JoAS Volume 9 No. 1 April 2024
DOI: https://doi.org/10.24073/joas.v9i1.54415
pISSN 2550-0910; eISSN 2579-4817
Available online at https://e-journal.unair.ac.id/JoAS

Journal of Aquaculture Science

Monitoring Diseases in Water of Vannamei Shrimp (Litopenaeus Vannamei), Banyuwangi District

Andhika Putra Ramadhan*, Ervina Wahyu Setyaningrum , Mega Yuniartik

Department of Fisheries Science, Faculty of Agriculture and Fisheries, Universitas 17 Agustus 1945 Banyuwangi

Article info:

Submitted: February 12, 2024
Revised: May 3, 2024
Accepted: May 6, 2024
Published: April 30, 2024

E-mail addresses:
dhikaputraramadhan19@gmail.com
*Corresponding author

ABSTRACT

Banyuwangi Regency has high potential for vannamei shrimp farming. Vannamei shrimp farming is susceptible to disease attack. Disease monitoring serves to determine the distribution of disease in vannamei shrimp ponds. The purpose of this study was to monitor disease in vannamei shrimp pond waters of Banyuwangi Regency. This research method uses descriptive method. Monitoring of water quality parameters insitu and exitu. Disease monitoring using Real-Time PCR Quant studio 5 to detect AHPND, WSSV, EHP, and IMNV. The analysis used was descriptive analysis. The results showed that the water quality in vannamei shrimp ponds in Banyuwangi Regency was an average temperature of 25.8°C, average salinity of 33.9 g.L^{-1}, average dissolved oxygen (DO) 7.15 mg.L^{-1}, average pH 8.15, average ammonia 0.257 mg.L^{-1}, average nitrite 0.04 mg.L^{-1}, average nitrate 4.9 mg.L^{-1}. As for the disease, there are three types, namely AHPND, EHP, and IMNV.

Keyword: aquaculture, disease, vannamei shrimp.

INTRODUCTION

Banyuwangi Regency is the largest regency in East Java Province with an area of 5,782.50 km^2 with a coastline length of 175.8 km and has ten islands (Setyaningrum et al., 2019). It is located between 7°43' - 8°46' N and 113°53' - 114°38' E, bordering Jember, Situbondo, and Bondowoso regencies. Industrial development, agribusiness areas, agro-industrial housing, transport, ports, and tourism are the main priorities in Banyuwangi Regency (Setyaningrum et al., 2020). The long coastline in Banyuwangi Regency is suitable for shrimp farming development because it relies on land that has not been fully utilised. Currently, at least 1,386 hectares of the total 1,417.2 hectares of pond potential in Banyuwangi Regency have been utilized, or there are still 31.2 hectares of land that have not been utilized and 605 hectares need to be rehabilitated (Rosyidah et al., 2020). Most of the pond land used in Banyuwangi is mainly for cultivating vannamei shrimp, both traditional and intensive methods. Vaname shrimp cultivation has advantages compared to other shrimp because it is more profitable than tiger shrimp (Isamu et al., 2018). However, water quality plays an important role in increasing pond productivity (Setyaningrum et al., 2023). Furthermore, a safe and controlled environment is a supporting factor for the development of vannamei shrimp cultivation (Purnamasari et al., 2017). Infectious diseases can occur if shrimp experience stress due to inadequate environmental ecology or an imbalance between pathogens, the environment and the host. In cultivating vannamei shrimp, there are water quality parameters such as temperature, salinity, dissolved oxygen (DO), pH, ammonia, nitrite and nitrate. The rapid development of the

Vannamei shrimp cultivation industry and changes in local climate have changed the ecological balance (Yuniartik *et al.,* 2022; Maryati *et al*., 2017).

One of the problems in vannamei shrimp farming due to fluctuations in water quality is the onset of disease. Diseases that appear include White Spot Syndrome Virus (WSSV), Acute Hepatopancreatic Necrosis Disease (AHPND), Enterocytozoon hepatopenaei (EHP), and Infectious Myonecrosis Virus (IMNV) (Sukenda *et al*., 2011). According to Lee *et al.,* (2022) In the last 20 years, shrimp diseases have caused critical economic losses that seriously threaten aquaculture practices, where White Spot Syndrome (WSS) is a deadly viral disease caused by White Spot Syndrome Virus (WSSV).

IMNV infection can cause shrimp population deaths of up to 70%. IMNV transmission occurs horizontally through water due to cannibalism, while vertical transmission occurs from parent to seed (Zaujat *et al*., 2016). Meanwhile, hepatorenal Enterocytozoon (EHP) is a microsporidian parasite that inhibits the growth of vannamei shrimp by attacking their hepatopancreas. On the other hand, reduced dissolved oxygen causes the decomposition process of organic matter to be hampered and increases nitrite (NO_2) and ammonia (NH_3), resulting in shrimp poisoning and even death (Nkuba et al., 2021). Acute hepatopancreatic necrosis disease (AHPND), first appeared in China in 2009, the outbreak spread to Vietnam (2010), Malaysia (2011), Thailand (2012), Mexico (2013), Philippines (2015) and South America (2016) (Kumar *et al*., 2021). Fluctuations in water quality parameters such as temperature, salinity and pH as well as non-compliance with water quality standards can lead to the occurrence of AHPND disease (Suryana *et al*., 2023).

Factors causing the decline in shrimp resistance to disease attack are due to poor aquatic ecology (Maryati *et al*., 2017). This continues to cause mass die-offs as well as population decline (Hadie & Hadie, 2017). Disease monitoring activities in vannamei shrimp are important in supporting the success of aquaculture. (Fatmala *et al*., 2019). The purpose of this study was to monitor disease in vannamei shrimp ponds in Banyuwangi Regency.

MATERIAL AND METHOD

Time and Location

The research was conducted from November 2023 to January 2024. The research was conducted in Banyuwangi Regency, namely vannamei shrimp ponds in Wongsorejo, Kalipuro, Banyuwangi, Kabat, Blimbingsari, and Muncar sub-districts (Figure 1).

Research Material

The research materials used in this study were vannamei shrimp pond water samples at DOC 20 and water quality parameters (temperature, salinity, pH, DO, TAN, nitrate, and nitrite).

Tabel 1. Measurement of Water Quality Parameters

Parameters	Method	Unit	Description Observation
Temperate	Termometer	°C	*In-situ*
Salinity	Refraktometer	$g.L^{-1}$	*In-situ*
pH	pH meter	-	*In-situ*
DO	DO meter	$mg.L^{-1}$	*In-situ*
TAN	Test Kit	$mg.L^{-1}$	Laboratorium
Nitrate	Test Kit	$mg.L^{-1}$	Laboratorium
Nitrite	Test Kit	$mg.L^{-1}$	Laboratorium

pISSN 2550-0910; eISSN 2579-4817
Available online at https://e-journal.unair.ac.id/JoAS

Data Collection Method

Data collected using surveys and direct observation, where the process of collecting data in the form of diseases is as follows.

Water Sampling

Water sampling in the pond by tying the sample bottle on the bamboo and inserted into the pond horizontally on the edge of the pond about 15 cm from the bottom of the pond. The first to third bottle rinses are discarded, then the fourth new collection in the special sample bottle of disease. Samples are then placed into a sample bottle with a size of 300 ml. Furthermore, the sample bottle is placed into a cool box to maintain the durability of the sample (Umami *et al.*, 2018). The samples were then taken to the laboratory for observation.

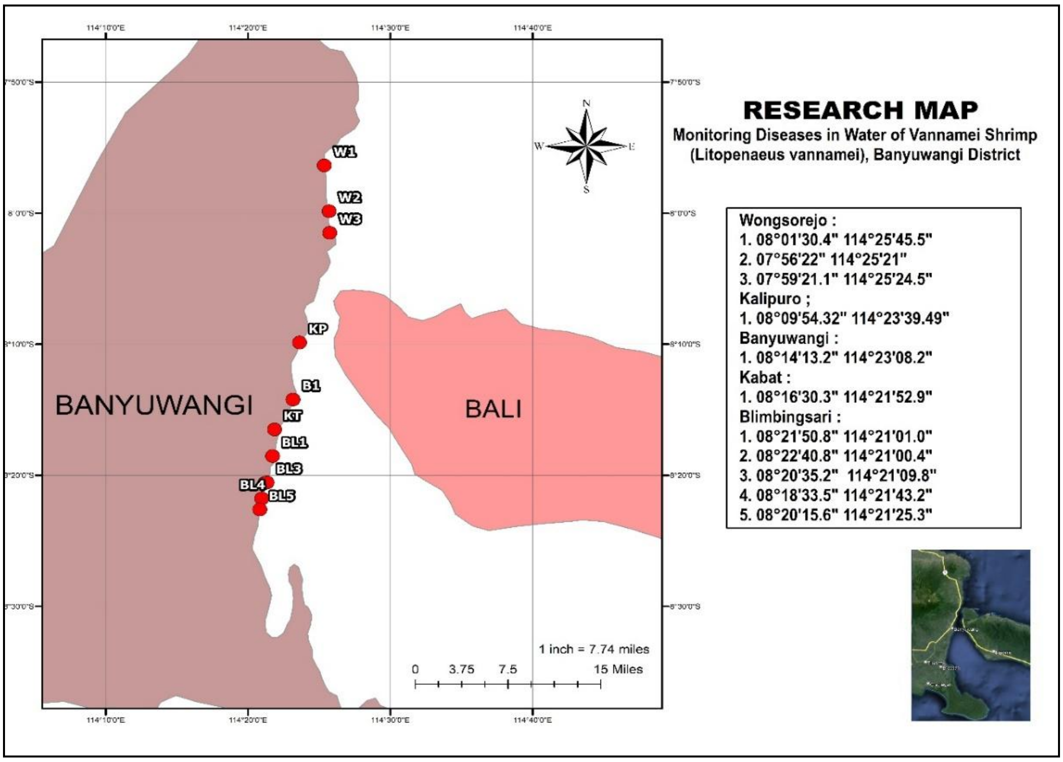

Figure 1. Vannamei Shrimp Pond Research Location Map

Disease Identification

Disease identification was analyzed using the Quant Studio 5 Real-Time PCR tool. Previously, 200 ml of prepared water samples were taken.

Data Interpretation

Based on CT value data from AHPND, WSSV, EHP, and IMNV diseases. The limit value for negative is >40, the value of 40 - 35 indicates a low positive, the value of 35-28 indicates a medium positive result, and the value <28 indicates a high positive result. The result of the analysis is that the more it is pulled to the center, the higher the potential for disease, conversely, the more it is pulled outward, the lower the potential for disease. Quant studio 5 Real-Time PCR test results are declared negative if the CT (cycle threshold) value exceeds 40, depending on each laboratory. This means that within 40 repetitions of PCR amplification, there is no viral genetic material in the sample being tested.

Data Analysis

This study used descriptive analysis. Descriptions were made of water quality and disease from PCR results which were then analyzed using a spider web. Where according to Permatasari *et al.* (2023), The use of the spider web method is an alternative learning method that can optimally improve results.

RESULT AND DISCUSSION

Water Quality

Water quality parameters are influential to Vannamei shrimp farming activities. Based on checking water quality (Table 2) in-situ and ex-situ get the following results. The results of measuring water quality parameters during the research were temperature ranging from 27 – 31°C, with an average of 25.8°C, salinity ranging from 5 – 35 g.L^{-1} with an average of 33.9 g.L^{-1}, DO ranging from 4 – 9.1 mg.L^{-1} with an average of 7.15 mg.L^{-1}. Meanwhile, pH values range between 6.5 – 9 with an average of 8.15, ammonia ranges from 0 – 1.21 mg.L^{-1} with an average of 0.257 mg.L^{-1}, and averages for nitrite and nitrate are 0.04 and 4, respectively. According to PERMEN KP No.75, (2016), the quality standard values for temperature are 28-30 °C, salinity 26-32 g.L^{-1}, DO >4 mg.L^{-1}, pH 7.5 - 8.5, ammonia ≤ 0, 1, nitrite ≤1, and nitrate ≤0.5. The average results in the table above do not comply with the quality standards: temperature, salinity, ammonia, and nitrate.

Table 2. Water Quality Check Result

Code	Temp (°C)	Salinity (g.L^{-1})	DO (mg.L^{-1})	pH	Amonia (mg.L^{-1})	Nitrite (mg.L^{-1})	Nitrate (mg.L^{-1})
W 2B	28.5	35.00	7.6	8.5	1.21	0	0
W 3B	28	24.00	4.9	8	0.12	0	0
KP 1B	29.2	20.00	9.1	8.4	0.233	0.091	2
B 1B	27	5.00	7.9	9	0	0.002	0
KT 1B	29.7	30.00	5.9	7.8	0.116	0	0
BL 1B	31	25.00	7.0	7.9	0.33	0.08	8
BL 2B	28.1	33.00	8.7	8.6	0.14	0.09	10
BL 3B	30	25.00	7.9	6.5	0.27	0.08	13
BL 4B	28	29.00	4.0	8.2	0	0.07	15
BL 5B	28	13.00	8.5	8.6	0.15	0	1
Average	25.8	23.90	7.15	8.15	0.257	0.04	4.9

Note. W : Wongsorejo; KP : Kalipuro; B : Banyuwangi; KT : Kabat; BL : Blimbingsari; B : days of cultivation

The results of the water quality measurements above serve to monitor the success of vannamei shrimp cultivation. This is by the statement by Ritonga et al., (2021), that water quality management plays an important role in the success of shrimp cultivation. Decreased water quality causes shrimp to become stressed and susceptible to disease, which often leads to failure in shrimp farming. The temperature in vannamei shrimp ponds in Banyuwangi Regency ranges between 27 – 31°C with an average of 25.8°C. The temperature of each pond in each sub-district varies due to changes in weather at each water sampling location and the presence of ponds. close to the mangrove area (Yuniartik, 2021). Temperature is a physical parameter that affects the growth rate and life of aquatic biota (Dewi & Yuniartik, 2019).

Salinity in vannamei shrimp ponds Banyuwangi Regency has a range of 5 - 35 g.L^{-1} with an average of 23.9 g.L^{-1}, salinity in each pond in each sub-district is different because the salinity used in cultivation is different in each pond. Each organism, especially vannamei shrimp have varying tolerance to changes in salinity, because it has an influence on the growth and survival rate of aquatic organisms (Renitasari & Musa, 2020).

The pH ranged from 6.5 to 9 with an average pH value of 8.15. This value meets the requirements of quality standards. Each pond in Banyuwangi Regency has a value that meets the requirements of quality standards. Each pond in Banyuwangi district has different values depending on meteorological factors and sedimentation of feed residue. Meteorological factors are influenced by moderate rainfall and sedimentation of feed residues. The increase in pH that occurs in aquaculture ponds is caused by the decomposition of waste and feed residues, while the decrease in pH is caused by the addition of water to the reservoir and the intrusion of rainwater into the pond compartment and does not drop suddenly (Purnamasari et al., 2017).

Dissolved oxygen (DO) in vannamei shrimp ponds ranged from 2.38 - 9.1 mg.L^{-1} with an average of 7.15 mg.L^{-1}. According to Edi *et al.*, (2021) the value of DO is related to the value of temperature if the low temperature then high dissolved oxygen levels, while high temperatures cause low dissolved oxygen levels. Meanwhile, ammonia ranges from 0 - 1.21 mg.L^{-1}with an average of 0.257. Ammonia has a major effect on shrimp survival. This agrees with that if the high concentration of ammonia identifies that the waters are polluted (Hamuna *et al.*, 2018). The nitrite value found in vannamei shrimp ponds in the range of 0 - 0.091 mg.L^{-1}with an average of 0.04 mg.L^{-1}. High nitrite content affects the growth rate of shrimp and shrimp susceptibility to disease. This agrees with Pasongli & Dirawan, (2016) that nitrite is a toxic substance in shrimp farming, especially in shrimp growth, the result of ammonia oxidation obtained from nitrite and *Nitrosomonas* bacteria. As for the value of nitrate that can range from 0 - 15 mg.L^{-1} with an average result of 4.9 mg.L^{-1}. This value exceeds the limit of aquaculture quality standards, thus affecting the ecology of the waters themselves. This is in line with the opinion of Hamuna *et al.*, (2018) Nitrate of more than 0.2 mg.L^{-1} is caused by enrichment and algae blooming, which is very detrimental because it can affect the local aquatic ecosystem.

Type of Disease

Disease is an obstacle for vannamei shrimp farmers. Based on laboratory observations using Real-Time PCR Quant studio 5 shows the results of disease identification in (Table 3) as follows. From the results of Real-Time PCR analysis, three farms were found to have indications of disease, namely IMNV with a CT value of 38.8 with low potential in ponds in Banyuwangi region, EHP was found in the first pond in Blimbingsari region with a CT value of 29.9 with low potential, and AHPND was found in the third pond in Blimbingsari region with a CT value of 37.2 with low potential. The following illustrates the results of the analysis using a spider web graph (Figure 2). In Banyuwangi region ponds were found to have very low salinity, resulting in high susceptibility of shrimp to IMNV infection. This statement was confirmed (Mita *et al.*, 2016) low salinity leads to increased susceptibility of shrimp to IMNV infection, so stress levels in shrimp will increase, leading to increased virus replication and shrimp mortality.

Table. 3 Disease Monitoring Data in Vannamei Shrimp Farms of Banyuwangi Regency.

DOC 20	Disease Name			
Farm Code	*AHPND*	*WSSV*	*EHP*	*IMNV*
W 2	40	40	40	40
W3	40	40	40	40
KP 1	40	40	40	40
B 1	40	40	40	38.8
KT 1	40	40	40	40
BL 1	40	40	29.9	40
BL 2	40	40	40	40

Continued in next page

BL 3	37.2	40	40	40
BL 4	40	40	40	40
BL 5	40	40	40	40

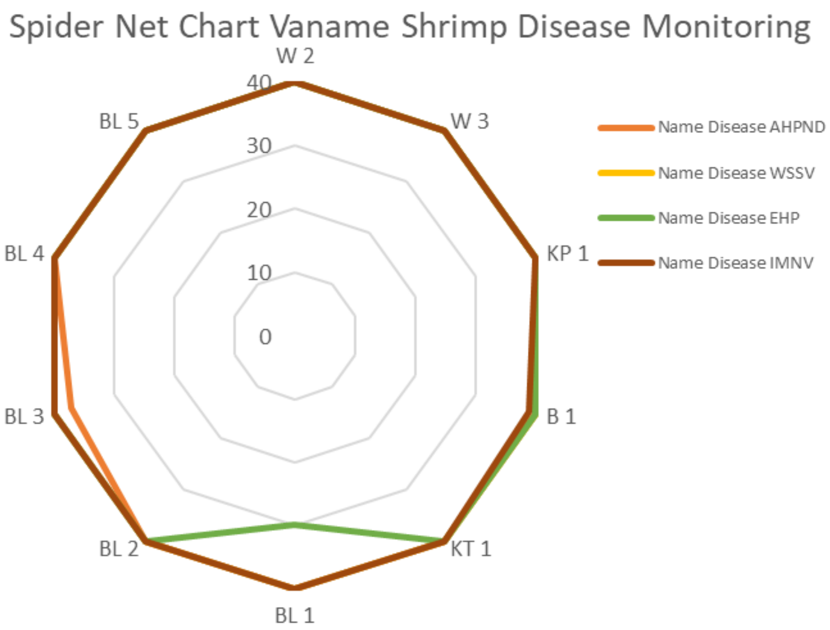

Figure 2. Disease Monitoring Results.

Based on research results Zaujat *et al.*, (2016) environmental factors and farming practices of vannamei shrimp that differ from region to region are thought to have a strong influence on the diversity of IMNV diseases in Indonesia. Natural exchange of IMNV between regions is also possible through vectors, shrimp carcasses, contaminated water, or atmospheric sources. Transmission involves poor water quality from inlets and outlets, and natural hosts on water bodies, while terrestrial transmission occurs through aquatic ecology. Research results Lee *et al.*, (2022) reinforces that the cause of the disease is due to poor ecology (unstable water quality fluctuations) and poor feed quality.

In the first pond in the Blimbingsari area, EHP was found due to high water temperatures. This statement agrees with Hamzah *et al.*, (2022) temperature affects oxygen consumption, growth, and survival of shrimp in the rearing environment. The effect of temperature alone is associated with increased mortality of disease-infected shrimp.. According to Aras *et al.*, (2023) EHP transfer occurs during transmission from broodstock, but it is also possible through feces, so that spores can be contaminated in naturally fed rearing tanks, especially in the late stages of naupli and larval hatching. Research results Jaroenlak *et al.*, (2018) stated that EHP is known to occur widely in Asia e.g. Thailand, China, India, Vietnam, Indonesia and Malaysia. As such, any treatment or management protocol that would stop or interrupt transmission would be an effective control measure.

According to Se *et al.*, (2023) shrimp reared at low temperatures can affect metabolism and appetite. Temperature affects the appetite and eating habits of shrimp. When the temperature is low, the shrimp's appetite decreases, so the vannamei shrimp becomes weak and does not actively swim until black spots appear on its body. Furthermore, in the third pond in the Blimbingsari area identified AHPND disease caused by water quality at low pH. This statement agrees with Farabi & Latuconsina (2023) pH in ponds affects the fertility and productivity of shrimp. pH is too low or acidic can affect the toxicity in water increases, so it can jeopardize the survival of shrimp. optimal pH for shrimp farming is the range of 7.5 - 8.5. From the results of the study stated that Farabi & Latuconsina (2023) AHPND disease can develop rapidly around 8 days or after seed stocking, and severe mortality occurs at 20 to 30 days post rearing. Research results Kumar *et al*., (2021), stated that dirty water conditions and low water quality can cause disease, for example, high nitrite and ammonia.

CONCLUSION

From the results of research conducted on vannamei shrimp ponds DOC 20 in Banyuwangi Regency, it can be concluded that the water quality is the average temperature of 25.8 °C, the average salinity of 33.9 g.L^{-1}, DO (Dissolved Oxygen) average 7.15 mg.L^{-1}, pH average 8.15, ammonia average 0.257 mg.L^{-1}, nitrite average 0.04 mg.L^{-1}, nitrate average 4.9 mg.L^{-1}. While the results of the Quantstudio 5 Real-Time PCR test contained three diseases, namely AHPND, EHP, and IMNV.

ACKNOWLEDGEMENTS

The authors would like to thank the Faculty of Agriculture and Fisheries, University of 17 August 1945 Banyuwangi and the Indonesian Cakrawala Conservation Foundation for the research facilities provided to the authors.

CONFLICT OF INTEREST

There is no conflict of interest in this manuscript between all authors upon writing and publishing this manuscript.

AUTHOR CONTRIBUTION

Ervina Wahyu Setyaningrum, Andhika Putra Ramadhan, and Mega Yuniartik contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

FUNDING INFORMATION

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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