Parasites Infection And Histopathological Changes Of Cultured Rabbit Fish *(Siganus guttatus)*

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Abstract- Fish disease caused by parasitic infection is an obstacle in aquaculture that often causes mass mortality of fish. This study aims to determine occurrence of parasitic infection and histopathological changes on gills of rabbit fish (Siganus guttatus). This study was conducted in April to June 2021. Fish samples were obtained from a Shrimp Hatchery Installation in South Sulawesi. Number of fish examined were 50 fish from the nursery tank and 10 fish from broodstock tank. Gills, fins, and body surface of the fish were examined and parasites found were identified based on their morphological characteristics. Histological samples were dehydrated in series of alcohol solution, cleared in Xylol, and stained with Hematoxylin and Eosin. Parasites found on fish from all locations were Pseudohaliotrema sp., Zoothamnium sp., and Copepods. The prevalence of Pseudohaliotrema sp. at the nursery tank was 14% and the broodstock tank was 100%, while the mean intensity for each location was 1.14 ± 2.08 and 56.4 ± 36.08, respectively. The prevalence of Zoothamnium sp. at the nursery tank was 24%, and the broodstock tank was 2%, whereas the mean intensity of each location was 1.41 ± 2.41 and 1.50 ± 0.70, respectively. The other parasite found, Copepods, occurred in low prevalence and mean intensity in the two locations. The histopathological changes observed were lifting and fusion of gill lamellae, and inflammatory cell infiltration. In conclusion, the high parasitic infection of fish in the aquaculture facilities and their severe pathological effects potentially causes fish mortality without proper treatment.

Index Terms- Marine parasite, Histopathology, Nursery tank, Broodstock.

I. INTRODUCTION

Rabbit fish (*S. guttatus*) is a widely distributed marine fish in the Indo-Pacific region (Anam et al., 2020). This fish inhabits coastal areas and can be found in seagrass beds, coral reefs, river mouths in the depth of 6 meters (Gorospe & Cesar, 2013). This fish is herbivorous, has a long intestine and thick stomach wall (Simora et al., 2015). In Indonesia, the rabbit fish has high potential for aquaculture since the fish possesses some biological characteristics suitable for aquaculture and also high demand of the fish from the local market. In 2014, the demand for the fish increased by 296,132 tons/year and tends to increase every year (Ministry of Marine Affairs and Fisheries, 2014). Fisheries statistics show the production of rabbit fish in the Makassar Strait fluctuates by year. In 2013, the production of Rabbit fish reached 471.7 tons/year, while in 2015 decreased by 382.9 tons/year. Marine fisheries production still depends on fishing, especially in the South Sulawesi (Parawansa et al., 2019). In an effort to rely on cultured Rabbit fish, the main source of seeds is obtained from the wild. Rabbit fish seeds from the wild are most likely to be infected with various parasites which will rapidly grow when transferred to a culture environment (Oni et al., 1983).

One of the most pathogenic parasites in Rabbit fish is Caligidae which is found on the surface of the fish's body or commonly known as sea lice. This parasite is common parasite in marine and freshwater fish throughout the world (Cruz-Lacierda et al., 2011). This parasite generally has a body shape consisting of a head that is fused with four thoracic vertebrae and has a round cephalothorax (Kabata, 1979). Copepods infection on fish is characterized by lethargic fish in the bottom of the tank and the occurrence of lesions on the body surface of fish (Lin et al., 1996). The infection of this parasite causes high massive mortality and losses in fish aquaculture (Boxshall & Defaye, 1993; Johnson et al., 2004; Lester & Hayward, 2006).

Other parasite that commonly found on cultured marine fish is the monogenean parasite, for instance, the infection of Pseudohaliotrema sp. was found on the gills of Rabbit fish in the Indo-Pacific region and one of the monogenean parasites from ordo dactylogyridae (Kritsky& Galli, 2007). The population of monogeneans can rapidly increase supported by environmental and host

factors (Rohde, 1982). The life cycle of monogeneans does not require an intermediate host, generally developing from eggs, larvae, and adults (Anshary, 2016). The infection of this parasite can occur and spread on fish cultivated with high stocking density and is affected by host size (Sailaja et al., 2017). In addition, environmental conditions such as high temperatures also affect parasitic eggs to develop rapidly (Silan & Maillard, 1989; Kim et al., 2001).

Common clinical symptoms due to parasitic invasion on their hosts are the fish become weak, no appetite, lesions on the body surface, weight loss, and abnormal swimming (Gusrina, 2008). In addition, parasitic infection on fish causes inhibition of growth and even mass mortality, resulting in a decrease in production and economic losses (Alifuddin et al., 2003). Therefore, studying parasitic infection and the impact on the histopathology of fish's gills is important are required. This study aimed to analyze the parasitic infection rate and the gills histopathological condition of the cultured Rabbit fish in nursery tanks and brood tanks.

II. MATERIALS AND METHODS

This research was carried out in April to June 2021 at the Shrimp Hatchery Installation, Barru Regency, South Sulawesi, and the Fish and Parasite Disease Laboratory, Faculty of Marine Sciences and Fisheries, Hasanuddin University Makassar. The fish samples were obtained from a fish nursery tank with a water capacity of 8 tons. The density of each tank ranges 300 – 400 fish. Fish stocked in nursery tanks are fish that are transferred from the hatching tank. The feed used is a commercial feed, pellet with the size of 3 mm and fish were fed 3 times a day ad-libitum. The rearing tank for broodstocks is a concrete tank measuring 1.5 x 2 x 1 m3 with a capacity of 3 tons. The broodstocks were captured from the wild and then selected and domesticated to be used as broodstock. The broodstock is the first generation broodstock (G1) which was selected from the earth pond with a density of 50 fish. The feed used for Rabbit fish was a 5 mm pellet and was given 3 times a day ad-libitum.

Fish Examination. Rabbit fish examined here were obtained from the nursery tanks and the broodstock tanks. Total amount of 50 fish from the nursery tank was examined with a total length of 9.5 – 14 ± 1.33 cm and a weight of 20 – 47 ± 8.85 grams, and 10 broodstocks were examined with a total length of 25.5 – 30 ± 1.21 cm and a weight of 375 – 496 ± 31.63 grams. For parasitology examination, the fish were taken from the rearing tank and placed on a tray and visually observed for presence of macro parasites. Then, measure the length and weight of each fish examined and investigate parasite on the fins, mucus, and gills of fish. The fish organs were placed on a petri dish containing the same water where the fish were taken except for the mucus which was placed directly on a glass slide and observed under a stereomicroscope and a compound microscope. The parasites found were recorded for their morphological characteristics. Parasite was Identified morphologically using references for parasite identification by Kabata (1985), and various journal references accordingly.

Histopathological Preparations. The infected gills were removed, put in tissue cassette, preserved into 10% Buffer Formalin, and then stored in 70% alcohol solution before pursuing the next histological process. The samples were dehydrated in gradient alcohol series: 70%, 80%, 90%, and 95%) for 24 hours in each solution, and in 100% alcohol I, II, III for 24 hours each. For the clearing process, the samples were put into Xylol I (1 hour), Xylol II (30 minutes), Xylol III (30 minutes), and the first 15 minutes at room temperature while the next 15 minutes was incubated at 56^0C. The sample was then immersed in paraffin and cutting using a microtome with 5 µm thickness. Before staining in H & E solution, the samples were subjected to deparaffinization process with immersing the tissue in Xylol I and II solutions for 30 minutes each and followed by 100%, 95%, 80%, and 70% alcohol for 30 minutes, respectively. Sample was immersed in distilled water for 5 minutes and followed by staining with hematoxylin and eosin solution for 10 minutes, added 5 drops of distilled water and immersed in eosin solution for 20 minutes. Then the sample was put into 70%, 80%, 90%, 95%, 100% alcohol for 1 hour and Xylol solution for 30 minutes each. Finally, the sample was mounted with entellan and covered with a cover glass and observed under a microscope.

Parasite Infestation. Parasite on the fish sample was calculated to determine parasite infection rate using the prevalence and the mean intensity formula according to Bush et al., (1997), namely:

$$\mathbf{P} = \frac{\boldsymbol{N}}{\boldsymbol{n}} \times \mathbf{100}\%$$

Where:
P = Prevalence (%)
N = Number of infected fish
n = Number of examined fish

$$\mathbf{I} = \frac{\Sigma \mathbf{P}}{\mathbf{N}}$$

Where:
I = Mean intensity (parasite/fish)
P = Number of parasites
N = Number of infected fish

Water Quality. Water quality measurements were carried out in the morning. Water quality parameters measured were salinity (hand refractometer), temperature (thermometer), Hydrogen potential (pH-meter), dissolved oxygen (DO-meter), NH_3, NO_2, NO_3, and Total Organic Matter using chemical solution titration method.

III. RESULTS AND DISCUSSION

Parasitological investigation showed that some parasites infected the Rabbit fish reared in different locations, the parasites were *Pseudohaliotrema* sp., *Zoothamnium* sp., and Copepods. The parasitic infections rate in Rabbit fish from nursery tank are presented in Table 1.

Table 1. Parasite Infection Rate inRabbit Fish from Nurseries Tank

Length: 9,5–14 ± 1,33 (cm)	**Weight:** 20–47±8,85 (g)	**n:** 50 fish
Parasite	**Prevalence (%)**	**Mean intensity±SD (parasites/fish)**
Pseudohaliotrema sp.	14	1,14±2,08
Zoothamnium sp.	24	1,41±2,41
Copepods	2	1±0

Table 1. showed prevalence of parasite *Pseudohaliotrema* sp. was 14%, *Zoothamnium* sp. was 24%, and Copepods was 2%. According to Syukran et al., (2017) this indicate the infection of *Pseudohaliotrema* sp. is classified as ”often” infected, *Zoothamnium* sp. infection as “common” and copepods occur occasionally. The mean intensity of *Pseudohaliotrema* sp. was 1.14 parasites/fish, *Zoothamnium* sp. was 1.41 parasites/fish, and Copepods was 1 parasites/fish. Based on mean intensity, the infection of *Pseudohaliotrema* sp., *Zoothamnium* sp., and Copepods are classified as low infection (Syukran et al., 2017).The parasitic infections rate from broodstock tank are presented in Table 2.

Table 2. Parasite Infection Rate on Rabbit Fish from broodstock tank

Length: 25,5-30±1,21 (cm)	**Weight:** 375-496±31,63 (g)	**n:** 10 fish
Parasite	**Prevalence (%)**	**Mean intensity±SD (parasites/fish)**
Pseudohaliotrema sp.	100	56,4±36,08
Zoothamnium sp.	2	1,5±0,70
Copepods	4	1,25±1,15

Table 2 showed that the prevalence of *Pseudohaliotrema* sp. reached 100%, *Zoothamnium* sp. was 2%, and Copepod was 4%. These data indicate that the infection of *Pseudohaliotrema* sp. classified as often and very severe, whereas the infection of *Zoothamnium* sp. and Copepods is classified as low according (Syukran et al., 2017). The mean intensity of *Pseudohaliotrema* sp. was 56.4 parasites/fish, followed by *Zoothamnium* sp. was 1.5 parasites/fish, and the Copepods was 1.25 parasites/fish. This indicates the infection of *Pseudohaliotrema* sp. is classified as severe infections while *Zoothamnium* sp. and Copepods are classified as low infection (Syukran et al., 2017).

This study reported 3 types of parasites on Rabbit fish maintained in nursery and broodstock tanks. The parasites were *Pseudohalitrema* sp., *Zoothamnium* sp., and Copepods. The parasites are mostly found on gills and mucus of fish. *Pseudohaliotrema* sp. is a monogenean parasite that often infecting the fish of the family Siganidae and is common in seawater fish, particularly found on the gills (Kritsky & Galli, 2007). Furthermore, this parasite has a fusiform body consisting of a head, eyes, anterior bilobus, a large vaginal pouch connected to the sperm pouch, and a haptor. These morphological characteristics of the parasite found this study is similar with the monogenean studied by Lim (2002), which states that *Pseudohaliotrema* has eyespot on its body, scattered granules (bilobed) randomly on the inside of the body, and are shaped like a tube.

The other parasite on Rabbit fish is *Zoothamnium* sp. This parasite usually infects the caudal and fins of its hosts. *Zoothamnium* sp. is a ciliates-protozoa and mainly found in low-quality waters in the cultured environment. *Zoothamnium* sp. is a conical parasite and practically spherical, contractile, white in color, has macro and micronucleus. Furthermore, generally branched and live in colonize. This is similar with Z*oothamnium* studied by Muttaqin et al., (2018) which states *Zoothamnium* has a body shape nearly inverted bell, contract, colonies by branches on each stalk, and are transparent.

The other parasite found from the two cultured site was unidentified Copepods. Majority of copepod parasite causes severe damage to the host's fins and implantation which develops. The impacts of sea lice infection in farmed marine fishes have been recently reviewed, with reported disease outbreaks and high mortalities (Johnson et al., 2004; Lester and Hayward, 2006). Copepod parasites usually infect the caudal, dorsal, and pectoral fins (Bharadhirajan et al., 2013). Copepod affects the health and growth rate of the host due to a lack of appetite. In addition, the occurrence of lesions on the body surface of the host causes the degradation of the osmotic condition and mortality (Freeman et al., 2013).

Based on Tables 1 and 2, the highest infection rate was an infection of *Pseudohaliotrema* sp. while the lowest was the Copepods. Parasite attack in broodstock is higher than in nursery tank. This is due to the difference in host size. That level sizes infection effect of parasite *Pseudohaliotrema* sp. Where, the life cycle of this parasite does not require an intermediate host to develop. Most of this group

of parasites release eggs, after hatching they produce larvae which then swim freely in the water column in search of a host and move to the gills or other organs (Anshary, 2016).

Differences in parasitic infestation and species are most likely caused by biotic factors (size, species, age, pathogen) and abiotic factors (temperature, pH, salinity, and other environmental factors). The high prevalence in all cultured locations is due to stress from the high stocking density in the culture tank. According to Anshary (2011), high stocking densities in fish cultured and lower water exchange systems allow the presence of parasites to grow quickly. in addition, high stocking densities will cause stress to fish which makes them susceptible to diseases, especially parasites. The investigation by Ode (2014), stated the prevalence of parasites was influenced by the fish size and changing seasons. Fish age cause affects the fish size, changes in morphology, and physiology.

Mean intensity for all parasites showed a low category of infection level and was usually influenced by the weight of the fish. The larger fish generally cause an increase in the parasitic infection rate. Afterward, the fish disease is caused by the imbalance of carrying capacity of the environment and the quality of production in a culture area, in this case, the relationship between the host, pathogen, and the environment. Examination of the histopathology of fish gills showed a serious pathological effect. The histopathological condition of Rabbit gills showed the presence of parasites in the secondary lamella and cysts in the secondary lamella. Figures 1 a and b shows parasitic cysts or nodules on the gills cause discoloration in the host.

Histopathological conditions on the gills of rabbit fish (*S. guttatus*) in the two cultured locations show serious damage with the presence of parasites cysts, lifting or fusion of gill lamellae (Figures 1 a and b).

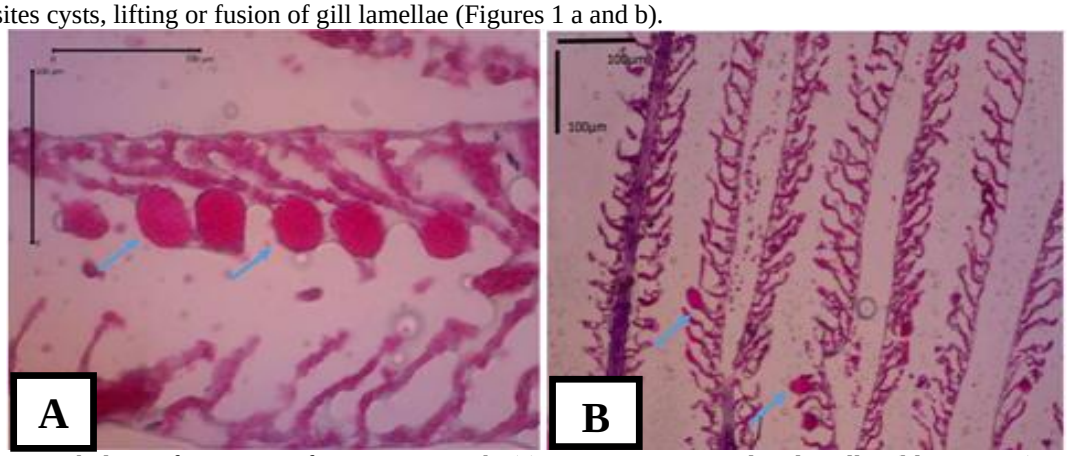

Figure 1. Gills histopathology of *S. guttatus* from nursery tank. (a) Parasites on secondary lamellae (blue arrows), 40x magnification; (b) Parasite cysts on secondary lamellae (blue arrows), 10x magnification.

Gills histopathological changes of *S. guttatus* from broodstock tank showed bleeding in the primary lamellae and the occurrence of inflammatory cell infiltration (Figure 2). Histopathological changes show the occurrence of bleeding in the primary lamellae, congestion in the secondary lamellae, and inflammatory cell infiltration (necrosis) which is caused by acute cells due to tissue damage. According to Plumb (1994), necrosis is characterized by cell or tissue death accompanied by cells degeneration. According to Woo & Buchman (2012), necrosis describes a condition of decreased tissue activity characterized by the gradual loss of cell parts.

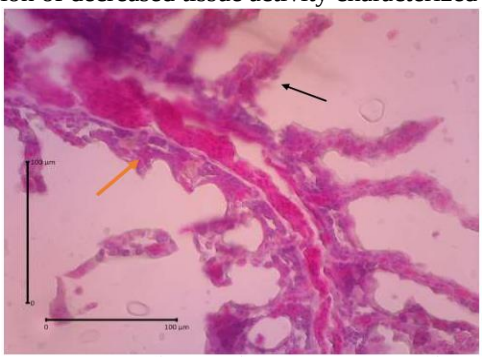

Figure 2. Primary lamellae haemorrhage (yellow arrow), congestion on secondary lamellae (black arrow), and inflammatory cell infiltration, (40x magnification).

Water quality measurement in several cultured environments are present in Table 3. Water quality measurements show that fewer conditions are less than optimal but are still suitable for the culture of Rabbit fish. In fish farming, water quality affects fish growth and defence against pathogens. Generally, fish metabolism has a strong relationship with temperature. Optimal metabolism occurs at high temperatures within the normal range while low temperatures cause fish to be more susceptible to parasites

Table 3. Water Quality

Location	pH	Temperature (^{0}C)	Salinity(ppt)	DO (ppm)	NH_3 (ppm)	NO_2 (ppm)	NO_3 (ppm)	BOT (ppm)
Nursery	5	32	30	6	0,037	0,022	0,060	56,88
Broodstock	5	30	28	5	0,063	0,130	0,236	120,08

IV. CONCLUSION

Parasites on Rabbit fish from all cultured locations are *Pseudohaliotrema* sp., *Zoothamnium* sp., and Copepods. The parasitic infection rate attacks fish is classified as often and generally found to be infected culture fish. The histopathological conditions of Rabbit gills showed damage and caused serious impacts. Water quality measurements show suitable for Rabbit fish cultured.

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The preferred spelling of the word “acknowledgment” in American English is without an “e” after the “g.” Use the singular heading even if you have many acknowledgments.

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