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Prevalence and intensity of protozoan ectoparasite infestation on nursery of humpback grouper (*Cromileptes altivelis*) in hatchery and floating net cage

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Abstract. Ectoparasites which often attack humpback groupers are protozoa from the Ciliate class, *Trichodina* sp. and *Cryptocaryon* sp. This study studied the differences in the prevalence and intensity of protozoan ectoparasites in nursery of humpback grouper in hatcheries and floating net cages. The results showed that there was a significant difference ($p < 0.05$) between the prevalence and intensity of protozoan ectoparasites in nursery of grouper which were kept in hatchery and floating net cages. The prevalence nursery of humpback grouper infested from hatcheries and floating net cages is *Trichodina* sp. ectoparasites are 83.3% and 76.6%. Prevalence of *Cryptocaryon* sp. ectoparasites are 76.6% in hatchery and the prevalence of floating net cages is 50%. From the statistical test shows that the intensity of the *Trichodina* sp. ectoparasites at the hatchery is 12.76 individuals / head was significantly different ($p < 0.05$) on the intensity of the *Trichodina* sp. ectoparasites. In floating net cages of 4.2 individuals / head. Meanwhile, the results of statistical tests on the intensity of ectoparasite *Cryptocaryon* sp. at the hatchery is 4.47 individuals / head significantly different ($p < 0.05$) on the intensity of ectoparasites *Cryptocaryon* sp. in floating net cages there are 1.73 individuals / head.

1. Introduction

The development of humpback grouper (*Cromileptes altivelis*) nursery using floating net cages and hatchery is an alternative to overcome obstacles that arise during maintenance. According to SNI [1] that the size of humpback grouper nursery in hatcheries is 2-3 cm, 4-7 cm and 8-10 cm. Nurseries with a size of more than 10 cm will experience a transition period from a closed culture system in a hatchery to an open culture system in the floating net cages or called a nursery system.

Humpback grouper hatchery experiences several problems including inadequate maintenance environmental conditions, high density with a narrow tub size and the rest of the feed causing changes in water quality [2]. The problem with the development of humpback grouper fish in floating net cages in Situbondo is that water quality parameters are rarely measured unless there is a research activity. Other problems that occur are trash fish and management techniques [2]. Open cultivation systems cause water to become a source of infectious disease [3].

Ectoparasites protozoa are one of the infectious agents that cause disease in humpback grouper nursery. According to [4] that ectoparasites that attack the surface of the body are thought to have greater potential for spread. This is because ectoparasites are free to move from one host to another. Prevalence is the number of organisms in the population that have a particular disease, disorder or



condition at a time related to the large population from which the case originated. Intensity is a category of levels of the number of parasites found in examined and infected fish [5].

One case of protozoan ectoparasites found in humpback grouper in floating net cages in Jepara waters is the protozoan parasite *Trichodina* sp., *Cryptocaryon* sp. and *Epistylis* sp. [6]. Cases of protozoan ectoparasites found in humpback grouper fish in the hatchery and floating net cages waters of Endau, Malaysia are *Cryptocaryon* sp. with a prevalence of 35% floating net cages and 70% hatchery, and *Trichodina* sp. with a prevalence of 5% floating net cages, 80% hatchery. The intensity value in the hatchery is 7.1 individuals / head and the intensity value in the floating net cages is 3.2 individuals / head [7]. Umasugi dan Burhanuddin, (2015) [8] added that *Trichodina* sp. and *Cryptocaryon* sp. is a type of protozoan ectoparasites in the tropics that are able to infest humpback grouper. This shows that the prevalence and intensity of the protozoan ectoparasites in Endau waters, Malaysia are included in the medium category level.

2. Material and methods

2.1 Time and location

This research was conducted at the Educational Laboratory of the Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya in September 2018 - January 2019.

2.2 Research methods

The research method used in this study is a survey method. [9] Survey method is an attempt to collect information from a portion of the population that is considered to represent a certain population.

2.3 Research materials

Equipment used for research sampling included plastic packing, styrofoam boxes, plastic buckets and rubber. The tools used for the temporary container before the fish were observed include 40 x 50 x 60 cm³ aquarium, aerator, filter and rock woll. The tools used for the protozoan ectoparasite examination process include calipers, digital scales, scalpels, surgical scissors, tweezers, petri dishes, glass objects, glass cover, tweezers, microtube, trays and binocular microscopes. Equipment to measure water quality include a thermometer, pH meter, Dissolved Oxygen meter, refractometer and ammonia test kit, nitrate test kit, nitrite test kit.

Fish that used in this study were humpback grouper measuring 8-10 cm totaling 30 fishes taken from the UPBL Situbondo hatchery and 13-20 cm totaling 30 fishes taken from floating net cages BPBAP Situbondo. The materials used for sampling are ice cubes and water for maintenance from each location. The materials used for ectoparasite examination are test sample fish, distilled water and tissue.

2.4 Work procedures

2.4.1 Sampling

Samples of humpback grouper nursery were taken from the hatchery of the Situbondo Aquaculture Management Unit (UPBL), East Java and the floating net cages belonging to the Situbondo Aquaculture Fisheries Center (BPBAP), East Java.

According to [10] states that the large number of samples taken for correlational descriptive research is 27-30 individuals per population in an area. Sampling of grouper nursery of 8-10 cm taken from two UPBL Situbondo hatcheries measuring 3x2x1.5 m³ with 2000 stocking densities / tub. Samples of grouper nursery size of 13-20 cm were taken from two floating net cages BPBAP Situbondo plots with stocking densities of 400 individuals / unit.

2.4.2 Examination of Ectoparasite

Ectoparasites examination in humpback grouper using the native method is scrapping the body surface and gills by examining it using a microscope with a magnification of 100x and 400x. Ectoparasites include body surfaces and gills on fish size of 8-10 cm and 13-20 cm. Examination of

humpback grouperis are done by scrapping the skin from the head, body surface and fins with a scapel and placed into a petri dish [11]. The results of this scraping are placed on the glass object and closed by the glass cover. Ectoparasites in humpback grouper were calculated as prevalence and intensity values. The morphological and protozoan ectoparasites found were based on the key identification [12].

3. Results and discussion

3.1 Protozoa Ectoparasites Examination in Humpback Grouper

The results of examination of 30 samples of humpback grouper from hatchery and floating net cages were found ectoparasites which infested humpback grouper namely *Trichodina* sp. and *Cryptocaryon* sp.

3.1.1 *Trichodina* sp.

The results of the examination of the genus *Trichodina* sp. found on the surface of the body from the head to the caudal fins. Observations using a 100x and 400x magnification microscope showed that *Trichodina* sp. looks circular with a size of 10-120 μm .



Figure 1. *Trichodina* sp. a. Cilia, b. Denticle

3.1.2 *Cryptocaryon* sp.

Examination results obtained ectoparasites *Cryptocaryon* sp found on the surface of the body from the head to the caudal fins and gills. Observations using a 100x and 400x magnification microscope showed that *Cryptocaryon* sp. looks solitary (individual). *Cryptocaryon* sp. looks blackish brown and is shaped like a pear.

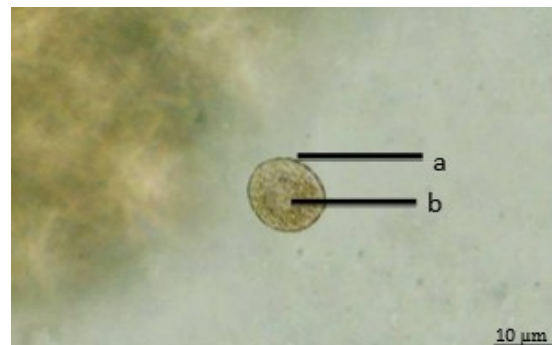


Figure 2. *Cryptocaryon* sp.
a. Cilia, b. Macronucleus

Table 1. The prevalence of humpback grouper infected with *Trichodina* sp. and *Cryptocaryon* sp.

Location	Fish total that examined	Protozoa that founded	Mix infestation	Number of fish that infested	Prevalence (%)
<i>Hatchery</i>	30	<i>Trichodina</i> sp.	390	26	86,66
<i>Floating net cages</i>	30	<i>Cryptocaryon</i> sp. <i>Trichodina</i> sp. <i>Cryptocaryon</i> sp.	47	29	96,6

Table 2. Intensity of protozoan ectoparasites of humpback grouper in hatcheries

Protozoa Ectoparasite	Sample (fish)	Fish infested (fish)	Number of ectoparasite (individual)	Intensity (ind/fish)	Category
<i>Trichodina</i> sp.	30	25	319	12,76	Heavy
<i>Cryptocaryon</i> sp.	30	29	103	3,55	Low

The results showed that the humpback grouper nursery from hatchery and floating net cages found the ectoparasites of *Trichodina* sp. and *Cryptocaryon* sp. *Trichodina* sp. cause skin diseases with itchy symptoms in fish which generally infects the outside including the skin, fins and fish gills. According to [13] *Cryptocaryon* sp. cause white spots on the surface of the body and gills. *Cryptocaryon* sp. is ciliate moves with vibes hair. *Trichodina* sp. and *Cryptocaryon* sp. can develop optimally because the hatchery has high water quality and stocking density can support the growth of ectoparasites. The problem of water quality in floating net cages is caused by leftover fish feed and the change of the net is rarely done. Nursery in floating net cages has fluctuating temperatures. This is because floating net cages are in open waters that depend on nature. Besides that, in floating net cages, decomposition of leftover fish feed results in high organic matter.

Table 3. Intensity of protozoan ectoparasites of humpback grouper in floating net cages

Jenis ektoparasit protozoa	Sample (fish)	Fish infested (fish)	Number of ectoparasite (individual)	Intensity (ind/fish)	Category
<i>Trichodina</i> sp.	30	20	84	4,2	Moderate
<i>Cryptocaryon</i> sp.	30	15	26	1,73	Low

Table 4. Results of measurements of water quality in hatcheries and floating net cages

Parameter	<i>Hatchery</i>	Floating net cages	SNI (2011)
Temperature (°C)	24-26	30-31	28-32
DO (mg/L)	7-9	7-9	>5
pH	7	7	7,5-8,5
Salinity(ppt)	30-31	30-31	28-33
Ammonia (mg/L)	1	3-5	≤ 0,01

The prevalence in hatchery is 86.6% (Table 1) with the number of humpback grouper nursery infected by 26 of the 30 humpback grouper nursery examined included the usual infection prevalence criteria, whereas in floating net cages was 96.6% (Table 1) with the number of infected humpback groupers 29 out of 30 animals examined including infection prevalence criteria almost always. Furthermore, from the results of calculating the prevalence by Chi-square statistical test there was no significant difference in the prevalence of hatchery and floating net cages, $p > 0.05$. According to [14] that, the high and low prevalence rates are caused by factors such as endurance or conditions of the host body that are able to adapt to changes in water quality or due to an attack of parasitic infections. Aquatic conditions that do not support fish life are able to cause fish to become stressed and cause the

prevalence of parasitic infections to be high [15]. The prevalence of parasitic infections in waters is influenced by habitat consisting of components such as temperature, oxygen content, and others [16].

Ectoparasites intensity *Trichodina* sp. the results obtained were 12.76 individuals / fish in the hatchery whereas, the intensity of ectoparasites *Trichodina* sp. in floating net cages of 4.2 individuals / fish. [17] states that fish are susceptible to parasites if the oxygen content in waters is > 4 mg / l per day. Life cycle of *Trichodina* sp. is influenced by the oxygen content in the waters (> 3 ppm). Ectoparasites intensity *Cryptocaryon* sp. the results obtained were 3.55 individuals / fish in hatchery whereas, the intensity of ectoparasites *Cryptocaryon* sp. in floating net cages of 1.73 individuals / fish. This can occur because of ciliate class ectoparasites such as *Cryptocaryon* sp. commonly found in sea water fish. This is in accordance with research conducted by [8] obtained the results of protozoan ectoparasites which infest humpback grouper fish in tropical regions including Indonesia, including *Cryptocaryon* sp. with an intensity value of 4.0 individuals / fish. This is probably due to *Cryptocaryon* sp. can develop due to high organic matter in the waters and fluctuating water temperatures. The high intensity of *Trichodinasp.* in the hatchery caused by the hatchery which has a high organic material content, the base and walls of the pond in the form of concrete, while *Trichodina* sp. grow quickly because one of the properties of these ectoparasites can live in a wide range of salinity. This is in accordance with the opinion of [18] which states that some of the factors supporting the existence of this protozoa include the presence of high organic matter in waters, fluctuating water temperatures, oxygen levels less than the normal limit of <4 ppm and the presence of transmission through water and contact directly between healthy and infested fish *Trichodina* sp.

The high intensity of protozoan ectoparasites in the hatchery may also be due to the fact that the concrete pond has solid walls and the bottom of the pond so that it can be used as a sticking site for *Trichodina* and *Cryptocaryon* ectoparasites. This is in accordance with the opinion of [13] which states that *Trichodina* and *Cryptocaryon* ectoparasites are attached to the substrate in the form of walls and bottom of ponds which contain ammonia content ≤ 0.01 mg / L and pH <7 . Low intensity of protozoa ectoparasites in the incoming water floating net cages depends from natural conditions so that large currents will cause ectoparasites to be wasted. This is in accordance with the opinion of [18] which states that currents play a very important role in water circulation, in addition to carriers of dissolved and suspended materials, currents also affect the amount of oxygen solubility in water.

Based on the results of statistical tests the calculation of the intensity of the hatchery and floating net cages showed significantly different ($p < 0.05$). Ectoparasites intensity *Trichodina* sp. the hatchery was 12.76 individuals / fish included in the severe category and *Cryptocaryon* sp. in the hatchery of 3.55 individuals / fish in the low category. Mixed ectoparasite infestation could possibly occur due to *Trichodina* sp. and *Cryptocaryon* sp. is a ciliate that lives normally in good waters, but will increase if there is a decrease in water quality. This is in accordance with the opinion of [19] states that ciliates such as *Trichodina* can live normally in good waters, but this ciliate protozoa will increase its population in waters whose water quality begins to decline. Water quality in hatcheries is rarely measured in terms of water quality parameters and there is residual food that settles to the bottom of the maintenance pond which causes high organic matter. This can cause fish to be stressed and vulnerable to disease. *Trichodina* sp. can grow optimally at a temperature of 25° - 30° C, salinity 0-35 ppt, pH 7-8, and DO 4-5. Meanwhile, *Cryptocaryon* sp. can grow optimally at temperatures 29 - 35° C, salinity 29-33 ppt, pH 7-8, and DO 4-5.

The results of the water quality showed that the measurement of water quality at the time of sampling at each location was in good condition and still in the standard of grouper seed breeding according to [1]. Temperature measurements at all hatchery locations yielding 24 - 31° C are still within the safe range for the cultivation process which is around 24 - 29° C. DO measurements yield 7 - 9 mg / L and are still above the standard requirement of > 5 mg / L. The measurement of pH to get results 7 is still in the safe range for cultivation which is around 7-8. Salinity measurements get 31-32 ppt, while the standard for salinity is 30-33 ppt. The possibility of using sea water has caused the discovery of ectoparasites *Trichodina* sp. and *Cryptocaryon* sp.

4. Conclusion

Ectoparasites protozoa that infest humpback grouper from the Situbondo UPBL hatchery and the floating net cages Situbondo BPBAP are from the genus *Trichodina* and *Cryptocaryon*, with prevalence degree almost always. Infection degree of *Trichodina* sp. ectoparasites in Hatchery UPBL Situbondo is acute and low for floating net cage BPBAP Situbondo. Infection degree of *Cryptocaryon* sp. ectoparasite in hatchery UPBL Situbondo and floating net cage is low category. There is no significant difference between the prevalence of humpback grouper nursery infected with protozoan ectoparasites from the Situbondo UPBL hatchery and floating net cages Situbondo BPBAP. There is a significant difference between the intensity of ectoparasites *Trichodina* sp. and *Cryptocaryon* sp. in the Situbondo UPBL hatchery and floating net cages Situbondo BPBAP.

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