The abundance of *Vibrio* sp. bacteria on *lithopenaeus vannamei* grow out - pond in CV. Lautan Sumber Rejeki Banyuwangi

A Asmarany¹,², S Jayanti¹, N U Mahbubah¹

¹Marine and Fisheries Polytechnic of Sidoarjo, Department of fish pathology handling technique, Sidoarjo 61253, East Java, Indonesia

²Corresponding author: anja.asmarany17@gmail.com

**Abstract.** Vibriosis is one of the bacterial diseases that caused by a group of *Vibrio* bacteria. The total abundance of *Vibrio* bacteria that exceed the normal threshold can cause the mass death in vannamei shrimp (*Litopenaeus vannamei*) aquaculture. The study aimed to analyze the abundance of *Vibrio* sp. in vannamei shrimp rearing ponds. The abundance of *Vibrio* bacteria was analyzed using total plate count agar methode on TCBS and CHROMagar *Vibrio* selective media. Histopathological analysis was also carried out to determine the effect of *Vibrio* sp. abundance to hepatopancreas organ damage in vannamei shrimp. Three species of *Vibrio* bacteria founded in C1, C2 and C3 ponds and identified as *V. parahaemolyticus*, *V. vulnificus* and *V. alginolyticus*. The total abundance of *Vibrio* bacteria in the C3 pond at 77 days of culture was higher than C2 and C3 ponds i.e 2.75 x 10² CFU/mL with 66,67% of hepatopancreas organ damage. *Vibrio* bacteria abundance in aquaculture ponds affects the percentage of shrimp hepatopancreas damage.

1. **Introduction**

Vannamei shrimp (*Litopenaeus vannamei*) is an alternative aquaculture commodity besides tiger shrimp (*Penaeus monodon*) and white shrimp (*Panaeus merguensis*) with promising business prospects, so it is widely cultivated in Indonesia. Vannamei shrimp has been officially introduced in Indonesia since 2001 due to a decrease in the quality and production of tiger prawns [14,16]. Vannamei shrimp has several advantages including being more resistant to disease, can live in a wide salinity range, high survival rate, faster growth with relatively short enlargement time and can be cultivated with high stocking density [15,17].

Vannamei shrimp aquaculture with high stocking rates must be accompanied by proper management of feed and water quality. Water quality conditions will affect the survival rate of shrimp because it can trigger disease attacks. Vibriosis is a disease that often attacks vannamei shrimp and can cause mass death. Vibriosis is caused by infection with the *Vibrio* bacteria. Several species of vibrio bacteria that attack shrimp are *V. alginolyticus*, *V. campbellii*, *V. Penaeicida*, *V. vulnificus*, *V. damsela*, *V. parahaemolyticus*, and *V. Harveyi* [13,20,24]. Clinical symptoms that appear in shrimp infected by vibriosis include red intestine, stomach, uropods, and telopods, hepatopancreas and brownish red images, peeling hepatopancreas epithelial cells, tubular epithelial necrosis and hemocytic infiltration [24].

The presence of vibrio bacteria can be influenced by conditions of water parameters such as temperature, pH, salinity, dissolved oxygen, and nutrient content in aquaculture [13,24]. The abundance...
of vibrio bacteria in aquaculture with an amount exceeding $10^4$ CFU/mL causes White Feces Disease (WFD), vibriosis and mass mortality in shrimp [19]. This study aims to determine the abundance of bacteria in vannamei shrimp enlargement ponds in CV. Lautan Sumber Rejeki Banyuwangi, East Java. Analysis of Vibrio bacteria abundance in vannamei shrimp enlargement activities is expected to prevent vibriosis disease attacks and increase the productivity of vannamei shrimp aquaculture.

2. Materials and methods
This research was conducted on vannamei shrimp grow out – pond in CV. Lautan Sumber Rejeki Banyuwangi, East Java, which has an area of 7 hectares with a total of 42 ponds. The average area of each pond is 4,547 m$^2$. The type of cultured shrimp is vannamei (Litopenaeus vannamei) with the observation time being done when the shrimp at 56-91 days of age.

2.1. Water Sampling
Water sampling was carried out on C1, C2 and C3 ponds. Water was taken using sterilized glass bottles. The sample bottle is immersed in the pond plot with the bottle neck tilted downwards. The sample bottle is immersed to a depth of ± 20 cm.

2.2. Isolation of Vibrio sp. Bacteria
Isolation of Vibrio sp. performed using selective media Water samples from C1, C2 and C3 ponds were diluted to $10^{-2}$ and grown as much as 100 L on 8.8% TCBS media and 7.47% CHROMagar Vibrio. Bacterial culture for each pond was carried out in duplicate. Bacterial cultures were incubated in an incubator at 30 °C for 24 hours. After 24 hours, observations were made on the morphology of the colonies growing on the media for the identification and calculation of the number of bacterial colonies.

2.3. Total Abundance of Vibrio sp. Bacteria
The method that used to count the number of Vibrio sp. is the Total Plate Count (TPC) method which refers to the 2006 Indonesian National Standard regarding determination of total plate number in fishery products. This method counts the number of colonies of Vibrio sp. grown on selective media. The number of colonies growing on the selective media was recorded at each level of dilution. The formula that used in calculating the total plate number is as follows [21].

$$N = \frac{\sum C}{[(1 \times n1) + (0,1 \times n2)] \times (d)}$$

- $N$ = Number of product colonies
- $C$ = Number of colonies in each cup that calculated
- $n1$ = Number of cups in the first dilution that calculated
- $n2$ = Number of cups in the second dilution that calculated
- $d$ = The first dilution that calculated

2.4. Water Quality Measurement
Water quality testing was carried out by checking the parameter values of pH (pH meter), temperature (thermometer), nitrite (spectrophotometer), and total organic matter (spectrophotometer) on water samples from C1, C2 and C3 pond. Parameters of pH, nitrite and ammonium were measured once a week, while temperature and salinity were measured 2 times in 1 week.

2.5. Microscopic Examination of the Shrimp Hepatopancreas
Examination of the shrimp hepatopancreas was carried out microscopically to determine the level of shrimp hepatopancreas damage at 56-91 days of culture. A total of 0.1 grams of hepatopancreas was included in 0.9 mL of NaCl solution. The sample was homogenized slowly, then taken and dropped on a glass object to be observed microscopically. The percentage (%) of hepatopancreas histological damage was analyzed using the equation (2).
Total of Damage (100%) = U x (n)LP x maximum score

U = number of repetitions
(n) LP = number of field view repetition

3. Result and Discussion

The results of the bacterial colonies identification isolated from water samples on C1, C2 and C3 ponds showed that there were 3 colonies with different morphology on TCBS agar media, namely green, yellow, and translucent colonies. The yellow colonies were suspected to be *V. alginolyticus*, green colonies were suspected to be *V. parahaemolyticus* and translucent colonies were suspected to be *V. vulnificus* (Figure. 1a). *V. parahaemolyticus*, *V. vulnificus* and *V. alginolyticus* are a group of opportunistic bacteria that cause vibriosis in shrimp [4,24].

![Figure 1. Bacterial colonies on (a) TCBS media and (b) CHROMagar Vibrio media](image)

Identification of vibrio bacterial colonies on TCBS agar media still requires further confirmation using CHROMagar *Vibrio* media so that the results obtained are more accurate. Colonies of *V. parahaemolyticus* on TCBS media were visually difficult to distinguish from other bacterial colonies because the bacteria were covered by a yellow color produced by bacteria that ferment sucrose, especially *V. alginolyticus* [2,6,10]. Confirmation of vibrio bacterial species using CHROMagar Vibrio showed that there were 3 different color colonies growing on the media. Visually, the colony morphology of *V. parahaemolyticus* was mauve, *V. vulnificus* was blue-green and *V. alginolyticus* was cream (Figure. 1b). CHROMagar Vibrio media is widely used in the process of identifying groups of vibrio bacteria to distinguish between species of bacteria *V. parahaemolyticus* and other viral bacteria such as *V. alginolyticus*, *V. vulnificus* and *V. cholerae*. Media CHROMagar Vibrio contains chromogenic which is a specific substrate of the enzymes -glucosidase and -galactosidase. These two enzymes are only possessed by the bacterium *V. parahaemolyticus* [6,10,23]. When compared with TCBS agar media, CHROMagar Vibrio media has a specificity level of 95% and an accuracy of 88% [2].

3.1. Vibrio Bacteria Abundance

Vibrio is a normal bacterial flora in shrimp farming activities and will become an opportunistic pathogen when shrimp experience stress due to poor water quality or lack of nutrients [9]. The total abundance of vibrio bacteria in the three pond plots fluctuated during the rearing period of 56-91 days old shrimp. The C1 pond had a total abundance of Vibrio bacteria with the highest number of $2.75 \times 10^2$ CFU/mL at the 77 days of culture, while the lowest number of vibrio was found in the C2 pond is $0.26 \times 10^2$ CFU/mL (Figure. 2). The total vibrio bacteria in all pond met the water quality standards for shrimp aquaculture. Based on SNI 7772: 2013, the safe limit for total vibrio bacteria in shrimp aquaculture is $10^3$ CFU/mL. Vibrio bacteria will be pathogenic if their abundance in maintenance water reaches $8.35 \times 10^4$ CFU/mL or more [8]. When the population of vibrio bacteria is higher than the number of other bacteria, it can reduce the survival of shrimp during the stocking and grow-out period [26].
3.2. Water Quality Analysis

In aquaculture, environmental parameters affect the distribution of bacteria in the water. Parameters that affect the population of *Vibrio* bacteria in water include, temperature, pH, salinity, organic matter, ammonia, dissolved oxygen, and total nitrogen content [3,9,25,26]. Pond water quality are also closely related to shrimp health condition.

The temperature parameters in ponds C1, C2 and C3 were still in optimum conditions for shrimp growth with values ranging from 31 to 32 °C (Figure 3). The highest temperature occurred in the C2 pond at 84 days of culture, which was 32.2 °C. Temperature is a chemical factor that is difficult to control because it is influenced by location and weather [18]. Vannamei shrimp will grow in optimal
conditions at temperatures ranging from 28 – 33 °C, while the temperature value that can affect the growth of *Vibrio* sp. which is at temperature ranging from 20 - 40 °C [21].

pH fluctuation in aquaculture can affect shrimp growth. The optimal pH value for the growth of vannamie shrimp is between 7.5–8.5, pH value in the C1, C2 and C3 pond indicate that the results are still include in the optimum value range. The highest pH value occurred in the C3 pond at 56 days of culture, which was 8.35.

![Figure 4. Nitrite and ammonium parameter in C1, C2 and C3 ponds](image)

High levels of nitrite and ammonium compounds in water can cause shrimp stress. It will make the shrimp more susceptible to *Vibrio* attack [7]. The optimum value of nitrite (NO$_2$^-) for survival and growth in shrimp is < 0.45 mg/L and good nitrite content is < 0.01 mg/L [5,21]. The content of organic matter in the water can affect the amount of nitrite (NO$_2$^-) which can then be toxic to shrimp. The nitrite values in the C1, C2 and C3 ponds were known to exceed the standard value of optimum nitrite content in water, the highest nitrite value came from the C1 pond when DOC 84 was 0.411 mg/L while the lowest nitrite value came from the C2 pond when the DOC 70 was 0.039 mg/L.

The highest ammonium concentration was found in the C1 pool, which was 1.8 mg/L at 70 – 77 days of culture. Ammonium concentration in the water correlated with the total abundance of vibrio bacteria. Pond C1 has a total abundance of vibrio bacteria with the highest number compared to other ponds, which is 2.75 x 10$^2$ CFU/mL at the 77 days of culture. Ammonia stress can decrease the vannamie shrimp immune response to *V. alginolyticus* bacteria such as decreased phagocytic activity and production of pentaedine antimicrobial compounds [11]

3.3. Hepatopancreas Damage Analysis

From microscopic observations of the hepatopancreas organ of shrimp located in the pond plots C1, C2 and C3 at 56, 63, 70, 77, 84 and 91 days of culture, it was found that damage to hepatopancreas cells in the form of cell rupture, some damage to the cell nucleus, organ tissue structure hepatopancreas looks loose and there is a cavity (Figure 5).
On examination of the total percentage of microscopic hepatopancreas damage from shrimp on C1, C2 and C3 ponds, it was found that the highest damage to the hepatopancreas organ microscopically came from shrimp in C1 pond in DOC aged 77 days that was 66, 67% and the percentage of damage to hepatopancreas the lowest came from shrimp in the C3 pond at the DOC aged 56 days, which was 10% (Table 1)

**Table 1. Average Total Percentage of Shrimp’s Hepatopancreas Damage**

| Days of Culture (DOC) | Pond | Total average of hepatopancreas damage | % Total average of hepatopancreas damage |
|-----------------------|------|----------------------------------------|----------------------------------------|
| 56                    | C1   | 2                                      | 33,33                                  |
|                       | C3   | 1.2                                    | 20,00                                  |
|                       | C2   | 1.4                                    | 23,33                                  |
| 63                    | C1   | 2.6                                    | 43,33                                  |
|                       | C3   | 0.6                                    | 10,00                                  |
|                       | C2   | 1.6                                    | 26,67                                  |
| 70                    | C1   | 2.8                                    | 46,67                                  |
|                       | C3   | 3.2                                    | 53,33                                  |
|                       | C2   | 2.4                                    | 40,00                                  |
| 77                    | C1   | 4                                      | 66,67                                  |
|                       | C3   | 2.8                                    | 46,67                                  |
|                       | C2   | 2.2                                    | 36,67                                  |
| 84                    | C1   | 3.2                                    | 53,33                                  |
|                       | C3   | 3.6                                    | 60,00                                  |
|                       | C2   | 2.4                                    | 40,00                                  |
| 91                    | C1   | 3.4                                    | 56,67                                  |
|                       | C3   | 3.6                                    | 60,00                                  |
|                       | C2   | 2.6                                    | 43,33                                  |

Hepatopancreas damage can be caused by infection with *V. parahaemolyticus* bacteria, but these bacteria exist as strains that cause AHPND and non-AHPND [1]. Damage to the hepatopancreas of shrimp infected with AHPND is mediated by receptors owned by *Vibrio parahaemolyticus strain* AHPND, namely LvAPN1 which is involved in the pathogenesis of AHPND and acts as a toxin receptor that causes damage to the hepatopancreas organ [12]. The bacteria initially colonize in the stomach, where they begin to produce the PirABvp toxin. At the initial time point of infection with *Vibrio parahaemolyticus* strain AHPND PirBvp toxin can cause cell damage, then at a later time point, the PirAvp and PirBvp bacteria and toxins are detected in the [1].
4. Conclusion
Total abundance of vibrio bacteria increased along with the growth period of the shrimp. Water quality factors can affect the susceptibility of shrimp to attack by vibrio bacteria. The results showed that the total abundance of vibrio bacteria was directly proportional to the percentage of hepatopancreas damage in shrimp.

5. References
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