The effectiveness of sand and red tilapia rearing in absorbing nitrogen and phosphorus of liquid waste from *Litopenaeus vannamei* culture

[1]Rustadi, [2]Gamal M. Samadan, [1]Djumanto, [1]Murwantoko

[1] Department of Fisheries, Gadjah Mada University, Bulaksumur, Yogyakarta, Indonesia; [2] Fisheries and Marine Science Faculty, Khairun University, Ternate, Indonesia. Corresponding author: Rustadi, rustadi@ugm.ac.id

Abstract. The culture of vannamei shrimp (*Litopenaeus vannamei*) in sandy soils can produce very high profits in a short period. However, liquid waste discharged into the surrounding environment contains high concentrations of total nitrogen (N) and total phosphorus (P), which cause pollution and can triggers outbreaks of white feces disease. This study aimed to evaluate the use of sand and red tilapia culture to reduce total N and total P concentrations of liquid waste from vannamei shrimp aquaculture. The sand filter experiment used a 1x1x1 m^3 pond, while the red tilapia density treatment used a 2x1x1 m^3 pond. The sand thickness treatments tested were 20 cm, 40 cm, and 60 cm. The stocking densities of red tilapia were 20, 30, and 40 fish m^{-2}. Each treatment was repeated three times. The vannamei shrimp culture liquid waste flows into the experimental pond. The total N and P concentrations from shrimp culture liquid waste and water from experimental ponds were collected. In addition, at the end of the study, the number of individuals, length, and weight of fish were determined. Environmental parameters measured at the beginning and end of the experiment included temperature, total suspended solids (TSS), salinity, dissolved oxygen, and alkalinity. Nutrient reduction in the sand filter was calculated based on the difference in N and P concentrations at the effluent output and in the experimental pond. The composition of N and P in the carcass at the beginning and end of culture was measured to determine the amount of N and P absorbed. Data were analyzed descriptively, and the mean was tested with a significance level of 95%. The experimental results show that the sand filter can reduce the total P and TSS. The sand thickness of 20 cm was the most effective ($p<0.05$) in reducing P among the sand thickness, while reducing N in small quantities. The higher stocking density of red tilapia produced higher effectivity of N nutrients, but reduced P nutrients (by being converted into the fish carcasses).

Key Words: fish, nutrients, sandy land, waste.

Introduction. Intensive shrimp culture has proven to return investment quickly, but the impact of waste disposal on the surrounding environment is a global issue (Djumanto et al 2016; Nguyen et al 2020). Vannamei shrimp (*Litopenaeus vannamei*) culture on the sandy lands of the southern coast of Java has doubled in recent decades. The pond area grows, reaching about 10 km from the shoreline. The pond construction consists of sandy land covered with plastic mulch, and the water source is pumped from shallow wells at a depth of between 20 to 40 m and with a salinity of 10-20 ppt; the waste is disposed to the surrounding area (Samadan et al 2018, Priyono et al 2019).

Waste originating from intensive shrimp culture consists of liquid and solid materials. The liquid waste of vannamei shrimp culture is dominated by organic matter consisting of excretes, uneaten feed and moulting skin that can be dissolved and particulate. Organic waste will decompose into nitrogen (N) and phosphorus (P) nutrients (Davidson et al 2008). In addition to nutrients, the decomposition of organic waste decreases water quality, such as oxygen depletion, toxic gas generation, and an increase in suspended solids (Nguyen et al 2007; Sonnenholzner 2008; Sun & Boyd 2013). Since the development of sandy ponds shrimp, liquid waste has been disposed of to the environment without treatment. Therefore, the high concentration of N and P nutrients causes environmental pollution, eutrophication and stimulates disease in shrimp culture

(Nguyen et al 2007; Shinn et al 2018). In addition, it decreases the groundwater quality as a source of shrimp culture in sandy areas.

A practical and friendly treatment to reduce liquid waste is physical filtering and biofilter. Sand is widely used to filter dissolved organic particles, reducing turbidity and organic matter (Davidson et al 2008). Sand has improved water quality with varying results (Lefrancois et al 2010; Thomas & Kani 2016). Some researchers use aquatic biota to utilize nutrients and improve wastewater quality before being discharged into the surrounding environment (Castine et al 2013; Hidayati et al 2020). Shrimp culture liquid waste contains N and P, which help culture milkfish (*Chanos chanos*) (Djumanto et al 2018), seaweed (*Gracilaria verrucosa*), green mussels (*Perna viridis*) (Widowati et al 2021) or other aquatic biota. Red tilapia (*Oreochromis* sp.) is a superior strain in growth, resistant to low water quality. It supports a water salinity of 18 ppt (MMAF-RI 2012), and can become resistant to higher salinities with acclimatization. This study aims to evaluate the effectiveness of sand thickness and red tilapia density in reducing total nitrogen (TN), total phosphorous (TP) and organic particulates, and improving the quality of shrimp culture wastewater.

Material and Method

Description of the study sites. The research was carried out in two separate stages, namely improving water quality physically and biologically. Physical improvement of water quality used sand, while biological improvement used fish in the rearing media. Both studies were conducted simultaneously in a sand pond. The research was conducted from August to December 2017 at the Collaborative Pond belonging to the Department of Fisheries, Gadjah Mada University, located in Keburuhan Village (7°51'08.1″S, 109°55'00.3″E), Ngombol District, Purworejo Regency, Indonesia.

Liquid waste originating from shrimp culture in ponds measuring 3x4x1 m^3 was collected in a holding pond of 2x1x1 m^3 and then distributed according to the treatment of sand thickness and fish stocking density. The container for filtering liquid waste was a pond measuring 1x1x1 m^3, made by digging sand soil, with six units. The pond was lined with a plastic sheet of mulch to prevent leakage. The pond's bottom was composed of a mixture of gravel wrapped in a fine net (waring), then backfilled with sand until it reached a thickness according to treatment. The thickness of the sand was 20, 40, and 60 cm.

Tilapia rearing used a pond measuring 2x1x1 m^3, made in the same way as for sand thickness, numbering six units. The walls of the pond were covered with plastic mulch to prevent leakage and fish pests. The pond was provided with a 2 m high pararoof to avoid birds and reduce exposure to direct sunlight.

The two stages of the study were arranged using a completely randomized design with two replications. The sand filter experiment consisted of 3 thickness treatments, namely 20, 40, and 60 cm. The tilapia rearing experiment consisted of 3 stocking density treatments, namely low (20), medium (30), and high (40) individuals m^{-2}. The positions of the treatment ponds were arranged randomly. Red tilapia with a weight between 1.67-1.88 g and a total length of 5-7 cm was obtained from a fish hatchery in Yogyakarta and transported to the study site using a sealed plastic bag given oxygen. The fish were acclimatized to the new environment and salinity for two weeks. In the fish stocking treatment, the fish were not fed during the experiment, the only feed source being from the liquid waste of the shrimp culture.

Data collection. Data consisted of total nitrogen (TN) and total phosphorous (TP) representing dominant organic N and P pools, including plankton in ponds. TN and TP data, total suspended solids (TSS) in sand thickness and fish culture experiments, were taken from the water entering (inlet) and leaving the pond (outlet) for each treatment. The TN in liquid waste and fish carcasses was analyzed with the Kjeldahl method, and TP by sulfuric acid-nitric acid digestion and were measured using a spectrophotometer (Shimadzu UV 1650 PC). In the fish culture treatment, the fish water quality parameters including salinity, pH, dissolved oxygen (DO), nitrite-N, nitrate-N, ammonia-N, and total organic matter (TOM) were monitored for 15 days during the 75 days of the experiment.

Water temperature was measured using a mercury thermometer, salinity was measured using a refractometer, DO was measured using a DO meter (YSI 556 MPS), and water pH was measured using a pH meter. Samples were collected and preserved *in situ*, and the analysis of some parameters in the laboratory followed standard methods (APHA 2005). The fish length was measured using a ruler, while individual weight was measured using an electric scale (0.1 g accuracy).

The effectiveness of the sand in reducing N and P contents was determined based on the difference of the TN and TP concentration between the inlet ("liquid waste") and outlet water from the ponds with the sand thickness treatments. It also represents the effectiveness of red tilapia culture in reducing N and P nutrients. The effectiveness (FE, %) of the treatment in reducing TN and TP uses the formula (Drennan II et al 2006; Eding et al 2006):

FE (%) = {(TN, TP liquid waste) - TN, TP outlet water)/TN, TP liquid waste)}x100

The growth rate of each stocking density of red tilapia was calculated by the formula GR=(Wt-W0)/t, where GR was the growth, Wt and W0 were the final weight and initial weight, respectively, and t was the length of time for culture. The survival rate was calculated according to the formula SR=Nt/N0x100.

Statistical analysis*.* The effectiveness of sand thickness and stocking density of red tilapia culture was analyzed using ANOVA with an F test at a 95% confidence level. Duncan's difference test was conducted to determine the significant difference between treatments. Apart from the effectivity of absorbing TN and TP contents, the data of survival and growth of red tilapia, and water quality were analyzed. All data are presented as mean ±SD.

Results and Discussion

The effectiveness of the physical filtration of sand. Sources of total N and P and TSS from liquid waste from vannamei shrimp culture varied. Total N ranged from 0.200 to 0.408 mg L^{-1}, while total P ranged from 0.0009 to 0.0066 mg L^{-1}. TSS values ranged from 0.0015 to 0.2116 mg L^{-1}. At the treatment pond of the sand thickness outlet, the total N values ranged from 0.152 to 0.504 mg L^{-1}. The total P ranged from 0.0004 to 0.0027 mg L^{-1}, and TSS ranged from 0.108 to 0.0996 mg L^{-1}. The total nutrient concentrations of N and P, and TSS are presented in Figure 1.

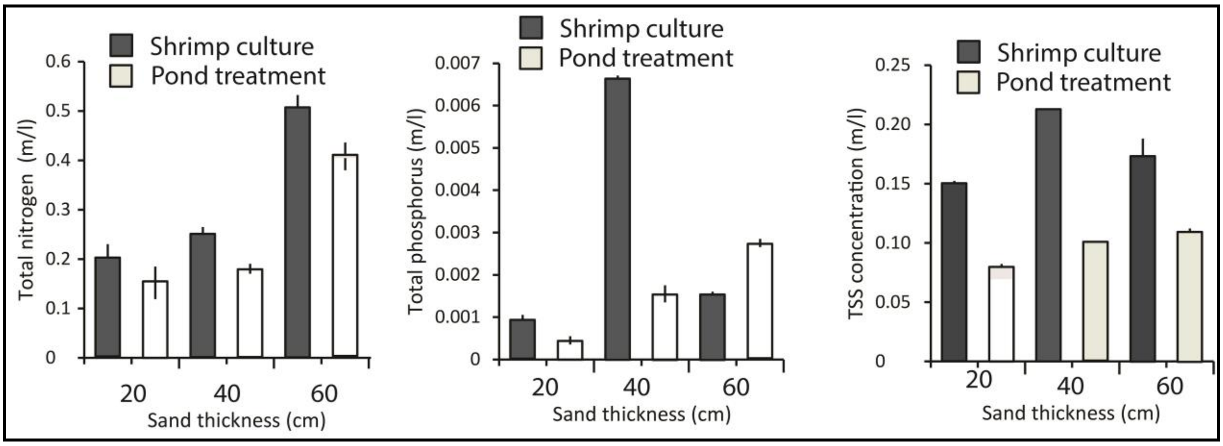

Figure 1. Concentration of total N, total P and total suspended solids (TSS) in the outlet of each treatment of sand filter thickness. The bars represent the standard deviation.

The thickness of the sand filter affects the total N efficiency with results of varying percentages. A thicker sand lowers the efficiency level. Sand filters at a thickness of 20 cm, 40 cm, and 60 cm produced efficiencies of 25.98%, 23.61%, and 22.32%, respectively. The three thickness treatments were categorized as less efficient in filtering N-containing liquid waste (Figure 2). Based on the analysis of the efficiency of total N absorption, there was no difference between the three treatments ($p<0.05$).

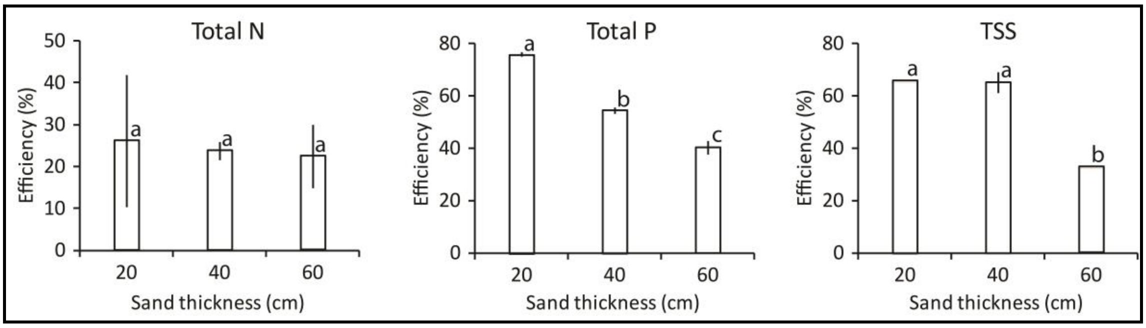

Figure 2. The filtration efficiency of total N, total P, and TSS using a sand filter at a thickness of 20, 40 and 60 cm. Different letter notations on the bars show a significant difference ($p<0.05$).

Filtration of total P using in the sand experiment showed varying percentages of efficiency. The use of a thicker sand filter will result in lower efficiency. A sand thickness of 20 cm produces an efficiency of 75.13%, while a thickness of 40 cm produces an efficiency of 54.17%. At a thickness of 60 cm, the treatment efficiency is 40.02% (Figure 2). The analysis of the mean value of P filtering efficiency showed significant differences among the three treatments ($p<0.05$).

The highest filtration efficiency for TSS resulted in the sand filter thickness of 20 cm, 65.58%, followed by the thickness of 40 cm, with 64.91%, and then the thickness of 60 cm, with an average of 32.62% (Figure 2). Sand filter thicknesses of 20 and 40 cm are efficient in filtering TSS from vannamei culture liquid waste. On the other hand, the 60 cm thickness treatment was less efficient in filtering TSS from shrimp culture liquid waste. The thickness treatments of 20 and 40 cm were similar in efficiency, but both significantly different from the efficiency of the thickness treatment of 60 cm ($p<0.05$).

The effectiveness of the biofilter. The average TN and TP in liquid waste as input were 0.200±0.033 and 0.0009±0.0001 mg L^{-1} in the 20 ind m^{-2} density treatment, 0.2478±0.017 and 0.0066±0.00006 mg L^{-1} in the 30 ind m^{-2} treatment, and 0.4080±0.028 and 0.0015±0.00005 mg L^{-1} at 40 ind m^{-2} (Figure 3a and 3b).

The TN and TP contents of fish culture ponds from the outlet are presented in Figure 3c and 3d. The concentrations of TN and TP in the outlet were 0.1520±0.033 and 0.0004±0.0001 mg L^{-1} in the 20 ind m^{-2} stocking density, respectively. They were 0.1760±0.0001 and 0.0015±0.0002 mg L^{-1} in the 30 ind m^{-2} stocking density, respectively, and 0.5040±0.028 and 0.00027±0.0001 mg L^{-1} in the 40 ind m^{-2} stocking density, respectively. The TN and TP stored in red tilapia carcasses in the 20 ind m^{-2} stocking density were 2.64±0.099 and 0.31±0.0012 mg L^{-1} respectively, 2.72±0.087 and 0.31±0.011 mg L^{-1} in the 30 ind m^{-2} stocking density, respectively, and 2.57±0.065 and 0.32±0.027 mg L^{-1} in the 40 ind m^{-2} stocking density, respectively (Figure 3e and 3f).

The effectiveness of TN and TP in the red tilapia experiment, which function as a biofilter, is shown in Figure 3e and 3f. The average filtration effectivity of TN in the 20 ind m^{-2} density was 24±1.7%, in the 30 ind m^{-2} density it was 29±6.9%, and in the 40 ind m^{-2} density was 36.4±1.3%. The higher density of red tilapia, the higher the filter effectiveness. The three treatments were not significantly different ($p<0.05$).

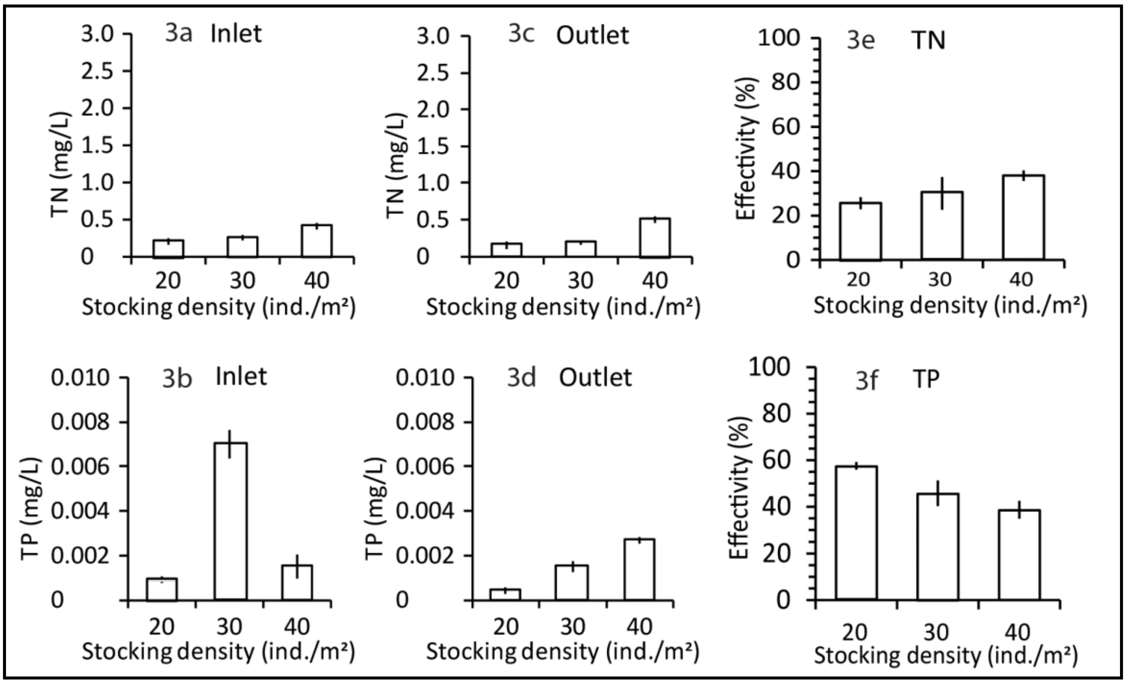

Figure 3. Average TN and TP in inlet, outlet of red tilapia pond and effectivity at different fish densities 20, 30 and 40 ind m^{-2}.

The TP retention value in the 20 ind m^{-2} density was 55.6±1.1%, in the 30 ind m^{-2} density it was 43.80±5%, and in the 40 ind m^{-2} density it was 36.7±3.30%. Higher density proved a higher filtration effectiveness of TP. These results indicate that red tilapia is classified as less effective filtering N than P from liquid waste, and there are no significant differences (p>0.05) among different densities.

Red tilapia could accumulate N and P in the form of fish carcasses (Figure 4). The average TN in carcass in the 20 ind m^{-2} density was 2.64±0.099 mg L^{-1}, in the 30 ind m^{-2} density it was 2.72±0.087 mg L^{-1}, and in the 40 ind m^{-2} density it was 2.57±0.065 mg L^{-1}. The average TP in carcass in the 20 ind m^{-2} density was 0.31±0.012 mg L^{-1}, in the 30 ind m^{-2} density it was 0.31±0.011 mg L^{-1}, and in the 40 ind m^{-2} density it was 0.32±0.027 mg L^{-1}.

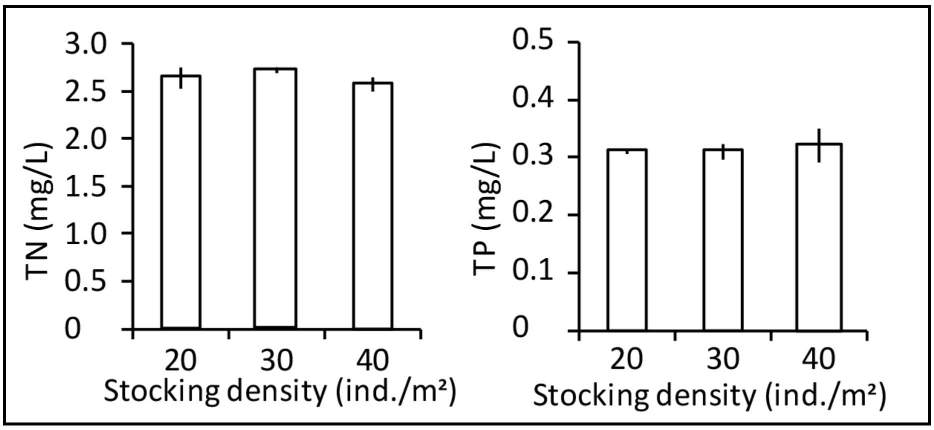

Figure 4. Average TN and TP in red tilapia carcass at different fish densities (20, 30 and 40 ind m^{-2}).

Red tilapia culture performance. The individual weight of red tilapia at the beginning of culture ranged from 1.67 to 1.88 g, then after a culture period of 15 days, it increased to 18.4-18.7 g (Figure 5). After 45 days of culture, the average weights were between

38.2-49.2 g in the three stocking densities. The fish had the highest weight at the 30 ind m^{-2} density. After 45 days, the average weight of fish ranged from 47.9 to 76.4 g. The highest average weight was in the 30 ind m^{-2} density, while the lowest was in the 40 ind m^{-2} treatment. Furthermore, after 60 days, the average weight ranged from 74.5 to 83.0 g. The stocking density of 30 ind m^{-2} had the highest average weight, while the 20 ind m^{-2} and 40 ind m^{-2} density treatments were not significantly different. At the end of culture, the average weight in the density of 20 ind m^{-2} was the highest, 102.0 g ind^{-1}. The densities of 30 and 40 ind m^{-2} produced similar values, 94.2-95.5 g ind^{-1}.

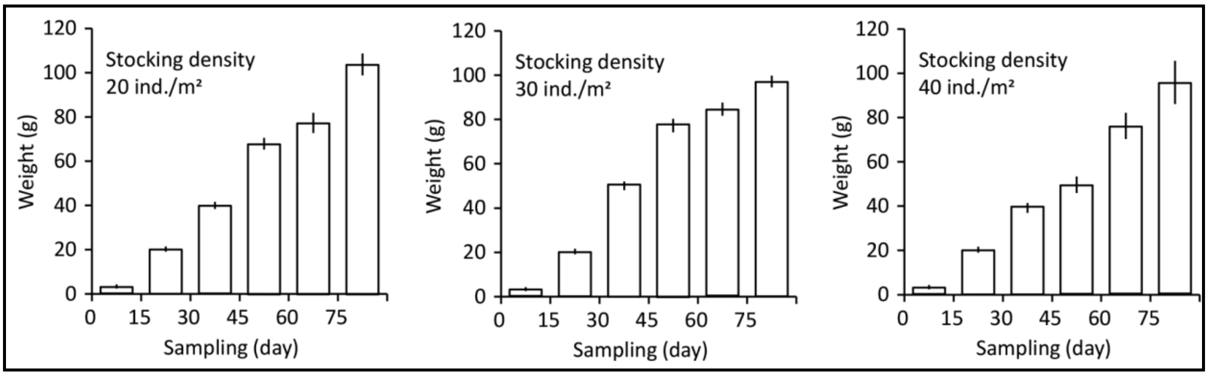

Figure 5. The average weight of red tilapia at different densities in ponds that received liquid waste from vannamei shrimp cultivation.

Data on the growth performance of red tilapia during the experiment are presented in Table 1. A sampling at the beginning and end of the study showed that the mean body weight, daily growth, and survival were different among treatments ($p<0.05$).

Table 1

The fish stock, growth, survival and biomass of red tilapia in different stocking density

Parameters	*Units*	*20 ind m^{-2}*	*30 ind m^{-2}*	*40 ind m^{-2}*
Fish stock	individual	40	60	80
Average length	mm	4.94	5.05	4.96
Initial average weight	g	1.67	1.88	1.86
Initial biomass	g	66.8	112.8	148.8
Survival rate	%	90.59 ± 2.89^a	73.39 ± 3.05^b	60.24 ± 8.58^c
Final average weight	g	102.0 ± 4.5^a	95.5 ± 2.2^b	94.15 ± 9.3^c
Final biomass	g	1306.89	4205.25	4537.28
Daily growth rate	g day^{-1}	0.87 ± 0.0^a	0.56 ± 0.2^b	0.81 ± 0.2^c

Note: different superscripts on the same line show significant differences ($p<0.05$).

In the treatment of 20 ind m^{-2}, the highest SR was 90.59±2.89%, followed by the density treatment of 30 ind m^{-2} with 73.39±3.05%, and the lowest SR was in the density treatment of 40 ind m^{-2} with 60.24±8.58% (Table 2). SR decreased as stocking density increased, and all the treatments were significantly different ($p<0.05$). Although growth and SR decreased with increasing density, the red tilapia biomass increased.

Water quality in the three treatments of fish stocking density during culture varied. The average value of water quality parameters such as temperature, salinity, pH, DO, NO_2, NH_3, total organic matter and alkalinity were still in the standard range for tilapia life in ponds because red tilapia could tolerate it (Table 2). Water quality parameters such as temperature, salinity, pH, DO, nitrate, nitrite, ammonia, and alkalinity, were not significantly different. However, total organic matter (TOM) at 20 and 30 ind m^{-2} stocking densities were different from those in the high stocking density ($p<0.05$).

Table 2
Water quality parameters of red tilapia ponds at different densities during the experiment

Parameter	*Units*	*20 ind m^{-2}*	*30 ind m^{-2}*	*40 ind m^{-2}*
Temperature	^oC	27.4 ± 0.28^a	27.8 ± 0.42^a	28.3 ± 0.21^a
Salinity	ppt	20.0 ± 1.41^a	20.0 ± 1.41^a	20.0 ± 1.41^a
pH	unit	9.7 ± 0.49^a	9.6 ± 0.64^a	9.5 ± 0.92^a
O_2	mg L^{-1}	3.5 ± 0.10^a	3.2 ± 0.08^a	2.8 ± 0.66^a
NO_2	mg L^{-1}	0.01 ± 0.01^a	0.01 ± 0.02^a	0.01 ± 0.00^a
NH_3	mg L^{-1}	0.03 ± 0.04^a	0.01 ± 0.01^a	0.03 ± 0.02^a
TOM	mg L^{-1}	49.2 ± 19.77^{ab}	72.9 ± 13.75^{ab}	102.1±6.87bc
Alkalinity	mg L^{-1}	100.3 ± 74.11^a	120.8 ± 49.91^a	204.9 ± 78.05^a

Note: the same superscript on the same row shows a non-significant difference (p>0.05).

Liquid waste from shrimp pond cultivation was collected in one holding pond, but sampling was carried out in the inlet of each experimental pond. The TN and TP input value in each experiment varied, but the value was not significantly different (p>0.05). The slight difference was due to technical difficulties in laboratory collection, storage, and analysis. However, the results were lower than in the study conducted by Teichert-Coddington et al (2000). This difference was due to the waste used in this study only as much as 10 L per week. TN and TP in the outlet water in each treatment showed lower values than in the inlet. It was due to the presence of filtering by sand and the utilization of liquid waste by red tilapia. The decrease in N and P input to output was assessed as filtering effectiveness.

Lower sand thickness had a significant effect (p<0.05) in filtering TP and TSS, while for TN, it was not significant. The sand thickness experiment was more effective for TP than TN. The thicker the sand, the less effective it is. The treatment of 20 cm thickness of sand resulted in a TP filtration effectiveness of 75.13%, more than those of the 40 cm and 60 cm thicknesses (54.17% and 40.02%). At thicknesses of 20 cm and 40 cm of sand, the TSS of liquid waste produces effectiveness of 65.58% and 64.91%. The sand thickness of 60 cm has an efficacy of 32.62%, so it is less effective. The lower effectiveness of high sand thickness in filtering TP could occur because P exists in the bound form of calcium phosphate in alkaline water (Sun & Boyd 2013). These particles can be retained by the pores between the sand grains with a 0.15-0.3 mm diameter. Some particles that can pass through the pores of the media will be bound to each other and form larger grains to be stuck in a deeper layer (Logsdon 2008). Furthermore, there is a reduction of particles smaller than suspended particles such as colloids and dissolved particles in the adsorption process. Meanwhile, waste in the form of N undergoes eutrophication into dissolved N nutrients, so that it is slightly filtered, while ammonia and nitrite gases will evaporate.

Red tilapia can utilize nitrogen reasonably efficiently, the effectiveness being 36.4% in the 40 ind m$^-$2 density treatment. At the same time, the treatments of 20 ind m^{-2} and 30 ind m^{-2} stocking densities resulted in the effectiveness of 24% and 29%, respectively. It was classified as less effective. However, N absorption in the three treatments showed that red tilapia could filter well the waste from vannamei shrimp culture.

Compared with nitrogen, the filtration effectivity of red tilapia of TP had a higher percentage, but a lower density had a higher effectivity in P absorption. Effectivity of the red tilapia was 55.6% in 20 ind m^{-2}, 43.8% in 30 ind m^{-2}, and 36.7% in 40 ind m^{-2}, respectively.

The effectiveness of N absorption is lower than that of P because N is more easily absorbed by plankton and evaporates in the form of ammonia, nitrite and N_2 gas. Furthermore, plankton is used by fish as natural food. The P nutrients are particles bound into the sediment and settle at the bottom of the pond. A higher stocking density presents more agitation and movement, stirring the sediment and releasing more P.

The TN converted to red tilapia carcass was quite large at 2.57-2.72 mg L^{-1}, which was not significantly different between stocking density treatments. Meanwhile, the converted TP in fish meat was around 0.31-0.32 mg L^{-1}. Red tilapia can utilize waste from vannamei shrimp cultivation directly (Cruz et al 2008). In addition, the results of the decomposition of liquid waste through the nitrification process will decompose into nitrate and ammonium nutrients and phosphate, which can be absorbed by phytoplankton. Furthermore, red tilapia can take advantage of phytoplankton that grow as natural food (Rustadi et al 2002; Cruz et al 2008). The decomposition of liquid waste produces toxic nitrite and ammonia gas, but red tilapia has a high resistance to these harmful gasses. The deterioration of liquid waste requires oxygen, so that the DO concentration in the red tilapia culture pond becomes very low. However, red tilapia is very tolerant of low oxygen conditions. When the DO concentration is low, red tilapia can carry out air-breathing, taking oxygen from the air (Johannsson et al 2014). The mortality of red tilapia tended to increase with the stocking density, but still remained within reasonable limits.

Red tilapia can survive and grow well using liquid waste from vannamei shrimp cultivation. Red tilapia culture in ponds that only serve liquid waste show growth in length and weight. Red tilapia can utilize liquid waste from shrimp ponds very well. Liquid waste from vannamei shrimp culture mainly consists of uneaten feed, natural food, and other protein ingredients (Nguyen et al 2007). Vannamei shrimp culture waste consists of liquid waste and sediment containing high levels of N and P. Liquid waste sediments contain inedible feed particulates (Yanfeng et al 2015). Red tilapia species are omnivorous fish that eat various natural foods, including small aquatic animals, plankton, and decaying organic matter. As an omnivorous species, red tilapia requires lower protein levels than shrimp. Vannamei shrimp culture waste is also used for milkfish cultivation (Djumanto et al 2018). In this study, although the percentage of absorption of total P is lower than for total N, red tilapia can be used as a waste filter organism or bio-filter.

Conclusions. Sand can be used as an effective physical filter for total phosphorus waste and total suspended solids, but it is less effective in filtering total nitrogen. The thickness of the sand filter was best at 20 cm for filtering. In addition, red tilapia can effectively filter nitrogen waste but is less efficient for filtering phosphorus waste. Tilapia can take advantage of the rests of feed from the vannamei shrimp culture liquid waste.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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Authors:
Rustadi, Gadjah Mada University, Faculty of Agriculture, Department of Fisheries, 55281 Yogyakarta, Indonesia, e-mail: rustadi@ugm.ac.id
Gamal Mustik Samadan, Fisheries and Marine Science Faculty, Khairun University, 97719 Ternate, Indonesia.gm.samadan74@gmail.com
Djumanto, Gadjah Mada University, Faculty of Agriculture, Department of Fisheries, 55281 Yogyakarta, Indonesia, e-mail: lely4192@yahoo.com
Murwantoko, Gadjah Mada University, Faculty of Agriculture, Department of Fisheries, 55281 Yogyakarta, Indonesia, e-mail: murwantoko@ugm.ac.id

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