

Correlation between Density of Vibrio Bacteria with *Oscillatoria sp.* Abundance on Intensive *Litopenaeus vannamei* Shrimp Ponds

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Abstract The abundance of Vibrio bacteria and the presence of an excess **KEYWORDS** Oscillatoria sp plankton type are the main problems that often arise in intensive vanamei (Litopenaeus vannamei) shrimp culture. To examine this problem, the Intensive ponds purpose of this study was to determine of model approach interaction between the Litopenaeus abundance of Vibrio and Oscillatoria sp with the shrimp growth rate in ponds. This vannamei research was conducted with the ex-pose facto design on intensive shrimp culture Oscillatoria sp operations in Bayeman Village, Probolinggo. The results data from field research Vibrio bacteria variable are analyzed using a dynamic modeling system. From the modeling analysis results, showed that the Oscillatoria sp abundance pattern tended to increase over with shrimp culture period as following the pattern of tropical status dynamics by the nutrient increase load of 16.37%/week in ponds ecosystem. Meanwhile, the increase of Vibrio colonies density in ponds continued to increase aggregately by 0.99%/week on nine weeks and 12.5%/week on the last eight weeks of shrimp culture periods. So, it can be concluded that the fluctuations density of vibrio and Oscillatoria sp bacteria in ponds is are bioecological responses from increased nutrient loads and other micromaterials in ponds due to the longer period of shrimp culture.

Introduction

Litopenaeus vannamei shrimp culture is the strategic pillars from fisheries production commodities that are very promising in several tropical Asian countries (Ashton, 2007 and Syah, 2017). Since it was introduced and developed in Indonesia from at 2000s decade, the production and development of vanname shrimp on aquaculture activities have continued to increased (Romadhona *et al*, 2016 and Yi *et al*, 2016). Data from the report by Directorate General of Aquaculture (2016), the last production of vanname shrimp in Indonesian reached 488,019 tons and is predicted to increase at 10% per year.

On the shrimp culture bussines, increase of shrimp harvest productivity due to intensive on shrimp culture system, has a negative impact on the increasing prevalence of spreading germs, which is caused by increasing cultivation waste (Jayanthi *et al*, 2018 and Mohan *et al*, 2019). This is usually characterized by a decrease in the quality standards of pond water quality. Water quality parameters are vital components that should role playing in the viability of vannamei shrimp culture (Rahman *et al.*, 2015 and Zafar *et al.*, 2015). some variables of water quality that have a significant influence to ecosystem dynamics in vannamei shrimp farming ponds are the presence of *Blue Green Algae* (BGA) plankton and uncontrolled abundance of vibrio bacteria (Boyd and Tucker, 1998; Weidemann, 2002; Cardenas *et al.*, 2017; and Stalin *et al.*, 2017).

Blue Green Algae (BGA) plankton or cyanobacteria such as *Oscillatoria sp* in the event of blooming will cause shrimp to become off-flavour due to high levels of geosmine Ariadi et al. Correlation between Density of Vibrio Bacteria with

(Lovell *et al.*, 1985 and Tucker, 2000). Whereas vibrio is a type of pathogenic bacteria that is the cause of various serious problems in aquaculture (Chatterjee and Haldar, 2012). From the description of the problem, the purpose of this study was to determine interaction model between the abundance of vibrio bacteria with presence of *Oscillatoria sp* plankton on the growth rate of *Litopenaeus vannamei* shrimp in intensive pond culture.

Materials and methods

This research was conducted on intensive ponds in Bayeman Village, Tongas District, Probolinggo Regency used by ex post facto design research on a 400 m² vannamei shrimp pond during the operational cycle period of shrimp culture or precisely from July to November 2018. structured once every week from the start post until total harvest periods. So, the data will be collected according to the time series of the cultivation age. The research variables measured were water quality parameters which included TAN, NO₂, TOM, temperature, plankton density, and total vibrio bacteria. While the shrimp biological parameters observed were average body weight (ABW) and average daily gain (ADG) which were determined based on periodic sampling.

The method measurement of the concentration TAN and NO₂ parameters was determined by spectrophotometry methods, while measurement of TOM parameters was determined by titrimetry methods at analyzed in Training Center Laboratory CP Prima Company, Paiton, Probolinggo. And all of them refer to applying procedure by APHA (1980), while temperature parameters are measured *in situ* on ponds using a Hg Thermometer.

Vibrio bacterial samples were analyzed in the Training Center Laboratory. CP Prima Company, Paiton, Probolinggo. Bacterial samples from the pond were taken as much as 50 ml, then before planting in media, a gradual dilution was carried out (up to 10⁻²), and planting was done on TCBS media so that in the petri disk with spread method. After that incubation at room temperature for 24 hours. Then the next step is to counted at the number of TVC (Total Vibrio Count) colonies prefer by method of Prescott *et al.* (2002).

Analyzed the density and diversity of plankton species were calculated and identified using hemocytometer microscopically with Olympus Type cx22 microscope based applied procedure by APHA (1980). Then plankton abundance is calculated by the formula:

 Σ cell/ml = N x 10⁻¹/ 1 x 10⁴ cm³

Where: N is the number of plankton calculated, 10^{-1} is the value of the diluent factor, and 1×10^4 cm³ is the size of the volume in the hemocytometer box.

To determine the diversity of composition and number individual plankton in the pond, it was determined using the Shannon-Wiener diversity index (Michael, 1995) using the formula:

H′ = -∑ **pi ln pi;** pi = ni/N

Where: **H'** is the value of the diversity index, **pi** is the number of individuals of type **i** (ni) divided by the total number of individuals in the sample (N).

The technique used for collect growth parameters of shrimp is done by random sampling using nets then weighed from several pond points to obtain shrimp body weight (ABW), then to determine the level of growth rate (ADG) of shrimp then calculated using formula by Jiao *et al.* (2014) as follows:

Then, to construct an interaction model between the abundance of vibrio bacteria with presence of *Oscillatoria sp* on the shrimp growth rate, it was processed into a modeling analysis with StellaTM software Ver. 9.12.

Results and discussions

Profile of Ponds Culture

The operational cycle of shrimp culture in the implementation of this study lasted 85 days. From the process of shrimp culture periods, the profile of the average shrimp growth rate (ADG) was obtained as an indicator of growth and

water quality variables which included the average concentration of nitrite parameters (NO₂), Total Ammonia Nitrogen (TAN), temperature, total vibrio, total organic matter (TOM), as well as the Blue Green Algae abundance of type *Oscillatoria sp.* as an indicator of pond environment (Table 1).

DOC	ADG	Feed/day	NO₂	TAN	Temperature	TOM	TVC	<i>Oscillatoria sp zbun</i>
(day)	(gr)	(kg)	(mg/L)	(mg/L)	(°C)	(mg/L)	(CFU/ml)	(cell/ml)
85	0.27	53.58	0.02	0.140	29.3	113.51	5.87E+02	4.69E+05

The value of average daily gain (ADG) by shrimp on the study shows that shrimp growth rates are running normally with weight gain per day at 0.27 gr, this result is better than results by Junda (2018) which gets an ADG value 0, 26 in intensive vanname shrimp ponds during 112 days. The value of the water quality variable for the nitrite (NO₂) parameter of 0.02 mg/L detected is still low compared to the results by Rochin *et al.* (2017) study of 0.28-0.62 mg/L which is also a toxic level by the compound nitrite. Likewise, the TAN value, which is calculated to be 0.140 mg/L, is still lower than the results of previous studies by Gross *et al.* (2004) of 0.2 mg/L. Temperature parameters measured at 29.3°C are the optimal range of parameters for the growth of vanname shrimp cultivation (Singh *et al.*, 2013). While high levels of organic matter are caused by input feed input and fertilization that continues to grow throughout the cultivation period (Schober *et al.*, 2007).

The abundance of vibrio bacteria in ponds research average still tends to be lower (10^2 CFU/mI) than compared with the abundance vibrio bacteria results of detected on intensive shrimp ponds in India by Gopal *et al.* (2005), which obtained an abundance of vibrio bacteria on an average at 10^4 CFU/mI from 15 pond sample points on the west and east coasts of India. The level of vibrio pathogenicity and abundance in ponds is strongly influenced by conditions of pond water quality parameters, such as temperature and dissolved oxygen concentration (Orozco *et al.*, 2007). While the high density of *Oscillatoria sp* plankton which is a Blue Green Algae species caused by the condition from pond water parameters that tend to be eutrophic or even hyper-eutrophic. In addition, the type of *Oscillatoria sp* can be fix capture Nitrogen from diffusion so that the population will be easy to *Bloom* (Aliviyanti *et al.*, 2017).

Water Quality Characteristic

The Concentration of TAN and Nitrite

TAN concentration during culture periods ranged from 0.000-0.713 mg/L while Nitrite (NO₂) concentrations ranged from 0.006-0.042 mg/L (Figure 1). The standard optimum of TAN and Nitrite (NO₂) concentrations according to Edhy *et al.* (2010) is 0.10 mg/L and 0.2 mg/L. The highest increase TAN concentration occurred at 63 days with 0.713 mg/L and the lowest at 28 and 49 days with 0.0 mg/L. Because at 28 days periods can be siphoning activity was first performed after the period of *blind feeding* shrimp farming and at the age 49 days there was a recirculation activity. While the maximum concentration of Nitrite (NO₂) occurred at 42 days by 0.042 mg/L and the lowest at 77 days by 0.006 mg/L. This is suspected on 42 days culture periods is a biological synthesis of changes

in Ammonia (NH_3) compounds into Nitrite (NO_2) by *Nitrosomonas sp* strain bacteria intens on the nitrification cycle.

While the decline minimum value at 77 days culture is caused by a decrease amount of feed given at that periods. High TAN concentrations are caused by increased management and increasing frequency of feeding with poor management of pond bottom sludge (Hopkins *et al.*, 1994 and Jescovitch *et al.*, 2017). Variations of TAN and NO₂ concentration can also be due to the fertilization process. Fertilizing activities can increase the level of inorganic nutrient on pond ecosystems (Suwanpakdee *et al.*, 2010).

The level of concentration TAN and Nitrite (NO_2) in the pond ecosystem, naturally is also

influenced by the effectiveness of the nitrification cycle that is taking place. Nitrification is part of the biogeochemical cycle of nitrogen changes in aquaculture ecosystems that remodel ammonia (NH₃₋) to Nitrite (NO₂) by *Nitrosomonas* sp bacteria and convert of Nitrite (NO₂) to Nitrate (NO₃) by Nitrobacter (Hastuti, 2011). Nitrogen biogeochemical processes in shrimp farming which are dominated from nitrification cycle, besides being determined by nitrifying bacteria activities are also influenced of water quality stability conditions and the use of paddle wheels aerators as aquaculture engineers (Hargreaves, 1998 and Fernandes et al., 2010).

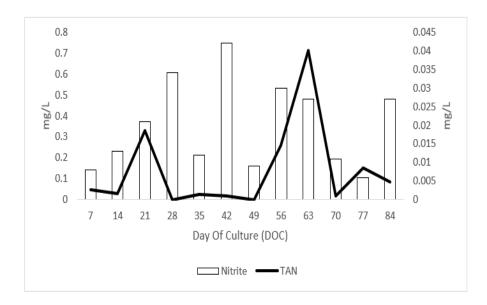


Figure 1. Concentration of TAN (Total Amonia Nitrogen) and Nitrite (NO₂) in Ponds.

The proportion of TAN and Nitrite (NO₂) levels will increase with pH and temperature conditions. High TAN and Nitrite (NO₂) concentrations in water will affect the permeability of body cells on aquatic organisms and reduce of the ions concentration level (Edhy *et al.*, 2010). To manage so that the TAN and Nitrite (NO₂) parameters in the farm remain below the threshold and are not toxic, it can be used to add probiotic treatment and maintaince the stability of daily fluctuations in water quality parameters such as pH, dissolved oxygen, temperature, and salinity (Nurhatijah *et al.*, 2016 and Jaganmohan *et al.*, 2018).

Organic Materials and Vibrio Bacteria

Organic matter concentration in the ponds during intensive shrimp culture periods measured by parameters of Total Organic Matter (TOM) ranged from 76.07-186.50 mg/L. While density vibrio

bacteria were measured by the abundance of Total Vibrio Count (TVC) on TCBS media. The count was calculated to be between 50-2,230 CFU/ml (Figure 2). The optimum standards for organic matter on intensive ponds and the abundance of vibrio bacteria according to regulation by the Minister of Marine and Fisheries of the Republic Indonesia No. 75 tahun 2016, concerning General Guidelines for Enlargement of *Penaeus monodon* and *Litopenaeus vannamei* shrimp is \leq 90 mg/L for TOM concentration and \leq 1.000 CFU/ml for abundance vibrio bacteria. The highest concentration of organic matter occurs on 77 days shrimp culture at 186.50 mg/L, this is thought to be caused by the amount of organic material such as particle suspension, turbidity level, and accumulation of material that settles in the bottom sediment pond which is increasing due to the old cultivation periods.

While the lowest levels of organic matter were detected on 14 days culture periods at 76.07 mg/L, the phenomenon seemed reasonable because at these conditions it was the initial period of maintenance so that addition of cultivation inputs was still considered small. Meanwhile, the highest vibrio bacterial density was observed at 28 days as many as 2.230 CFU/ml.

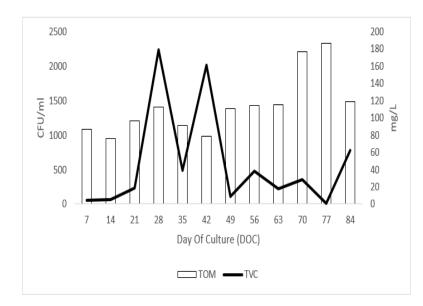


Figure 2. Concentration of TOM (Total Organic Matter) and Vibrio Abundance in Ponds

This result was suspected because at that age it was a *blind feeding* period so that there was no control over feed inputs, so that it allegedly had an indirect impact on increasing levels of waste load in the ecosystem pond. Meanwhile, the lowest density of vibrio bacteria at the 77 days culture periods is 10 CFU/ml, which is possible because at that periods there has been a decrease in the number of inputs to aquaculture ponds.

The high concentration of organic matter and the density of vibrio bacteria that are

pathogenic in pond ecosystems is an indication of the accumulation of waste loads due to aquaculture activities, which are usually caused by increased feed inputs, phytoplankton blooms, poor water quality management practice and unsupportive to environmental factors (Lekshmi et al., 2014). Vibrio bacteria that are sensitive to environmental conditions will be very opportunistic if their abundance is threshold above the (Heenatigala and Fernando, 2016). So that, it will cause various multiple diseases in cultivated shrimp such as

white feces syndrome, Enterocytozoon hepatopenaei (EHP) infection, and mass death caused by physiological symptoms caused by Vibrio bacterial infections (Jayasree et al., 2006; Sriurairatana et al., 2014; Mastan, 2015; and Aranguren et al., 2017).

From Figure 2, it can be seen that the distribution of vibrio bacteria decreases after 42 days shrimp periods, while the level of organic matter in ponds ecosystem shows a graph of the increase in 42 days shrimp periods. It can be synthesized that at the 42 days culture periods there was a dominance competition between vibrio and plankton due to bioaugmentation through periodic treatment by probiotics bacteria. The bioaugmentation process will be beneficial in maintaining the stability of water quality and suppressing the presence of pathogenic bacteria without having to reduce the ratio nutrients for phytoplankton (Janeo et al., 2009). In addition, the decrease of vibrio

35

30

25

20 Celcius

15

10

5

0

7 14 21 28 35 42 49 56 63 70 77 84

bacteria density of at the age of 42 days it can also due to other factors such as the presence of water circulation, effective feed management, and stabilizing pond alkalinity levels (Mangampa, 2015).

Daily of Temperature Fluctuations

Temperature is a water quality parameter that plays an important role in the course of the vanname shrimp culture system (Abdelrahman et al., 2018). In ponds culture, the level of temperature is strongly influenced by weather and environmental conditions (Culberson and Piedrahita, 1996). In this study, the temperature level in the morning ranged from 26.6-29°C with an average of 28.37°C, while during the day it ranged from 28.7-32°C with an average temperature at 30.28°C and overall average temperature values in the farm range from 29.3°C (Figure 3).

> 31 30.5

30

28.5

28 27.5

27

26.5

26



In the vannamei shrimp culture, the level of temperature fluctuations greatly affects the metabolic rate and the physiological response of shrimp to the environment as well as the decomposition process of organic matter by aquatic microorganisms (Chakravarty et al., 2016). In addition, temperature fluctuation effects also affect to dynamics of water quality parameters and the dominance composition of plankton in ponds (Palafox et al., 2019). The temperature values in farms vary depending on the shape of the region's topography and weather conditions (Islam *et al.*, 2004).



The close relationship between temperature and weather conditions will indirectly have an impact on the productivity of shrimp harvest (Rimi *et al.*, 2013). Due to unpredictable weather changes will increase vulnerability and poor adaptation of shrimp to erratic environmental conditions (Soto *et al.*, 2018). To keep the shrimp from being stressed due to temperature fluctuation effect, farmers will usually grow phytoplankton to a density of 10⁶ cell/ml in hopes of absorbing sunlight radiation during the day, so that at night the pond water conditions remain warm (Edhy *et al.*, 2010).

Dynamics of Oscillatoria sp Abundance

Plankton Oscillatoria sp is a type of cyanobacteria that cause HABs in aquatic ecosystems (Deep et al., 2013). Oscillatoria sp on aquatic environment will produce toxins such as neurotoxin, anatoxin, and hepatotoxins which cause shrimp to become off-flavour (Smith, 1996; Rodgers, 2008; and Tho et al., 2012). Oscillatoria sp and several other types of Blue Green Algae plankton can grow with nutrient-poor in pond waters because Oscillatoria sp has buoyancy and heterocyst cells that can take free nutrients from the air (Edhy et al., 2010). From the research data, it shows that the abundance of Oscillatoria sp varies during the period of shrimp culture, with an abundance of at least 0 cell/ml at the beginning of the cultivation period and the most 1.38×10^6 seen at 21 days cultured. It is possible that age there is no activity siphon and cultivation is still dependent on blind feeding system, so it is very possible to occurs nutrient imbalance in shrimp pond ecosystem. Oscillatoria sp abundance always exists from DOC 14 days until the harvest. While plankton abundance in ponds also varies from DOC 7 days to harvesting with the lowest amount of 2.5×10^3 cell/ml (Figure 4).

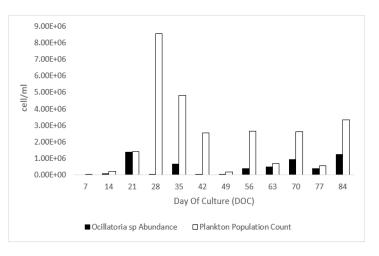


Figure 4. Plankton Populations and Existence Dynamics of Oscillatoria sp in Ponds.

Oscillatoria sp and cyanophyta are harmful algae species and mostly avoided in intensive vanname shrimp culture (Yusoff *et al.*, 2001 and Jia *et al.*, 2014). *Oscillatoria sp* and cyanophyta species that cause mud smell shrimp can grow at a ratio of N: P 1:15 or below 1:20 (Aziz *et al.*, 2015). So, it is desirable to manage N and P levels in pond waters as a limiting factor for the growth of Oscillatoria sp (Xu et al., 2010). In addition, the abundance and dominance of certain plankton type in the waters are also influenced by environmental physical-chemical factors such as temperature and dissolved oxygen (Bassat, 2008 and Palafox et al., 2010). To overcome the blooming of Oscillatoria sp or other types of Blue Green Algae, can be used chemical materials such as CuSO₄, Simazine, and potassium ricinoleate (Supono, 2015). Option other without chemical materials, by changing water, minimizing of nutrient input to the pond, and mixing through with aeration mechanism (Rodgers, 2008).

Model Interaction Between Oscillatoria sp with Vibrio Bacteria Abundance and Shrimp Growth

The model interaction between the existence of Oscillatoria sp plankton and abundance of Vibrio sp bacteria and its correlation to the shrimp growth rate of ponds can be seen in Figure 5.A. From interaction model that occurs of pond ecosystem, it can be interpreted based on the model validation results in Figure 5.B that an increase of shrimp biomass in ponds estimated at 6.45% per week will affect of aquatic tropics status levels to increased nutrient load. Where in this study estimated at 16.37% from an increase nutrient load on the pond per week. The increase nutrient load in intensive ponds is naturally caused by an increasing amount from a feed that continues to grow in line with the increase of shrimp growth rate (Thakur and Lin, 2003).

Changes of trophic status levels in pond aquaculture ecosystems will lead to dynamic conditions in some parameters of the shrimp culture indicator. In this study, it was shown that changes in the status tropic waters correlated positively with the increasing abundance of *Oscillatoria sp.* as seen in Figure 5.B *Oscillatoria sp.* is a cyanobacteria strain that is very tolerant from fluctuations of changes on aquatic tropics levels (Aliviyanti *et al.*, 2017).

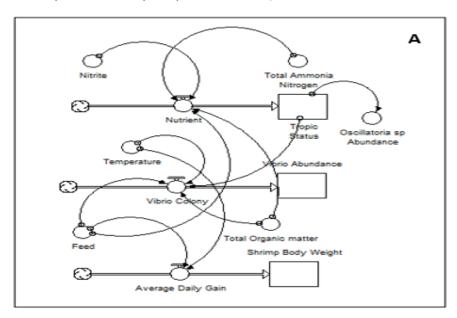
During the operational cycle of shrimp culture, the abundance of vibrio bacteria continues to increase at aggregative and temporal. Starting from the initial 3.75 weeks of shrimp culture periods there is a mild increase in the number of Vibrio populations or as much as 6.20% per week. And it increased to 12.12% per week in the last 8.25 weeks of shrimp culture periods. eventuality, an increase in vibrio abundance is caused by the longer age of shrimp culture. So that, it will produce a lot of organic material load waste on the pond ecosystems (Nimrat *et al*, 2008). As well as the dynamic fluctuations in water quality throughout the shrimp culture cycle, it also contributes to the level of pathogenicity and development of Vibrio bacteria in ponds (Kopprio *et al.*, 2017).

The Oscillatoria sp abundance can be said, that the index of growth in the pond is very closely influenced by the level of fertility on the pond ecosystem. This is in according with Pirzan and Masak (2007) who said that in natural conditions, an increase in primary productivity in pond aquaculture would be followed by an increase in the diversity of plankton such as cyanophyta and chlorophyta. The graph of the Vibrio sp bacteria increased from the results of conceptual validation models has an aggregative pattern of increased rate following the length of the shrimp culture periods. In intensive shrimp ponds with high stocking densities, Vibrio sp is often found with an average abundance between 10¹-10³ CFU/ml and depends on the condition of the pond waters (Tompo, 2016).

So that, it can be analyzed from the Vibrio sp and Oscillatoria sp abundance on intensive ponds is very much influenced by the increasing of aquaculture inputs and daily fluctuations of water quality (Figure 5.B), where the number and concentration continue to increase and dynamically respond to the aquaculture operational period. from increasing shrimp biomass and growing shrimp culture periods. In addition, the presence of Oscillatoria sp can be used as a biological parameter for the determined of status tropics in aquatic ecosystem, because accorded of biologycal analyzed, cyanobacteria strains can be used as implications for bioreporters or in situ bioindicators in aquatic environment (Mateo et al., 2015).

Oscillatoria sp is a Cyanophyta strain that is cosmopolite or easily develops and adapts

moderately to intensive ponds with pH, salinity, dissolved oxygen and varying temperatures (Utojo, 2015 and Harris, 2016). The Oscillatoria sp abundance and other types of Cyanophyta plankton will continue to tend to increase with addition age of shrimp culture periods and correlate very closely with phosphate concentration, turbidity, and salinity in ponds (Widigdo and Wardiatno, 2013). Increased diversity of Cyanophyta and Chlorophyta types of plankton which usually tends to be followed by an increase of primary productivity in pond waters. Making these conditions, sometimes will have a negative impact both ecology, economy or environmental health (Tarunamulia *et al.*, 2016).



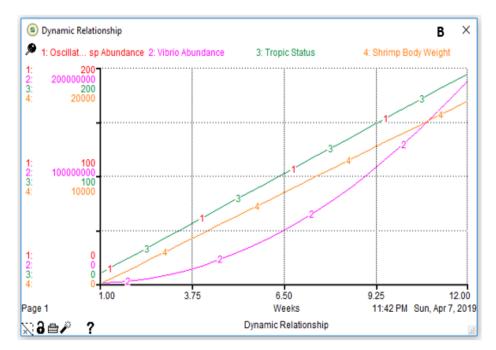


Figure 5. Model of the Relationship Between Vibrio sp, Oscillatoria sp, and Shrimp Growth by Stella[™] Ver. 9.12 (A) Model Conceptual, (B) Model Validation

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Whereas Vibrio sp is a type of gram-negative bacteria that is opportunistic pathogenic towards vannamei shrimp (Cardenas et al, 2017). Shrimp infected with clinical vibriosis is characterized by the red carapace, melanosis of the skin, necrosis on tail and hepatopancreas are dark red and lesions are found (Sarjito et al., 2015 and Han et al., 2018). The physical-chemical quality factor of poor water is the main trigger for the vibrio abundance in intensive ponds (Kharisma and Manan, 2012). Management of handling poor water quality is the main reason why the existence of vibrio is so diverse and abundant in intensive aquaculture waters (Bintari et al., 2016 and Utami et al., 2016). To overcome the level of pathogenicity vibriosis on ponds, it can be carried out both chemically, physically, and biologically according to environmental conditions, culture methods and financial capabilities (Hatmanti, 2003).

Specific actually, an increasing amount of Vibrio bacteria abundance at week 3.75 and Oscillatoria sp aggregate (Figure 5.B) can be overcome by pond habitat manipulating with maintaining the stability of C : N : P Ratio. Increased and stabilizing of levels by C : N : P Ratio will increase the growth of heterotrophic bacteria and regulate the dominance of plankton communities and then to reduce of the inorganic nitrogen concentration in ponds (Masithah et al, 2016). C:N ratio strategy is the most potential method for growing heterotrophic bacterial communities that are beneficial in aquaculture systems (Avnimelech, 1999). The optimum ratio of C : N to maintain for the stability of water quality and shrimp farming productivity is 15 (C) : 1 (N) (Panjaitan, 2011). While adjusting of N: P ratio according to the pond ecosystem will determine for type and plankton abundance that exist in ponds (Daruti et al., 2017). The ratio optimum for N : P in shrimp culture is 16 (N) : 1(P) (Redfield et al., 1963).

Conclusions and suggestions

The conclusions from the results of this study indicate that the water quality dynamic experiences fluctuating levels of concentration over 85 days shrimp culture periods of shrimp maintenance. Whereas, the relationship between the existence and abundance of *Oscillatoria sp* and vibrio bacteria in ponds is strongly influenced by the longer age shrimp culture periods.

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