Virulence of *Vibrio* sp. on whiteleg shrimp (*Litopenaeus vannamei*) in different salinity ranges

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Abstract. Whiteleg shrimp farming activities frequently face several challenges, including fluctuations in water quality, which can cause disease outbreaks when optimal conditions are not met. This study aims to determine the effect of salinity on the virulence of *Vibrio* sp. on whiteleg shrimp. The test animals used were prawns with pathogen-free certificates reared in water with different salinity concentrations. There were six treatments with three replications: A - 10 mg L^{-1} salinity; B - 15 mg L^{-1} salinity; C - 20 mg L^{-1} salinity; D - 25 mg L^{-1} salinity; E - 30 mg L^{-1} salinity; F - no bacterial infection. Before use, both the containers and the water were sterilized. *Vibrio* sp. was obtained from the Laboratory of Systems and Technology, Department of Aquaculture, Khairun University, Indonesia. Bacteria at a concentration of 10^6 cells mL^{-1} innoculated healthy whiteleg shrimp by immersion. The shrimp were observed for 33 hours. The results showed that salinity greatly affects the virulence of the *Vibrio* sp. in whiteleg shrimp farming. The highest virulence level of salinity occurred at 15 mg L^{-1} salinity.
Key Words: salinity condition, shrimp disease, vibriosis, water quality.

Introduction. Whiteleg shrimp (*Litopenaeus vannamei*) is a commodity with high economic value and an aquaculture sector export product. In recent decades, the yield of whiteleg shrimp aquaculture production has been the highest in the crustacean category (FAO 2020).

Efforts to increase aquaculture production frequently face several challenges, including fluctuations in water quality (Pietoyo et al 2016). Out of the water quality parameters, salinity influences disease incidence because it correlates with bacteria ability to cause disease (Feliatra et al 2019). One of the diseases that attack shrimp is vibriosis caused by *Vibrio* sp. In the cultivation system of whiteleg shrimp, *Vibrio* sp. was reported as a cause of acute hepatopancreatic necrosis disease (Kewcharoen & Srisapoome 2019).

Salinity fluctuations are one of the issues that can lead to disease outbreaks. Salinity is known to be closely related to osmotic pressure adjustment, survival, distribution, and growth of aquatic organisms. Whiteleg shrimp can live in a salinity range of 2-45 mg L^{-1}, but grow best in a salinity range of 20-30 mg L^{-1} (Pazir et al 2020). In the whit eleg shrimp culture system, salinity is an important factor that needs to be considered. The salinity of whiteleg shrimp culture ponds is recommended to be maintained at the optimal range for preventing *Vibrio* bacterial infection. This bacterial infection is reported to be higher at low salinity conditions (Li et al 2010). This study aims to determine the effect of salinity on the virulence of *Vibrio* sp. in whiteleg shrimp.

Material and Method. Whiteleg shrimp fry weighing an average of 0.11 g were used as test animals. The fry was purchased from PT Benur Kita in South Sulawesi and have been reared for 30 days at the Wet Laboratory, Faculty of Fisheries and Marine Affairs, Khairun University, Indonesia. During the study, feed (Feng Li Gold - Starter 1) was administered 4 times a day at a rate of 15% of the total biomass.

The tanks used were 16 plastic containers (5 L each), each tank with 15 whiteleg shrimp. The water was placed in containers based on the salinity treatment with a volume of 2 L per container and aerated. The salinity concentration in each treatment was determined using a dilution formula:

$$M_1.V_1 = M_2.V_2$$

Where: M1 - initial liquid concentration; M2 - final liquid concentration after dilution; V1- initial water volume; V2 - final water volume after dilution.

The *Vibrio* sp. strains used in this study came from the Laboratory of Systems and Technology of Aquaculture Study Program, Khairun University, Indonesia. Bacteria at a concentration of 10^6 cells mL^{-1} were prepared. One loop of bacteria was first cultured on TCBSA media for 18 - 24 h and incubated at 30°C to obtain the concentration of bacteria according to the treatment.

The test bacteria grown on TCBSA media were inoculated into 200 mL of nutrient broth (NB) liquid culture medium, in five loops. It was then incubated for 24 h at 110 rpm in a shaker incubator. The yield of bacterial growth on NB media was then collected in 50 mL increments and centrifuged at 4000 rpm for 10 min in an Eppendorf tube. The SS solution was used to wash the bacterial cells 3 times.

After obtaining the desired concentration of bacteria, healthy whiteleg shrimp were infected with bacteria by the immersion system. Observations were performed for 33 h. At every 1 h interval, the number of whiteleg shrimp mortalities was determined. Clinical symptoms that showed morphological changes (of the hepatopancreas, swimming and walking legs, and tail) and total mortality were observed. The survival rate was calculated with the formula:

$$S = \frac{Mt}{Mo} x\ 100\%$$

Where: S - survival (%); Mt - number of live shrimp at the end of the study; Mo - number of live shrimp at the beginning of the study.

During the study, water quality conditions were monitored, by determining salinity (mg L^{-1}), temperature (°C), pH and dissolved oxygen (mg L^{-1}) with a multi-parameter water quality checker (Horiba U-50). A completely randomized design (CRD) was used, which included six treatments and three replications: A - 10 mg L^{-1} salinity; B - 15 mg L^{-1} salinity; C - 20 mg L^{-1} salinity; D - 25 mg L^{-1} salinity; E - 30 mg L^{-1} salinity; F - no bacterial infection.

The obtained data were analyzed using analysis of variance (ANOVA) with a confidence interval of 95%. The differences in the effect between the treatments were evaluated by the least significant difference test (LSD). Data analysis was performed using Microsoft Excel and SPSS software.

Results and Discussion. Whiteleg shrimp infected with *Vibrio* sp. displayed behavioral and morphological changes. Healthy shrimp are usually active at first, but after a bacterial infection, they become less active and prefer to congregate around the aeration. Furthermore, because the shrimp had a decreased response to food, the majority of the feed given was not consumed. Meanwhile, macroscopic observation of hepatopancreatic discoloration, swimming and walking legs, and the tip of the tail presented damage.

According to Utami et al (2016), clinical symptoms of *V. harveyii* bacterial infection in whiteleg shrimp included changes in behavior such as movement toward aeration, decreased feed response, and decreased activity. Meanwhile, morphological changes such as redness of the swimming legs and telson, necr osis of the tail, melanosis of body segments, and necrosis of some hepatopancreas cells have been observed. *V. parahaemolyticus*, the causative agent of AHPND, also damages the hepatopancreas of whiteleg shrimp (Liu et al 2018; Kewcharoen & Srisapoome 2019).

Salinity influences *Vibrio* sp. virulence. Virulence was observed for 33 h during the study, and the shrimp mortality is presented in Figure 1.

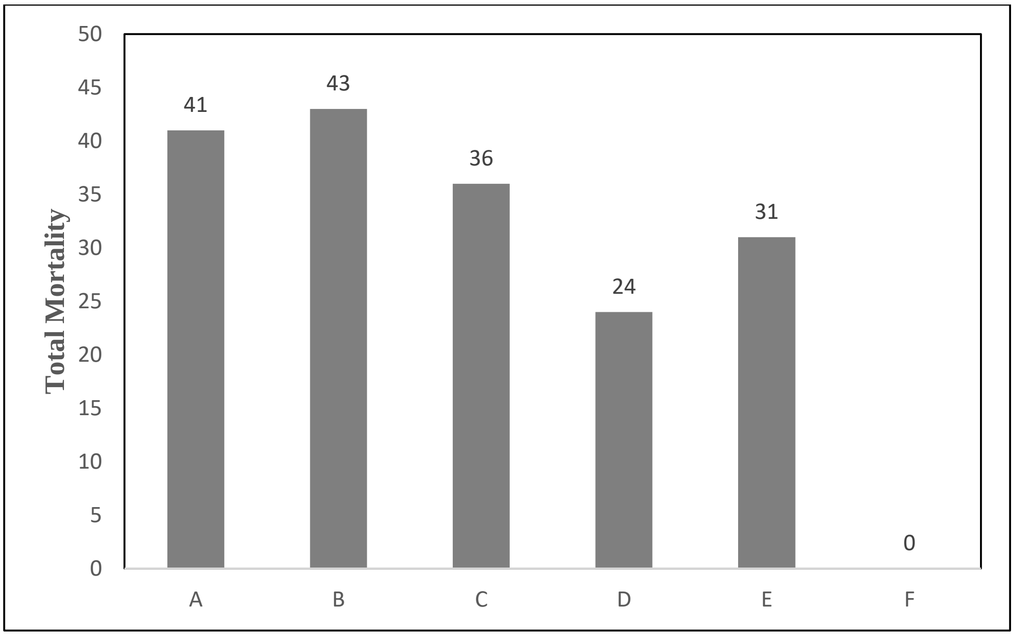

Figure 1. Total mortality of whiteleg shrimp by challenge test of *Vibrio* sp; A - 10 mg L-1 salinity; B - 15 mg L-1 salinity; C - 20 mg L-1 salinity; D - 25 mg L-1 salinity; E - 30 mg L-1 salinity; F - no bacterial infection.

The total mortality value demonstrates that salinity has an effect on *Vibrio* sp. virulence in whiteleg shrimp. *Vibrio* sp. virulence was highest in the B treatment, with a total mortality of 43 shrimp. The total mortality of treatment A was 41 shrimps, treatment C had 36 dead shrimp, treatment D presented a mortality of 24 shrimp, and treatment E a mortality of 31 shrimp. The control without *Vibrio* sp. bacterial infection presented no mortalities (Figure 2). The observation of total mortality coincides with the survival of the whiteleg shrimp.

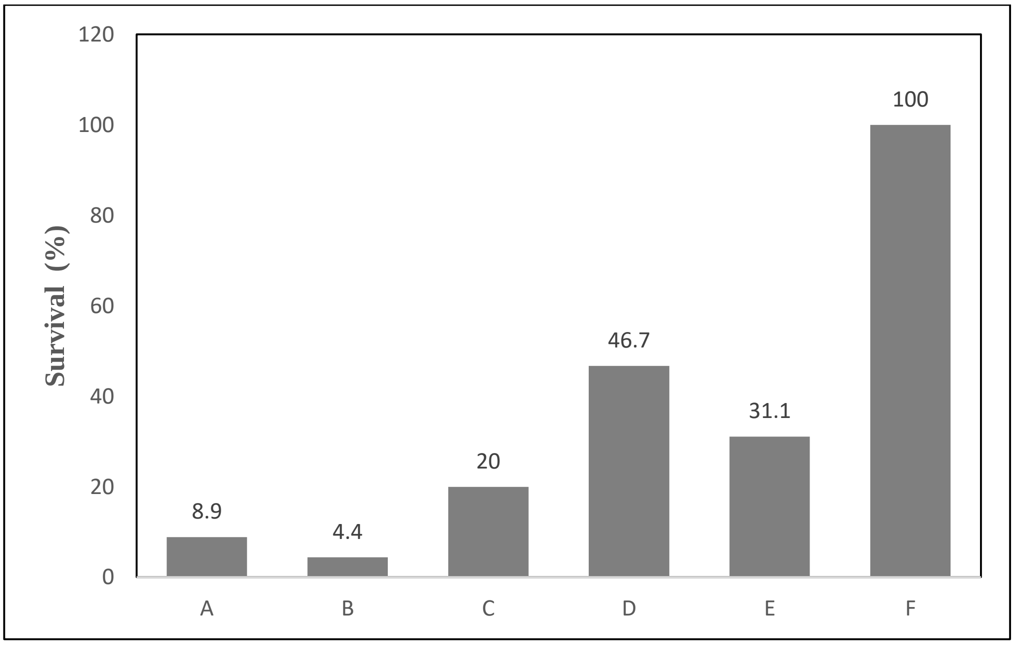

Figure 2. Whiteleg shrimp survival after being tested against *Vibrio* sp. A - 10 mg L-1 salinity; B - 15 mg L-1 salinity; C - 20 mg L-1 salinity; D - 25 mg L-1 salinity; E - 30 mg L-1 salinity; F - no bacterial infection.

Figure 2 reveals that the bacterial infection of *Vibrio* sp. at different salinities resulted in decreased survival. Treatment B showed the lowest survival, namely 4.4%. Treatments A, C D and E had a survival rate of 8.9, 20, 46.7 and 31.1%, respectively. Analysis of variance indicated that salinity treatment had a significant effect on the virulence of *Vibrio* sp. in whiteleg shrimp ($p<0.05$). B treatment had the most influence. This condition is in accordance with the results of Li et al (2010), where *V. alginolyticus* infection in whiteleg shrimp increased when the salinity was low. Meanwhile, *V. harveyi* infection at a salinity lower than 20 mg L^{-1} resulted in a decrease in the survival of whiteleg shrimp up to 50% (Utami et al 2016).

Salinity conditions significantly affect the extracellular production of *Vibrio* sp. Virulence itself is the ability of a microorganism to cause disease through extracellular products, especially toxins such as pear A, pear B, haemolin, and flagellin that have been identified in the pathogenic bacterium *Vibrio* (Bauer et al 2020). In the whiteleg shrimp culture system, *Vibrio* attacks have recently been reported as the main agent causing AHPND outbreaks. *Vibrio* bacteria produce pear A and pear B toxins, causing damage to the hepatopancreas and ultimately death. This disease shows a decrease in whiteleg shrimp production due to mortality up to 100% (Liu et al 2018; Kewcharoen & Srisapoome 2019).

The emergence of *Vibrio* sp. is typically caused by stressful conditions, including changes in environmental quality. These fluctuations can lead to an increase in disease attacks. *Vibrio* diseases weaken the immune system and can raise blood glucose levels in fish (Mankiewicz et al 2020). A weakened immune system increases the likelihood of infection with *Vibriosis*-causing agents. Furthermore, increased attacks can be supported by cortisol secretion, which has been shown to suppress and inhibit immunity (Harris & Bird 2000).

One of the most critical factors in the success of whiteleg shrimp culture is water quality (Table 1). Water temperature is one of the essential factors in regulating life processes and organism distribution in bodies of water (Kale 2016). The ideal temperature range for whiteleg shrimp growth is 25-30°C (Abdelrahman et al 2018).

Table 1

Water quality observations

Parameter	*Observation results*
Temperature (°C)	25–30
Salinity (mg L^{-1})	15–30
pH	7.5–8.5
Dissolved oxygen (mg L^{-1})	>4

The pH concentration of the water affects the shrimp's appetite. A water pH below the tolerance range disrupts the molting process, the skin becomes soft, and viability is low (Perez-Velazquez et al 2012). The optimal water pH range for the growth of whiteleg shrimp is 7.5-8.5 (Furtado et al 2016). Dissolved oxygen is needed by aquatic organisms for several physiological activities. Shrimp can grow with the optimum dissolved oxygen concentration >4 mg L^{-1} (Duan et al 2013). Salinity conditions have a significant impact on the osmoregulation process, which controls the balance of water and ions between the body and its environment. Salinity also affects the survival, distribution, and growth of aquatic organisms; its concentration is influenced by temperature and weather conditions (Kale 2016). Whiteleg shrimp in low salinity cannot grow optimally (Zheng et al 2018; Pazir et al 2020). This increases energy requirements, and large amounts of metabolic processes occur. Various sources of body energy are synthesized to meet these energy needs, reducing growth (Martin 2009).

Conclusions. Based on the study's findings, salinity has a significant impact on the virulence of *Vibrio* sp. in whiteleg shrimp culture. The highest salinity virulence level occurred at 15 mg L^{-1}. Therefore, to prevent *Vibrio* sp. infecting whiteleg shrimp, it is recommended to maintain the salinity range at 25 mg L^{-1}.

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Conflict of Interest. The authors declare that they have no conflict of interest.

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