The correlation between ectoparasite infestation and total Vibrio parahaemolyticus bacteria in Pacific white shrimp (Litopenaeus vannamei) in Super Intensive Ponds

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The correlation between ectoparasite infestation and total *Vibrio parahaemolyticus* bacteria in Pacific white shrimp *(Litopenaeus vannamei)* in Super Intensive Ponds

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Abstract. This study aimed to determine the type, intensity, degree of ectoparasite infestation and its correlation to the total *V. parahaemolyticus* bacteria in super-intensive pacific white shrimp culture. In this study, sampling was carried out in 3 super-intensive pacific white shrimp pond areas spread out in East Java, namely Bangil, Tuban, and Lamongan with 50 shrimps (PL30-PL 40). The obtained data underwent regression and correlation analysis. Based on the results, there were three types of ectoparasites, namely *Zoothamnium* sp., *Epistylis* sp. and *Vorticella* sp. High ectoparasite intensity was found in pacific white shrimp from Lamongan and Tuban ponds, namely 76 and 55 individuals/shrimp, respectively, showing the heavy infestation. High total V. parahaemolyticus bacteria was found in Tuban (1.16×10^5 CFU/gr) and Lamongan (1.16×10^5 CFU / gr) ponds. Based on the results, the coefficient value was $R = 0.807$ showing positive correlation of *V. parahaemolyticus* with the increasing parasite intensity and low oxygen levels.

1. Introduction

Pacific white shrimp *(Litopenaeus vannamei)* is one of the potential crustacean species to be developed to industrialize the aquaculture sector around the world [1]. Pacific white shrimp is one of the highest economic value commodities and is most widely cultivated by contributing more than 70% of shrimp production worldwide [2]. A report from the Food and Agriculture Organization (FAO) states that the increase in consumption of Pacific white shrimp has an impact on world shrimp production. Based on data from the Badan Pusat Statistik (BPS) processed by the Directorate General of PDS-KKP, shrimp production in Indonesia in early 2019 was able to generate USD 1462.09 million or around 46.87% of the total Indonesian fishery product exports.

However, the development of uncontrolled cultivation system can increase shrimp disease by pathogenic bacteria and viruses [3–5]. The super-intensive system (±150 fish / m^2 density) with a high level of feed consumption can increase particulate organic matter and microorganism population [6]. Uncontrolled microorganism population can take up an abundance of oxygen in the water to maintain metabolic activity during the breakdown of organic matter [7]. Decreased dissolved oxygen can cause aquatic animals to be in hypoxic or even anoxic conditions due to organism respiration and decomposition of food waste and feces ultimately impacting oxygen competition between shrimp and microorganisms [8].

The aquaculture pond intensification increases the accumulation of organic matter, competition for feed, oxygen, and a place to live which ultimately decreasing shrimp immunity and survival rates [9]. Therefore, this can cause pacific white shrimp cultivation failure due to Vibriosis [10]. Vibriosis is caused by bacteria from the genus Vibrio and is the main bacteria causing disease in shrimp [11]. Vibriosis in shrimp can occur suddenly and spread rapidly over a few days to 2 weeks [12]. Previous studies showed that *Vibrio parahaemolyticus* causes mass death in young to juvenile shrimp [13]. Similar incident also occurred in shrimp ponds in Thailand lossing approximately 26 million dollars in 2015 due to *Acute hepatopancreatic necrosis disease* (AHPND) or *Early Mortality Syndrome* (EMS) caused by the pathogenic organism *V.parahaemolyticus* [14].

Apart from vibriosis, poor water quality is an ideal condition causing disease such as parasites in aquatic animals [15]. The high ectoparasite number on pacific white shrimp in super-intensive culture system are *Zoothamnium, Epistylis*, and *Vorticella*. These parasites prefer a low oxygen content cultivation environment (<3 ppm) with a high organic matter accumulation [16].

This study was conducted in super intensive pacific white shrimp ponds in 3 cities namely Bangil, Tuban, and Lamongan, East Java. The locations were determined due to the incidence of ectoparasite infestation and their position as Pacific white shrimp cultivation center area in East Java Province [17]. Based on the description above, this study aimed to determine the correlation between ectoparasite infestation intensity and total *V.parahaemolyticus* bacteria in pacific white shrimp cultivated in super-intensive ponds. This study is expected to be an informative reference regarding environmental health management for aquaculture, especially for shrimp cultivators, related fisheries practitioners, and the marine and fisheries office, especially in East Java.

2. Materials and methods

2.1 Materials

The equipments used for water quality measurement were plastic tub, anco, pH pen, thermometer, DO meter, refractometer, and ammonia test kit. The tools used for ectoparasite examination of pacific white shrimp were sectio, scalpel, binocular microscope, object-glass, cover glass, pipette, label paper, tissue, camera, and stationery. The material used in this study was pacific white shrimp with PL-30 to PL-40 post larva age. The materials used for identification of *Vibrio* bacteria with Total Plate Count (TPC) were distilled water, Plate Count Agar (SigmaAldrich,USA), Trypticase Soy Agar (TSA) (SigmaAldrich,USA), 2.5% NaCl (SigmaAldrich, USA), Tryptic Soy Broth (TSB) (SigmaAldrich, USA), Thiosulfate-citrate-bile salts- media sucrose agar (TCBS) (SigmaAldrich, USA), and 70% alcohol.

2.2. Method

Sampling was carried out in three (3) locations of pacific white shrimp cultivation centers, namely Bangil, Tuban, and Lamongan, East Java. Sampling was carried out at several points in the pond randomly on 50 shrimps with 2 replications.

Ectoparasite examination of shrimp was carried out using the native method on the swimmerets, walking legs, tails, and gills. The target part was placed on the glass object, added with little physiological NaCl, then covered with a glass cover. The sample was observed using a microscope of 100x and 400x magnification [17].

The parameters observed were 1) ectoparasite identification, 2) ectoparasite infestation degree determination, 3) ectoparasite intensity calculation, 4) V. parahaemolyticus Total Plate Count (TPC) calculation and 5) water quality examination including dissolved oxygen (DO), ammonia levels, temperature, pH, salinity, nitrates, and nitrites.

Infestation degree describes the severity caused by ectoparasite infestation. The infestation degree can be divided into 3 categories, namely 1) mild (5-25 zooids), 2) moderate (26-50), and 3) heavy (> 50 zooids) [16] based on Dyer [18] (Table 1).

Table 1. Parasite infestation category.

Intensity	Category
<1	Very Mild
1-5	Mild
6-55	Moderate
51-100	Heavy
>100	Awful
>1,000	Super infestation

The ectoparasite infestation (individual/shrimp) refers to the following formula:

$$Intensity = \frac{\Sigma \text{parasite found}}{\Sigma infested\ shrimp} \quad (1)$$

The TPC testing process used *Plate Count Agar* (PCA) as a medium for planting 1 gram of thawed sample into a Petri dish for later incubation. The bacterial morphology observation was carried out using the gram stain technique under a microscope with 1,000x magnification. Furthermore, *V. parahaemolyticus* colonies can be seen based on the appearance of "green or turquoise" color on the TCBS agar medium as a "selective medium" [19].

Water quality were measured and analyzed based on dissolved oxygen with a DO meter, temperature with a thermometer, salinity with a refractometer, acidity level (pH) with a pH meter, and ammonia levels with an ammonia test kit.

2.3. Data analysis
The water quality measurement was presented in table form. The data obtained were processed using Analysis of Variance (ANOVA) (SPSS 23.0, IBM USA). The relationship between parameters were analyzed using regression and correlation analysis. The coefficient (R) approaches 1 meaning higher relationship.

3. Result and discussion

3.1 Ectoparasites
Ectoparasites found were *Zoothamnium, Vorticella*, and *Epistylis* mild, moderate, and severe infestation degree, respectively. These ectoparasites were found in the walking legs, swimmerets, tail, and gills of pacific white shrimp. The identifying process refers to Lynn in Mahasri et al. [20]. In this study, *Zoothamnium* has an oval shape and colonies, whitish color, and branched. *Vorticella* is ciliated, unbranched, solitary, and bell-like. *Epistylis* resembles Vorticella but colonized. These ectoparasites also have cilia for moving, but the non-contractile branches [12].

3.2 Infestation degree and ectoparasite intensity
The infestation degree shows the number of parasites infesting Pacific white shrimp [15]. Based on Table 2, it can be seen that ectoparasite infestations in Bangil ponds were in the "moderate" and "mild" categories. On 50 samples examined, the intensity values were 39 (moderate infestation) and 6 (mild infestation). Meanwhile, in Lamongan ponds, the infestation was "moderate" and "heavy" with intensity values of 27 and 76 respectively. Meanwhile, the highest infestation degree was found in the Tuban ponds with the intensity values of "low" (8) and "heavy" (55). Heavy or mild intensity and degree of ectoparasite infestation are closely related to stocking density and organic matter in the waters. The high stocking density can increase the disease spread due to lesions/wounds of shrimp body to spread horizontally.

Table 2. Results of ectoparasite examination, intensity, and infestation degree.

Location	N samples	Result	Intensity (Individual /shrimp)	Infestation degree
Pond 1	50	Positive : *Zoothamnium*, *Epistylis* & *Vorticella*	39	Moderate
Pond 2	50	Positive : *Zoothamnium*, *Epistylis* & *Vorticella*	6	Mild
Pond 3	50	Positive : *Zoothamnium*, *Epistylis* & *Vorticella*	27	Moderate
Pond 4	50	Positive : *Zoothamnium*, *Epistylis* & *Vorticella*	76	Heavy
Pond 5	50	Positive: *Zoothamnium*, *Epistylis* & *Vorticella*	8	Mild
Pond 6	50	Positive: *Zoothamnium*, *Epistylis* & *Vorticella*	55	Heavy

3.3 Total Plate Counts (TPC)

The *V.parahaemolyticus* examination results are presented in Table 3 showing the total bacteria of Pacific white shrimp. Based on the TPC calculation, the highest number of *V. parahaemolyticus* was found in Lamongan and Tuban Regencies, namely 1.16 × 10^5 CFU/gr. In these ponds, the high ectoparasite intensity, the heavy infestation degree, and the low dissolved oxygen (DO) were found. Meanwhile, *V. parahaemolyticus* from Bangil pond was lower than the two regencies, namely 1.3 × 10^4 CFU / gr and 0.38 × 10^3 CFU/gr. The examination results are shown in Tables 2 and 3.

Table 3. Abundance of *Vibrio parahaemolyticus*.

Location	N samples	Result	TPC (CFU/gr)
Pond 1	6	Negative *V. parahaemolyticus*	1.3 x 10^4
Pond 2	6	Negative *V. parahaemolyticus*	0.38 x 10^3
Pond 3	6	Negative *V. parahaemolyticus*	1.3 x 10^4
Pond 4	6	Positive *V. parahaemolyticus*	1.16 x 10^5
Pond 5	6	Negative *V. parahaemolyticus*	0.3 x 10^3
Pond 6	6	Positive *V. parahaemolyticus*	1.16 x 10^5

High amount of feed increases particulate organic matter and ammonia levels which tend to be toxic in aquaculture waters. In addition, the organic matter decomposition from requires oxygen, so that the continuous addition of organic material can decrease dissolved oxygen in the ponds. This is very dangerous because oxygen is the main requirement for metabolism, supporting growth, and forming an immune system for pacific white shrimp. Therefore, feed management is suggested to control this situation as in line with a report from Jayanthi [6] that the decreasing water condition can cause physiological stress and reduce shrimp immunity to disease attacks.

3.4 Water quality

Based on the water quality measurement (Table 4) in Bangil, Lamongan, and Tuban, there were several water parameters below standard, especially dissolved oxygen (DO) levels. The lowest oxygen levels were found in Lamongan and Tuban ponds with < 4 ppm oxygen level, while Bangil pond had ideal condition.

Table 4. Water quality in super intensive pacific white shrimp ponds in East Java.

Ponds	DO (mg/L)	Temperature (ºC)	pH	Salinity (ppt)	Nitrate (mg/L)	Nitrite (mg/L)	Ammonia (mg/L)
1	4.2	29	7.5	17	15	0.3	0.5
2	4.1	29	7.2	17	10	0.5	0.5
3	4.2	28.5	7	17	15	0.2	0.5
4	3.8	29	6.8	19	20	1.0	1.2
5	4.0	28.5	7.5	17	15	0.5	0.5
6	3.7	29	6.8	18	20	1.2	1.0

Dissolved oxygen (DO) is a very important parameter of water quality due to its relation to the survival, health, and growth rate of pacific white shrimp [8]. The minimum oxygen solubility to support shrimp life is around 4–8 ppm.

3.5 Regression analysis

Based on the regression and correlation analysis, a strong relationship was found between the infestation degree and the total *V. parahaemolyticus* with R correlation coefficient value of 0.807 (Figure 1).

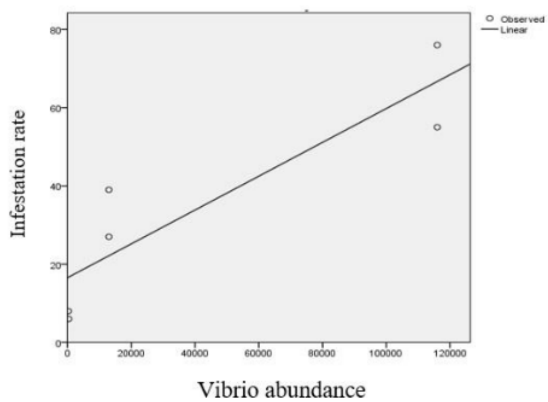

Figure 1. Regression curve of ectoparasite infestation level with *V. parahaemolyticus* abundance.

The maximum limit of Vibrio bacteria in shrimp ponds is around 10^4 CFU / ml [21]. The total bacteria of Tuban and Lamongan ponds (10^5 CFU/gr) was higher than Bangil ponds (10^4 CFU / gr). There was a relevant relationship between high intensity and infestation degree, low dissolved oxygen, and high Vibrio bacteria. This relationship was strengthened by the results of regression and correlation analysis. The higher the ectoparasite infestation, the higher the potential to increase Vibrio triggering vibriosis disease. Vibriosis disease can have a serious impact, especially the productivity level. The vibriosis varies according to attack intensity, shrimp resistance, and rearing water. Understanding the biological characteristics of shrimp, the amount and frequency of effective feeding need to be performed so that the shrimp can be utilized properly. In addition, it can be important so as not to add to the pile of organic matter which actually worsens the condition of pond waters by implementing this system.

4. **Conclusion**

The ectoparasites found in Pacific white shrimp, *Litopenaeus vannamei* with a super intensive system were *Zoothamnium, Epistylis, and Vorticella* with the infestation degree respectively mild to moderate in Bangil, Tuban, and Lamongan. Regression and correlation analysis showed close relationship between the ectoparasite infestation degree with the abundance of *V. parahaemolyticus* and the decrease of dissolved oxygen (DO). The authors recommended to maintain the quality of pond waters, to control the feed frequency, and disease management to maintain productivity. Further studies on the abundance of plankton (*Phyto / Zooplankton*) are needed because it is closely related to the occurrence of toxic algae blooming in aquaculture waters.

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CLAIM

Take an arguable position on the scientific topic and develop the essay around that stance.

ADVANCED	The essay introduces a precise, qualitative and/or quantitative claim based on the scientific topic or text(s), regarding the relationship between dependent and independent variables. The essay develops the claim and counterclaim fairly, distinguishing the claim from alternate or opposing claims.
PROFICIENT	The essay introduces a clear, qualitative and/or quantitative claim based on the scientific topic or text(s), regarding the relationship between dependent and independent variables. The essay effectively acknowledges and distinguishes the claim from alternate or opposing claims.
DEVELOPING	The essay attempts to introduce a qualitative and/or quantitative claim, based on the scientific topic or text(s), but it may be somewhat unclear or not maintained throughout the essay. The essay may not clearly acknowledge or distinguish the claim from alternate or opposing claims.
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EVIDENCE

Include relevant facts, definitions, and examples to back up the claim.

ADVANCED	The essay supplies sufficient relevant, accurate qualitative and/or quantitative data and evidence related to the scientific topic or text(s) to support its claim and counterclaim.
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DEVELOPING	The essay supplies some qualitative and/or quantitative data and evidence, but it may not be closely related to the scientific topic or text(s), or the support that is offered relies mostly on summary of the source(s), thereby not effectively supporting the essay's claim and counterclaim.
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REASONING

Explain how or why each piece of evidence supports the claim.

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PROFICIENT	The essay applies scientific reasoning in order to explain how or why the cited evidence supports the claim. The essay demonstrates logical reasoning and understanding of the scientific topic and/or text(s). The essay's explanations attempt to anticipate the audience's knowledge level and concerns about this scientific topic.
DEVELOPING	The essay includes some reasoning and understanding of the scientific topic and/or text(s), but it does not effectively apply scientific ideas or principles to explain how or why the evidence supports the claim.
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Focus your writing on the prompt and task.

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ORGANIZATION

Organize your writing in a logical sequence.

ADVANCED	The essay incorporates an organizational structure throughout that establishes clear relationships among the claim(s), counterclaims, reasons, and evidence. Effective transitional words and phrases are included to clarify the relationships between and among ideas (i.e. claim and reasons, reasons and evidence, claim and counterclaim) in a way that strengthens the argument. The essay includes an introduction and conclusion that effectively follows from and supports the argument presented.
PROFICIENT	The essay incorporates an organizational structure with clear transitional words and phrases that show the relationship between and among ideas. The essay includes a progression of ideas from beginning to end, including an introduction and concluding statement or section that follows from and supports the argument presented.
DEVELOPING	The essay uses a basic organizational structure and minimal transitional words and phrases, though relationships between and among ideas are not consistently

	clear. The essay moves from beginning to end; however, an introduction and/or conclusion may not be clearly evident.
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LANGUAGE

Pay close attention to your tone, style, word choice, and sentence structure when writing.

ADVANCED	The essay effectively establishes and maintains a formal style and objective tone and incorporates language that anticipates the reader's knowledge level and concerns. The essay consistently demonstrates a clear command of conventions, while also employing discipline-specific word choices and varied sentence structure.
PROFICIENT	The essay generally establishes and maintains a formal style with few possible exceptions and incorporates language that anticipates the reader's knowledge level and concerns. The essay demonstrates a general command of conventions, while also employing discipline-specific word choices and some variety in sentence structure.
DEVELOPING	The essay does not maintain a formal style consistently and incorporates language that may not show an awareness of the reader's knowledge or concerns. The essay may contain errors in conventions that interfere with meaning. Some attempts at discipline-specific word choices are made, and sentence structure may not vary often.
EMERGING	The essay employs language that is inappropriate for the audience and is not formal in style. The essay may contain pervasive errors in conventions that interfere with meaning, word choice is not discipline-specific, and sentence structures are simplistic and unvaried.