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Intensive *Litopenaeus vannamei* Pond Performance with Irrigation System Based on Distribution of *Vibrio* spp.

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ABSTRACT

Intensive shrimp ponds are characterized by the high stocking density of shrimp and artificial feeding and are equipped with an irrigation network system. The purpose of this study was to analyze the intensive *L.vannamei* pond with an irrigation system based on *Vibrio* spp. distribution to determine environmental quality degradation in terms of the treat of pathogenic bacteria. This study was carried out for one production cycle from March to July 2021 in an intensive shrimp farm in Sarjo Village, Pasangkayu Regency, West Sulawesi. The parameters analyzed in this study consisted of Total Bacterial Count (TBC), Total *Vibrio* Count (TVC), yellow and green colony *Vibrio* spp. in water using the spread plate method. Water samples for bacterial analysis were taken from 1) source water: (a) surface (0–50 cm depth) and (b) pump mouth; 2) treatment pond; 3) aquaculture pond; 4) Waste Water Treatment Ponds (WWTP); 5) outlet (before discharge into public waters). The results showed that the distribution of bacteria from seawater to WWTPs pond still increased the abundance of *Vibrio* spp., although the abundance of *Vibrio* spp. is still safe or below the maximum limit of <10^4 CFU/mL. Distribution of *Vibrio* bacteria in Intensive *L.vannamei* Pond Sarjo Village, West Sulawesi gave a relatively good pond performance because it was characterized by an abundance of *Vibrio* bacteria in the pond irrigation system from inlet to outlet which was still below the maximum limit and high pond productivity.

Introduction

Rapid population growth and limited land availability require humans to be more creative in producing food products with high intensity, but not damaging the environment. Intensification in various fields of food production, in addition to being able to meet the food needs of the community, on the other hand, has also caused negative impacts on the environment. This is because more intensive production activities will also produce greater waste (Coldebella *et al.* 2017).

One of the intensive production activities in aquaculture and growing quite rapidly in Indonesia is the intensification of shrimp farming in ponds. Currently, the intensification of shrimp ponds especially *Litopenaeus vannamei* is one of the government's programs to increase national shrimp production (Kayandi *et al.* 2020).

According to Wasielesky *et al.* (2013), intensive shrimp farming is characterized by the high stocking density of shrimp, high artificial feeding, and is equipped with an irrigation network system. High organic matter can reduce water quality and increase the growth of pathogenic bacteria such as *Vibrio* spp. (Alfiansah *et al.* 2018; Widigdo *et al.* 2020).

Vibrio spp. is bacteria that can live in saline waters, and are opportunistic pathogen for shrimp that can cause an disease in shrimp when shrimp conditions are weak and environmental factors are poor. (Sarjito *et al.* 2016; Asaad *et al.* 2019; Han *et al.* 2020). Poor management of aquaculture will lead to an increase in the population of *Vibrio* bacteria during the aquaculture process and wastewater discharged into

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public waters. Therefore, Waste Water Treatment Pond (WWTP) are very necessary in intensive shrimp ponds to improve the waste water quality before discharged into public waters. Based on these problems, this study aims to analyze the intensive *L.vannamei* pond with an irrigation system based on *Vibrio* spp. distribution to determine environmental quality degradation in terms of the treat of pathogenic bacteria. The information obtained from the results of this study is expected to be used as a reference for the management of shrimp aquaculture areas and coastal areas.

Materials and Methods

Location and time of study

This study was carried out for one production cycle from March to July 2021. The study location and sample analysis were carried out in an intensive shrimp farm in Sarjo Village, Pasangkayu Regency, West Sulawesi (Figure 1). Sampling locations were carried out at several stations which can be seen in Table 1.

Table 1. Sampling point.

Sampling point	Sampling location
1	Source water
2	Treatment pond
3	Aquaculture pond
4	WWTP 1
5	WWTP 2
6	WWTP 2 discharge
7	Outlet (mangrove)

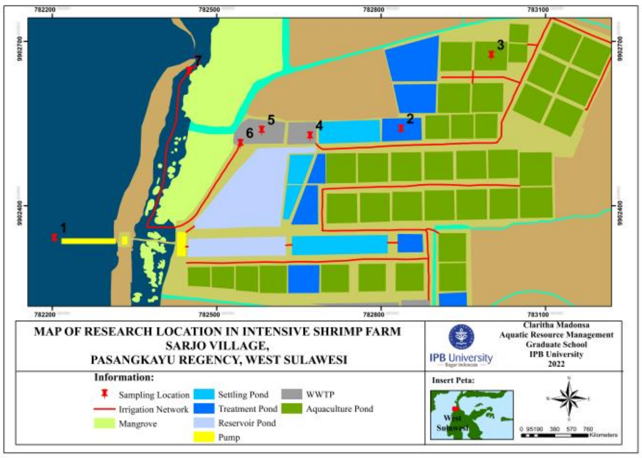

Figure 1. Map of study location.

Data collection

The data collected during the study consisted of primary data and secondary data. Primary data includes the abundance of bacteria in water. A sampling of water samples for analysis of bacterial abundance was carried out once every two weeks and for sampling point in source water is carried out at high and low tide during shrimp culture period using a 15 mL glass bottle that had been pre-sterilized (Table 2). The abundance of bacteria observed in this study consisted of Total Bacterial Count (TBC), Total *Vibrio* Count (TVC), yellow colony *Vibrio*, and green colony *Vibrio*.

Secondary data collected includes information on the volume of water exchange from each pond every day obtained from pond technicians and pond performance data. Information on the volume of water discharged from each pond will be used to calculate the retention time of WWTP.

Sample analysis

Sample analysis for bacterial cells was carried out by inoculation of bacteria using the spread plate method. Bacterial inoculation was started by diluting the sample water using a physiological saline solution. The dilution that will be used for total bacterial inoculation is 10^{-1}-10^{-5} and for *Vibrio* bacteria inoculation using a 10^{-0}-10^{-2} dilution. Each sample for inoculation was carried out in duplicate.

After that, 0,1 mL of the diluted sample water was taken using a micropipette to inoculate the bacteria on the agar medium in a petri dish. The agar medium used for total bacterial inoculation was Tryptic Soy Agar (TSA) added with NaCl and for *Vibrio* using Thiosulphate Citrate Bile-salt Sucrose Agar (TCBSA). The Petri dishes were then incubated in an incubator at 37°C for 24 hours. After 24 hours of incubation, the bacterial colonies that grew were counted and entered into the Total Plate Count (TPC) formula.

Data analysis

Analysis of the abundance of bacteria

The abundance of bacteria calculated in this study consisted of Total Bacterial Count (TBC), Total *Vibrio* Count (TVC), yellow colony *Vibrio*, and green colony *Vibrio* in water. The abundance of bacteria was calculated using the Total Plate Count (TPC) method. Counting the number of colonies in Petri dishes can be done using the following formula (Madigan *et al.* 2003):

$$\text{Total Bacteria (CFU/mL)} = \sum \text{colony} \times \frac{1}{d} \times \frac{1}{n}$$

Information:
d = Calculated dilution factor
n = Sample volume (mL)

Volume of the Wastewater Treatment Plant (WWTP)

The data needed to calculate the volume of the WWTP consists of data on the length of the WWTP canal, the width of the WWTP canal, and the depth of the WWTP. The volume of WWTP can be calculated using the following formula:

$$V\ (m^3) = p\ (m) \times l\ (m) \times h\ (m)$$

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Information:
V = Volume of the WWTP (m^3)
p = Length (m)
l = Width (m)
h = Depth (m)

Wastewater discharge entering the WWTP

Water discharge is the water flow rate (in the form of water volume) flowing in a particular cross-section every unit of time (Hillel *et al.* 2015). Waste Water Treatment Pond (WWTP) is a structure specifically designed to process and manage waste water so that the discharged wastewater does not have a negative impact on the aquatic environment. The calculation of the wastewater discharge entering the WWTP is obtained from secondary data. Secondary data obtained is from technicians who provide information on the volume of water discharged (water change) from each aquaculture pond every day. The calculation of the wastewater discharge entering the WWTP will be used to calculate the retention time.

Retention time of WWTP

Retention time or residence time is the average time required to empty water through the outflow. The formula used to calculate retention time is as follows:

$$\tau \text{ (day)} = \frac{V\ (m^3)}{Q\ (m^3/day)}$$

Information:
τ = *Retention time* (day)
V = Volume of the WWTP (m^3)
Q = Wastewater discharge entering the WWTP (m^3/hari).

Pond productivity

Productivity is the biomass produced per area of maintenance. The formula used to calculate pond productivity is as follows:

$$P \text{ (kg/ha)} = \frac{B \text{ (kg)}}{L \text{ (ha)}}$$

Information:
P = Pond productivity (kg/ha)
B = Biomass produced (kg)
L = Area used (ha)

Survival Rate (SR)

Survival Rate (SR) is one of the parameters to determine the survival rate of an organism expressed in percent (%). The SR can be calculated using the following formula:

$$SR\ (\%) = \frac{Nt}{No} \times 100\%$$

Information:
SR = Survival Rate (%)
Nt = Number of harvested shrimps (tail)
No = Number of stocked shrimps (tail)

Table 2. Sampling the abundance of bacteria in water.

Bacteria Sampling Location	Sampling Time	Analysis Method
Source water (inlet): water surface and pump mouth	Once every two weeks, at high and low tide	Total Plate Count (TPC)
Treatment pond	After treatment	
Aquaculture pond	Once every two weeks	
WWTP 1	Once every two weeks	
WWTP 2	Once every two weeks	
WWTP 2 discharge	Once every two weeks	
Outlet (mangrove)	Once every two weeks	

Results

The abundance of bacteria in source water at high and low tide

Observations on the abundance of bacteria in source water were carried out to determine environmental health and aquaculture in the Intensive Shrimp Pond in Sarjo Village, Pasangkayu Regency, West Sulawesi from a bacterial threat perspective. Observations were made at different depths, namely at the surface and at the depth of the pump mouth at high tide and low tide to see when seawater was taken for shrimp pond. The results of the abundance of TBC and TVC in source water can be seen, respectively, in Figure 2 and Figure 3.

The results showed that the depth and tides caused differences in the abundance of TBC and TVC in source water. At high tide, the depth of the pump mouth reaches 3 m, while at low tide the depth of the pump mouth reaches 1.5 m. The abundance of TBC and TVC at the pump mouth at a depth of 3 m has a lower abundance than at the pump mouth at a depth of 1.5 m and the Surface at a depth of 0-50 cm. The abundance of TBC and TVC at the pump mouth at a depth of 3 m is in the range of (5,1 ± 0,5) x 10^3 CFU/mL to (8,0 ± 0,6) x 10^4 CFU/mL and (6,6 ± 0,3) x 10^1 CFU/mL to (1,3 ± 0,3) x 10^2 CFU/mL.

Meanwhile, the abundance of TBC and TVC at high tide was lower than at low tide. The abundance of TBC and TVC at high tide was in the range (5.1 ± 0.5) x 10^3 CFU/mL to (1.9 ± 0.07) x 10^4 CFU/mL and (6.6 ± 0 ,3) x 10^1 CFU/mL to (1.9 ± 0.08) x 10^2 CFU/mL. The abundance of TBC and TVC at low tide were in the range (1.0 ± 0.01) x 10^4 CFU/mL to (2.8 ± 0.06) x 10^4 CFU/mL and (8.5 ± 0 ,6) x 10^1 CFU/mL to (3.2 ± 0.09) x 10^2 CFU/mL.

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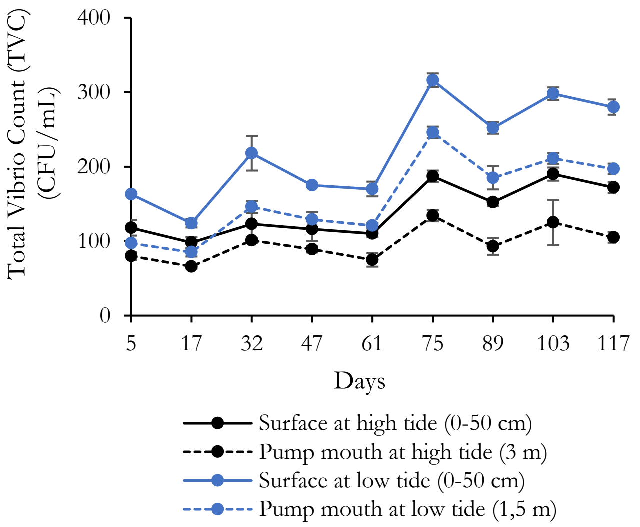

Figure 2. The abundance of TVC in source water.

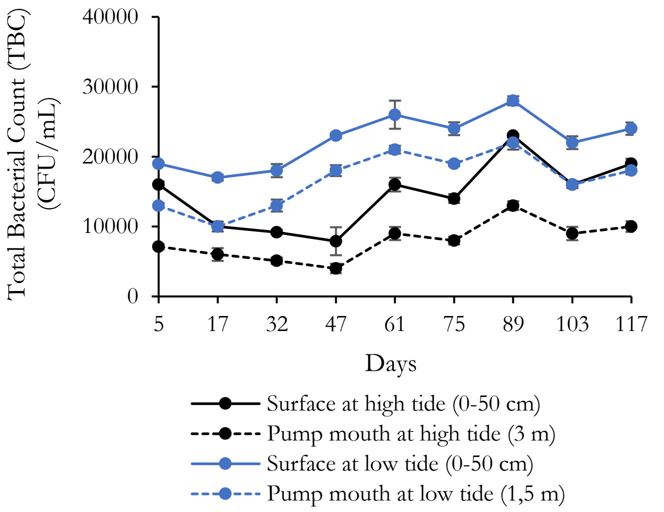

Figure 3. The abundance of TBC in source water.

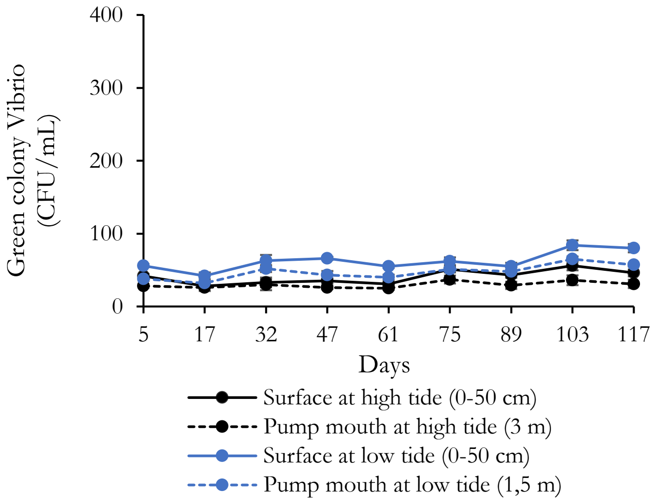

Figure 4. The abundance of green colony *Vibrio* in source water.

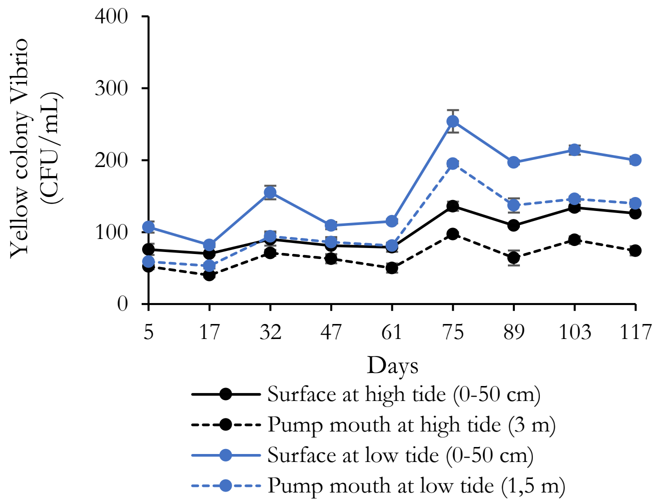

Figure 5. The abundance of yellow colony *Vibrio* in source water

Based on the results in Figure 4 and Figure 5, depth and tides also caused differences in the abundance of yellow and green *Vibrio* colonies in source water. The abundance of yellow and green *Vibrio* colonies at the pump mouth at a depth of 3 m was lower than the abundance at the pump mouth at a depth of 1.5 m and on the surface at a depth of 0-50 cm. The abundance of bacteria in the pump mouth at a depth of 3 m ranged from (4.0 ± 0.3) x 10^1 CFU/mL to (8.9 ± 0.5) x 10^1 CFU/mL for yellow *Vibrio* colonies and (2.5 ± 0.2) x 10^1 CFU/mL to (3.7 ± 0.5) x 10^1 CFU/mL for green *Vibrio* colonies.

At high tide, the abundance of yellow and green *Vibrio* colonies was lower than at low tide. The abundance of yellow and green *Vibrio* colonies at high tide ranged from (4.0 ± 0.3) x 10^1 CFU/mL to (1.4 ± 0.06) x 10^2 CFU/mL and (2.5 ± 0.2) x 10^1 CFU/mL to (5.6 ± 0.6) x 10^1 CFU/mL. The abundance of yellow and green *Vibrio* colonies at low tide ranged from (5.3 ± 0.4) x 10^1 CFU/mL to (2.5 ± 0.6) x 10^2 CFU/mL and (3.2 ± 0.2) x 10^1 CFU/mL to (8.4 ± 0.8) x 10^1 CFU/mL.

The abundance of bacteria in pond water

The following shows the results of the abundance of TBC and TVC during the study in Figure 6 and Figure 7. The distribution of bacteria in ponds starts from seawater which is pumped into the treatment pond to be treated with chlorine. Chlorine treatment is given to kill bacteria and eliminate various pathogens that can cause disease in shrimp. It can be seen in the table below, the abundance of TBC and TVC in the treatment pond has a very low abundance compared to the other ponds due to the influence of the chlorine treatment.

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After passing the treatment pond, the water will flow to the aquaculture pond. At DOC 5 to 32, the abundance of TBC in pond water was lower than the other DOCs, but at DOC 47 to DOC 117 the abundance of TBC increased from (2.3 ± 0.6) x 10^4 CFU/mL to a maximum (2, 3 ± 0.09) x 10^5 CFU/mL at DOC 89. The increase in the abundance of TBC in DOC 47 was followed by a decrease in the abundance of TVC. Likewise, at DOC 89, the abundance of TBC reached the highest abundance followed by a decrease in the abundance of TVC which also reached the lowest abundance during the study. The lowest abundance of TVC was (5.2 ± 0.6) x 10^2 CFU/mL.

Then from the aquculture pond the water will flow to WWTP 1 and continue to WWTP 2. In WWTP 1, the abundance of TBC and TVC is still high and even reaches a maximum abundance of (2.4 ± 0.09) x 10^5 CFU/mL and (2.9 ± 0.09) x 10^3 CFU/mL. However, in WWTP 2, the abundance of TBC and TVC decreased from (2.4 ± 0.09) x 10^5 CFU/mL to (1.2 ± 0.1) x 10^4 CFU/mL for TBC and from (2.9 ± 0 .09) x 10^3 CFU/mL to (4.0 ± 0.7) x 10^2 CFU/mL for TVC. In WWTP 2 discharge, the abundance of TBC and TVC continued to decrease and reached the lowest abundance of (1.1 ± 0.9) x 10^4 CFU/mL for TBC and (2.0 ± 0.8) x 10^2 CFU/mL for TVC.

Pond wastewater from WWTP 2 discharge does not enter seawater directly but flows through the mangrove area. In this outlet (mangrove), the abundance of TBC and TVC fluctuated but still had a lower abundance than in the aquaculture pond. The lowest bacterial abundance at the outlet (mangrove) before entering seawater was (1.4 ± 0.4) x 10^4 CFU/mL for TBC and (1.8 ± 0.6) x 10^2 CFU/mL for TVC.

The abundance of yellow and green colony *Vibrio* can be seen in Figure 8 and Figure 9. The results showed that during the study the abundance of yellow colony *Vibrio* tended to be higher than that of green colony *Vibrio*, but both had a decreasing trend during the study. The abundance of yellow and green colony *Vibrio* in the treatment pond was lower than the other ponds due to the presence of chlorine treatment. In the aquaculture pond, the abundance of yellow and green colony *Vibrio* was higher than in the treatment pond, but with a decreasing graph trend. In WWTP 1, the abundance of yellow and green colony *Vibrio* was still high, but at WWTP 2, the abundance of yellow and green colony *Vibrio* decreased from (1.8 ± 0.08) x 10^3 CFU/mL to (3.3 ± 0.4) x 10^2 CFU/mL for yellow colony *Vibrio* and (1.0 ± 0.7) x 10^3 CFU/mL to (7.0 ± 0.3) x 10^1 CFU/mL for green colony *Vibrio*.

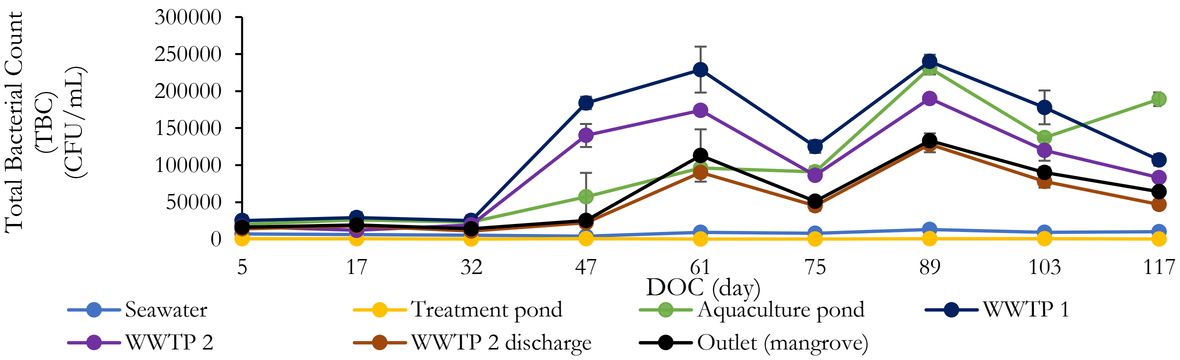

Figure 6. The abundance of TBC in pond water.

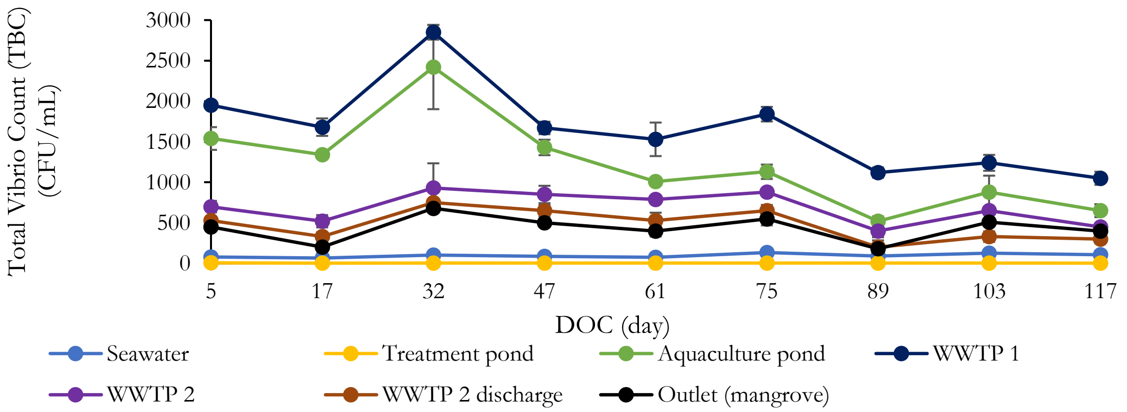

Figure 7. The abundance of TVC in pond water

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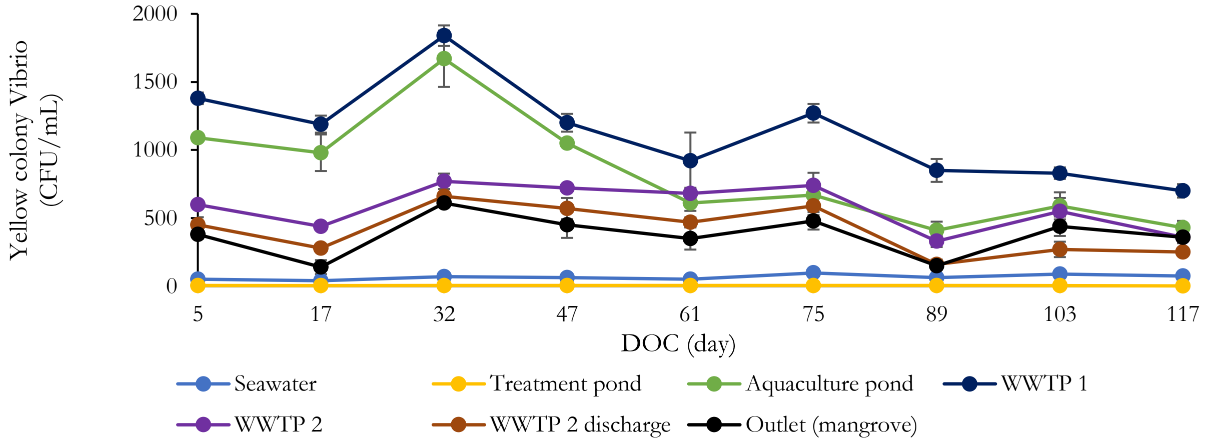

Figure 8. The abundance of yellow colony *Vibrio* in pond water.

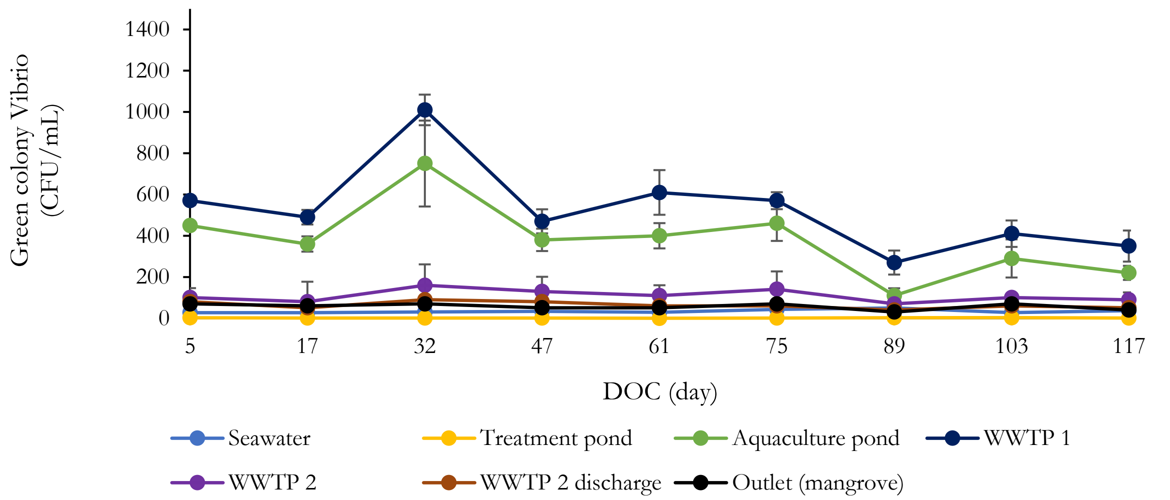

Figure 9. The abundance of green colony *Vibrio* in pond water.

After that, at WWTP 2 discharge, the abundance of yellow and green colony *Vibrio* continued to decrease and reached the lowest abundances respectively of (1.6 ± 0.4) x 10^2 CFU/mL and (4.0 ± 0.4) x 10^1 CFU/mL. The abundance of yellow and green colony *Vibrio* at the outlet (mangrove) was already lower than the WWTP 2 discharge, which was (1.4 ± 0.5) x 10^2 CFU/mL for yellow colony *Vibrio* and (3.0 ± 0.5) x 10^1 CFU/mL for green colony *Vibrio*.

Retention time of WWTP

The results of the calculation of the retention time of WWTP in intensive shrimp farms can be seen in Table 3. The wastewater treatment process at the Intensive Shrimp Farm in Sarjo Village, Pasangkayu Regency, West Sulawesi is carried out biologically with a pond and is designed like a long maze (Figure 10). Based on these results it can be said that the conditions of bacteria like this can be achieved in the water change process starting from DOC 30 as much as 11.250 m^3/day, and with the volume of WWTP 1 is 25.886 m^3 and the volume of WWTP 2 is 16.000 m^3, so the retention time of WWTP 1 and WWTP 2 is 4 days. The 4-day retention time in the pond WWTP was able to reduce the abundance of TBC from (2.4 ± 0.09) x 10^5 CFU/mL to (1.1 ± 0.9) x 10^4 CFU/mL, the abundance of TVC from (2, 9 ± 0.09) x 10^3 CFU/mL to (2.0 ± 0.8) x 10^2 CFU/mL, the abundance of yellow colony *Vibrio* from (1.8 ± 0.08) x 10^3 CFU/mL to (1, 6 ± 0.4) x 10^2 CFU/mL, and the abundance of green colony *Vibrio* from (1.0 ± 0.7) x 10^3 CFU/mL to (4.0 ± 0.4) x 10^1 CFU/mL.

Table 3. Retention time of WWTP.

	Volume of WWTP (m^3)	Water discharge that enters the WWTP (m^3/day)	Retention time (day)
WWTP 1	25.886	11.250	2,3
WWTP 2	16.000	11.250	1,4
		Total	3,7 = 4

Table 4. Pond performance indicators.

Indicators	Standard	Reference	Result
Pond productivity	10-15 ton/ha	KEP.75/MEN/2016	56 ton/ha
Survival Rate (SR)	> 75 %	SNI 01.7246-2006	76 %
Food Convertion Ratio (FCR)	< 1,5	Tahe *et al.* (2014)	1,55
Harvest size	20-40	KEP.75/MEN/2016	27

Figure 10. WWTP design at the Intensive Shrimp Farm in Sarjo Village.

The pond performance

Success in intensive shrimp farm production can be seen from the successful performance of the pond. The indicators that can be used to see the pond performance can be seen in Table 4. From the results of the abundance of bacteria obtained during this study, the performance of the pond is classified as good because pond productivity > 15 tons/ha, SR > 70%, FCR 1.55, and harvest size is in the range of 20-40.

Discussion

The abundance of bacterial cells (TBC, TVC, and *Vibrio* colonies yellow and green) in the source water indicates that tides and water depth can affect the abundance of bacteria. The abundance of bacteria at high tide is lower than at low tide (Figure 2, 3, 4, and 5). Maslukah *et al.* (2014) and Sembel and Manan (2018) state that tides can affect the presence of organic material which will then have an impact on fluctuations in the abundance of bacteria in seawater. The pattern of tidal currents which has an alternating pattern will affect the pattern of distribution and supply of organic material originating from the river. When low tide conditions, the mass of river water will be more dominant and cause the content of organic matter in seawater to be higher. Contrariwise, during high tide conditions, organic material becomes low due to the dilution of the water mass from the sea, so that the abundance of bacteria at high tide is lower than at low tide (Chen *et al.* 2019; Jubaedah *et al.* 2021). This is also supported by the study of Martineac *et al.* (2021) which stated that the abundance of bacterial cells at high tide was lower than at low tide.

The abundance of bacteria in the pump mouth is lower than on the surface of the water. This is in accordance with the study of Juantari *et al.* (2013) which states that as the depth increases, the bacterial population decreases. The abundance of bacteria on the surface of the water can be influenced by the presence of phytoplankton. This assumption is in accordance with the study of Nuchsin (2007) and Osterholz *et al.* (2018) which states that bacteria are positively correlated with phytoplankton. After the phytoplankton die, the phytoplankton cells are degraded by bacteria under aerobic conditions. In the degradation process, bacteria remineralize nutrients, especially phosphorus and nitrogen which can be reused by phytoplankton as a source of nutrients (Noerdjito 2019).

According to KEP.75/MEN/2016, the maximum limit of the abundance of TVC for aquaculture water sources is < 1 x 10^3 CFU/mL. The results of the abundance of TVC in seawater in this study were still below the maximum limit of < 1 x 10^3 CFU/mL. Although at low tide there was an increase in the abundance of TVC, the increase was still below the maximum limit. This shows that the seawater extraction site in this study location from the point of view of the threat of *Vibrio* bacteria has not been polluted. Thus, seawater extraction for aquaculture in this study location can still be carried out, both at high tide and at low tide. However, if in ponds the abundance of TVC is high or there are indications of vibriosis, it is advisable to only pump water at high tide, because the abundance of bacteria is lower at high tide than at low tide.

The abundance of bacteria in pond water during the study fluctuated in each pond. The abundance of TBC, TVC, yellow, and green colony *Vibrio* in the Treatment Pond decreased drastically, due to chlorine treatment to kill bacteria. However, in the aquaculture pond, the abundance of TBC, TVC, yellow and green colony *Vibrio* was increased. The increase in the abundance of TBC, TVC, yellow, and green colony *Vibrio* in the aquaculture pond was due to the higher the Day of Culture (DOC), the age of the shrimp would also increase so that the feed given was also increasing. The consequences of high artificial feeding can lead to an increase in organic matter from feed residues and shrimp manure. High organic matter in pond water can be a substrate for bacterial growth, so the abundance of TBC, TVC, yellow, and green colony *Vibrio* can increase during the aquaculture process (Widigdo *et al*. 2020; Widigdo *et al*. 2021).

The increase in the abundance of TBC in the aquaculture pond was also caused by the addition of probiotics (*Bacillus* sp.) which was given twice a week during the aquaculture process. Bacteria that can grow on Triptic Soy Agar (TSA) media for TBC are heterotrophic bacteria (including *Bacillus* sp.) that can utilize organic compounds as a carbon source for growth, so that the increase in the abundance of TBC during aquaculture is thought to be caused by high organic compounds and the addition of probiotic *Bacillus* sp.

The increase in the abundance of TBC at DOC 47 was followed by a decrease in the abundance of TVC, as well as yellow and green colonies *Vibrio* in the aquaculture pond, which was caused by competition between heterotrophic bacteria and *Vibrio* bacteria. Lestari *et al*. (2016) stated that heterotrophic bacteria can inhibit the growth of pathogenic bacteria such as *Vibrio* spp. This is in accordance with the study of Ernawati (2016) and Artha *et al*. (2019) which states that heterotrophic bacteria can produce antibacterial compounds in the form of lactic acid, peroxide, and bacteriocin so that they can inhibit the growth of pathogenic bacteria. Some examples of heterotrophic bacteria found in ponds and even used as probiotics are *Bacillus subtilis* and *Pseudomonas maleii* (Lestari *et al*. 2016).

Based on Figure 7, the abundance of TVC after 40 days of DOC began to decrease. This was due to the effect of water changes (20-35%) in the aquaculture pond after 40 days of DOC. Widigdo (2013) stated that water change aims to dilute or dispose of excretory waste (feces) and toxic metabolic wastes (decomposition of feed residues and dead plankton), and can reduce the abundance of bacteria.

Until DOC 40 days, the water used in the aquaculture pond is water that has been given chlorine (sterilization) in the treatment plot, but after DOC 40 days, the water used for water changes is no longer sterilized. Water that is not sterilized again after 40 days of DOC is because after 40 days of DOC the shrimp are more resistant to disease. Rohmin *et al*. (2017) stated that the age of shrimp under 40 days is more susceptible to disease because the shrimp immune system will increase as the age of the shrimp increases.

Diluting or removing pollutants in the pond by changing water provides more ideal conditions for shrimp life, compared to only sterilizing but the amount of water used to dilute pollutants in pond water is not enough. Thus it can be said that water dilution/change is more important than just keeping water sterilized from bacteria.

Meanwhile, the abundance of yellow colony *Vibrio* in the aquaculture pond during the study was always higher than the abundance of green colony *Vibrio*. The presence of *Vibrio* bacteria must always be monitored during the aquaculture process because if the population has reached quorum sensing it can cause disease in shrimp. The ability of *Vibrio* bacteria to perform quorum sensing is strongly influenced by the bacterial population in the water (Suwoyo and Tampangallo 2015). Quorum can be achieved quickly if there is a dominance of one type of bacteria, especially green *Vibrio* bacteria. Some studies say that in shrimp ponds it will be more dangerous if the dominant bacteria are green *Vibrio* bacteria. Green *Vibrio* bacteria that are pathogenic for shrimp include *Vibrio parahaemolyticus* (Wicaksono *et al*. 2020), *Vibrio harveyi* (Utami *et al*. 2016), *Vibrio shilonii* (Wicaksono *et al*. 2020), and *Vibrio vulnificus* (Sarjito *et al*. 2016).

Taslihan *et al*. (2004) stated that the maximum limit for the presence of *Vibrio* spp. for aquaculture is 10^4 CFU/mL and the maximum limit for total bacteria is 10^6 CFU/mL. Based on this, it can be said that the management of *Vibrio* spp. during the aquaculture process in this pond, it was good, because the abundance of TVC was still below the maximum limit of $< 10^4$ CFU/mL, and the abundance of TBC was also still below the maximum limit of $< 10^6$ CFU/mL.

After being in the aquaculture pond with the existing processes, the water flows to the Wastewater Treatment Ponds (WWTP). The wastewater treatment plant in this pond consists of 2 ponds, called WWTP 1 and WWTP 2. At WWTP 1, the abundance of bacteria is still higher, but at WWTP 2 the abundance of bacteria has decreased and even decreased in WWTP 2 discharge.

The wastewater treatment process at the Intensive Shrimp Farm in Sarjo Village, Pasangkayu Regency, West Sulawesi is carried out biologically with a pond and is designed like a long maze (Figure 10). According to Said and Utomo (2018), the biological wastewater treatment process with a pond is to accommodate wastewater in a large or long pond with a long residence time, so that with the activity of microorganisms that grow naturally, it can reduce the abundance of pathogenic bacteria. This is in accordance with the study results obtained. The result of calculating the retention time of WWTP in intensive shrimp farms before being discharged into public waters is 4 days. Pond waste water with a retention time of 4 days in WWTP has decreased the abundance of TBC, TVC, yellow and green *Vibrio* colonies before the wastewater is discharged into public waters.

Pond wastewater from WWTP 2 does not enter seawater directly but flows through the mangrove area, where the abundance of bacteria has decreased. Based on these results, it can be said that the area of microbiological processes that occur in these intensive shrimp farms contributes greatly to reducing TVC from maximum (2.9 ± 0.09) x 10^3 CFU/mL to (2.0 ± 0.8) x 10^2 CFU /mL, the abundance of yellow colony *Vibrio* from (1.8 ± 0.08) x 10^3 CFU/mL to (1.6 ± 0.4) x 10^2 CFU/mL, and the abundance of green colony *Vibrio* from (1.0 ± 0.7) x 10^3 CFU/mL becomes (4.0 ± 0.4) x 10^1 CFU/mL.

Idami and Nasution (2020) stated that the abundance of *Vibrio* bacteria in pond wastewater should be equal to or lower than the abundance of *Vibrio* bacteria in source water (intake). Although the abundance of *Vibrio* bacteria in WWTP 2 effluent was able to reduce the abundance of *Vibrio* bacteria, especially green colony *Vibrio* from (1.0 ± 0.7) x 10^3 CFU/mL to (4.0 ± 0.4) x 10^1 CFU/mL, and this abundance is still higher than in source water (intake), but the abundance of green colony *Vibrio* of (4.0 ± 0.4) x 10^1 CFU/mL has met the quality standard of shrimp pond effluent from KEP.28/MEN/2004, namely < 10^2 CFU/mL.

The distribution of *Vibrio* bacteria in ponds like this during the aquaculture process provides a relatively good pond performance, because the pond productivity is very high, namely 56 tons/ha, SR 76%, FCR 1.55, and the size of shrimp at harvest is 27 tail/kg. Based on these results, it can be said that water management in ponds by application of probiotic *Bacillus* sp., changing water during the aquaculture process, and treating wastewater in WWTPs are several ways that can be used to suppress the growth of *Vibrio* pathogenic bacteria, so as to increase the productivity of intensive shrimp pond.

Although the results of the performance of this pond are quite good, some management suggestions are still needed, especially to reduce the abundance of *Vibrio* bacteria in WWTP 2 discharge so that it is lower than in source water. One alternative that can be done to reduce the abundance of *Vibrio* in WWTP 2 discharge is to create conditions dominated by aerobic heterotrophic bacteria. The presence of aerobic heterotrophic bacteria can reduce the abundance of *Vibrio*, so that an adequate DO concentration is needed to support the activity of heterotrophic bacteria in the WWTP 2 pond.

Based on this, suggestions that can be done to increase DO concentration in WWTP 2 ponds are to use aquatic plants, such as *Lemna minor* and *Hydrilla verticillate*. Safitri *et al.* (2019) stated that aquatic plants can function as oxygen suppliers from the photosynthesis process and the direct diffusion process from the air that enters the water. In addition, aquatic plants can also reduce the nutrient content of pond wastewater. Thus, the use of aquatic plants in WWTP 2 ponds is one suggestion that can be used because it does not require large wastewater treatment costs but provides great benefits for improving the quality of intensive pond wastewater.

Conclusion

Distribution of *Vibrio* bacteria in intensive *L.vannamei* pond in Sarjo Village, West Sulawesi gave a relatively good pond performance because it was characterized by an abundance of *Vibrio* bacteria in the pond irrigation system from inlet to outlet which was still below the maximum limit and high pond productivity.

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