during the experiment in all treatments, while it decreased slowly in the control (Figure 6).

Table 7

Initial, final and reduction values of BOD, COD and TSS in three treatments (M-20, M-25 and M-30) and the control (CM).

	BOD			COD			TSS		
	Initial (mg/L)	Final (mg/L)	Removal (%)	Initial (mg/L)	Final (mg/L)	Removal (%)	Initial (mg/L)	Final (mg/L)	Removal (%)
M-20	45,6	12.6±0.8	72±1.8[a]	78,9	25.3±2.1	68±2.5[a]	312	166.0±1.0	47±0.3[a]
M-25	45,6	7.7±0.1	83±0.3[b]	78,9	15.2±0.5	81±0.6[b]	312	144.5±0.5	54±0.2[b]
M-30	45,6	8.8±0.7	72±2.3[a]	78,9	18.4±0.8	77±1.0[b]	312	169.5±4.5	46±1.4[a]
CM	45,6	33.1±0.3	28±0.7[c]	78,9	53.9±0.7	32±0.8[c]	312	281.5±3.5	10±1.1[c]

Different superscripts in the same column indicate that the values are different ($p<0.05$).

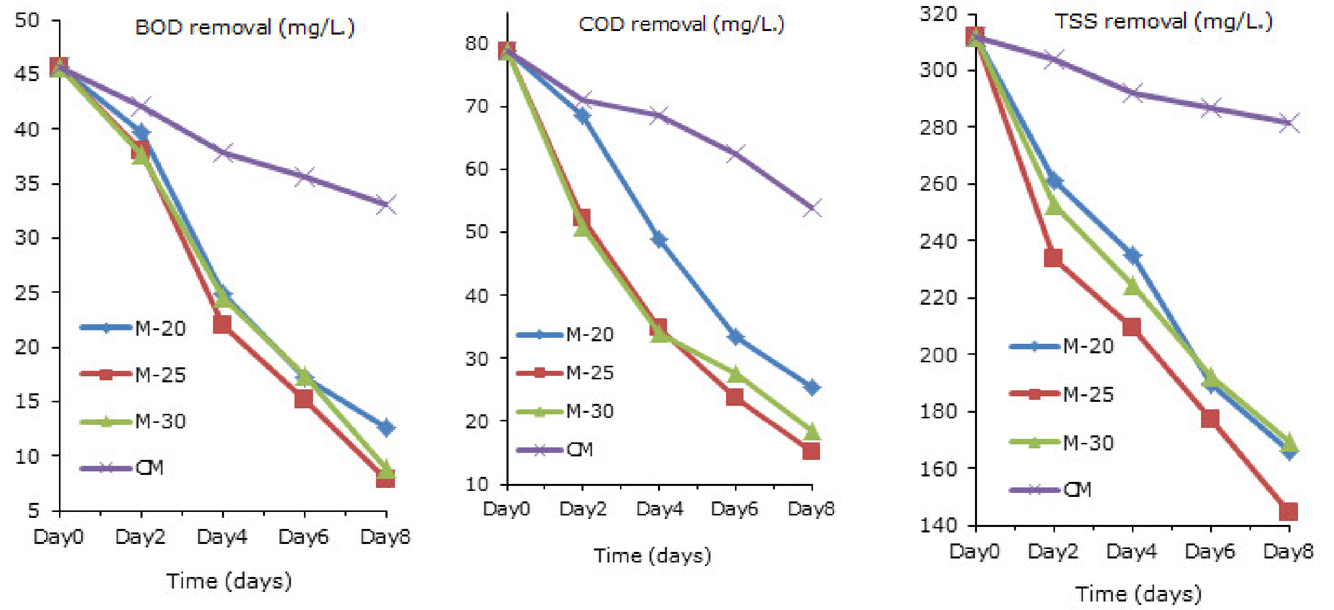


Figure 7: Reduction of BOD, COD and TSS by *M. cephalus* in three treatments (M-20, M-25 and M-30) and the control (CM).

Within the treatments, the highest achieved NO_3-N level was at the lowest stocking density and the lowest achieved NO_3-N level was at the highest stocking density. The concentration of PO_4-P greatly increased from 0.61 mg/L to >1.5 mg/L (increase about 154-160%) in all treatments and in the control during the 8-day-treatment process, but no significant differences were found among different *M. cephalus* stocking densities.

The BOD and COD concentrations were reduced in all treatments with various densities of *M. Cephalus* (Table 7). The BOD and COD removal efficiency was highest at medium stocking density of *M. cephalus*. This removal rate met the required wastewater standard (BOD≤30 mg/L; COD≤50 mg/L) after 4 days of treatment at all three stocking densities (Figure 7).

The reduction of TSS in effluent from intensive shrimp farms varied with *M. cephalus* stocking densities; the highest reduction of TSS was achieved at medium stocking density of 25 fish/m^3. However, the final level of TSS at these three mullet densities was higher than the threshold (50 mg/L) of the wastewater standard.

Conclusion

This study indicated that *O. niloticus* and *M. cephalus* showed more capacity than *S. guttatus* to remove OM and reduce COD and BOD from shrimp farm effluent; both also exhibited wider toxicity tolerance. The different initial levels of shrimp wastewater from 50% to 100% did not significantly affect the survival rate of tilapia and mullet. However, increasing shrimp wastewater ($P<0.05$) significantly decreased the survival rate of *S. guttatus*.

The removal rates of BOD, COD and TSS were significantly different when the stocking densities of tilapia and mullet were changed in the range of 20-30 fish/m^3. BOD, COD and TSS contents were removed at 66–83%, 68–81% and 30–54%, respectively.

The highest removal was achieved at the medium stocking density (25 fish/m^3 equivalently 500 g of fish) (P>0.05). Although the TSS level remained above the threshold for wastewater, the medium stocking density of *M. cephalus* (25 fish/m^3) was the most effective for maximizing the removal of organic matter from intensive shrimp farm effluent.

Acknowledgments

The authors acknowledge the financial support of the Vietnamese Ministry of Education and Training (MOET), and the University of South Australia, Australia.

Literature

1. American Public Health Association (APHA), 2005, Standard methods for the analysis of water and wastewater, Washington, DC: American Public Health Association.
2. Boopathy R., 2009, Biological treatment of shrimp production wastewater, *J. Ind.Microbiol. Biotech.,* **36**, 7, 989-992.
3. Erler D., Pollard P., Duncan P. & Knibb W., 2004, Treatment of shrimp farm effluent with omnivorous finfish and artificial substrates, *Aquacult. Res.*, **35**, 9, 816-827.
4. Mook W.T., Chakrabarti M.H., Aroua M.K., Khan G.M.A., Islam M.S. & Hassan M.A., 2012. Removal of total ammonia nitrogen (TAN), nitrate and total organic carbon (TOC) from aquaculture wastewater using electrochemical technology: A review, *Desalination,* **285**, 1-13.
5. Morris D.J. & North A.W., 1984, Oxygen consumption of five species of fish from south Georgia*, J. Exp. Mar. Biol. Ecol.*, **78**, 1-2, 75- 86.
6. Troell M., Rönnbäck P., Halling C., Kautsky N. & Buschmann A., 1999, Ecological engineering in aquaculture: use of seaweeds for removing nutrients from intensive mariculture, *J. Appl. Phycol.*, **11**, 1, 89-97.
7. Tian X., Li D., Dong S., Yan X., Qi Z., Liu G. & Lu J., 2001, An experimental study on closed- polyculture of penaeid shrimp with tilapia and constricted tagelus. Aquacult., **202**, 1-2, 57-71.
8. Whitfield A., Panfili J. & Durand J.D., 2012, A global review of the cosmopolitan flathead mullet Mugil cephalus Linnaeus 1758 (*Teleostei: Mugilidae*), with emphasis on the biology, genetics, ecology and fisheries aspects of this apparent species complex, *Rev. Fish Biol. Fish.*, **22**, 3, 641-681.
9. Zhu Wei, Lei Wan & Lianfang Zhao, 2010 Effect of nutrient level on phytoplankton community structure in different water bodies, *J. Environ. Sci.*, **22**, 1, 32-39.

Lich Q. Nguyen, Vietnamese, PhD, Hue University, College of Agriculture and Forestry, Head department of Civil Engineering, Phung Hung, Hue City, Vietnam.

N. Bolan, Australian, PhD, Professor, The University of Newcastle (UON), University Drive, Faculty of Science and Environmental Chemistry, Global Centre for Environmental Research, Information Technology, Callaghan, Australia.

M. Kumar, Australian, PhD, Adjunct Professor, Flinders University of South Australia, School of Biological Sciences, Ottoway, Australia.