DEPIK
Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan
Journal homepage: www.jurnal.usk.ac.id/depik

Identification of species, intensity, and prevalence of Vannamei shrimp (*Litopenaeus vannamei*) ectoparasites in traditional ponds North Gorontalo

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ARTICLE INFO

Keywords:
Ectoparasites
Prevalence
Intensity
Vannamei shrimp

DOI:10.13170/depik.12.1.27071

ABSTRACT

Vannamei shrimp (*Litopenaeus vannamei*) production continues to decline due to parasitic attacks on ponds in North Gorontalo Regency. Ectoparasites are one of the factors that can cause failure in the cultivation of vannamei shrimp. This study aims to determine the type of ectoparasites, intensity, and prevalence of vannamei shrimp cultured in traditional ponds in the North Gorontalo Regency. The sampling of the research was carried out at five research stations. The sampling of this research was taken at random in 5 plots of ponds and the total number of shrimps as many as 150 tails. At each station, samples were taken of 30 vannamei shrimp. Sampling using fishing gear in the form of nets. The observed samples were 8-10 cm in size alive. Observations of ectoparasites were carried out microscopically using a Zeis Binocular microscope with 10x magnification. Data analysis used descriptive analysis of the types of ectoparasites, intensity, and, the prevalence of ectoparasites found. The results of the study found four types of ectoparasites found in vannamei shrimp, namely *Zoothanium* sp., *Epistylis* sp., *Carchesium* sp., and *Vorticella* sp. The intensity of ectoparasite attack on shrimp was categorized as low to moderate infection, namely 4-7 individuals/shrimp. The prevalence rate is 80-100% with the category of moderate to very severe infection.

Introduction

Vannamei Shrimp (*Litopenaeus vannamei*) is an introduced species cultivated in Indonesia. Vannamei shrimp come from Central American waters, countries in Central and South America such as Ecuador, Venezuela, Panama, Brazil, and Mexico have long cultivated a type of shrimp known as Pacific white shrimp (Hafidloh and Sari, 2019). Vannamei shrimp was officially introduced to the farming community in 2001 after the decline in production of tiger shrimp (*Penaeus monodon*) due to various problems encountered in the production process, both technical and non-technical problems (Subyakto *et al*., 2009). This species is relatively easy to breed and cultivate, so vannamei shrimp is one of the mainstay species in shrimp culture in several countries around the world. In addition, vannamei shrimp is also of economic value and has become one of the leading national commodities (Fendjalang *et al*., 2016). The potential for fisheries in the North Gorontalo district includes capture fisheries sub-sector, and aquaculture sub-sector which includes marine cultivation, ponds, floating nets, and pool. The potential for fisheries in North Gorontalo Regency is expanding, along with production achievements which have increased significantly every year. Capturing fisheries prouduction this district since 2015-2018 has seen a decline of 550,31 tons. In 2015, the production of fisheries catch was 25,563.60 tons, whereas in 2018 the production was 25,013.29 tons. In 2018, the production of aquaculture is far higher than fisheries catch that is 17,033,964 tons (Central Bureau of Statistics North Gorontalo Regency, 2016).

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p-ISSN 2089-7790; e-ISSN 2502-6194
Received 22 July 2022; Received in revised form 21 November 2022; Accepted 7 January 2023
Available online 21 April 2023

The production of aquaculture in 2018 consists of 14,487,507 tons marine cultivation, 2,119,585 tons ponds, 419,348 tons pool, a 7,524 tons floating net (Central Bureau of Statistics North Gorontalo Regency, 2016). The vannamei shrimp production in 2020 (semester I) reached 98,266 tons (Department of Fisheries and Marine Affairs, North Gorontalo Regency, 2020).

These data indicate that the potential development of vannamei shrimp culture is not optimal. The quality of cultivation of vannamei shrimp can suffer a decraese as it develops against pests and diseases. Also, if the water conditions are poor, it can lower immunity so that the shrimp are more susceptible to disease (Junaidi *et al*., 2020). The results of the observations showed that the problem that is often faced by the vannamei shrimp farming community in the traditional ponds of the Mootinelo village is the presence of parasites that can inhibit the growth of shrimp so that it can result in crop failure.

Parasites are organisms that live with the help of other organisms without any reward (Pangaribuan *et al*., 2012). Parasite attack can be one of the predisposing factors for infection with more dangerous pathogenic organisms such as viruses and bacteria. Losses caused by ectoparasite attacks can be in the form of damage to external organs, namely the skin and gills. Susilo *et al.* (2018) also stated that high levels of ectoparasite attack can cause acute mortality even in the absence of symptoms in the organism. The high number of parasites can inhibit the growth of shrimp because the parasites suck the blood and nutrients of the shrimp. Therefore, it is necessary to take action to overcome the existing problems. Knowledge of the identification of ectoparasites in shrimp is very basic and important because the types of parasites that are often encountered have the potential to become obstacles in the cultivation *L. vannamei*.

This study aims to describe the types of Ciliophora and their attack level, as well as clinical symptoms of vannamei shrimp infected with Ciliophora ectoparasites on land that has many polyculture ponds.

Materials and Methods

Location and time of research

This research activity was carried out in March-July 2021. There are two locations for this research, namely the location for sampling *L. vannamei* in Mootinelo Village, Kwandang District, North Gorontalo Regency and for examining *L. vannamei* samples at the Laboratory Aquaculture, Feed and Disease, Faculty of Fisheries and Marine Sciences, State University of Gorontalo.

Vannamei shrimp sampling locations are located at five stations (ponds) in Mootinelo Village. Station I is located at the coordinates 0°48'19.11"N 122 °52'34.70"E. Station I is located near a resident's plantation and adjacent to a pond that is no longer in use. The Station I area is ±7000 square meters with a stocking density of 30,000 individuals. Station II is located at the coordinates 0°48'20.94"N 122 °52'28.27"E, near people's plantations and far from seawater estuaries. The area of the pond at station II is ± 1 ha with a stocking density of 50,000 fish, the color of the water at station II is brownish. Station III is located at the coordinates 0°48'25.31"N 122 °52'30.96"E adjacent to station II and station IV. Station III pond area is ±2 ha with a stocking density of 25,000. Seawater sources are far from this station. Station IV is located at the coordinates 0°48'27.44"N 122°52'26.42"E. This pond is adjacent to station III in this pond where there are mangrove trees that have been cut down. The area of the pond at this station is ±1 ha with a stocking density of 35,000 individuals. Station V is located at the coordinates of 0°48'59.22"N 122°52'29.54"E. This station is located near the mangrove area and sea estuary compared to other ponds. The area of the pond at Station V is ±2 ha with a stocking density of 25,000 individuals.

Sampling in this study was taken randomly at 5 stations and the total number of shrimp was 150. At each station, 30 samples of *L. vannamei* were taken. Sampling using fishing gear in the form of nets and shrimp were sampled alive.

The identification of ectoparasites in vannamei shrimp refers to the research of Hafidloh and Sari, (2019). The stages of identification of ectoparasites were carried out during the research, namely (1) Preparing the tools and materials to be used, (2) Taking the shrimp from the storage container, (3) Weighing and measuring the length of the shrimp, (4) Cleaning the cutting board as a place for shrimp that has been cleaned. cut, (5) kills the shrimp by cutting the head of the shrimp. Then cut out the parts that you want to observe, namely the tail, swimming legs, carapace and gills, (6) Place the observation sample on a glass slide, (7) Take water from the live medium for shrimp. using a pipette then dripping on the observation sample, (8) Observing ectoparasites under a Zeis Binocular Microscope with a magnification of 10x10, (9) Recording the results and taking documentation on a microscope (10) Calculating the prevalence and intensity of *L. vannamei*.

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Data analysis

The prevalence and intensity of ectoparasites in shrimp were calculated following Kabata (1985) and Rahmayanti and Marlian (2018). The type, number and organ where the ectoparasites were found were recorded and the prevalence and intensity values were calculated using the following formula:

$$\text{Prevalence} = \frac{\sum infected\ shrimp}{\sum inspected\ shrimp} x100\%$$

$$\text{Intensity} = \frac{\sum ectoparasites\ found}{\sum infected\ shrimp}$$

Results

The results of the research on the identification of ectoparasites in vannamei shrimp, with a total sample of 150 individuals obtained from five traditional shrimp pond stations in Mootinelo Village, Kwandang District, North Gorontalo Regency, found types of ectoparasites belonging to the protozoa phylum, namely *Zoothamnium* sp., *Epistylis* sp., *Carchesium* sp., and *Vorticella* sp.

The types of ectoparasites that often attack vannamei shrimp seeds are *Vorticella* sp., *Zoothamnium* sp., and *Epistylis* sp. The highest attack rate comes from *Zoothamnium* sp. with a prevalence value of 53% and an intensity of 38 individuals/shrimp.

Figure 1. *Zoothamnium* sp. (10x Magnification).

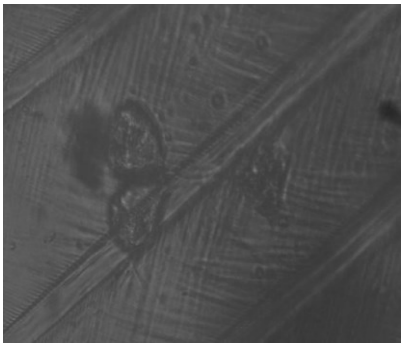

Figure 2. *Epistylis* sp. (40x magnification).

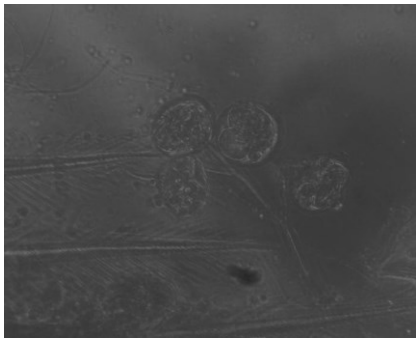

Figure 3. *Carchesium* sp. (40x magnification).

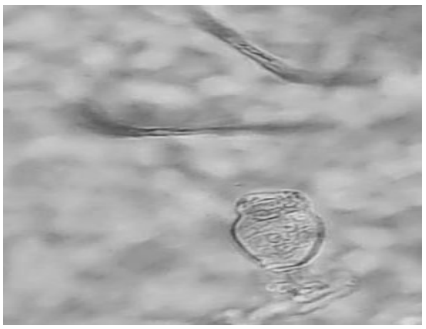

Figure 4. *Vorticella* sp. (10x magnification).

The results of the calculation of the prevalence and intensity of parasitic attacks on vannamei shrimp at the five stations can be seen in the image below.

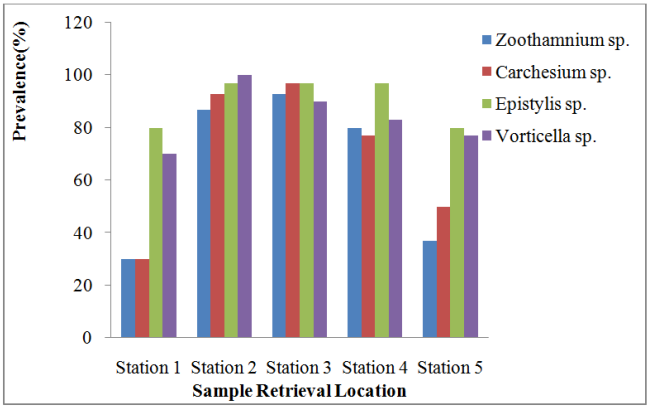

Figure 5. Graph of parasite attack prevalence on vannamei shrimp at each station.

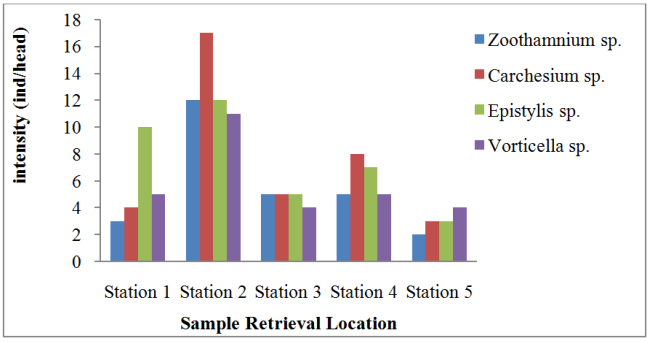

Figure 6. Graph of parasite intensity on vannamei shrimp at each station.

Discussion

Types of ectoparasites found in vannamei shrimp

Zoothamnium sp.

Zoothamnium sp. is an ectoparasite belonging to ciliates that can live normally in quality waters (Herlina, 2018). *Zoothamnium* sp. that was found was shaped like a bell, with the cilia on the peristome living in colonies having contractile stalks that branched and moved simultaneously and were transparent in color (Irvansyah *et al*., 2012: Handayani and Rozikin, 2019). *Zoothamnium* sp. ectoparasites. has a body shape like an inverted bell, is contractile, lives in colonies with many branches on each stem, and is transparent in color. *Zoothamnium* sp. is a parasite that often attacks cultured shrimp. *Zoothamnium* sp. can be seen in Figure 1.

The shrimp that are attacked by the *Zoothamnium* sp. parasite are characterized by their bodies covered with a white membrane and brown infected gills, and shrimp usually swim to the surface of the water. This is by following Zulkarnain (2011) who states that the body of shrimp infected with *Zoothamnium* sp. is covered with a kind of white or brown membrane and the shrimp has difficulty breathing because the host is covered in parasites.

Epistylis sp.

Epistylis sp. identified in this study has a bell-like shape but is slightly slender (Figure 2). *Epistylis* sp. has a transparent body and lives in colonies (Muttaqin *et al*., 2018). In addition, *Epistylis* sp. also has branches, is not contractile (not moving), and has cilia (vibrating hairs) on the peristome, while the stalks and branches of this ectoparasite colony cannot move (Pamenang *et al*., 2020).

According to Mahasri *et al*. (2019), the shrimp that are attacked by the *Epistylis* sp. parasite include moving slowly, the appearance of the shrimp becomes unattractive, the body looks brown and the gills of the shrimp are brown. Kakoolaki *et al*., (2017) stated that *Epistylis* sp. that attacks the shrimp body will experience a change in body color such as mossy with a brownish color caused by the attachment of this parasite. This parasite also attacks the gills so that the gills are black, and the movement becomes slow.

Carchesium sp.

The type of parasite *Carchesium* sp. (Figure 3) was found to have the same shape and characteristics as *Epistylis* sp. but what distinguishes the stalk of *Carchesium* sp. that is contractile (moving). This type of parasite most commonly attacks the tail of the shrimp, reaching 519 species of *Carchesium* sp. While the least attacked were on the gill organs, namely 36 individuals. the Movement that occurs in one branch of *Carchesium* sp. can trigger other branches of the main stalk to move along. This is reinforced by Hoar *et al*., (2020) who states that *Chachesium* sp. forms colonies of more than 3 individuals and can resemble trees with many trunks.

Vorticella sp.

Vorticella sp. identified color is yellowish, has vibrating feathers, has a bell-like shape with a long stalk, is not branched, does not colonize and is contractile (Figure 4). The shape of this ectoparasite almost resembles *Epistylis* sp. and *Carchesium* sp., but *Epistylis*sp. and *Carchesium* sp. live in colonies while *Vorticella* sp. solitary. Widiani and Ambarwati (2018) stated that *Vorticella* sp. has a zooid shape like a bell and in the peristome there are cilia. *Vorticella* sp. transparent in color found solitary life with contractile stalks cylindrical in shape, and unbranched.

The type of ectoparasite *Vorticella* sp. was found in all organs of the shrimp that were taken as observation samples. The most abundant shrimp organs found in *Vorticella* sp. ectoparasites were 379 individuals on the tail, then 287 individuals on the swimming legs, 65 individual carapace and 18 individuals on the tail. Nurlaila *et al*. (2016), stated that *Vorticella* sp. is a parasite that attacks the cranium, swimming legs, gills and tail of Vannamei shrimp.

Vannamei shrimp prevalence and intensity

Based on the results of the calculation of the prevalence and intensity of vannamei shrimp from five different ponds (stations) with a total sample of 150 individuals showing different results.The prevalence value was obtained from the number of shrimp infected with the parasite divided by the number of samples obtained in times of one hundred percent. While the intensity value is obtained from the number of parasites found divided by the number of shrimp that are infected with the parasite.

The results of the analysis on the prevalence and intensity of parasites that attack vannamei shrimp in traditional ponds in Mootinelo Village, Kwandang District, North Gorontalo Regency are presented in Figures 5 and 6. infected with protozoan parasites (*Zoothanium* sp. 9 individuals, 9 Carchesium sp., 24 *Epistylis* sp., and 21 *Vorticella* sp.). Station 2: *Zoothanium* sp. 26 individuals, 28 *Carchesium* sp., 29 *Epistylis* sp. and 30 *Vorticella* sp. Station 3: *Zoothanium* sp. 28 individuals, 29 *Carchesium* sp., 29 *Epistylis* sp., and 27 *Vorticella* sp. Station 4: *Zoothanium* sp. 24 individuals, 23 *Carchesium* sp., 29 *Epistylis* sp. and 25 *Vorticella* sp. Station 5 *Zoothanium* sp. 11 individuals, 15 *Carchesium* sp., 24 *Epistylis* sp. and 23 *Vorticella* sp.

For the number of parasites found in pond I, the number of parasites was 410 individuals which were

divided into several types of parasites including *Zoothanium* sp. 28 individuals, *Carchesium* sp. 36 individuals, *Epistylis* sp. 240 individuals, and *Vorticella* sp. 106 individuals. Pond II found the total number of parasites of 1467 individuals, including *Zoothanium* sp. 316 individuals, *Carchesium* sp. 471 individuals, *Epistylis* sp. 342 individuals, and *Vorticella* sp. 338 individuals. In pond III the total number of parasites found was 509, including *Zoothanium* sp. 131 individuals, *Carchesium* sp. 141 individuals, *Epistylis* sp. 135 individuals, and *Vorticella* sp. 102 individuals. In pond IV the number of parasites found was 629 individuals which were divided into several types of parasites, *Zoothanium* sp. 123 individuals, *Carchesium* sp. 185 individuals, *Epistylis* sp. 199 individuals, and *Vorticella* sp. 122 individuals. And in pond V the number of parasites found was 226 individuals, including *Zoothanium* sp. 21 individuals, *Carchesium* sp. 47 individuals, *Epistylis* sp. 77 individuals, and *Vorticell*a sp. 81 individuals.

The results of the calculation of the prevalence and intensity of vannamei shrimp based on the observed vannamei shrimp organs can be seen in Table 1.

Table 1. Prevalence and intensity of vannamei shrimp based on organs attacked.

Observed Organ	Prevalence (%)	Intensity (ind/shrimp)
Tail	92.00	12.31
Swimming legs	78.67	10.06
Carapace	36.00	5.31
Gills	16.67	2.72

The highest prevalence percentage in vannamei shrimp organs observed was found in the tail organ with a prevalence percentage of 92% then the second highest was in the swimming legs with a prevalence percentage of 78.67%, followed by the carapace and gills, respectively, the prevalence percentages were 36% and 16.67%. So that the tail and swimming legs are classified as almost always and usually, which means that the tail has a severe infection and the swimming leg is moderately infected. Meanwhile, the highest intensity was found in the tail organ, which was 12.31 ind/shrimp (medium category) while the lowest was found in the gill organ, 2.72 ind/shrimp (low category).

The high parasite attack on the tail and swimming legs is thought to be because these two organs are organs that often touch the substrate when moving. The results of Nurcahyo (2018), found the highest prevalence and intensity were found in the caudal fin organ of Vannamei shrimp where the value reached 56.6% and the highest intensity was 17.5 ind/shrimp, the highest was found in the walking legs. The high prevalence and intensity values are thought to be because walking legs are always used to make slow movements, this can trigger parasitic organisms in the waters to easily attach to the organs so that parasites spread quickly and the intensity level is getting higher, especially if the water quality is not good. and high organic matter content in water, parasites will stick to the shrimp organs (Setiyaningsih *et al*., 2014).

The relationship between parasite intensity and age of shrimp was grouped according to three patterns, namely independent of shrimp age, decreasing with increasing age of shrimp and with increasing age of shrimp. It is important to know the intensity value to predict the health condition of the shrimp. Due to disturbance in the host due to parasitic infection in general due to the high density of parasites. Parasite intensity was lower and tended to decrease with changes in shrimp length increasing (Rahmayanti and Marlian, 2018)

The attack of ectoparasites in large numbers will cause the death of shrimp. This is due to the weak condition of the shrimp, difficulty in breathing, decreased appetite, the molting process being hampered due to being filled with adherent organisms. The states that shrimp are attacked by this type of parasite experience disturbances such as decreased shrimp appetite and passive or weak body. Mahasri *et al.* (2019), stated that shrimp infected with ectoparasites will experience disturbances during the molting process, weakness and difficulty breathing and decreased shrimp appetite.

Water Quality

The results of the measurement of water quality parameters at each station ranged between 30 – 31°C, dissolved oxygen ranged from 2.3 – 3 mg/l, pH v6.5 – 7.3, salinity 29 -30 ppt and COD > 1500 mg/l. From the Regulation of the Minister of Maritime Affairs and Fisheries of the Republic of Indonesia Number 75/Permen-Kp/2016 concerning General Guidelines for Enlargement of Windu Shrimp (*Penaeus monodon*) and Vannamei Shrimp (*L. vannamei*) water temperature in rearing vannamei shrimp is 28 - 32°C, dissolved oxygen >3 mg /l, pH 7.5 – 8.5, salinity 5 - 40 ppt and COD <40 mg/l. This indicates that the values of dissolved oxygen, pH and COD are in the bad category for the maintenance of vannamei shrimp.

Ectoparasites that attack cultured organisms including Vanname shrimp are thought to be related to environmental conditions suitable for the life of

several types of ectoparasites, such as *Zoothamnium* sp., *Epistylis* sp., *Carchesium* sp., and *Vorticella* sp. (Samsundari and Adhy, 2013). The content of dissolved oxygen and COD (Chemical Oxygen Demand) has a relationship with the occurrence of pollution in the pond water environment where if the oxygen content decreases then COD increases. This increase in COD is thought to be due to the high organic matter in these waters, the lower the dissolved oxygen value, the higher the level of pollution of an aquatic ecosystem (Sun *et al.* 2006; Tamyiz, 2015).

Conclusion

The types of ectoparasites that attack vannamei shrimp in traditional ponds in Mootinelo Village, Kwandang District, North Gorontalo Regency are from protozoa, namely *Zoothamnium* sp., *Epistylis* sp., *Carchesium* sp., and *Vorticella* sp. The highest prevalence of parasitic attack was found at each station of the *Epistylis* sp. parasite, *Carchesium* sp., and *Vorticella* sp. with a prevalence value of 80-100% with the category of moderate to very severe infection. Meanwhile, the highest parasitic attack intensity was *Zoothamnium* sp., *Epistylis* sp., *Carchesium* sp., and *Vorticella* sp. ranging from 4 to 17 ind/shrimp with a low to moderate infection category.

Acknowledgements

Made as a gratitude to Faculty of Fisheries and Marine Science University of Gorontalo who has provided assistance so this study about *L. vannamei* can proceed well.

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How to cite this paper:
Juliana, J., Y. Koniyo, I.N. Dangkua, I.A. Rahman. 2023. Identification of species, intensity, and prevalence of Vannamei shrimp (*Litopenaeus vannamei*) ectoparasites in traditional ponds North Gorontalo. Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan, 12(1): 62-68.