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EFFECT OF NEGATIVE REDOX POTENTIAL OF DRY SEDIMENT ON THE INFECTIVITY OF *Vibrio parahaemolyticus,* GROWTH AND HEALTH STATUS OF PACIFIC WHITELEG SHRIMP

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ABSTRACT

Sediment serves as a site for the accumulation of aquaculture waste. Oxidation-Reduction Potential (ORP) is an index indicating anaerobic conditions. A combination of host, pathogen, and suboptimal environmental quality can lead to disease outbreaks. One such disease is Acute Hepatopancreatic Necrosis Disease (AHPND), caused by Vibrio parahaemolyticus. This study was aimed to analyse the impact of sediment drying on infectivity of V. parahaemolyticus growth and health status of Pacific whiteleg shrimp. The sediment ORP used at the initial study was -37 mV. The sediment was dried for 120 hours at a temperature of 40.75 ± 2.73 *°C. A total of 15 shrimp/aquarium with an average body weight of 2.35±0.22 g/individual were reared in aquarium sized 20x25x30 cm^3 containing dried and undried sediment for 120 hours, then contaminated with 10^5 CFU/mL V. parahaemolyticus through immersion from the start of rearing until 20 days later. The results showed that sediment drying was able to reduce bacterial abundance and increase ORP sediment. The longer duration of cultivation, the more the sediment ORP decreased. Sediment ORP decreased with increasing shrimp farming duration. The highest bacterial abundance in the sediment on thrid phase of study was observed in the positive control treatment on D+10 ((2.02±0.39)x10^4 CFU/g), then it decreased until the end. Meanwhile, bacterial abundance in water and hepatopancreas decreased until the end of the study. Survival rates, immune responses, and growth parameters of shrimp with sediment drying were higher than the positive control ($p<0.05$) but not significantly different from the negative control. The histopathology of shrimp hepatopancreas profile in the sediment drying treatment was better compared to the positive control.*

KEYWORDS: *Litopenaeus vannamei*, redox potential, sediment drying, *V. parahaemolyticus*

INTRODUCTION

Pacific whiteleg shrimp *Litopenaeus vannamei* is a major aquaculture species cultured in Indonesia. According to the FAO (2023), global production of this shrimp, reached 6,324,579 tons in 2021. By 2024, Indonesian government increased shrimp production target to two million tons (Rahayu, 2022). However, achieving this target faces several challenges, including diseases that cause significant losses in aquaculture. Shrimp farming patterns in Indonesia include traditional, semi-intensive, intensive, and super-intensive systems, with many farms shifting from soil-based ponds to concrete or HDPE-lined ponds, which significantly influence productivity, disease outbreaks, and cultivation management. Currently, traditional ponds remain a preferred option for farmers due to their lower costs compared to intensive farming systems (Mira *et al.,* 2022). In 2022, the total area of traditional ponds in Indonesia reached 747,952 hectares (KKP, 2023).

Diseases arise from a combination of host, pathogen, and environmental factors (Hamed *et al.,* 2018). Bacterial diseases in shrimp are typically caused by *Vibrio* spp., which are found in water, sediment, and shrimp (Zeng *et al.,* 2021). *Vibrio parahaemolyticus* is a major pathogen responsible for Acute Hepatopancreatic Necrosis Disease (AHPND), which has a high mortality impact (Peña-Navarro *et al*., 2020). Tran *et al.* (2013) reported that the pirA and pirB toxins produced by certain strains of *V. parahaemolyticus* can cause up to 100% mortality within 20-30 days post-stocking in the post-larval stage. AHPND outbreaks in earthen shrimp ponds have been recorded in Thailand and Vietnam (Boyd & Phu 2018; Chainark *et al.,* 2024).

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Sediment serves as an accumulation site for farming waste, rich in organic and inorganic materials. The farming environment, including water and sediment, is crucial to monitor (Zeng *et al.,* 2021), as shrimp are benthic organisms (Zhang *et al.,* 2019). Changes in sediment quality can directly affect shrimp health and increase the risk of disease outbreaks. According to PERMEN KP NO. 75 of 2016, optimal sediment conditions include a redox potential (ORP) more than +50 mV, organic matter content of less than 5%, and a pH of 5.5 to 7 (Regulation of the Minister of Marine and Fisheries No.75 Year 2016). Sediment texture also plays a crucial role in the decomposition of waste by bacteria (Wahyuningrum *et al.,* 2016).

Redox potential is an index of the degree of oxidation or reduction in a chemical system, serving as an indicator of anaerobic conditions in sediment. According to Chien (1989), redox potential is categorized into four levels: oxidation (400-700 mV), medium oxidation (100-400 mV), reduction (-100 to +100 mV), and high reduction (-300 to -100 mV). Over time, sediment redox potential tends to decrease, reaching negative values by the end of the farming period. A pond sediment may undergo a significant reduction in ORP, dropping as low as -300 mV (Suwoyo *et al.,* 2015). Waste decomposition under anoxic conditions can lead to the increase of harmful substances like ammonia, hydrogen sulfide, methane, and certain organic acids (Zhou *et al.,* 2009).

Shrimp farming in suboptimal sediment conditions can lead a low productivity, poor growth, and increased vulnerability to disease (Wiyoto *et al.,* 2017; Torun *et al.,* 2020). Sediment drying is an effective way to improve sediment quality and reducing pathogen viability. For instance, the viability of WSSV in pond sediment decreased and was unable to infect shrimp after the sediment was dried for 21 days under direct sunlight (Kumar *et al.,* 2013). Therefore, improving the farming environment, particularly the pond bottom, is crucial for successful aquaculture. Pond drying has been implemented in the field as an effort to improve pond quality. While sediment conditions are known to affect microbial activity in aquaculture systems, there is limited information regarding how sediment ORP influences the abundance or virulence of *Vibrio parahaemolyticus* associated with AHPND. Thus, the relationship between sediment redox status and the occurrence of AHPND remains largely unexplored.

This research aims to investigate the dynamics of *Vibrio parahaemolyticus* in the aquaculture environment and to evaluate the effectiveness of sediment drying as a mitigation strategy. Specifically, the objectives were to (1) analyze the spread of *V. parahaemolyticus* in pond water and sediment, (2) determine the impact of drying sediment with negative oxidation-reduction potential (ORP) on the abundance of *V. parahaemolyticus*, and (3) assess the potential of this treatment to improve environmental quality and enhance shrimp growth also health status.

MATERIALS AND METHODS

Preparation of Culture Media and Test Animal

The experiment was conducted from January to August 2024 at the Department of Aquaculture, Bogor Agricultural University, using shrimp (2.35 ± 0.22 g/individual). Sediment samples were collected from ponds in Bakauheni, South Lampung, that had been used for over five years and were no longer under cultured condition (The sediment ORP used at the initial study was -37 mV, then sterilized by autoclave (121^{0}C, 15 minutes) and characterized for texture by soil texture triangle method. Experiments were carried out in 20 × 25 × 30 cm^3 aquarium containing 5 cm sediment and 10 L sterile seawater (28 ppt), with salinity maintained by adding freshwater. The experimental procedures of Pacific white leg shrimp in this study were handled under Indonesian accreditation SNI 8117:2015.

The experiment used a completely randomized design (CRD) in three phases. In the first phase, tested control (PBS) and contaminated (10^5 CFU/mL *V. parahaemolyticus*) water for 20 days to assess bacterial presence in sediment. The second phase using two treatments and three replicates: a control group (K2) with PBS and a contamination group (B2) with *V. parahaemolyticus* in the sediment from the highest *Vp*Rf^R abundance from the first phase of study. For this phase, 150 g of sterile pond sediment was placed into six aluminium foil cups (160 mL each) examined contaminated sediment drying (40.75 ± 2.73 °C, 120 h). Phase three, evaluated shrimp performance in dried and undried sediments under PBS (K-), positive control (K+), and drying treatments (O) contaminated with *V. parahaemolyticus* 10^5 CFUmL^{-1}, lasting for 20 days. Three replicates were used to observe shrimp growth and cumulative mortality, while the other two replicates focused on immune response, bacterial abundance, histopathology, and PCR testing.

Preparation for Feed and Test Bacteria

In the third phase experiment, 15 shrimp with an average size of 2.35 ± 0.22 g/individual were placed in each 15 aquarium. The shrimp were fed a diet containing 35% protein at a feeding rate of 6%, provided four times daily at 06:00, 10:00, 14:00, and 18:00. The

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bacteria used was *V. parahaemolyticus* rifampicin-resistant 50 µg/100 mL(Rf^R), were sourced from the Aquatic Organism Health Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University. The challenge test, conducted through immersion, involved a bacterial concentration of 10^5 CFU/mL (Chonsin *et al.,* 2015) and lasted for 20 days from the start of maintenance.

Sample Collection

During the first phase experiment, water quality parameters such as dissolved oxygen (DO), pH, temperature, and salinity were monitored daily. Water and sediment samples were collected after contaminated by *V. parahaemolyticus* to measure total *Vibrio* sp. (TVC) and *V. parahaemolyticus* Rf^R (*Vp*Rf^R) population. Sediment samples were taken using a two cm diameter PVC pipe. In the second phase experiment, sediment samples were dried for up to 120 hours to measure *Vp*Rf^R population and sediment ORP using a Lutron WAC-2019SD. In the third phase experiment, cumulative mortality and water quality (DO, pH, temperature, and salinity) were observed daily. Shrimp hemolymph, hepatopancreas and weight, also the environmental aspects (water and sediment) including analyses of water (Total Ammonia Nitrogen/TAN, nitrite, nitrate, Total Organic Matter/TOM), sediment (ORP, pH, organic matter), samples were collected after challenge test with *V. parahaemolyticus*. Hemolymph was used to assess the immune response of shrimp, while water, sediment, and hepatopancreas samples were analyzed for TVC and *Vp*Rf^R population. Moribund shrimp were examined by histopathology and PCR.

Data Collection and Calculation

Growth Performances

Growth performances were calculated using the following formula: Average Body Weight (ABW) = total weight/total number of shrimps (Parlina *et al*., 2018). Specific Growth Rate (SGR) (%) = [(ln final shrimp weight (g) – ln initial shrimp weight (g))/ experiment duration (days)] x 100 (Halver & Hardy 2002). Feed Conversion Ratio (FCR) = feed intake/ ((biomass of shrimp at the end of rearing (g) + biomass of dead shrimp (g)) – biomass of shrimp at initial of rearing (g)) (Foysal *et al.,* 2020), and Survival Rate (SR) (%) = (final shrimp number/initial shrimp number) x 100 (Liu *et al.,* 2019).

Cumulative Mortality

The calculation of cumulative mortality can be used to illustrate shrimp mortality trends. Cumulative mortality of shrimp is calculated using the formula: = (final shrimp number/initial shrimp number) x 100 (Effendie, 1997).

Clinical Symptoms

Clinical symptoms of shrimp affected by *V. parahaemolyticus* are observed visually, especially through changes in hepatopancreas condition, pale body color, decreased feeding behavior, and weak swimming movements. Observation of clinical symptoms includes the initial, acute, and terminal phases (Soto-Rodriguez *et al.,* 2018).

Immune Response

A total of 0.1 mL of shrimp hemolymph was collected from the ventral sinus using a 1 mL syringe pre-filled with anticoagulant solution at a ratio of 1:2. Total haemocyte count is calculated by hemocytometer. THC = (Number of observed cells) / (Number of observed squares) × 1 / (Volume of large square) × dilution factor (Huynh *et al*., 2018). Phagocytic Activity (%) = (phagocytizing cells) / (phagocytes) × 100%, A 50 µL shrimp hemolymph sample was mixed with 50 µL of *Staphylococcus aureus* suspension (10w cells/mL in PBS) in a microplate and incubated for 20 minutes. A 5 µL aliquot was then smeared on a glass slide, air-dried, fixed in 100% methanol for 5 minutes, then stained with 10% Giemsa solution for 15 minutes, followed by rinsing with distilled water and drying (Anderson & Sawicki 1995). Respiratory burst is measured according to Cheng *et al.* (2004), Phenoloxidase activity is measured based on the method by Liu & Chen (2004) using microplate reader.

Bacterial Count

The abundance of TVC and *Vp*Rf^R population in water, sediment, and hepatopancreas is calculated using the total plate count method (Madigan *et al*., 2014). Water samples of 0.1 mL and sediment and hepatopancreas samples of 0.1 g were homogenized and diluted with 0.9 mL PBS. *Vibrio* was cultured in Thiosulfate Citrate Bile Sucrose (TCBS) Agar, while *Vp*Rf^R were cultured in TCBS Agar+Rif. Bacteria incubated at 37°C for 18-24 hours.

Histopathology

Histopathology samples from the hepatopancreas of dead shrimp from treatments K-, K+, and O were prepared as histopathological specimens following the procedure by Lightner & Redman (1977). The specimens were observed under a microscope with a magnification of 40x100 to examine cell integrity or damage.

Polymerase Chain Reaction

Sample Preparation and DNA Extraction

The PCR samples were freshly dead shrimp. The shrimp were rinsed with distilled water, and the hepatopancreas was dissected using a dissecting set. DNA extraction from the shrimp's hepatopancreas was conducted using the gSYNC™ DNA Extraction Kit.

Amplification and Electrophoresis

The amplification procedure began by preparing the reaction mixture for First PCR and Nested PCR according to the number of samples (K-, K+, and O). The primers used were AP4. AP4-F1 (5'-ATG-AGT-AAC-AAT-ATA-AAA-CAT-GAA-AC-3'), AP4-R1 (5'-ACG-ATT-TCG-ACG-TTC-CCC-AA-3'), AP4-F2 (5'-TTG-AGA-ATA-CGG-GAC-GTG-GG-3'), and AP4-R2 (5'-GTT-AGT-CAT-GTG-AGC-ACC-TTC-3') (Dangtip *et al.,* 2015). The total volume PCR reagent mixture in one PCR tube was 10 μL, containing 5 μL of Redmix PCR, 0.8 μL of Primer Forward, 0.8 μL of Primer Reverse, 2.4 μL of Nuclease Free Water (NFW), and 1 μL of DNA template. Thermocycle for first and nested PCR (Table 1). The desired DNA amplicon size using this method is 230 bp. The amplification process was carried out on a peqSTAR XS Thermocycler (The peqSTAR, Germany). The PCR products were run in 1.5% agarose gel with 0.6 μL of fluorescence added, 1xTBE buffer was added to cover the gel in an electrophoresis device. PCR products, Marker DNA ladder 100 bp, positive control, and negative control were placed into the agarose wells, with each well containing 2 μL. Electrophoresis was carried out for 40 minutes at 100 volts. The agarose was then placed on a UV Trans-Illuminator to read the final results.

Data Analysis

Table 1. Thermocycle for first and nested PCR

First Step		*Nested*	
Predenaturation	94 °C, 2 min	Predenaturation	94 °C, 2 min
30 cycles		30 cycles	
Denaturation	94 °C, 30 sec	Denaturation	94 °C, 20 sec
Annealing	60 °C, 30 sec	Annealing	55 °C, 20 sec
Extension	72 °C, 30 sec	Extension	72 °C, 30 sec
Final Extension	72 °C, 2 min	Final Extension	72 °C, 1 min
Hold/End	20 °C, 0 sec	Hold/End	20 °C, 0 sec

The abundance of total *Vibrio* sp. (TVC) and *V. parahaemolyticus* Rf^R (*Vp*RF^R), also the measurement of water and sediment parameters in experiment phase 1 and phase 2, clinical symptoms, histopathology, cumulative mortality, and PCR confirmation in experiment phase 3 were analyzed descriptively. Meanwhile, bacterial abundance TVC and *Vp*RF^R, immune response, and growth performance were tested for normality and homogeneity, then statistically analyzed using Analysis of Variance (ANOVA) with a 95% confidence interval through SPSS 23 software. If there were significant differences ($P<0.05$), a further test was conducted using Duncan's test.

RESULTS AND DISCUSSIONS

First Phase of Study

Sediment Texture and Bacteria Count

The sediment texture used in this study was clay, consisting of 13.79% sand, 25.16% silt, and 61.07% clay. Clay texture aligns with the standard water quality criteria for shrimp farming. The abundance of *Vp*Rf^R bacteria was higher in the sediment compared to the water. The highest bacterial *Vp*Rf^R abundance in the sediment was observed in treatment B1 D+5 ($(1.26 \pm 0.57) \times 10^5$ CFU/g), but this decreased over time (Table 2). The bacterial abundance in all treatments decreased by the end of the observation period due to the absence of shrimp and feed input, leading to minimal organic matter as a nutrient source for bacteria in both water and sediment.

Water Parameters

The results indicate that all water quality parameters were within the normal range according to standard criteria for pacific whiteleg shrimp farming (Table 3). Maintaining stable water quality in shrimp farming can enhance production and reduce the risk of disease outbreaks (Ariadi *et al.,* 2019).

Second Phase of Study

Bacterial Count

Results indicate that in the contaminated treatment, the highest abundance of *Vp*Rf^R bacteria occurred at hour 0 ($(1.91 \pm 0.12) \times 10^5$ CFU g^{-1}) and then decreased by hour 120 (Table 4). The decrease in *Vp*Rf^R abundance in the sediment during the second stage of the study refers to previous research. Specifically, drying WSSV-positive sediment (211,500 WSSV counts g^{-1}) under sunlight for 19 days was able to reduce

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WFD infection in shrimp, even though quantitative RT-PCR detection still showed positive WSSV in both the sediment (4,420 WSSV counts g^{-1}) and the shrimp (Kumar *et al.*, 2013). Possible limitations include the short 120-hour trial duration under laboratory conditions in this study, which may not fully represent actual pond environments. However, the researchers attempted to approximate the total sunlight exposure time equivalent to about 20 days of sediment drying under natural sunlight, assuming an average of 6 hours of sun exposure per day.

Table 2. Bacterial count in water and sediment

Sample	Time of Sampling	K1		B1	
		*Vp*Rf^R	TVC	*Vp*Rf^R	TVC
Water (CFU/mL)	D0	0	$(2.67 \pm 0.08)x10^3$	0	$(2.67 \pm 0.08)x10^3$
	D+5	ND	$(1.6 \pm 0.33)x10^3$	$(7.4 \pm 0.20)x10^3$	$(1.46 \pm 0.30)x10^4$
	D+10	ND	$(2.72 \pm 4.07)x10^3$	$(5.22 \pm 3.78)x10^3$	$(1.15 \pm 0.83)x10^4$
	D+15	ND	$(0.63 \pm 0.03)x10^3$	$(0.4 \pm 0.32)x10^3$	$(0.13 \pm 0.02)x10^4$
	D+20	ND	$(0.16 \pm 0.04)x10^3$	$(0.23 \pm 0.36)x10^3$	$(0.56 \pm 0.32)x10^3$
Sediment (CFU/g)	D0	0	$(1.15 \pm 0.04)x10^5$	0	$(1.15 \pm 0.04)x10^5$
	D+5	ND	$(6.13 \pm 1.27)x10^5$	$(1.26 \pm 0.57)x10^5$	$(1.84 \pm 0.60)x10^6$
	D+10	ND	$(5.45 \pm 4.91)x10^5$	$(0.35 \pm 0.24)x10^5$	$(5.17 \pm 0.97)x10^5$
	D+15	ND	$(1.51 \pm 0.67x10^5$	$(0.71 \pm 0.06)x10^4$	$(4.53 \pm 0.83)x10^5$
	D+20	ND	$(0.94 \pm 0.36)x10^5$	$(0.16 \pm 0.09)x10^4$	$(3.87 \pm 0.53)x10^5$

Description: *Vp*RF^R: *V. parahaemolyticus* Rf^R; TVC: *Total Vibrio Count*; ND: Not Detected. Control (K1), Contaminated (B1).

Table 3. Water quality during first phase of study

Parameters	Treatment		Quality Standard
	K1	B1	
pH	7.5-7.91	7.5-7.92	7.5-8.5*
Temperature ($^{\circ}$C)	27.3-29.5	27-28.6	>27**
DO (mgL^{-1})	3.8-5.9	3-6.4	≥3.0*
Salinity (ppt)	28-32	28-32	10-35*

Description: *Regulation of the Ministry of Marine and Fisheries No.75 Year 2016, **Chrisnawati *et al.,* 2018. Control (K1), Contaminated (B1).

Table 4. Bacterial count in dried sediment during second phase of study

Drying Hour-	*Vp*Rf^R CFU/g	
	K2	B2
0	0	$(1.91 \pm 0.00)x10^5$
24	ND	$(1.37 \pm 0.37)x10^3$
48	ND	$(1.01 \pm 0.37)x10^3$
72	ND	$(0.48 \pm 0.18)x10^3$
96	ND	$(0.53 \pm 0.31)x10^2$
120	ND	ND

Description: *Vp*RF^R: *V. parahaemolyticus* Rf^R; Not Detected (ND). Control (K2), Contaminated (B2).

Sediment Redox Potential

The measurement of sediment redox potential in both control and contaminated treatments showed an increase by the end of the observation period. In both treatments, the redox potential increased by 24-25 mV, from an initial –37 mV to –(12-13) mV. However, these values still far from the minimum standard for sediment ORP quality. According to Boyd (1995) and Regulation of the Minister of Marine and Fisheries No.75 Year 2016, optimal pond sediment conditions require a minimum redox potential of (+) 50 mV, with a normal pH slightly towards weak alkalinity. The increase in redox potential suggests that drying the pond sediment promotes higher oxidation states because of oxygen diffusion (Marsi *et al.,* 2024) and facilitate biochemical transformation processes.

Table 5. Redox potential measurements in second phase of study

Drying Hour-	Redox Potential (mV)	
	K2	B2
0	-37	-37
24	-32	-34
48	-25	-26
72	-20	-21
96	-16	-15
120	-12	-13

Description: Control (K2), Contaminated (B2).

Third Phase of Study

Growth Performance

The growth performance of shrimp reared in dried and undried sediment with negative redox potential, and challenged with *V. parahaemolyticus* in the K+ treatment, showed significant differences compared to K- and O in all parameters ($p<0.05$). The growth performance of shrimp is influenced by environmental quality and the presence of pathogens. Pathogenic bacteria and unfavorable environmental conditions cause shrimp to experience stress, preventing them from utilizing feed efficiently. A high feed conversion ratio indicates that less of the consumed feed is absorbed by the shrimp's body (Supono *et al*., 2021). Excess feeding occurs when shrimp are stressed due to poor environmental conditions or disease outbreaks. Increasing organic matter in the sediment contributes to sediment thinkening (Tabel 6).

Table 6. Growth performance of shrimp reared in dried and undried sediment

Parameters	Treatment		
	K-	K+	O
ABW (g/shrimp)	5.0 ± 0.5^{b}	3.7 ± 0.5^{a}	5.1 ± 0.4^{b}
TFC (g)	42.42 ± 4.43^{b}	19.33 ± 4.10^{a}	39.65 ± 5.09^{b}
SGR (%/Day)	3.88 ± 0.48^{b}	2.47 ± 0.62^{a}	3.95 ± 0.97^{b}
FCR	1.33 ± 0.12^{a}	4.06 ± 1.77^{b}	1.32 ± 0.15^{a}
SR (%)	68.89 ± 3.85^{b}	13.33 ± 6.67^{a}	60.00 ± 13.33^{b}

Description: Different superscripts letters following the mean (±SD) indicate significant differences (Duncan $p<0.05$). Average body weight (ABW); total feed consumption (TFC), specific growth rate (SGR), feed conversion ratio (FCR), survival rate (SR). Negative control (K-), positive control (K+), dried (O).

Bacterial Count

The highest abundance of *Vp*RF^{R} bacteria in water was observed in the K+ treatment on D+3 ($(2.86 \pm 1.73)\times10^{4}$ CFU/mL). The highest *Vp*RF^{R} abundance in sediment occurred in the K+ treatment on day D+10 ($(2.02 \pm 0.40)\times10^{4}$ CFU/g), while the highest *Vp*RF^{R} abundance in the hepatopancreas was also in the K+ treatment on D+3 ($(2.99 \pm 0.07)\times10^{4}$ CFU/g) (Tabel 7). According to Sirikhairin *et al*. (2014), if the bacterial density of *V. parahaemolyticus* in the hepatopancreas exceeds 10^{4} CFU/mg, it can cause lesions and dysfunction in the hepatopancreas. The decrease in bacterial abundance is due to the bacteria dying off when they cannot find a host, as they do not receive sufficient nutrients to survive (Valente & Wan, 2021).

Clinical Symptoms

Shrimp with AHPND infection showed changes in the hepatopancreas and intestines of infected shrimp, which appeared pale, with empty intestines, slow swimming, and pale bodies, tending toward whiteness (Figure 1). The clinical symptoms of AHPND infection in shrimp consist of initial, acute, and terminal phases (Soto-Rodriguez *et al*., 2018). In the initial phase, shrimp still appear normal, but changes start to occur, such as a pale hepatopancreas and partially or completely empty digestive tracts. In the acute phase, shrimp experience anorexia, lethargy, empty digestive tracts, a pale hepatopancreas, and some fluid present in the middle part of the body when dissected. In the terminal phase, the hepatopancreas turns white, the body becomes pale, the shrimp sink, and death ensues (Hong *et al*., 2016). The post-infection hepatopancreas of shrimp shows lesions and hydropic degeneration as a result of *V. parahaemolyticus* infection.

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Table 7. Bacterial count in water, sediment, and shrimp hepatopancreas during third phase of study

Sample	Time of Sampling	K-		K+		O	
		*Vp*Rf^{R}	TVC	*Vp*Rf^{R}	TVC	*Vp*Rf^{R}	TVC
Water (CFU/mL)	D0	0	$(0.30\pm0.14)\times10^{2}$	0	$(0.30\pm0.14)\times10^{2}$	0	$(0.30\pm0.14)\times10^{2}$
	D+3	ND^{a}	$(2.04\pm1.04)\times10^{4a}$	$(2.86\pm1.73)\times10^{4b}$	$(1.7\pm0.20)\times10^{5b}$	$(2.14\pm0.57)\times10^{4b}$	$(2.01\pm0.55)\times10^{5b}$
	D+10	ND^{a}	$(2.66\pm0.17)\times10^{4a}$	$(0.65\pm0.13)\times10^{4b}$	$(1.33\pm0.04)\times10^{5c}$	$(0.44\pm0.09)\times10^{4b}$	$(0.85\pm0.16)\times10^{5b}$
	D+20	ND^{a}	$(7.8\pm1.98)\times10^{3a}$	$(0.28\pm0.28)\times10^{3b}$	$(1.18\pm0.06)\times10^{5b}$	$(0.70\pm0.71)\times10^{2b}$	$(1.86\pm1.41)\times10^{4a}$
Sediment (CFU/g)	D0	0	$(7.60\pm0.85)\times10^{3b}$	0	$(7.60\pm0.85)\times10^{3b}$	0	$(0.40\pm0.28)\times10^{2a}$
	D+3	ND^{a}	$(2.04\pm0.40)\times10^{5a}$	$(5.83\pm0.13)\times10^{3c}$	$(1.33\pm0.30)\times10^{6b}$	$(2.33\pm1.12)\times10^{3b}$	$(1.24\pm0.06)\times10^{5a}$
	D+10	ND^{a}	$(1.24\pm0.08)\times10^{6a}$	$(2.02\pm0.40)\times10^{4c}$	$(0.90\pm0.23)\times10^{7b}$	$(5.29\pm0.78)\times10^{3b}$	$(3.66\pm1.78)\times10^{6ab}$
	D+20	ND^{a}	$(8.74\pm6.02)\times10^{5a}$	$(3.03\pm2.01)\times10^{3b}$	$(2.03\pm0.52)\times10^{6a}$	$(1.23\pm0.16)\times10^{3b}$	$(2.18\pm1.03)\times10^{6a}$
Hepato-pancreas (CFU/g)	D0	0	$(1.07\pm0.24)\times10^{4}$	0	$(1.07\pm0.24)\times10^{4}$	0	$(1.07\pm0.24)\times10^{4}$
	D+3	ND^{a}	$(8.70\pm0.71)\times10^{5a}$	$(2.99\pm0.07)\times10^{4b}$	$(2.70\pm0.42)\times10^{6b}$	$(3.31\pm1.54)\times10^{4b}$	$(1.12\pm0.28)\times10^{6a}$
	D+10	ND^{a}	$(6.30\pm0.42)\times10^{5b}$	$(0.85\pm0.04)\times10^{4c}$	$(8.80\pm0.85)\times10^{5c}$	$(5.80\pm3.22)\times10^{3b}$	$(2.04\pm0.09)\times10^{5a}$
	D+20	ND^{a}	$(1.61\pm0.67)\times10^{5a}$	$(0.12\pm0.06)\times10^{4b}$	$(4.92\pm1.10)\times10^{5b}$	$(0.6\pm0.28)\times10^{3b}$	$(1.25\pm0.18)\times10^{5a}$

Description: Different superscripts letters following the mean (±SD) indicate significant differences (Duncan $p<0.05$). *Vp*RF^{R}: *V. parahaemolyticus* Rf^{R}; TVC: *Total Vibrio Count*; ND: Not Detected. Negative control (K-), positive control (K+), dried (O).

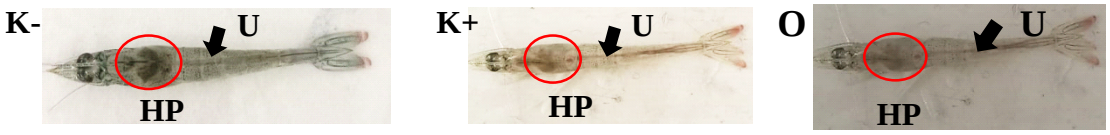

Figure 1. Clinical symptoms of shrimp post-infection with *V. parahaemolyticus*. Description: HP: Hepato-pancreas; U: Gut. Negative control (K-), positive control (K+), dried (O).

Cumulative Mortality

The highest cumulative mortality occurred in the K+ treatment group at 86.67±6.67%, followed by the O treatment group at 40±13.33%, with the lowest in the negative control group at 31.11±3.85% (Figure 2). These mortality rates refer to Hong *et al.* (2016), which reported that Pacific whiteleg shrimp infected with AHPND have a mortality rate ranging from 40% to 100%. The cumulative mortality in the K+ group peaked on day 5 post-infection. This suggests that shrimp challenged with *V. parahaemolyticus* infection and untreated sediment had a higher mortality rate compared to those infected with *V. parahaemolyticus* and treated sediment. However, mortality also occurred in the K- group at 31.11±3.85%, likely due to suboptimal sediment quality and shrimp cannibalism. According to Soto-Rodriguez *et al.* (2019), environmental factors such as salinity and temperature can slow bacterial regeneration time, and these conditions can increase bacterial virulence due to stress factors.

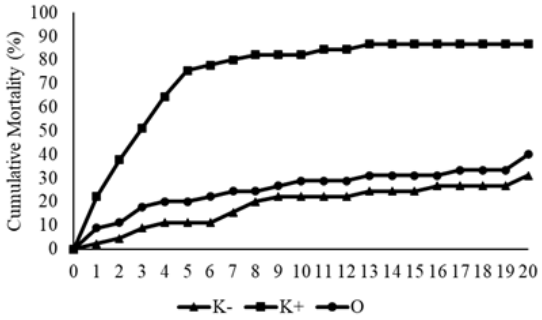

Figure 2. Cumulative mortality pattern of shrimp post-infection with *V. parahaemolyticus*. Description: Negative control (K-), positive control (K+), dried (O).

Histopathology

Normal shrimp tissues showed intact tubules, while infected shrimp showed into the terminal phase, marked by lumen dilation and increased hemocytic infiltration (Morales-Covarrubias *et al.*, 2018; Soto-Rodriguez *et al.*, 2018).

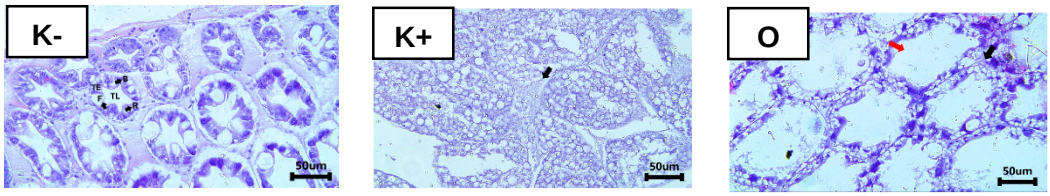

Figure 3. Histopathological profile of hepatopancreas of healthy shrimp and and post-infection by *V. parahaemolyticus* in *L. vannamei.* Description: Tubular lumen (TL); B cell (B); R cell (R); F cell (F); Negative control (K-), positive control (K+), dried (O). Red arrow: lumen dilation; black arrow: hemocytic infiltration.

Immune Responses

The THC values on D+3 post-infection showed significant differences ($P<0.05$). On D+10, THC levels increased across all treatments, as well on D+20, in K- and O showing significant differences compared to K+ ($P<0.05$). The immune response parameter AF on D+3 increased in all treatments, with K- and O significantly different, but not significantly different from K+ ($P<0.05$). On D+10, AF in the K- treatment was significantly different from K+ and O ($P<0.05$). On D+20, AF decreased in all treatments. The RB immune response parameter decreased on D+3, with the lowest value in K+, significantly different from K- but not significantly different from O ($P<0.05$). On D+10 and D+20, RB values increased across all treatments. The AF immune response parameter decreased on D+3, with K- significantly different from K+ and O. On D+10 and D+20, PO values increased in all treatments (Table 8).

Table 8. Immune response of shrimp during thrid phase of study

Parameters	Treatment	Time of Sampling			
		D0	D+3	D+10	D+20
Total Haemocyte Count (10^5 CFU/mL)	K-	6.9±0.85	8.85±1.06^{b}	13.65±1.48^{b}	15.60±0.30^{b}
	K+		4.95±0.64^{a}	7.80±1.70^{a}	12.12±0.68^{a}
	O		5.7±0.85^{a}	10.95±0.21^{ab}	16.19±1.55^{b}
Phagocytic activity (%)	K-	47.91±0.04	48.53±0.02^{a}	52.62±0.01^{a}	44.68±0.03^{a}
	K+		57.35±0.02^{ab}	61.71±0.02^{b}	45.70±0.03a
	O		65.46±0.05^{b}	62.91±0.02^{b}	56.62±0.03^{b}
Respiratory Burst λ 630 nm (10 μL^{-1})	K-	0.89±0.05	0.71±0.05^{b}	0.91±0.04^{b}	1.55±0.17^{b}
	K+		0.38±0.06^{a}	0.59±0.08^{a}	0.84±0.05^{a}
	O		0.53±0.08^{ab}	0.81±0.09^{ab}	1.71±0.12^{b}
Phenoloxidase λ 490 nm (10 μL^{-1})	K-	0.13±0.06	0.1±0.011^{b}	0.12±0.005^{b}	0.18±0.008^{b}
	K+		0.07±0.003^{a}	0.09±0.004^{a}	0.10±0.011^{a}
	O		0.08±0.007^{a}	0.12±0.008^{b}	0.14±0.025^{ab}

Description: Different superscripts letters following the mean (±SD) indicate significant differences (Duncan $p<0.05$). Not Detected (ND). Negative control (K-), positive control (K+), dried (O).

The shrimp immune response, both cellular and humoral, was influenced by sediment conditions and pathogen infection, leading to decreases in THC, RB, and PO Wiyoto (2016). The decrease in THC was caused by haemocytes migrating to the infected tissue, concentrating the immune response at that site (Hamsah *et al.*, 2019). Phagocytic activity plays a role in the immune response mechanism of shrimp against pathogen infections and physiological processes like tissue repair (Liu *et al.*, 2020). The decrease in RB was believed to be due to the migration of haemocyte cells, particularly hyaline cells, to the infection site for phagocytosis and to initiate the defense response. Additionally, pathogen infection in the body can cause biological stress, resulting in changes such as decreased ROI and oxidative stress within cells, leading to a reduction in RB values (Febrianti *et al.*, 2016).

PCR

The PCR results showed that the hepatopancreas organ form K+ and O groupdisplayed a DNA band of 230 bp, aligning with the positive control (Dangtip

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et al., 2015) (Figure 4). This result confirms that the bacteria used during the challenge test were *V. parahaemolyticus* with the AHPND strain.

Water and Sediment Parameters

Water and sediment parameters including pH, temperature, DO, salinity, and nitrate, were within the normal standard quality range. However, the water quality parameters for TAN and TOM exceeded the standard thresholds. The ORP and organic matter exceeded the referenced standards (Table 9) (Regulation of the Minister of Marine and Fisheries No.75 Year 2016: Chrisnawati *et al.,* 2018).

Organic matters both the water and sediment were increased due to feed input, feed waste, and shrimp excretion. Redox potential during the study remained within the tolerance range, reaching up to (-100) mV. According to research by Wiyoto (2016), sediment ORP in shrimp ponds affects the survival and growth rates of shrimp infected with WSSV.

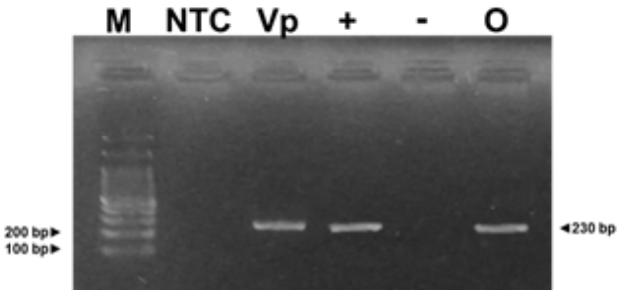

Figure 4. PCR electrophoresis confirmed AHPND in shrimp post-infection *V. parahaemolyticus*. Description: Marker DNA ladder 100 bp (M); no template control (NTC); *V. parahaemolyticus* (*Vp*)*;* positive control (+), negative control (K-), dried (O).

Table 9. Water and sediment parameters during third phase of study

Parameter	Treatment			Quality Standard
	K-	K+	O	
Water				
pH	7.51-7.74	7.5-7.63	7.51-7.66	7.5-8.5*
Temperature (°C)	27.73-29.43	28-29.2	28.07-29.17	>27**
DO (mg/L)	3.43-4.6	3.47-4.87	3.5-4.67	≥3.0*
Salinity (ppt)	28-32	28-31,3	28-31,3	10-35*
TAN	0.03-0.40	0.033-0.44	0.03-0.34	≤0.1*
Nitrite	0.25-0.857	0.25-0.91	0.25-0.84	≤1*
Nitrate	0.13-0.905	0.13-0.89	0.13-0.802	-
TOM (mg/L)	40.3-151.2	40.8-181.4	40.8-156.2	≤90*
Sediment				
pH	6-6,5	6-6,5	6-6.5	5.5-7*
ORP (mV)	(-37)-(-47)	(-37)-(-44)	(-12)-(-23)	> +50*
Organic Matter (%)	5.08-11.28	5.08-13.51	5.02-9.67	<5*

Description: *Regulation of the Ministry of Marine and Fisheries No.75 Year 2016: **Chrisnawati *et al.,* 2018. negative control (K-), positive control (K+), dried (O).

CONCLUSION

The number of *V. parahaemolyticus* bacteria in the sediment gradually decreased over the observation period. The sediment drying with negative redox potential for 120 hours at a temperature of 40.75 ± 2.73 °C effectively reduced the bacterial abundance of *V. parahaemolyticus* , such as the main target of sediment drying on the infectivity of *V. parahaemolyticus* while improving the sediment's ORP. This drying process under similar conditions also contributed to improving growth parameters (ABW, SGR, FCR, and SR) in shrimp infected with *V. parahaemolyticus*. Farmers should conduct sediment drying for at least 120 hours or 20 days for sediments contaminated with *V. parahaemolyticus*, ensuring an initial ORP of -37 mV before initiating a shrimp farming cycle

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REFERENCES

Anderson, D. & Sawcki, A. (1995). Basic Haematology and Serology for Fish Health Program. In *Second Symposium on Diseases in Asia Aquaculture "Aquatic Animal Health and The Environmental."* Phuket. hlm 17.

Boyd, C. E. & Phu, T. Q. (2018). Environmental factors and acute hepatopancreatic necrosis disease (AHPND) in shrimp ponds in Viet Nam: Practices for reducing risks. *Asian Fisheries Sciences,* 31S, 121-136. https://doi.org/10.33997/j.afs.2018.31.S1.009

Chainark, S., Chainark, P., & Soonsan, P. (2023). Differences in shrimp pond bottom soil properties and bacterial load between acute hepatopancreatic necrosis disease (AHPND)-infected ponds and AHPND-free ponds and their relation to AHPND. *J World Aquac Soc,* 55(3), 1-26. https://doi.org/10.1111/jwas.13071

Cheng, W., Hsiao, I. S., Hsu, C. H., & Chen, J. C. (2004). Change in water temperature on the immune response of Taiwan abalone *Haliotis diversicolor supertexta* and its susceptibility to *Vibrio parahaemolyticus*. *Fish Shellfish Immunol*, 17(3), 235–243. https://doi.org/10.1016/j.fsi.2004.03.007

Chien, Y. (1989). The Management of Sediment in Prawn Ponds. In the: *The Third Brazillian Shrimp Farming Congress*. Joao Pessoa, PB, Brazil. hlm 219–243.

Chonsin, K., Matsuda, S., Theethakaew, C., Kodama, T., Junjhon, J., Suzuki, Y., Suthienkul, O., & Iida, T. (2018). Genetic diversity of *Vibrio parahaemolyticus* strans isolated from farmed Pacific white shrimp and ambient pond water affected by acute hepatopancreatic necrosis disease outbreak in Thailand. *FEMS Microbiol Lett*, 363(2), 53–54. https://doi.org/10.1093/femsle/fnv222

Chrisnawati, V., Rahardja, B. S., & Satyantini W, H. (2018). The effect of administering probiotics at different times on the reduction of ammonia and total organic matter in the rearing media of Pacific white shrimp *Litopenaeus vannamei*). *Journal of Marine and Coastal Science*, 7(2), 68–77. https://doi.org/10.20473/jmcs.v7i2.20715

Dangtip, S., Sirikharin, R., Sanguanrut, P., Thitamadee, S., Sritunyalucksana, K., Taengchaiyaphum, S., Mavichak, R., Proespraiwong, P., & Flegel. T. W. (2015). AP4 method for two-tube nested PCR detection of AHPND isolates of *Vibrio parahaemolyticus*. *Aquac Rep*, 2, 158–162. https://doi.org/10.1016/j.aqrep.2015.10.002

Effendie, M. I. (1997). Biologi Perikanan. Yogyakarta (ID): Yayasan Pustaka Nusantara.

FAO. (2023). GLOBEFISH: Information and Analysis on Market Trade of Fisheries and Aquaculture. [Access on 2023 Jun 10]. https://www.fao.org/in-action/-%0Aglobefish/market-reports.

Febrianti, D., Yuhana, M., & Widanarni, W. (2016). Dietary synbiotic microcapsule influence the immune responses, growth performance and microbial populations to white spot syndrome virus in Pacific white shrimp (*Litopenaeus vannamei*). *J Fish Aquat Sci*, 11(1), 28–42. https://doi.org/:10.3923/jfas.2016.28.42

Foysal, M. J., Fotedar, R., Siddik, M. A. B., & Tay, A. (2020). *Lactobacillus acidophilus* and *L. plantarum* improve health status, modulate gut microbiota and innate immune response of marron (*Cherax cainii*). *Sci Rep*, 10(1), 1–13. https://doi.org/10.1038/s41598-020-62655-y

Halver, J. E., & Hardy, R. W. (2002). *Fish Nutriton*. Ed ke-3. California (CA): Academic Press, Inc.

Hamed, S. Ben, Ranzani-Paiva, M. J. T., Tachibana, L., Dias, D. de C., Ishikawa, C. M., & Esteban, M. A. (2018). Fish pathogen bacteria: Adhesion, parameters influencing virulence and interaction with host cells. *Fish Shellfish Immunol*, 80 (March), 550–562. https://doi.org/10.1016/j.fsi.2018.06.053

Hamsah, H., Widanarni, W., Alimuddin, A., Yuhana, M., Junior, M. Z., & Hidayatullah, D. (2019). Immune response and resistance of Pacific white shrimp larvae administered probiotic, prebiotic, and synbiotic through the bio-encapsulation of Artemia sp. *Aquaculture International*, 27(2), 567–580. https://doi.org/10.1007/s10499-019-00346-w

Hong, X., Lu, L., & Xu, D. (2016). Progress in research on acute hepatopancreatic necrosis disease (AHPND). *Aquaculture International*, 24(2), 577–593. https://doi.org/10.1007/s10499-015-9948-x

Huynh, T.G., Cheng, A.C., Chi, C.C., Chiu, K.H., Liu, C.H. (2018). A synbiotic improves the immunity of white shrimp, *Litopenaeus vannamei*: Metabolomic analysis reveal compelling evidence. *Fish Shellfish Immunol*, 79:284–293. https://doi.org/0.1016/j.fsi.2018.05.031.

KKP (Ministry of Marine and Fisheries). (2016). Regulation of the Ministry of Marine and Fisheries of the Republic of Indonesia Number 75/Permen-KP/2016 concerning General Guidelines for Raising Tiger Prawns (*Penaeus Monodon*) and Vaname Shrimp (*Litopenaeus vannamei*) 22nd volume. Indonesiahlm 1–43. https://peraturan.bpk.go.id/Details/158666/permen-kkp-no-75permen-kp2016-tahun-2016.

KKP (Ministry of Marine and Fisheries). (2023). Area

 Copyright @ 2025, Indonesian Aquaculture Journal, p-ISSN 0215-0883; e-ISSN 2502-6577

of cultivation. [Access 2024 Jun 21]. https://portaldata.kkp.go.id/#panel-footer-kpda

Kumar, S. S., Bharathi, R. A, Rajan, J., Alavandi, S., Poornima, M., Balasubramanian, C., & Ponniah, A. (2013). Viability of white spot syndrome virus (WSSV) in sediment during sun-drying (drainable pond) and under non-drainable pond conditions indicated by infectivity to shrimp. *Aquaculture*, 402–403, 119–126. https://doi.org/:10.1016/j.aquaculture.2013.04.001

Lightner, D. V., & Redman, R. (1977). Histochemical demonstration of melanin in cellular inflammatory processes of penaeid shrimp. *J Invertebr Pathol*, 30(3), 298–302. https://doi.org/:10.1016/0022-2011(77)90137-9

Liu, F., Li, S., Yu, Y., Sun, M., Xiang, J., & Li, F. (2019). Effects of ammonia stress on the haemocytes of the Pacific white shrimp *Litopenaeus vannamei*. *Chemosphere*, 239, 124759. https://doi.org/:10.1016/j.chemosphere.2019.124759

Liu, C. H., & Chen, J. C. (2004). Effect of ammonia on the immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus*. *Fish Shellfish Immunol*, 16(3), 321–334. https://doi.org/:10.1016/S1050-4648(03)00113-X.

Madigan, M., Martinko, J., Dunlap, P., & Clark, D. (2014). Biology of Microorganisms. Ed ke-12. San Fransisco (CA): Pearson.

Marsi., Sabaruddin., Warsito., Jubaedah, D., Triovani, B., Fitria, E. A., Anggraini, L., & Nuranjani. (2024). Dynamics of pyritic soil ph due to changes in water conditions and ameliorant application. *J. Solum,* 21(1), 30-42. https://doi.org/:10.25077/jsolum.21.1.30-42.2024

Mira, M., Sujarwo, P. A., Triyanti, R., Shafitri, N., & Zulham, A. (2022). Comparative analysis of Pacific white shrimp farming using traditional, semi-intensive, and intensive techniques in coastal areas. *Jurnal Sosial Ekonomi Kelautan dan Perikanan*, 17(1), 51-62. https://doi.org/:10.15578/jsekp.v17i1.10228

Morales-Covarrubias, M., Cuellar-Anjel, J., Varela-Mejias, A., & Elizondo-Ovares. (2018). Shrimp bacterial infections in latin america: a review. *Asian Fish Sci*, 31S, 76–87. https://doi.org/:10.33997/j.afs.2018.31.s1.001

Parlina, I., Nasirin, N., Ihsan, I. M., Suharyadi, S., Syaputra, A., Budiani, S., & Hanif, M. (2018). Comparison of environmental management in Pacific white shrimp (*Litopenaeus vannamei*) farming with the application of chelated inorganic substances and probiotics.. *Jurnal Teknologi Lingkungan*, 19(1), 33-40. https://doi.org/:10.29122/jtl.v19i1.2533

Peña-Navarro, N., Castro-Vásquez, R., Vargas-Leitón, B., & Dolz, G. (2020). Molecular detection of acute hepatopancreatic necrosis disease (AHPND) in *Penaeus vannamei* shrimps in Costa Rica. *Aquaculture*, 523(735190), 1-8. https://doi.org/:10.1016/j.aquaculture.2020.735190

Rahayu, T. H. (2022). Export-oriented aquaculture and aquaculture villages based on the blue economy. In: Ministry of Marine Affairs and Fisheries, Directorate General of Aquaculture. page 1–23.

Sirikhairin, R., Taengchaiyaphum, S., Sritunyalucksana, K., Thitamadee, S., Flegel, T., & Mavichak, R. (2014). A new and improved PCR method for detection of AHPND bacteria. *Aquatic Animal Health Research Center*. https://enaca.org/?id=96

Soto-Rodriguez, S. A., Gomez-Gil, B., Lozano-Olvera, R., Bolanmejia, C., Aguilar-Rendon, K. G., & Enciso-Ibarra, J. (2018). Pathological, genomic and phenotypical characterization of *Vibrio parahaemolyticus*, causative agent of acute hepatopancreatic necrosis disease (AHPND) in Mexico. *Asian Fish Sci*. 31 Special Acute Hepatopancreatic Necrosis Disease (AHPND), 102–111, https://doi.org/:10.33997/j.afs.2018.31.s1.007

Soto-Rodriguez, S. A, Lozano-Olvera, R., Palacios-Gonzalez, D. A, Bolan-Mejia C., & Rendon-Aguilar, K. G. (2019). Characterization and growth conditions of *Vibrio parahaemolyticus* strains with different virulence degrees that cause acute hepatopancreatic necrosis disease in *Litopenaeus vannamei*. *J World Aquac Soc*, 50(5), 1002–1015. https://doi.org/:10.1111/jwas.12617

Supono, S., Pinem, R. T., & Harpeni, E. (2021). Performance of Pacific white shrimp (*Litopenaeus vannamei*) reared in a biofloc system with different carbon sources. *J Kelaut*, 14(2), 192–202. http://doi.org/10.21107/jk.v14i2.9191

Suwoyo, S. H., Tahe, S., & Fahrur, M. (2015). Characterization of sediment waste in super-intensive Pacific white shrimp (*Litopenaeus vannamei*) farming with varying densities. In the: *Prosiding Forum Inovasi Teknologi Akuakultur*, hlm 901–913.

Tran, L., Nunan, L., Redman, R. M., Mohney, L. L., Pantoja, C. R., Fitzsimmons, K., & Lightner, D. V. (2013). Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. *Dis Aquat Organ*, 105(1), 45–55. https://doi.org/:10.3354/dao02621

Torun, F., Hostins, B., Teske, J., De Schryver, P., Boon, N., & De Vrieze, J. (2020). Nitrate amendment to control sulphide accumulation in shrimp ponds. *Aquaculture*, 521:735010. https://doi.org/:10.1016/j.aquaculture.2020.735010

Valente, C. de S., & Wan, A.H. (2021). *Vibrio* and major commercially important vibriosis diseases in decapod crustaceans. *J Invertebr Pathol*, 181 May 2020, 107527. https://doi.org/:10.1016/j.jip.2020.107527

Wahyuningrum, E., Muskananfola, M., & Suryanto, A. (2016). Relationship between sediment texture, organic matter, and macrozoobenthos abundance in the Wulan Delta, Demak Regency. *Diponegoro Journal of Maquares*, 5(1), 46–51. https://doi.org/:10.2307/2324223

Wiyoto, W., Sukenda, S., Harris, E., Nirmala, K., & Djokosetiyanto, D. (2016). Water quality and sediment profile in shrimp culture with different sediment redox potential and stocking densities under laboratory condition. *Ilmu Kelautan*, 21(2), 65-76. https://doi.org/:10.14710/ik.ijms.21.2.65-76

Wiyoto, W., Sukenda, S., Harris, E., Nirmala, K., Djokosetiyanto, D., & Ekasari, J. (2017). The effects of sediment redox potential and stocking density on Pacific white shrimp *Litopenaeus vannamei* production performance and white spot syndrome virus resistance. *Aquac Res*, 48(6), 2741–2751. https://doi.org/:10.1111/are.13107

Zhang, X., Yuan, J., Sun, Y., Li, S., Gao, Y., Yu, Y., Liu, C., ... Xiang, J. (2019). Penaeid shrimp genome provides insights into benthic adaptation and frequent molting. *Nat Commun*, 10(1), 1-14. https://doi.org/:10.1038/s41467-018-08197-4

Zeng, S., Wei, D., Hou, D., Wang, H., Liu, J., Weng, S., He, J., & Huang, Z. (2021). Sediment microbiota in polyculture of shrimp and fish pattern is distinctive from those in monoculture intensive shrimp or fish ponds. *Science of the Total Environment*, 787, 147594. https://doi.org/:10.1016/j.scitotenv.2021.147594

Zhou, Q., Li, K., Jun, X., & Bo, L. (2009). Role and functions of beneficial microorganisms in sustainable aquaculture. *Bioresour Technol*, 100(16), 3780–3786. https://doi.org/:10.1016/j.biortech.2008.12.037

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