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Prevalence of Parasites and Water Quality Measurement in Vannamei Shrimp (*Litopenaeus vannamei* Boone) Ponds in Padang Pariaman Area, Indonesia

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Abstract

Background: *The vannamei shrimp (Litopenaeus vannamei Boone) is a marine species of high economic value. Meanwhile, vannamei shrimps are easily attacked by parasites that can cause failure in the cultivation. This study aims to investigate ectoparasites and endoparasites in vannamei shrimp ponds in the Padang Pariaman area in terms of their prevalence and the pond water quality.* **Methodology:** *This research was carried out by purposive random sampling method. The parasites identification were conducted at the Animal Taxonomy Laboratory, Biology Department, Universitas Andalas.* **Findings:** *Six species of ectoparasites were found, namely Acineta sp., Arcella sp., Epistylis sp., Trichodina sp., Vorticella sp., and Zoothamnium sp. Moreover, only one species of endoparasite was found, namely Enterocytozoon hepatopenaei (EHP). The prevalence of ectoparasite species was highest in Zoothamnium sp. in 40-day-old shrimp (80 %) and 63.3 % in 30-day-old shrimp. Further, the prevalence of endoparasites was highest in female shrimp (94.5 %), while it was 87.8 % in male shrimp. Furthermore, there were six parameters of shrimp pond water quality that do not meet the cultivation standards, namely pH, DO, BOD, COD, ammonia, and zinc, while the other two parameters meet the standards, namely temperature and salinity. In conclusion, the production of vannamei shrimp can be reduced and parasitic disease outbreaks can result from water quality that does not meet culture standards.* **Contribution:** *This study contribute to the shrimp ponds in the area in managing stocking density, feeding levels, and sanitation.*

Keywords: *Ectoparasites; Endoparasites; Prevalence; Vannamei shrimp; Water quality*

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INTRODUCTION

Padang Pariaman Regency is one of the regencies in West Sumatra Province, which is a buffer zone between Padang City and Pariaman City. The topographical conditions of this area consist of 40 % lowlands in the coastal area and 60 % hilly highlands up to Bukit Barisan (Dinas Lingkungan Hidup Kab. Padang Pariaman, 2007). Currently, several coastal areas in Padang Pariaman Regency have been widely used by the community as shrimp pond cultivation areas. Shrimp pond activities along the coast of Padang Pariaman Regency are found in 6 subdistricts and 15 villages with a total of 93 pond units (Hamdi et al., 2022). The rapid development of shrimp ponds occurs because the demand for shrimp in the domestic and global market is increasing every year (FAO, 2020).

Shrimp is one of the fishery products that has a high animal protein content (Dugassa & Gaetan, 2018). The shrimp species that is popularly cultivated is the vannamei shrimp (*Litopenaeus vannamei* Boone). Vannamei shrimp originate from the waters of Central and South America and have spread throughout Indonesia and been developed by farmers and the government. Vannamei shrimp is the main cultivated species and has an important market value in the world; in 2017, the production of vannamei shrimp reached 1.1 million tons. However, the export value of Indonesian vannamei shrimp is still lower than other world shrimp-producing countries, such as India, Vietnam, Ecuador, China, and Thailand. India is recorded as the country with the highest shrimp export value in the world, reaching US$3.70 billion, followed by Vietnam, Ecuador, China, Thailand, and Indonesia, each of which has an export value in US dollars of 2.71 billion, 2.60 billion, 2.16 billion, 1.98 billion, and 1.67 billion (KKP, 2017).

The development of vannamei shrimp cultivation is becoming increasingly rapid, replacing the cultivation of windu shrimp (*Penaeus monodon*). The main reasons for the commodity shift from windu shrimp to vannamei shrimp cultivation include its high productivity, its ability to utilize the entire water column from the bottom to the surface so that it can be maintained under high stocking density conditions, and it has a shorter maintenance time because its growth is relatively faster (Herawati et al., 2020). While the weakness of vannamei shrimp is susceptibility to diseases and parasites. The presence of parasitic infections in shrimp causes the shrimp to have difficulty changing their skin (molting), slow growth, and can lead to death (Pamenang et al., 2020).

Parasitic attacks can be a predisposing factor for infection by more dangerous pathogenic organisms such as viruses and bacteria. The high number of parasites can inhibit the growth of shrimp because the parasites suck the blood and nutrients of the shrimp. The losses caused by ectoparasite attacks can be in the form of damage to external organs, namely skin and gills. Patoka et al., (2016) also stated that high ectoparasite attacks can cause acute death even though there are no symptoms in the shrimp. One of the causes of the occurrence of parasitic protozoa in vannamei shrimp is the deterioration of water quality and non-compliance with farming standards. Poor environmental conditions can be caused by high stocking densities or leftover feed, which can increase ammonia levels in the water and waste accumulation in the culture areas, making shrimp more susceptible to disease. The presence of parasites in excess of normal limits can affect the health of the host. Water quality parameters to consider include pH, salinity, temperature, ammonia, zinc, biological oxygen demand (BOD), chemical oxygen demand (COD), and dissolved oxygen (DO).

Prevalence is the frequency of a parasite in the body of a given population

(Dang et al., 2021). Prevalence is related to the habitat and distribution of parasites in the host (Hidayat et al., 2021). The approach to the prevalence of parasites that infect shrimp will provide knowledge of the impact of parasites on the shrimp population in the pond. Therefore, based on the weaknesses of vannamei shrimp, it is very necessary to conduct a survey of the prevalence of ectoparasites and endoparasites and water quality measurement in vannamei shrimp (*L. vannamei*) ponds in the Padang Pariaman Area, West Sumatra Province, Indonesia, in order to provide information to shrimp farmers about the ectoparasites and endoparasites found so that they can determine policies in pond management.

METHOD

The place and Time of The Research

Sampling was conducted in vannamei shrimp ponds in Padang Pariaman, West Sumatra, Indonesia, and was carried out from October to December 2024. Sampling was collected using the purposive sampling method. Samples were taken in the afternoon to avoid stress on the shrimp and were carried out on 30-day-old and 40-day-old shrimp in six ponds. A total of 10 individual shrimp were collected in each pond. Then, the samples were put into 1-liter glass bottles and filled with pond water and oxygen to be stored in a cool box containing ice cubes to maintain freshness and inhibit changes in the rate of sample quality before being tested at the Animal Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Indonesia.

Ectoparasite Sample Identification

Shrimp samples were placed on a glass slide for identification under a Corona Binocular Microscope XSZ-107 BN. Parts identified included the rostrum, antennae, walking legs (pereiopod), swimming legs (pleopod), abdomen, and tail. Ectoparasites were observed and photographed with a 24 MP camera Vivo V15 smartphone. The ectoparasites found were identified based on their morphological characteristics and referring to Garcia (2016).

Endoparasite Sample Identification

The standard centrifugation floating method was used to extract parasites and/or their eggs from the shrimp hepatopancreas (Pyziel-Serafin et al., 2022). As much as 2 g of hepatopancreas sample from each shrimp (n= 60) was placed into 15 ml reaction tubes and diluted with distilled water until three-fourths of the tubes were filled. Tubes were spun using a Hettich Universal 1200 centrifuge at 2,000 rpm for 5 minutes. The supernatant layer formed atop the hepatopancreas solution was disposed of before refilling with saturated NaCl solution until three-fourths of the tubes were filled. The tubes were spun again for another 5 minutes, with saturated NaCl solution added afterward to top the tubes bulgingly. The test tubes were then slid onto the opening of the tubes, left for around 5 minutes, and lifted. Next, cover glasses were placed on the wetted side of each object glass before being examined with the Corona Binocular Microscope XSZ-107 BN at optimum magnification (100 x 400) to detect the presence of parasites, oocysts, or parasite eggs. Upon spotting parasites, oocysts or eggs were snapped with a 24 MP camera Vivo V15 smartphone. The measurements of parasites, oocysts, or eggs used the online GNU Image Manipulation Program (GIMP) software (available for free at https://gimp.org) and were expressed in micrometer (μm) units until two decimal precisions. The identification process was conducted by observing parametric measurements, morphological characters, and pictures of the objects, all guided with proper references, either for identifying parasites (Garcia, 2016) or the shrimp.

Water Quality Testing in The Shrimp Ponds

The water quality parameters of the culture media observed in this study were eight parameters: pH, salinity, temperature, ammonia, zinc (Zn), biological oxygen demand (BOD), chemical oxygen demand (COD), and dissolved oxygen (DO). Testing of the all parameters was carried out at Balai Standardisasi dan Pelayanan Jasa Industri (BSPJI), Padang City, West Sumatra, Indonesia.

Data Analysis for Prevalence and Intensity of Parasites

Prevalence is the percentage comparison between the number of samples invested with ectoparasites and the total number of samples identified. Intensity is the comparison between the number of individuals of a given parasite and the total number of shrimp infested with parasites. The results of the calculation of the prevalence and intensity of ectoparasites and endoparasites were then entered into the prevalence and intensity table.

RESULT AND DISCUSSION

Ectoparasite and Endoparasite Species Obtained in *Litopenaeus vannamei* Boone

Based on the results of research conducted at the Animal Taxonomy Laboratory, Department of Biology, Andalas University. Six species of ectoparasites were found attacking *L. vannamei* shrimp in the ponds in the Padang-Pariaman area, namely *Acineta* sp., *Arcella* sp., *Epistylis* sp., *Trichodina* sp., *Vorticella* sp., and *Zoothamnium* sp. While the endoparasite was one species, namely *Enterocytozoon hepatopenaei* (EHP).

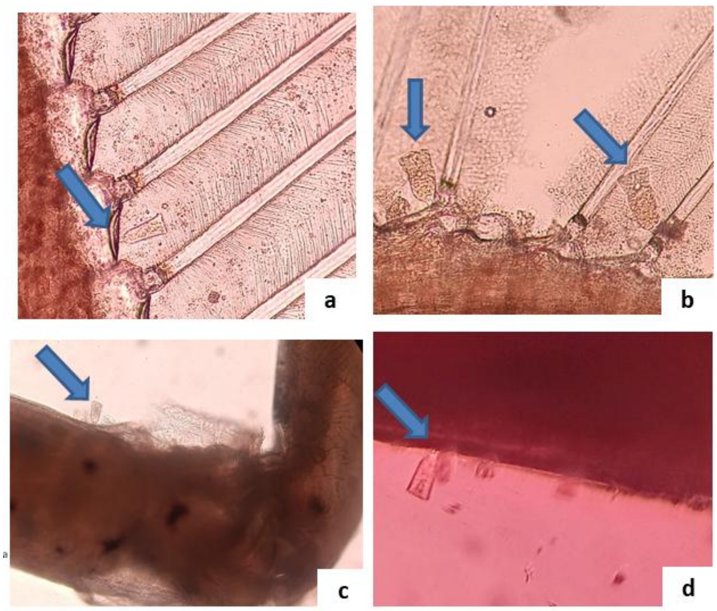

Figure 1. *Acineta* sp. with 10x magnification on a) tail; b) swimming legs; c) walking legs; d) rostrum

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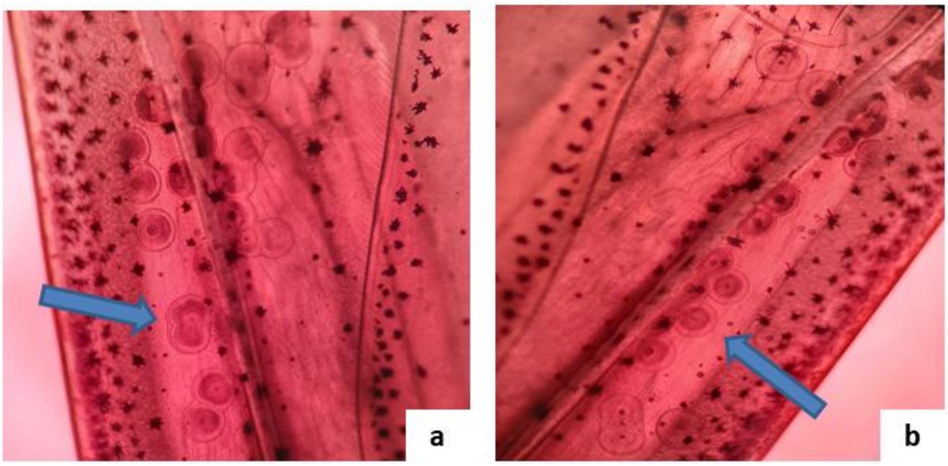

Figure 2. *Arcella* sp. with 10x magnification on a-b) tail

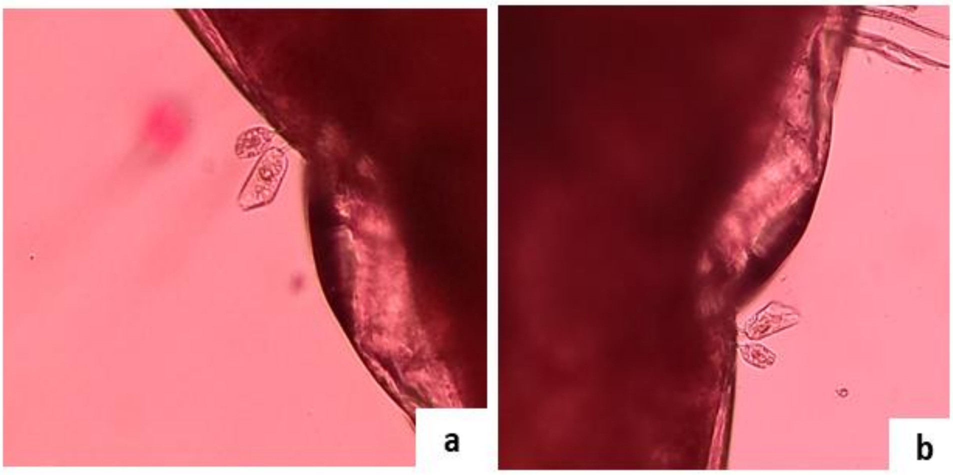

Figure 3. *Epistylis* sp. 10x magnification on a-b) walking legs.

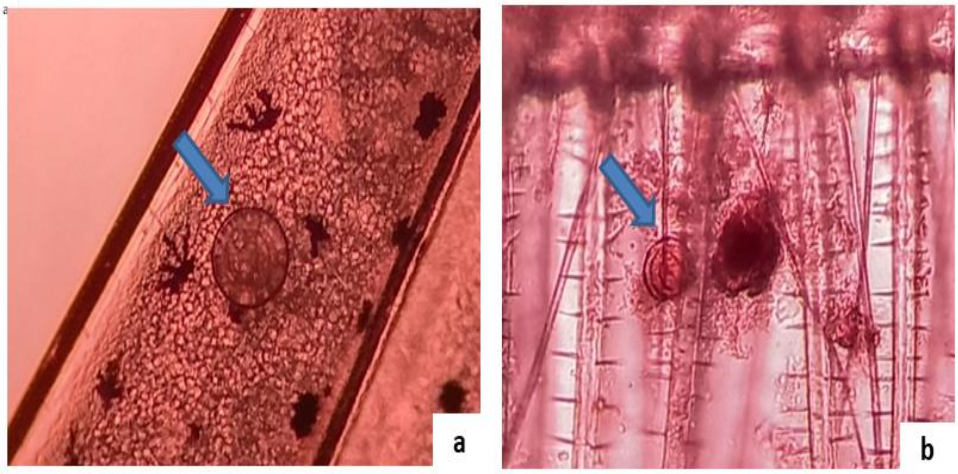

Figure 4. *Trichodina sp.* 10x magnification on a) tail; b) swimming legs

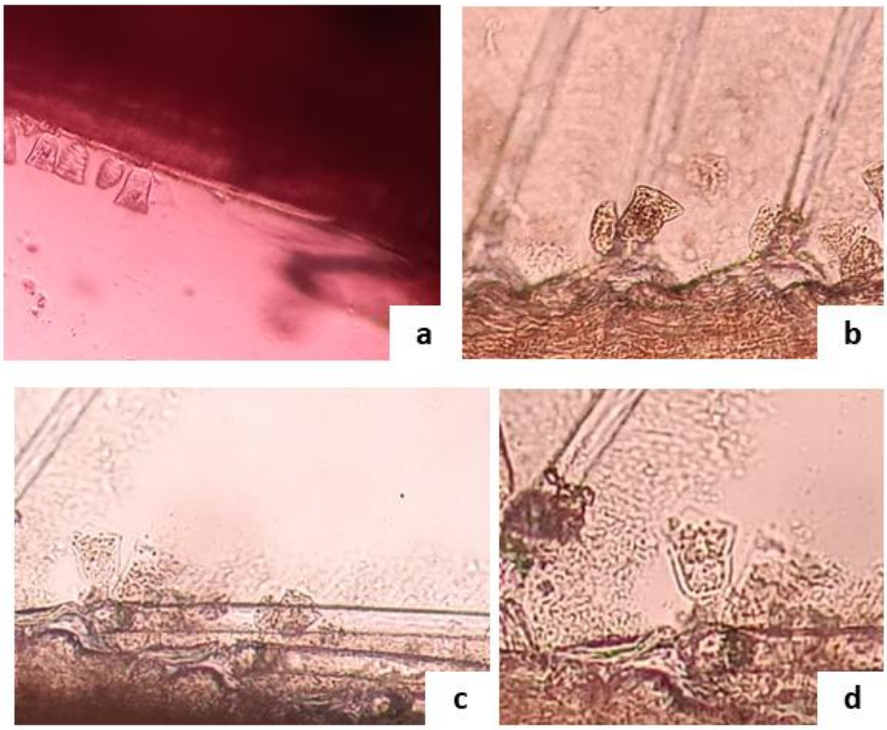

Figure 5. *Vorticella* sp. 10x magnification on a) rostrum; b) tail; c) swimming legs; d) walking legs

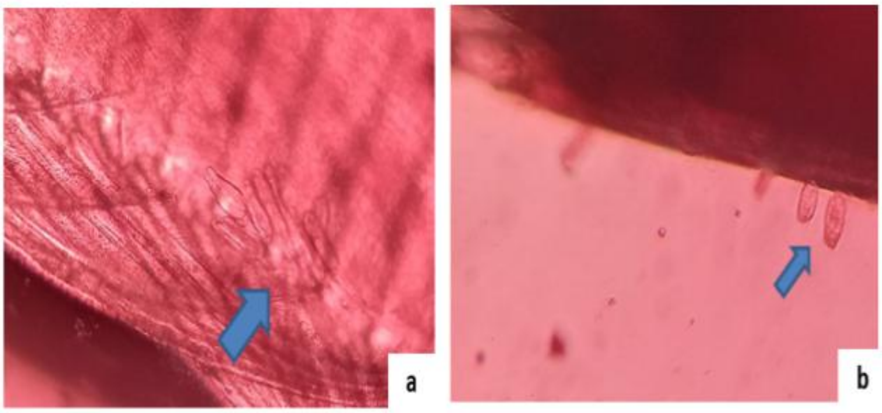

Figure 6. *Zoothamnium* sp. 10x magnification on a) swimming legs; b) rostrum

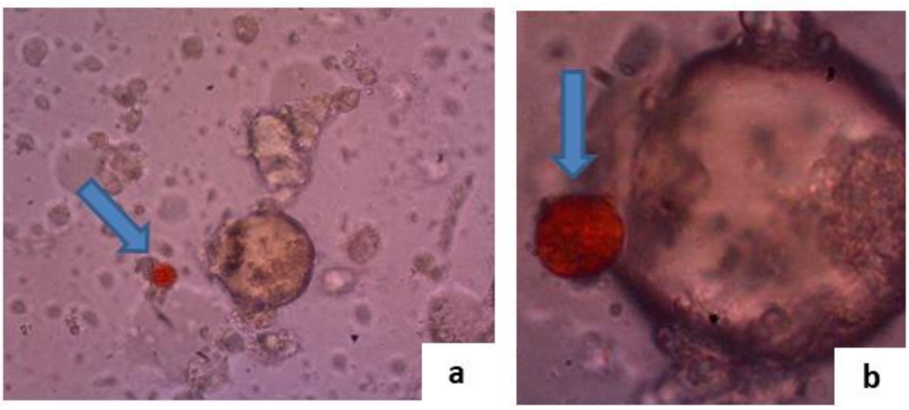

Figure 7. *Enterocytozoon hepatopenaei*, a) 40x magnification, b) 100x magnification

Prevalence and Intensity of Ectoparasites in *L. vannamei*

Table 1. Prevalence and intensity of ectoparasite species in *Litopenaeus vannamei* Boone

No	Species	30-day-old shrimp (n=30)		40-day-old shrimp (n=30)	
		Prevalence	Intensity	Prevalence	Intensity
1	*Acineta* sp.	36.6 %	9.5	56.6 %	14.8
2	*Arcella* sp.	23.3 %	22.7	40 %	15.9
3	*Epistylis* sp.	53.3 %	9.3	70 %	11.2
4	*Trichodina* sp.	6.6 %	1	16.6 %	2.2
5	*Vorticella* sp.	46.6 %	10	63.3 %	11.5
6	*Zoothamnium* sp.	63.3 %	8.4	80 %	13.7

Microhabitat of Ectoparasites in *L. vannamei*

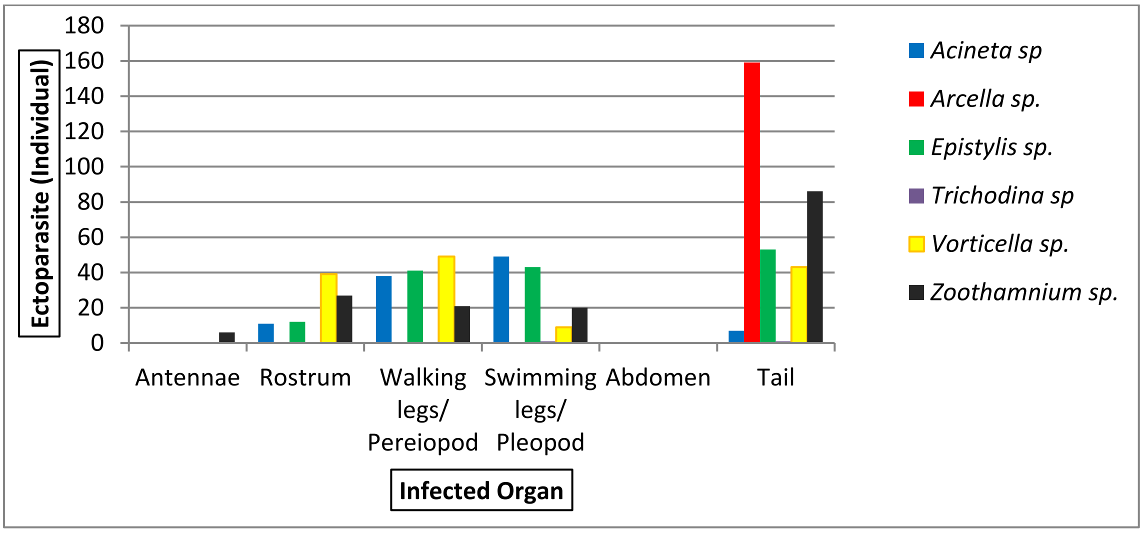

Figure 8. Microhabitat of Ectoparasites in 30-day-old *L. vannamei*

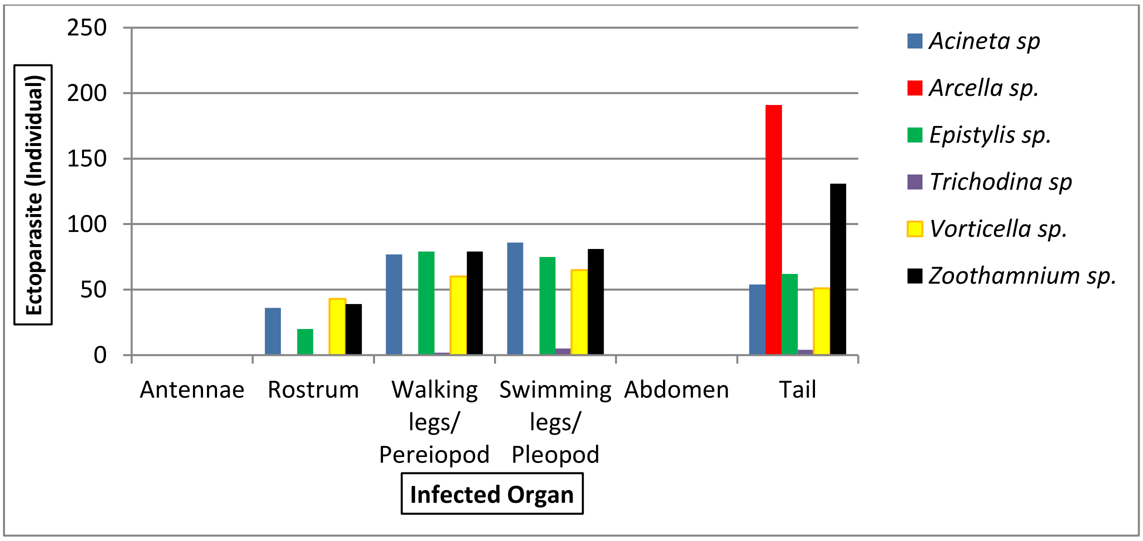

Figure 9. Microhabitat of Ectoparasites in 40-day-old *L. vannamei*

Prevalence and Intensity of Endoparasites (Enterocytozoon hepatopenaei-EHP)

Table 2. Prevalence and intensity of endoparasites (*Enterocytozoon hepatopenaei* (EHP) in *L. vannamei* based on sexs

Sex	Number of shrimp tested	Number of shrimp invested	Number of endoparasites	Prevalence (%)	Intensity
Male	33	29	197	87.80	6.79
Female	27	25	344	94.50	13.76

The Water Quality Measurements in The Shrimp Ponds of *L. vannamei*

Table 3. Results of The Water Quality Measurements in The *L. vannamei* Shrimp Ponds

Ponds	Temperature (°C)	Salinity (ppt)	pH (mg/L)	BOD (mg/L)	COD (mg/L)	DO (mg/L)	Ammonia (mg/L)	Zinc (mg/L)
Sea water (control)	29	22	7.94	1.0	74.71	6.94	0.1	0.116
1	31	21	6.84	16.36	2274.71	0.83	0.61	0.129
2	32	20	6.78	8.10	1018.48	1.11	0.1	0.132
3	29	20	6.89	4.81	1635.57	1.20	2.56	0.142
4	30	21	6.68	19.63	2362.86	0.74	0.69	0.130
5	31	20	7.01	24.06	3707.24	0.65	0.69	0.118
6	31	21	6.87	9.08	1608.02	1.20	3.90	0.140

Based on the identification results that have been carried out on a total of 60 samples of *L. vannamei* shrimp, the highest prevalence of ectoparasite was found in the *Zoothamnium* sp in 40-day-old shrimp (80 %) and 63.3% in 30-day-old shrimp, followed by *Epistylis* sp, *Vorticella* sp, *Acineta* sp, *Arcella* sp, and *Trichodina* sp (Table 1). *Zoothamnium* sp has a definitive host only in the type of shrimp, while *Epistylis* sp, *Vorticella* sp, and *Trichodina* sp are often found in freshwater fish farming (Yu et al., 2022). *Zoothamnium* sp is the type of parasite most often found as a cause of death in shrimp and has the ability to penetrate the shrimp carapace and cause damage to the inner skin surface (Mahasri et al., 2021). The factors that cause increased growth of this parasite are high stocking density, overfeeding, and also low oxygen content. *Zoothamnium* sp attacks shrimp at all stages from eggs, larvae, juveniles, and adults in water conditions with low dissolved oxygen levels (Mahasri et al., 2018).

Further, the level of infestation of *Zoothamnium* sp and *Epistylis* sp is more than other ectoparasites because they live in colonies, so that reproduction by colonies will be faster when compared to *Vorticella* sp, *Acineta* sp, and *Trichodina* sp which live solitary (Amalisa et al., 2021). Furthermore, *Trichodina* sp will mostly be present in the rainy season in semi-intensive ponds. *Acineta* sp. usually infects crustacean and tends to be higher in the rainy season (Kumar et al., 2020). This is in line with the time of the study, which was conducted during the rainy season.

The prevalence of all ectoparasites species that infest 40-day-old shrimp is more higher than 30-day-old shrimp (Table 1). This is supported by

Kakoolaki et al., (2016), who stated that the prevalence value in shrimp tends to increase along with the increasing size of the shrimp body and this is because small shrimp have a smaller cross-sectional area compared to larger shrimp. Further, the high and low factors of parasite prevalence rates are also caused by the parasite's ability to adapt to the host's body so that it can live and develop based on the quality of the supporting environment. Firdaus (2019) research stated that there is a difference in the number of parasites that infect between adult and young hosts. Older hosts contain a higher number of parasites.

According to Mahasri et al., (2019) stated the high intensity and prevalence of ectoparasites are also influenced by the high density of shrimp in the maintenance pond. In ponds with high shrimp density, shrimp will rub against each other so that ectoparasite transmission will occur quickly. Jayanthi et al., (2018) added ectoparasites are present in shrimp due to the molting factor. During molting, shrimp do not have antibodies to protect their soft body parts. Liao at al., (2018) stated that parasites in shrimp, such as *Epistylis* sp, *Zoothamnium* sp, and *Vorticella* sp, are parasites that often appear and stick to the exoskeleton of shrimp. In the molting phase, the old chitin will peel off from the body of the shrimp. The parasites will move from the old skin that peels off and sticks to the new shrimp skin to obtain food from its host. According to Juliana et al., (2023), disruption of the shrimp's physiological system and the biological balance of the maintenance water medium causes the shrimp's immunity to weaken. Weak shrimp larvae will be easily attacked by protozoan ectoparasites. If the disturbance is not severe and the shrimp larvae are still healthy when they molt, the shrimp can release the parasites.

Further, the organs most often attacked by ectoparasites were the tail, walking legs, and swimming legs (Figures 8 and 9). This is because the three organs that are attacked by ectoparasites have the most fine hairs (*setae*) so that the parasites stick more tightly to their hosts. According to Pamenang et al., (2020), the shrimp walking legs are always used to make slow movements and this can trigger parasitic organisms in the waters to easily stick to the organs. Another factor is because the swimming legs and walking legs are close to the substrate and are often moved on muddy bottoms. According to Mahasri et al., (2019), parasites attack shrimp that are at the bottom of the pond where there is an accumulation of organic matter and feed residue and the parasites spread rapidly to a higher level of intensity.

Furthermore, the prevalence of endoparasites in female shrimp (94.5 %) were higher than in male shrimp (87,8 %) (Table 2). This difference indicates that gender can affect the level of susceptibility to *Enterocytozoon hepatopenaei* (EHP) infection. This explanation can be associated with physiological and biological factors that differentiate male and female shrimp in terms of immunity and metabolism. According to Dang et al., (2021), female *L. vannamei* are known to have higher nutrient content such as lipids, proteins, and minerals, especially during the gonad maturation phase. This higher nutrient content can be a risk factor for EHP infection because this parasite utilizes host resources for its life cycle. Wiradana et al., (2019) stated that female shrimp tend to have more intensive feeding activities during the reproductive period to meet energy needs in gonad development. This higher feeding activity increases the possibility of exposure of female shrimp to EHP spores found in feed or pond bottom sediment. In addition, female energy allocation for gonad maturation can weaken the immune response, thus increasing their susceptibility to parasitic infections. A study by Gao et al., (2017) showed that females are more susceptible to parasitic infections and diseases in ponds, one of which is due to physiological changes associated with their reproductive cycle. The decreased activity of the immune system in females during the reproductive phase allows pathogens such as EHP to develop more rapidly in individual females.

The results of measuring eight water quality parameters show that there were six parameters that do not meet the ideal requirements for vannamei shrimp (*L. vannamei)* cultivation, namely pH, DO, BOD, COD, ammonia (NH_3), and zinc (Zn) levels, while the other two parameters meet the requirements, namely temperature and salinity (Table 3). The water temperature of the six vannamei shrimp ponds was 29 to 32 °C. Sheath et al., (2016) declared that the good water temperature for cultivation in the ponds ranges from 26.5 to 32 °C. However, at this optimum temperature, ectoparasitic protozoa will also grow well and have the potential to cause diseases in fish and shrimp, such as *Zoothamnium* sp., which reproduce rapidly at temperatures above 30 ºC (Hafidloh & Sari, 2019). According to Kakoolaki et al., (2016), temperatures above 30 °C can cause shrimp stress. So, at temperatures above 30 °C, vannamei shrimp and ciliophora ectoparasites begin to compete for dissolved oxygen to meet their respective metabolic needs. This is because temperature is a direct factor that affects the growth rate, survival, and increases the metabolic rate of organisms. Further, the salinity of the all ponds was 20 to 21 ppt and was relatively the same at the optimal concentration for shrimp growth. Research by Nurhasanah et al., (2021) suggests that young vaname shrimp (larvae and juveniles) require a salinity of 15-25 ppt for optimal growth.

However, ectoparasitic protozoa were found attacking vaname shrimp in the all ponds. This is because these parasites have a high tolerance to salinity, so they can be called euryhaline organisms. According to Kumar et al., (2020) A good pH value for vaname shrimp cultivation is 7.0-8.5. The results obtained by almost all shrimp ponds showed a pH value below 7, except for pond number five (7.01). The ability of shrimp to survive at different pH levels is because each organism has a varying pH tolerance limit and, in this case, also includes ectoparasite protozoa. A lower pH, such as the ponds in this study, which is acidic, can cause the shrimp's skin to become porous and soft so that it is easily removed and destroyed. Shrimp in conditions like this will be easily attached to parasites. According to Mahasri et al., (2021) *Zoothamnium* sp. increased in larvae reared with excessive feeding with pH below 7. In addition, low pH environments for long periods of time can cause metabolic stress in shrimp and reduce the efficiency of their immune system, thereby increasing susceptibility to endoparasites such as EHP. Under these conditions, EHP spores can survive longer because acidic environments tend to support spore stability. This shows that water quality parameters such as pH not only affect shrimp health but also indirectly increase the chances of pathogen infection.

The safe BOD value for water is 1-2 mg/L, while the BOD value obtained in the all ponds ranged from 4.81 to 24.06 mg/L. This indicates pollution in the water of the all ponds. Polyculture ponds that apply artificial feed have higher BOD levels than ponds with natural feed. Further, the COD values in the all ponds ranged from 1018.48 to 3707.24 mg/L, while the COD value of seawater (control) obtained was

74.71 mg/L. High COD values indicate high levels of organic pollution, which can have a negative impact on the aquatic environment and human health. High COD levels also cause the oxygen content in the water to decrease. According to Kumar et al., (2020), *Zoothamnium* sp., *Vorticella* sp., and *Epistylis* sp. can be a threat when there is too much pollution or drastic changes in water quality. Moreover, the safe DO content is more than 3.5 mg/L, meanwhile the DO obtained in the all ponds are far below the minimum threshold (<3) and the results of the study showed that the intensity and prevalence were quite high in the all ponds.

Ammonia (NH_3) indicates the content of organic matter in waters. Ammonia comes from the accumulation of artificial feed residues (pellets). According to Mahasri et al., (2019), the source of NH_3 in waters comes from urine and feces of aquatic organisms themselves, as well as the results of microbiological oxidation of organic matter. The optimal standard for NH_3 levels in cultivation is 0.05-0.1 mg/L, with a tolerance limit of 0.1-0.5 mg/L. The test results showed that NH3 levels in all ponds almost exceeded the optimum threshold because the value was above 0.5 mg/L except for pond number two (0.1 mg/L). High levels of NH_3 can also bind O_2 in the blood, inhibiting shrimp growth and being susceptible to disease and parasites. Research by Nkuba et al., (2021) showed that EHP can survive longer in environmental conditions filled with organic matter and with low oxygen levels.

The zinc (Zn) content values obtained in the all ponds ranged from 0.118 to 0.142 mg/L. Meanwhile, the zinc (Zn) heavy metal content value for marine biota is around 0.05 mg/L. However, if the levels of heavy metal zinc (Zn) exceed the established quality standards, it can cause pollution that is harmful to organisms (Kumar et al., 2020). This is thought to be because the shrimp ponds in this study are close to fishing areas and fishing boat activities. Zinc (Zn) heavy metal pollution can come from dyes in fishing boat paint, household waste activities (corrosion of water pipes, waste from consumer products such as in detergent formulas), and agricultural waste that uses a lot of pesticide fertilizers containing zinc (Zn) heavy metals.

CONCLUSION

In summary, the prevalence of ectoparasite species was highest in *Zoothamnium* sp. in 40-day-old shrimp (80 %) and 63.3 % in 30-day-old shrimp. Meanwhile, the lowest prevalence of ectoparasites was found in *Trichodina* sp., i.e., 16.6 % in 40-day-old shrimp and 6.6 % in 30-day-old shrimp. Further, the prevalence of endoparasites was highest in female shrimp (94.5 %), while it was 87.8 % in male shrimp. Furthermore, there were six parameters of shrimp pond water quality that do not meet the cultivation standards, namely pH, DO, BOD, COD, ammonia, and zinc, while the other two parameters meet the standards, namely temperature and salinity. Furthermore, water quality that does not meet culture standards can lead to outbreaks of parasitic diseases and have an impact on reducing the production of vannamei shrimp (*L. vannamei*). The findings of this study contribute to the shrimp ponds in the area in managing sanitation, feeding levels, and stocking densities. Further research will focus on the analysis of drug composition for the treatment of the shrimp infected by parasites or diseases and water quality management in the shrimp ponds.

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