# The use of *Perna viridis* to improve water quality of shrimp pond wastewater aquaculture

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**Abstract**. *Perna viridis* has economic value and ecological importance as a commodity in polyculture. This study aims to know the efficiency of *Perna viridis* to improve water quality of shrimp ponds wastewater aquaculture. This study used 4 treatments namely 0 (control), 2 g/L, 4 g/L, and 3 g/L in 35 L of shrimp pond wastewater aquaculture using a Complete Randomized Design (CRD). The parameters observed in this study were dissolved oxygen, biological oxygen demand (BOD), total organic matter (TOM), total suspended solids, salinity, pH, and water temperature. The result showed the following values: dissolved oxygen 5.26-6.92 mg/L; temperature 27.5-30.8°C; salinity 35-40‰; and pH 7.69-7.92. *P. viridis* was able to decrease TOM value on 7 day and BOD value on day 7 and 14. The highest growth rate of *P. viridis* for 28 days is 27.61% in density of 4 g/L. *Perna viridis* has potential as a commodity in polyculture and Integrated Multi-Trophic Aquaculture (IMTA) with shrimp ponds to improve waste water quality of shrimp wastewater aquaculture.
**Key Words**: bioremediation, BOD, effluent, green mussel, polyculture, TOM.

**Introduction**. The more intensive shrimp farming will increase the amount of waste generated. The impact of this waste affects the sustainability of shrimp farming because the waste discharged into the sea allows it to re-enter the pond environment. The accumulation of nutrients in the feed that is utilized as energy is in the range of 5-40% (Avnimelech & Ritvo 2003) and the rest will be suspended and settle to the bottom. One way to reduce aquaculture waste is to implement Integrated Multi-Trophic Aquaculture (IMTA). IMTA is the key to developing sustainable aquaculture using multiple organisms intended to optimize available nutrients and reduce solid waste (Crooker & Contretas 2010). Waste from one organism becomes a source of energy for other organisms, for example, waste from shrimp will become a source of food for filter feeder organisms and seaweed.

Green mussel (*Perna viridis*) is one of the species of shellfish that has high economic value and is widespread in Indonesian waters. *P. viridis* is included in the filter feeder category, which means organisms that get food by filtering substances suspended in the water (Cappenberg 2008). *P. viridis* is an appropriate organism for biomonitoring because it is widespread, sedentary, and belongs to filter feeder organisms capable of storing and accumulating organic and inorganic contaminants (Mamon et al 2016). *P. viridis* can survive and reproduce under high environmental pressure without feeding and has an important role as a biofilter that can improve environmental quality (Eshmat et al 2014). The use of *P. viridis* in IMTA serves to change the amount of feed from uneaten shrimp and their feces containing toxic ammonia to green mussel biomass and nitrate which are non-toxic and easily absorbed by seaweed.

*P. viridis* can be cultivated without additional feed providing benefits for farmers. *P. viridis* is able to remove particulate carbon and nutrient from aquatic environment (Srisunont & Babel 2015). Holdt and Edward (2014) stated that mussels have better ability as biofilter to absorb nitrogen than seaweed. *P. viridis* acts as a biofilter as well as biomass that can be harvested and has economic value. The use of *P. viridis* as a biofilter needs to be tested to determine the effective amounts of shrimp pond wastewater it can filter. The results of the research are expected to be information to support sustainable aquaculture and maintain the aquatic environment.

**Material and Method**. The research was conducted at the Brackish Water Aquaculture Center (BBAP) Jepara, Central Java from September 2019 to February 2020, during for five months.

***Preparation and acclimation***. The liquid waste is taken directly from the siphon channel of the shrimp pond, as much as 60 liters. Dilution of the waste is carried out by adding brackish water to a salinity of 30 ppt. The dilution is done so that the green mussels can live because the salinity range is between 15-45 ppt. Acclimatization aims to make *P. viridis* able to adapt to the new environment. *P. viridis* was first cleaned of dirt and attached animals. The acclimatization process was carried out for 7 days by keeping *P. viridis* in a fiber tub, given aeration and *Chlorella* feed. The water used during acclimatization has been adjusted to a salinity of 35 ppt.

***Main test***. The main test was carried out using a completely randomized design method with 3 replications. The treatment carried out was the use of different amounts of *Perna viridis* to know its ability and effectiveness to absorb shrimp wastewater. Implementation for 28 days by maintaining *P. viridis* in styrofoam boxes filled with shrimp pond waste water that has been diluted with brackish water so that the salinity is 35 ppt. The volume used is 35 liters with a concentration of 5 liters of saline wastewater and 30 liters of brackish water. The treatments that were tested had differences in the density of *P. viridis* which consisted of: control; 2 g/L; 4 g/L; and 6 g/L with three replications.

***Water quality measurements***. Water quality measurements include physical and chemical parameters. Physical parameters include water temperature, salinity, and total suspended solids (TSS). Chemical parameters include dissolved oxygen (DO), pH, ammonia, total organic matter (TOM), and biological oxygen demand (BOD). The growth rate of *P. viridis* was measured at the beginning and end of treatment for 28 days. The method used to test ammonia and organic matter is spectrophotometry with phenate solution for ammonia and potassium permanganate for organic matter. The TSS measurement method uses the gravimetric method using 500 mL of sample water. The BOD measurement water sample was taken directly from the maintenance container using an oxygen bottle and then closed using aluminum foil and a black plastic bag to keep the conditions inside the bottle always dark. BOD5 measurement was obtained from the difference between initial and final DO which had been incubated for 5 days in oxygen bottles in the dark.

***Growth rate of P. viridis***. The growth rate of *P. viridis* was measured at the beginning and end of treatment after 28 days.

***Data analysis***. The results of measurements of ammonia, organic matter, TSS, and BOD were analyzed by ANOVA using the SPSS version 23 application. ANOVA analysis was used to determine whether there was a significant difference between treatment and time. Data from these parameters were tested for homogeneity and normality, then ANOVA analysis was performed. The results of the ANOVA analysis which showed a significant difference were followed by the Tukey test with a 95% confidence level.

**Results and Discussion**. The BOD value which decreased on the $7^{th}$ and $14^{th}$ day, increased on the $21^{st}$ day, and fell again on the $28^{th}$ day related to TOM. The TOM value decreased on the $7^{th}$ day, increased on the $21^{st}$ day, and fell again on the $28^{t}$h day. The results of statistical analysis on the $7^{th}$ and $14^{th}$ days showed a significant difference. The $7^{th}$ day indicated a difference between the control and treatment of 2 g/L, 4 g/L, and 6 g/L while the $14^{th}$ day indicated a difference between the control and treatment 10; control with treatment of 2 g/L and 6 g/L; and 4 g/L treatment with 2 g/L and 6 g/L treatment. The treatments of 2 g/L and 6 g/L did not show any significant difference. The

BOD value is related to the water temperature because the higher the water temperature, the lower the BOD value. This happens because the decomposition takes place faster so that the BOD value decreases. BOD values during treatment are presented in Table 1.

Table 1

BOD concentrations during treatment

| *Density of P. viridis* | *BOD (mg/L) on day 0* | *7* | *14* | *21* | *28* |
|---|---|---|---|---|---|
| control | 4,9 | 3,51[a] | 3,8[a] | 3,84 | 2,85 |
| 2 g/L | 4,9 | 2,04[b] | 0,52[c] | 3,16 | 2,50 |
| 4 g/L | 4,9 | 1,98[b] | 2,01[b] | 3,97 | 1,78 |
| 6 g/L | 4,9 | 2,43[b] | 1,01[c] | 3,39 | 2,67 |

Note: Values in the column followed by the same letter show no significant difference at 95% confidence level.

Table 2

TOM concentrations during treatment

| *Density of P. viridis* | *TOM (mg/L) on day 0* | *7* | *14* | *21* | *28* |
|---|---|---|---|---|---|
| 0 | 112,61 | 107,2[c] | 121,59[c] | 195,6 | 128,24 |
| 5 | 112,61 | 72,05[a] | 48,55[a] | 206,89 | 100,02 |
| 10 | 112,61 | 82,85[a] | 105,70[b] | 209,08 | 107,79 |
| 15 | 112,61 | 74,53[b] | 77,94[b] | 222,14 | 106,97 |

Note: Values in the column followed by the same letter show no significant difference at 95% confidence level.

The results of statistical analysis showed that there was a significant difference on the 7$^{th}$ and 14$^{th}$ days. This indicated that the difference in the number of *P. viridis* had a significant effect on TOM (Table 2). The decreased TOM content on the 7$^{th}$ day occurred because *P. viridis* was able to filter out organic matter in the water and the decomposition process could proceed. The main food for *P. viridis* is in the form of microalgae and additional organic matter (Retnosari et al 2019). *Perna viridis* can digest feed residues and excrete metabolic products containing ammonia (NH3) then convert them into ammonium (NH4) and phosphate (PO4). Therefore, the use of *Perna viridis* helps to reduce ammonia in shrimp wastewater. Inorganic nutrients are utilized by microalgae to grow. The optimum temperature in decomposition is in the range of 26-32°C (Putra et al 2014) and observations are in the optimum temperature range. The increase in TOM that occurred on day 21 was caused by metabolic waste of *P. viridis* because the ammonia content on day 21 also increased and the moss attached to the test container began to detach. Decomposed moss can increase the value of TOM in water. According to Hamsiah (2002), the increase in the value of TOM and ammonia is caused by the metabolic waste products of aquatic organisms. The number of *P. viridis* used matters because the more *P. viridis* used in one container can reduce the filtration ability of individuals due to competition between individuals (Tantanasarit & Babel 2014). In the control treatment, organic matter can decrease due to the oxidation of organic matter which goes well in the presence of aeration. Decomposition of organic matter requires oxygen to convert organic matter into inorganic.

TSS affects the penetration of light into the water column. A high TSS value can inhibit the rate of photosynthesis of phytoplankton in water so that the presence of oxygen in the water is depleted. TSS measurement results are presented in Figure 1.

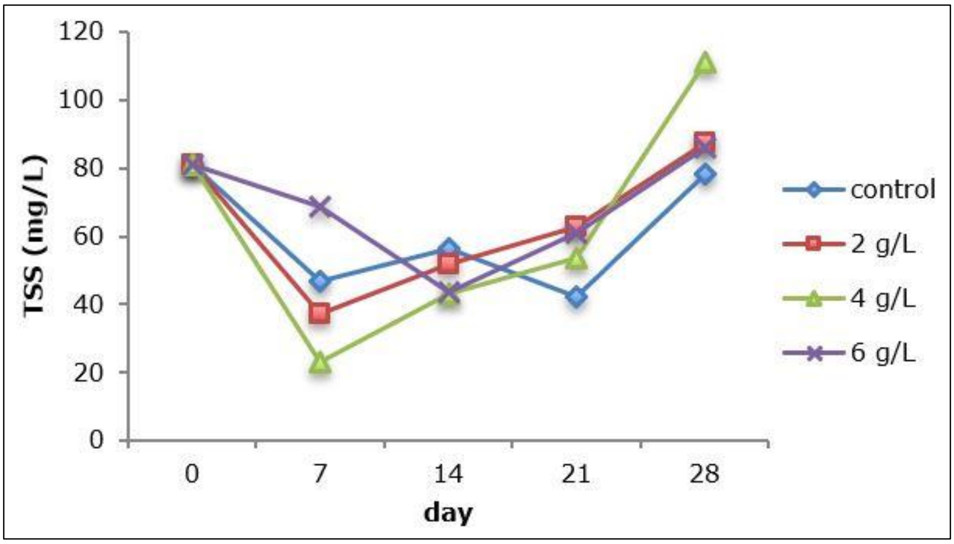

Figure 1. The dynamic of TSS concentration during treatments.

On the 7th day, all practices decreased, while on the 14th, 21st, and 28th days, most of them experienced an increase. Observation on the last day, it was known that the TSS value in all treatments increased, and the highest density was in the treatment of 4 g/L at 111.07 mg/L. Overall, the highest TSS mean value was found on the 28th day of 90.73 mg/L while the lowest was on the 7th day of 43.94 mg/L. The results of the statistical analysis presented no significant difference. This indicates that the treatment carried out has no effect on TSS. TSS affects the penetration of light into the water column. A high TSS value can inhibit the rate of photosynthesis of phytoplankton in water so that the presence of oxygen in the water is depleted. On the 7th day, the TSS content decreased in each treatment. The decreased TSS content occurred due to the deposition of particles at the bottom and *P. viridis* which was able to reduce suspended solids in the water. According to Wedsuwan et al (2016), *P. viridis* can clean particles with a diameter of more than 3μm with high efficiency. However, the increase in TSS occurred in most of the treatments on days 14, 21, and 28. This could be due to the low depth of the research vessel causing the solids that had settled on the bottom to rise. These solids include pseudofeces and mosses. According to Kusumawati et al (2015), the increase in TSS occurs because the particles accumulate in the labial palp thus inhibiting particle filtering. The ability of *P. viridis* is reduced and TSS cannot be decreased.

The water quality parameters observed to support the main parameters are dissolved oxygen (DO), water temperature, salinity and pH which are presented in Table 3.

Table 3

Water quality parameters during treatment

| *Parameter* | *Day* | | | | | | |
|---|---|---|---|---|---|---|---|
| | *0* | *7* | *14* | *21* | *28* | *Range* | *Mean* |
| DO (mg/L) | 6,92 | 5,18 | 5,39 | 5,46 | 5,26 | 5,26-6,92 | 5,64 |
| Water temp. (°C) | 30,8 | 27,5 | 30,2 | 30,2 | 29,1 | 27,5-30,8 | 29,63 |
| Salinity (ppt) | 35 | 35 | 37 | 39 | 40 | 35-40 | 37,2 |
| pH | 7,92 | 7,87 | 7,78 | 7,69 | 7,73 | 7,69-7,92 | 7,80 |

Based on Table 3 the DO results are in the range of 5.26-6.92 mg/L, and water temperature in the range of 27.5-30.8°C. In the observations made, the water temperature is in the optimum range. Increased water temperature does not have a negative effect on the growth and viability of clams (Acosta-Balbas et al 2019) but at temperatures <8°C and >42°C will cause death (Wang et al 2017).

The rearing area is outside the room which is shaded by a roof so that sunlight is blocked but can reach the bottom of the rearing container. Salinity was in the range of 35-40‰; and pH in the range of 7.69-7.92. Oxygen is needed for the growth and development of *P. viridis*, besides that it is needed for the overhaul of organic matter. *P. viridis* growth and development can be stunted due to lack of oxygen. The low dissolved oxygen in the water reduces the physiological energy of *P. viridis* to filter nutrients (Wang et al 2011). Dissolved oxygen is used by aerobic bacteria to break down organic matter. Factors that affect the dissolved oxygen content include organic matter, depth, and stocking density. The high content of organic matter causes the water to lack dissolved oxygen because it is used to remodel organic matter into inorganic. Depth affects dissolved oxygen because the deeper the water is, sunlight cannot penetrate to the bottom so that phytoplankton and algae cannot photosynthesize. In the observations made, the depth used is low so that sunlight can reach the bottom in addition to the presence of an aerator to help supply oxygen. Competition between individual shellfish for oxygen can affect the filtration rate and high density triggers more excretion. *P. viridis* can live optimally in the range of 15-32°C (Rajagopal et al 2006) and die at temperatures >41°C (McFarland et al 2015). Temperature is the main factor affecting the rate of respiration because an increase in water temperature can trigger an increase in metabolism and oxygen consumption (Gao et al 2008).

Salinity that increases every observation occurs because of the evaporation of water in the rearing container. Segini de Bravo (2003) stated that *P. viridis* has wide adaptability and tolerance to salinity. Even though, fluctuating salinity can trigger *P. viridis* stress and if it occurs for a long time can cause death (Layugan et al 2018). The effect of low salinity is that the strength of the byssus will decrease so that it cannot stick to the substrate, whereas if the salinity is high, it will reduce the survival rate (McFarland et al 2015). At 8.7‰ salinity *P. viridis* will stop filtering food and produce no excretion (Galimany et al 2018). According to Vijayavel (2010), an increase in pH to 11.5 can cause a mortality of 20%, while a decrease in pH to 5.5 causes a mortality of 90%. The pH value is related to the decomposition of organic matter. The decomposition process will be optimal if the pH is in the range of 7.5-8.5 (Beristain 2005). In observations, the pH value decreased from the first day of maintenance to the last day. This happened because the fluctuating content of TOM decomposed. Day 21 had the lowest pH value compared to other days because on day 21 the TOM content was the highest compared to other days.

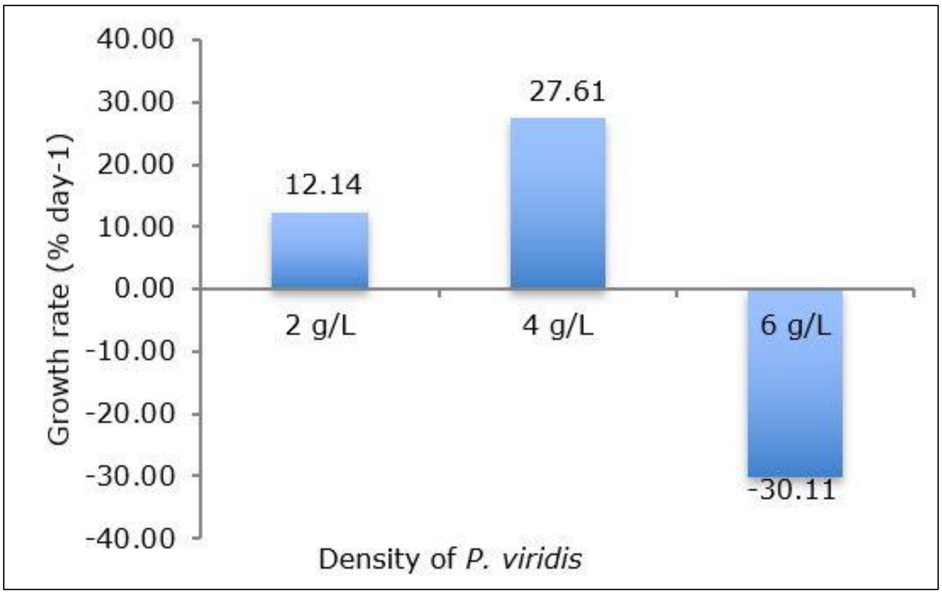

Figure 2. The growth rate of *P. viridis* during treatment.

Based on Figure 2, the highest RGR value was obtained in the 4 g/L density treatment of 27.61% and the lowest in the 6 g/L treatment of -30.11%. The growth of clams is influenced by the environment and the availability of food. Garcia et al (2016) stated that food availability and temperature are important factors in the physiology of shellfish. The value of the relative growth rate in the 6 g/L treatment was the lowest due to individual mortality so that the biomass was reduced. Another influencing factor is the higher density increases competition for food and higher excretion results. The accumulation of metabolic products can lead to death due to the toxic substances released.

**Conclusions**. Our results revealed that *P. viridis* able to reduce Total Organic Matter (TOM) and Biological Oxygen Demand (BOD) since day 7. The density 4 g/L of *P. viridis* has higher effectivity to reduce TOM, BOD, TSS, and has highest growth rate of biomass for 28 days treatment. *P. viridis* has high potential to be used in polyculture and Integrated Multi-trophic Aquaculture (IMTA) rearing system.

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

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