Identification of Ectoparasites in Pearl Catfish (*Clarias gariepinus*) with One and Three Months Age in Maclele Cultivation, Tuban District, Tuban Regency

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Abstract

This study aims to identify ectoparasites that attack pearl catfish and the incidence of ectoparasite infestations in 100 samples of one- and three-month-old pearl catfish. This research was conducted from January to March 2021 in Makalele Cultivation, Tuban District, Tuban Regency. The method used in this research is skin scraping and gill installation. Data analysis using Chi-Square Test with SPSS version 24.0 for windows. The results showed that there were 3 types of ectoparasites found, namely *Trichodina nobilis*, *Trichodina pediculus*, *Chilodonella* sp., *Dactylogyrus* sp. and the highest incidence of ectoparasites occurred in young fish compared to old fish with a percentage of 35% and 25%, respectively, and based on the Chi-Square test there was no significant difference (p≥0.05).

Keywords: *Clarias gariepinus*, incidence rate, *T. Nobilis, T. Pediculus, Chilodonella* sp.

Introduction

Tuban Regency is one of the regencies in East Java that has a high contribution to the fisheries business field and shows an increase from 2011 to now (RPJMD, 2016). Through fish farming business activities can reduce pressure on the utilization of marine resources. Catfish is one type of fish that has economic value and one type of freshwater fish that is often cultivated in the Tuban Regency. The type of catfish that is often cultivated in this area is the type of pearl catfish.

Pearl catfish cultivation can be found in Maculele Cultivation, Tuban District, Tuban Regency. This cultivation was established in 2017, This is due to the conditions in the area which have a stable temperature range among other areas. Murugaian (2008) stated that temperature is one of the factors that can affect the growth process of fish, as well as affect the fish's immune system (Ghufran, 2010). Problems that are often experienced in catfish farming are the incidence of disease and fish death.

The disease is an abnormality in fish caused by an unbalanced interaction between three components in an aquatic ecosystem, namely a weak host, a vicious pathogen, and a deteriorating environmental quality. Factors that play a role in the emergence of disease are internal and external. Internal factors are factors that come from the internal environment such as genetic disorders, immunity, and body metabolism. External factors that affect pathogenic diseases are parasites, viruses, fungi, and bacteria (Khotimah et al., 2018).

Ectoparasite attacks can cause acute high mortality, changes in fish morphology, decreased fish productivity, and fish death will often occur without showing symptoms and if not treated immediately will cause mass death because of the transmission and spread of ectoparasites is very fast. Ectoparasites that can infest catfish include Trichodina sp., Ichthyophthirius multiplies, Vorticella sp., Oodonium sp., Chilodenella sp., Dactylogyrus sp., and Gyrodactylus sp. (Hardiroseyani, 2006).

This study used pearl catfish aged one and three months. The age difference was chosen because the pearl catfish with the age of three months was still classified as mature age and ready for harvest, while the pearl catfish with the age of one month is included in the young age group.

Materials and Methods

Material

The material needed in this study was to use 100 live pearl catfish (Clarias gariepinus) samples, NaCl 0.9%, aquadest, formalin 0.1%, and oil immersion.

Tools

The research equipment used in the examination of the samples were gloves, masks, Styrofoam ice boxes, scrap tools (scalpel/wooden spatula), pipettes, surgical scissors, tweezers, Petri dishes, object-glass, cover glass, trays, toothpicks, light microscopes, and the Lucida microscope.

Sample and Sample Size

This research was conducted during January-March 2021. The sample size was calculated based on the sample size formula Solving (Husein, 2013):

Formula n $=\frac{N}{1+Ne^2}$

n = Number of samples; N = Population size; e = Sampling error.

n $=\frac{2000}{1+2000(0,1)^2}$

n $=\frac{2000}{21}$

n = 95.24

From the calculation results above, the sample size is rounded up to 100 samples.

Research Procedure

The method used is the method *cross-sectional study* and taking samples at the location directly in the than catfish Cultivation, Tuban District, Tuban Regency and taken by non-random sampling with the purposive method. Identification of ectoparasites will be conducted at the Parasitology Laboratory, Division of Veterinary Parasitology, Faculty of Veterinary Medicine, Airlangga University, Surabaya. Examination of ectoparasites on pearl catfish is carried out in two ways, namely, scraping or mounting gills. Identification of ectoparasites by scraping or scrapping method is done by scraping the skin of the fish, head to tail using a scalpel to get mucus. Taking fish slime is done with a strong push and carefully so as not to injure the fish's body. The mucus resulting from the scrapping was immediately placed on an object glass and dripped with physiological sodium chloride (0.9% NaCl) and then dried. Put into formalin 0,1% for 2-3 minutes of preservation then lid with cover glass. Observe with microscope with 400x-1000x magnification.

Data Analysis

Data from the identification of ectoparasites that infest pearl catfish (*Clarias gariepinus*) in the cultivation of Makalele, Tuban District, Tuban Regency presented descriptively, pictures, and tables. The results of the data to determine the difference in the incidence of ectoparasite infestation at the age of one and three months using the Chi-Square Test in the SPSS for Windows 24.0 program.

Results

Pool Temperature Check

Temperature checks in each pool were also carried out. Pools A1 and B1 have the same temperature and A2 and B2 have almost the same temperature. The water temperature in the range of 28 - 290C is still in the optimal temperature range for pearl catfish farming activities. Temperature is very influential on various chemical reactions in water and one of them is on the solubility of oxygen in the water and the metabolism of the fish's body, so it will affect the growth of fish.

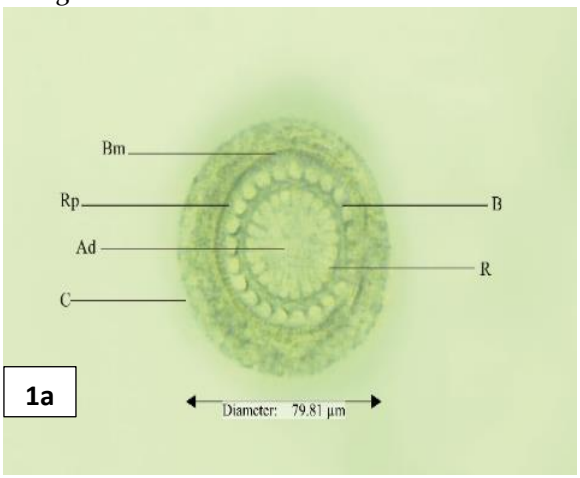
Ectoparasite Infestation Examination

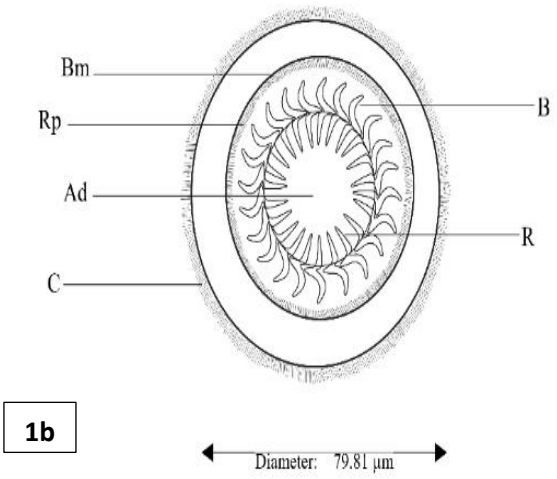
Based on the results of research that have been carried out on pearl catfish in Makalele Cultivation, Tuban District, Tuban Regency which was carried out from January to March 2021 on 100 samples of pearl catfish in which each pond was divided into 50, it was found that 60 fish were infested with ectoparasites using the native method, namely skin scrapings. (scrapping) and gill examination. From 100 samples of pearl catfish examined for skin and gills, positive results were obtained from 35 fish at one month and 25 at three months. Sampling carried out on the left and right gills and skin. One-month-old catfish found ectoparasite infestation as many as 32 positive samples on the left gill and as many as 31 positive samples on the right gill, 24 positive samples found on the left skin, and as many as 26 positive samples on the right skin. And in catfish aged three months, 23 positive samples were found on the left gills and 21 positive samples on the right gills, 20 positive samples were found on the left skin, and 21 positive samples on the right skin, the incidence of ectoparasite infestation in catfish One and three-month-old pearls. After being analyzed using the Chi-Square test with a significant value of 5% alpha to determine differences in the incidence of ectoparasite infestation in one and three-month-old pearl catfish, obtained p-value 0.066 (p0.05) non-significant. The results of this study showed that there was no difference in the level of ectoparasite infestation between the age of one and three months of pearl catfish.

Ectoparasite Microscopic Examination

The results of the examination of ectoparasites found through microscopic examination of the body of the pearl catfish, namely Trichodina sp., Chilodonella sp., and Dactylogyrus sp. On observation under a microscope, Trichodina sp. transparent and

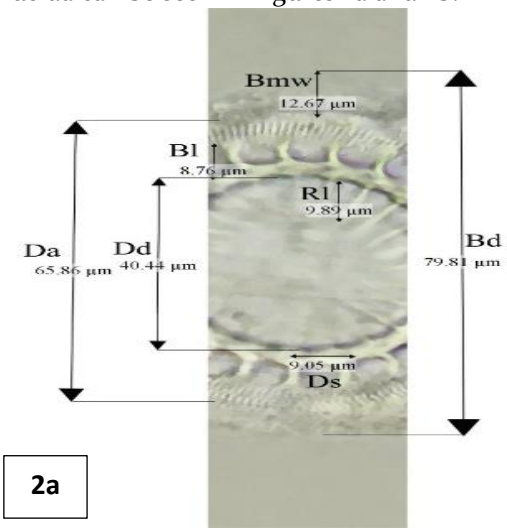
shaped like a ring filled with vibrating hairs. The characteristics of Trichodina sp. found in this study were found on the skin, fins, and gills. The shape of this parasite is spherical with the oral surface looking slightly concave and the outside of the cells seen surrounded by many cilia. This is by the research of Munawwaroh and Rahayu (2017)which states that Trichodina sp. has a concave body shape and has attachments located on the anterior and posterior parts. The diameter of Trichodina sp. found is 79.81µm. Image results of Trichodina sp. and camera lucida can be seen in Figures 1a and 1b.

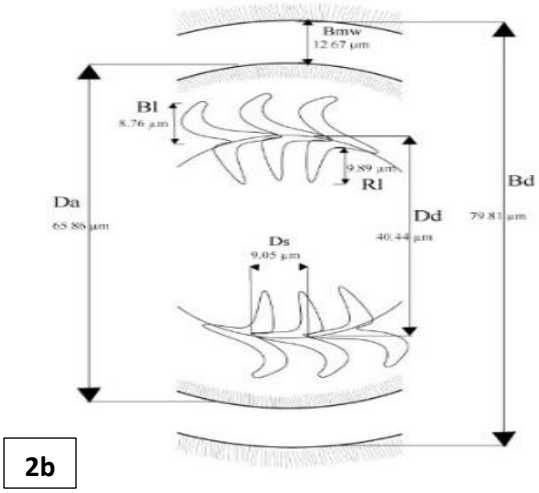




Figures 1 (a) *Trichodina* sp. **(b)** *Trichodina* sp. with with camera lucida with 400x magnification.*adhesive disk*(Ad); Blades (B); Border Membrane (Bm); Cilia (C); Ray (R); Radial pins (Rp). 100 m bar scale.

The type of Trichodina sp. What was found was *Trichodina nobilis* with a body diameter of 79.81µm. *Trichodina nobilis* was found to have a larger body size, sharp blade apex tip, slightly curved distal blade margin to the anterior blade margin, and visible long, straight, and pointed rays. Based on the calculation results, the body diameter of this parasite is 79.81 m. The adhesive disc is concave with a diameter of 65.86 m, surrounded by a border membrane with a width of 12.67 m. The denticle blade is pointed with a length of 8.76 m and 23 - 25 in number. The diameter of the denticle ring is 40.44 m. The ray is pointed at the tip with a length of 9.89 m and a width of the denticle span of 9.05 m. Size *Trichodina nobilis* and the results from the camera lucida can be seen in Figures 2a and 2b.

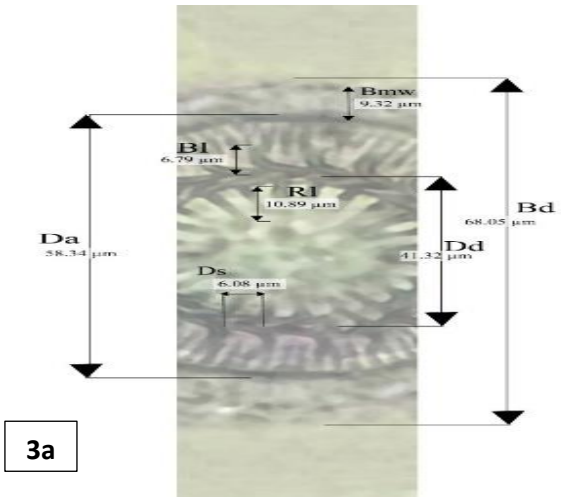


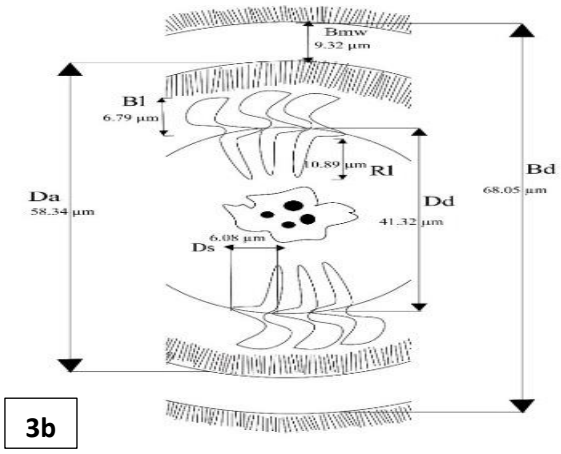


Figures 2 (a) *Trichodina nobilis* **(b)** *Trichodina nobilis* with camera lucida. *Body diameter* (BD); Blade length (Bl); Blade membrane width (Bmw); Diameter of adhesive disc (Da); *Denticle diameter* (Dd); Denticle span (Ds); *Ray length* (Rl).

The type of ectoparasite Trichodina sp. what was found next was Trichodina pediculus where the morphology was almost the same as *Trichodina nobilis*. In the results that have been researched obtained calculations for Trichodina pediculus showed the body diameter of this parasite 68.05 m. The adhesive disc is concave with a diameter of 58.34 m, surrounded by a border membrane with a width of 9.32 m. The denticle blade is pointed with a length of 6.79 m and a total of 26. The diameter of the denticle ring is 41.32 m. Rayon the end with a length of 10.89 m

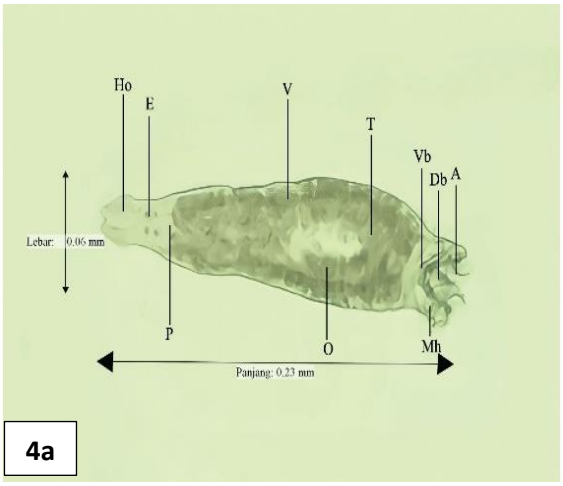
and a width of the denticle span of 6.08 m. Van and Basson (1986) in Woo (2006) stated that *Trichodina pediculus* has a wide distribution in fresh water and is generally found on the skin of adult fish and fish fry. Size *Trichodina pediculus* and the results from the camera lucida can be seen in Figures 3a and 3b.

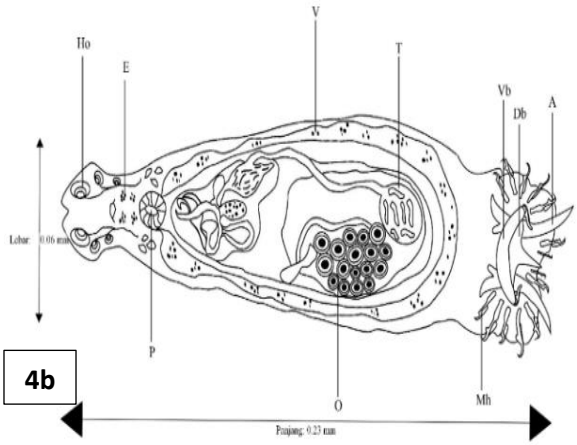




Figures 3 (a) Trichodina pediculus (b) Trichodina pediculus with camera lucida.*Blade length*(Bl); Blade membrane width (Bmw); Diameter of adhesive disc (Da); Denticle diameter (Dd); Denticle span (Ds); Ray length (Rl).

Other types of protozoa were also found, namely Chilodonella sp. This parasite is very much found on the skin, fins, and gills of pearl catfish. Chilodonella sp. is a protozoan that can reproduce rapidly by mitotic division but also by conjugation (Mahendra and Nurbadriati, 2019). The shape of the parasite found was oval with a convex dorsal and a flattened ventral had cilia, the size of the parasite found was 20.34 m long and 14.83 m wide, and the method of infestation of this parasite was seen in groups or colonies. *Chilodonella* sp. breed at a temperature of 0.5 - 200C. Under bad conditions, these parasites will form cysts. Chilodonella sp. cannot live without a host for more than 12 - 24 hours. The presence of ectoparasites Chilodonella sp. The high temperature can be caused because these ectoparasites have a low-temperature tolerance range so they can maintain their existence. Himage result of Chilodonella sp. and camera lucida can be seen in Figures 4a and 4b.

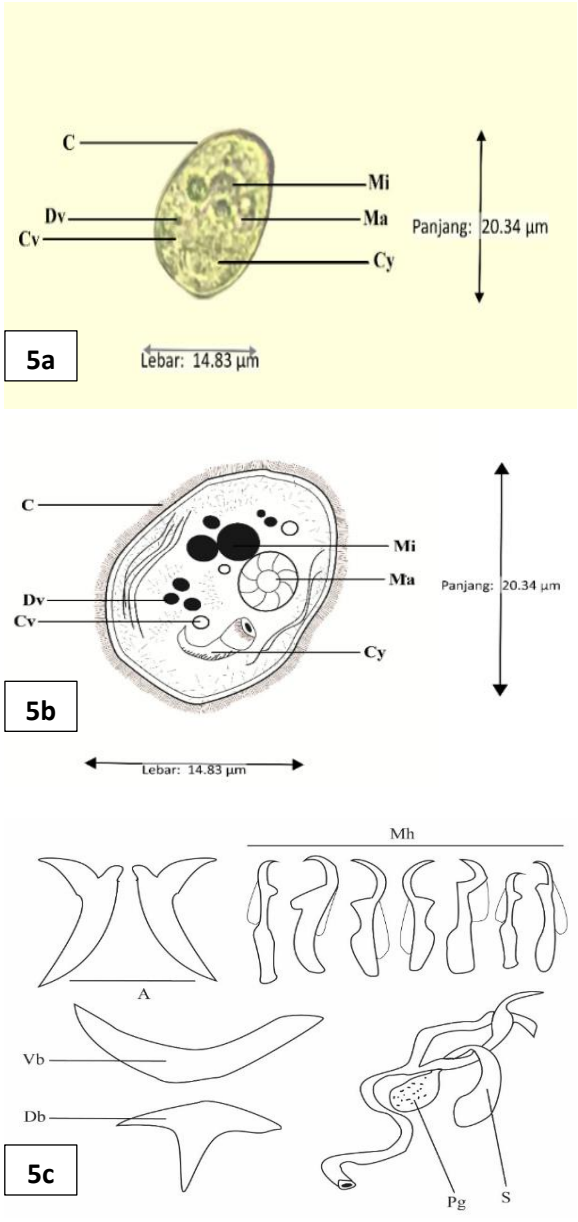




Figures 4 (a) *Chilodonella* sp. **(b)** *Chilodonella* sp. with lucida camera with 1000x magnification. Cilia (C); Contractile vacuole (Cv); Cytopharynx (Cy); Digested vacuole (Dv); Macronucleus (Ma); Micronucleus (Mi).

In addition to the protozoan parasites, this study also found monogenean parasites, namely Dactylogyrus sp. This parasite attaches to the gills of fish. The shape of this worm is dorsoventral flat and bilaterally symmetrical. On the dorsal side, there are prohaptor organs and eyespots, while on the ventral side there are opisthaptor organs. In the posterior part of the body, there is an opisthaptor with a pair of *median hooks*. The characteristics of the worms found are dark in color, with a body length of 0.23 mm and a width of 0.06 mm. On *Dactylogyrus* sp. The life cycle will start from an adult worm that lays eggs and hatches into a ciliated larva (onchomiracidium) which has more than one cilia and eye spot and

will swim until it attaches to the gills and then becomes an adult worm (Kabata, 1985). These worms are oviparous and have haptors, which are organs for attachment. Parasite *Dactylogyrus* sp. is capable of releasing 4-10 eggs every 24 hours and will increase in number with increasing temperature. Himage and organs from Dactylogyrus sp. and camera lucida can be seen in Figures 5a, 5b, and 5c.



Figures 5 (a) *Dactylogyrus sp.* **(b)** *Dactylogyrus sp.* with with camera lucida with 400x magnification. **(c)** Organs of *Dactylogyrus sp*. Anchor (A); Dorsal bar (Db); Head organs (Ho); Eyes (E); Marginal hooks (Mh); Ovaries (O); Pharynx (F); testes; Prostate gland (Pg); Stomatch (S); Testis (T); Vitellaria (V); Ventral bar (Vb).

Discussion

The results of research conducted on 100 samples of pearl catfish obtained from Makalele Cultivation, Tuban District, Tuban Regency from January to March 2021 were infested with ectoparasites where the age of one-month pearl catfish was 35% and the age of 3 months pearl catfish was 25%.To know differences in the incidence of ectoparasite infestation inone and three month old pearl catfish In this study, Chi Square analysis was carried out which showed no significant or non-significant difference ($p \geq 0.05$). This result is of course contrary to the opinionIrianto (2005) who stated that young fish are more susceptible to disease because the immune response in fish is fully formed when the fish are mature, in contrast to young fish, although young fish have an immune response but work less efficiently. In addition to age, several things affect the level of ectoparasite infestation in fish, including; species, temperature, nutritional conditions, physiological conditions, and population density (Hardi, 2015). Judging from the population of 1-month-old catfish ponds, there are more fish populations, but the size of 3-month-old catfish is much larger than 1-month-old catfish, resulting in a higher population density.

The high fish population density allows a decrease in the quality of aquaculture water (Shafrudin et al., 2006). This is by the statement of Sekar et al. (2016) that environmental conditions in the maintenance container and water quality can affect the increase in prevalence values and poor environmental conditions can cause fish conditions to become weak so that parasites will very easily attack the host. This will make the fish become stressed so that there is an unbalanced relationship between fish, the environment, and pathogens, and fish are easily infected with parasites. The water quality in Maclele Cultivation looks poor and there is still many fish floating on the surface of the water. The water condition of each pool looks a little cloudy. This of course can lead to easy ectoparasites that infest pearl catfish and can cause disease.

Based on the results of the identification found three types of ectoparasites infest pearl catfish in Makalele Cultivation, Tuban District, Tuban Regency. The infestation consisted of one and three months of age. The types of ectoparasites that have been found are Trichodina sp., *Chilodonella* sp., and *Dactylogyrus*sp. The percentage of pearl catfish in Makalele Cultivation, Tuban District, Tuban Regency aged one month consisted of 5% that were positive for Trichodina sp., 3% positive samples were infested *Chilodonella* sp.,5% positive samples were infested with Dactylogyrus sp., 5% positive samples were infested with Trichodina sp. +*Chilodonella* sp., and 17% of infected positive samples *Trichodina*

sp. + Dactylogyrus sp. In a three-month-old pearl catfish consists of6% positive infected with Trichodina sp., 9% positive sample infested *Chilodonella* sp., 1% positive samples were infested with Dactylogyrus sp., 5% infested positive samples *Trichodina sp. + Dactylogyrus sp.*, and 4% of the positive samples that were infested with *Trichodina* sp. + *Chilodonella* sp.

Total Trichodina sp. quite a lot found on the surface of the fish body because this parasite contains a lot of mucus and epithelial tissue which is a good place to live and a place to find food. This parasite attacks a lot and irritates the gills, fins, and skin. The possibility that can affect the parasitic attack height *Trichodina* sp. in this study, namely the content of organic matter and the density of bacteria in the waters. This corresponds to the statement Nugroho et al., (2012) content organic matter and density are biological indicators that are used to describe the quality of fish rearing in general.

The form of the parasite These rings are ciliated and move rapidly from one organ to another (Bell and Burt 2008). Men massage by Pramono and Syakuri (2008), *Trichodina* sp. can stick to the surface of the body and will rotate 3600 using cilia so that it will damage the surrounding cells and eat the destroyed epithelial cells to irritate the body surface.

The first *Trichodina nobilis* ectoparasite was identified by Chen (1963) found in Cyprin. fish*idae*. The results showed that *Trichodina nobilis* mostly infested the outer body of the fish and only a few infested the gills. This is following Vera et al. (2003) which states that *Trichodina nobilis* uses the host's body, especially on the skin for microhabitat.

This study also found *Trichodina pediculus* species, according to Dana et al. (2002). *Trichodina pediculus* attacks the skin and gills, irritating. The life cycle of *Trichodina nobilis* and *Trichodina pediculus is* quite fast and has only one definitive host and no intermediate hosts (Riko et al., 2012). *Trichodina nobilis* and *Trichodina pediculus* reproduce by dividing themselves or commonly called binary. When the denticle divides from the parent cell, it produces daughter cells. Transmission of Trichodina nobilis and *Trichodina pediculus* occurs through direct contact from infected fish to healthy fish.

Another type of protozoan ectoparasite found was Chilodonella sp. These ectoparasites mostly attack the skin, fins, and gills. The parasite found is in the form of oral and there are cilia on the entire surface of the body which is used as a means of locomotion. This is following the opinion of Mahasri and Kismiyati (2015) that the parasite *Chilodonella* sp. oval-shaped like a heart, the shape of the macronucleus is oval and the shape of the micronucleus is circular. How to infest this parasite in groups or colonies and often these ectoparasites are found in large numbers, when there is a decrease in temperature the fish will be easily infected with *Chilodonella* sp. The phylum Protozoa has a very fast life cycle and reproduction because it can reproduce in approximately 24 hours and therefore rapidly divides and is transmitted to fish.others (Amirullah et al., 2012). According to Tobler et al. (2007) Reproduction of *Chilodonella* sp. by mitotic division but also by conjugation. *Chilodonella* sp.

The ectoparasite worm *Dactylogyrus* sp. was also found in this study and found only on the gills, so it is referred to as gill worms and is one of the largest monogenean genera. Ectoparasite *Dactylogyrus* sp. has an opisthaptor, which is an organ to attach to the target organ of its host, which is equipped with two pairs of penetrating organs, which are like anchors. In this study, *Dactylogyrus* sp. is seen to have 2 pairs of eyes that look like black dots and have 4 indentations on the head. This is by the statement of Yuli et al. (2017) there are 2 pairs of black eyes on the anterior. Schmidt et al., (2006) explained the mechanism of the direct life cycle of monogenean parasites, which generally start with a single host, starting from an egg, becoming a ciliated larva oncomiracidium, then growing up. On *Dactylogyrus* sp. The life cycle will start from an adult worm that lays eggs and hatches into a ciliated larva (onchomiracidium) which has more than one cilia and eyespot and will swim until it attaches to the gills and then becomes an adult worm (Kabata, 1985). These worms are oviparous and have haptors, which are attachment organs that are equipped with two pairs of anchors and 14 lateral hooks.

Conclusion

The conclusion of the research on the identification of ectoparasites in pearl catfish (Clarias gariepinus) at the age of one and three months in Maclele Cultivation, Tuban District, Tuban Regency, is a type of ectoparasite that infests pearl catfish in Makalele Cultivation, Tuban District, Tuban Regency. The species are Trichodina nobilis, Trichodina pediculus, Chilodonella sp., and Dactylogyrus sp. On the incidence of ectoparasite infestation among pearl catfish aged one month and three months. there was no significant difference (p 0.05).

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