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Intensity and Predilection of Helminth Parasites of the Red Snapper (*Lutjanus argentimaculatus*)

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ABSTRACT

Marine fish, particularly the red snappers, are often exposed to helminth parasitic infestation. As a result of the parasitic infestation, the fish population, the fish weight, and the morphological changes in the fish are shrinking. The present research aimed to find out the intensity and predilection of the helminth ectoparasites over the infection of the red snapper (*Lutjanus argentimaculatus*) and employed the survey method for this purpose. The sampling was carried out by the purposive sampling technique. The sample obtained consisted of 30 fish, 20% of the total red snapper population of 150 fish reared in the floating net cages of Balai Besar Perikanan Budidaya Laut Lampung. The intensity of the fish infestation by a mixture of *Haliotrema epinepheli* and *Benedenia epinepheli* was 132.5 individuals/fish. *Neobenedenia girellae* and *Haliotrema epinepheli* infected fish with an intensity of 149.41 individuals/fish. The 66.7% of *Benedenia epinepheli* had a predilection for the dorsal fin, and 33.3% for the anal fin. In *Neobenedenia girellae*, 57.1% had a predilection for the body surface, 37.2% for the head surface, and 5.7% for the dorsal fin. In conclusion, all sampled fish were positively infected with helminth ectoparasites, including *Neobenedenia girellae*, *Haliotrema epinepheli*, and *Benedenia epinepheli*.

Keywords: Ectoparasite, Helminths, Infestation, Red snapper

INTRODUCTION

One of the main problems and challenges in red snapper cultivation is disease control (Hussan et al., 2016; Oliveira et al., 2020). Diseases of red snapper could occur in water due to imbalances in the environment, host, and pathogenic organisms (Engering et al., 2013; Hussan et al., 2016; Oliveira et al., 2020). Parasitic infestation and infection might also be attributed to high fish density and inadequate nourishment in fish, as well as poor water and environmental quality (Yadollahi et al., 2013; Hussan et al., 2016).

In nature, red snappers might be susceptible to a number of diseases or parasites (Cable et al., 2017; Oliveira et al., 2020). In the culture environment, the parasites might even be present in far greater numbers and could be lethal to fish (Omeji et al., 2011). Marine fish, particularly the red snappers, are often exposed to parasitic helminth infestation (Montoya-Mendoza et al., 2014; Oliveira et al., 2020). Visual results indicated that the most prevalent ectoparasitic worm in the red snapper was *Benedenia epinepheli*, which was found on the fin and skin (Jithendran et al., 2005; Loh et al., 2020). Meanwhile, *Neobenedenia girellae* (Kishimori et al., 2015), *Hatschekia poche* (Justine et al., 2012), *Haliotrema* species, and *Diplectanum squamatum* N. (Santos et al., 2002) were encountered on the gill.

A parasitic infestation could lead to economic losses. The economic impacts of parasites in fish include reduced fish population for consumption, decreased fish weight, and changes to fish morphology (Iwanowicz, 2011). The prevention and treatment of diseases had thus developed into an important cultivation component. Diseases might be caused by stress, pathogens (e.g., protozoans, bacteria, and viruses), environmental changes (e.g., algae bloom), overdose, and malnutrition (Pikarsky et al., 2004). Different causes could produce distinctive external symptoms of illness in fish, including sudden death, changes in behavior, loss of appetite, and bleeding in places where scales have peeled off (Madhun et al., 2015). Parasites infest or infect the red snapper in a number of ways by, for instance, parasitic contamination of the water in which red snappers are cultivated, direct contact, or rubbing with red snappers contaminated with parasites. In this case, the spread of the sick red snappers would have negative effects, especially if the density is too high. Furthermore, the equipment previously used to hand or transport parasite-contaminated red snappers would also cause parasite infestation or infection (Melianawati and Aryati, 2012).

Helminths are classified according to the International code. Every parasite belongs to particular phylum, class, order, genus, and species. Helminths per se are assigned to four different phyla, namely Platyhelminthes, Nematelminthes, Acanthocephala, and Annelida (Gibson et al., 2014; Morand et al., 2015).

The phylum Platyhelminthes is characterized by a dorsoventral flattening of the body and bilateral symmetry, whereby its organs are embedded within the parenchyma tissues, and there are no respiratory and circulatory systems. The phylum Platyhelminthes is composed of two classes, namely Trematode and Cestode (Hahn et al., 2014; Morand et al., 2015). The parasites of the Trematode class have an ovoid, leaf-like, unsegmented body, and an incomplete digestive tract (a sac with one or two suckers for attachment). Worms in this class have a hermaphrodite reproductive system. This class is divided into three subclasses of Monogenea, Aspidogastrea, and Digenea (Crotti, 2013). Parasites of the order Dactylogyridae of the class Trematode are parasites of aquatic vertebrates, for example, the Reptiles (crocodiles), Amphibians (frogs), and Pisces (fishes). The majority of the parasites in the order Dactylogyridae are ectoparasites with a direct life cycle. The order Dactylogyridae of the class Trematoda has a distinctive feature on its posterior part of the body, that is, a haptor with a pair of hooks to attach itself to its host (Gibson et al., 2014).

There are a number of ectoparasitic worm types that infect the red snapper, namely *Benedenia*, *Neobenedenia*, and *Diplectanum*. These three worms are described as follows. *Benedenia* is a colorless ectoparasite that attacks the surface of the body. In particular, *Benedenia* causes fish to lose their appetite and have abnormalities in the way they swim on the bottom or surface of the container or floating net cage. Severe infection with *Benedenia* could cause wounds or ulcers (a pus-like liquid) on the skin and eventually secondary bacterial and fungal infection (Zhang et al., 2017). *Neobenedeniagirellae* shows a higher degree of pathogenicity than *Benedenia epinepheli* because *Neobenedeniagirellae* infects not only the skin but also the eye and causes blindness. Severely infected fish lose their appetite, have dark body colors, and have irregular swimming patterns and movements (Zhang et al., 2017). *Neobenedenia melleni* shows clinical symptoms in the skin, such as hyperemia and bleeding, and often infects the eye, causing damages to the cornea and blindness of the fish (Trujillo-González et al., 2015). *Diplectanum* disrupts the respiratory function in fish. The resulting wounds would lead to secondary bacterial infection. The growth of fish might be delayed, and the body weight and appetite are reduced. The fish infected by *Diplectanum* had pale gills, which produced excessive amounts of mucous, and a pale body (Cecchini et al., 1998). These three worms (*Benedenia*, *Neobenedenia*, and *Diplectanum*) are responsible for the diseases that the red snappers suffer from. The present research was then conducted to figure out the intensity and predilection of the helminth ectoparasites of the red snappers.

MATERIALS AND METHODS

Ethical approval

The present research employed the survey method. The sample of red snappers (*L. argentimaculatus*) was obtained by the purposive sampling method according to specified criteria. The sample had sizes specified by the researchers from the location of the floating net cages for one week. In this case, the red snappers were found to be 25–35 centimeters long.

The red snappers enrolled as samples were taken from one of seven floating net cages at Balai Besar Perikanan Budidaya Laut (BBPBL) Lampung with the number 30, or accounting for 20% of the total population of 150 red snappers, which were reared in $1 \times 1 \times 1.5 \text{ m}^3$ polyethylene nets. The extracted sample was transported to the Laboratory of Fish Health and Environment of BBPBL Lampung, Indonesia, for helminth parasites examination.

The current research used a fishing net, a plastic bucket for fish sampling, and a Kemmerer bottle for water sampling. The instruments used to calculate the intensity and predilection of the helminth parasites were a set of surgical instruments (scalpel, surgical scissors, forceps, object-glass, and cover glass), pipette, towel paper, ruler, digital scale, digital camera, label paper, microtube, and trinocular microscope. Instruments used to measure water quality included a pH meter for pH measurement, a refractometer for measuring salinity, a Dissolved Oxygen (DO) meter for measuring dissolved oxygen, and a thermometer for measuring temperature.

The main materials used for the present research were the red snappers (*L. argentiaculatus*) and the seawater sample from the floating net cage location. Meanwhile, 250 mL of 5% glycerin was used to study the helminth parasite. The current research was undertaken from February through March 2016 in the Laboratory of Fish Health and Environment, BBPBL Lampung, and the Education Laboratory of the Faculty of Fisheries and Marine Affairs, Universitas Airlangga, while the sampling was performed at the location of the floating net cages of BBPBL Lampung.

RESULTS

The results of the present research concerned the intensity of the helminth ectoparasites, the predilection of helminth ectoparasites, the distribution of predilection of helminth ectoparasites, and the water quality parameters.

Intensity of helminth ectoparasites

From the investigation of the 30 samples of red snappers (*Lutjanus argentimaculatus*) that were cultivated in a floating net cage of BBPBL Lampung, helminth ectoparasites were found. The results of the sample examination indicated that a single sample fish could be infested with more than one type of helminth ectoparasite. Of the 30 sample

fish examined, 12 were only infested by *Haliotrema epinepheli* (sole infestation), including 1534 worms found with an intensity of 127.83 individuals/fish. Aside from those fish, 6 were infected with a mixture of *Benedenia epinepheli* and *Haliotrema epinepheli*, with 795 worms found with an intensity of 132.5 individuals/fish, and 12 were infested with a mixture of *Neobenedenia girellae* and *Haliotrema epinepheli*, having 1793 worms with an intensity of 149.41 individuals/fish.

Predilection of helminth ectoparasites

Observations on the predilection of the helminth ectoparasites were performed in the Laboratory of Fish Health and Environment of BBPBL Lampung. The observations were carried out on macroscopic and microscopic bases. The predilection test procedure was performed by scraping off all body parts of the fish, followed by observations under a microscope at 100 × and 400 × magnifications. Predilection sites for the helminth ectoparasites were the body surface of the fish and the fins, including the dorsal, pectoral, anal, and pelvic fins. The predilection study on the gills was conducted by observing the histopathological preparations of the fins infested by the ectoparasitic worms. The examination included changes in the structure and appearance of the organs infested by the ectoparasitic worms.

According to the examination results of the worms in the red snappers, *Benedenia epinepheli* had a round, elongated body with a length of 2.06 to 3.05 mm, a width of 0.67 to 1.51 mm, and a diameter of 0.50 to 0.78 mm. *Benedenia epinepheli* had attachment organs 0.16-0.25 mm long and 0.12 to 0.27 mm wide, a pharynx of 0.16-0.35 mm long and 0.18-0.25 mm wide, accessory sclerites of 0.07 to 0.11 mm long, anterior hamuli of 0.08 to 1.21 mm long, and posterior hamuli of 0.04 to 0.07 mm long.

Neobenedenia girellae was found to have a round elongated body with a length of 4.6 to 5.7 mm, and a width of 1.5 to 2.5 mm. In addition to an opisthaptor of 1.2-1.5 mm in size, *Neobenedenia girellae* also had a pair of attachment organs with the length of 0.14 to 0.22 mm and width of 0.17 to 0.29 mm, a pharynx with a length from 0.51 to 0.71 and a width of 0.39 to 0.42 as well as accessory sclerites with a length of 0.25 to 0.31 mm, anterior hamuli of 0.28-0.39 mm long, and posterior hamuli of 0.11-0.18 mm long. Meanwhile, *Haliotrema epinepheli* was found to be 200.8 μm in length and 48.12 μm in width, and also had a pharynx 15.40 to 18.71 μm in diameter.

Distribution of the predilection of helminth parasites

66.7% of the *Benedenia epinepheli* that infest the red snappers preferred the dorsal fin, and 33.3% preferred the anal fin. No *Benedenia epinepheli* infestation was found on the caudal, pectoral, and ventral fins, and head. For the *Neobenedenia girellae* infestation in the red snappers, a majority of 57.1% had a high predilection for the body surface, 37.2% a medium predilection for the head, and 5.7% a low predilection for the dorsal fin. No predilection was indicated for the caudal, pectoral, anal, and ventral fins. Meanwhile, a 100% predilection for *Haliotrema epinepheli* was indicated for the gills and none for other parts of the body. As for the histopathologic observations of the gills, the results indicated that *Haliotrema epinepheli* had a predilection for the lamellae part of the red snapper gills.

Water quality parameters

The measurement of the water quality in terms of pH, DO, and salinity was performed once a week in the morning. The results of the observation of some indicators of water quality in the floating net cages of BBPBL Lampung in 2016 indicated that the water was in a normal condition for the life of the cultivated fish. The data indicated that the values were within normal ranges, where the temperature was between 30°C and 31°C, salinity was reported as 5 ppt, pH was estimated at 7, DO was between 6 and 8.37 mg/L, and the ammonia was also between 0.044 and 0.221 mg/L.

DISCUSSION

Based on the results of the examination of the body surface and the gills of red snappers (*Lutjanus argentimaculatus*), it was found that three parasitic worm species infest the red snappers in the floating net cages of BBPBL Lampung, namely *Benedenia epinepheli*, *Neobenedenia girellae*, and *Haliotrema epinepheli*. The helminth parasites *Benedenia epinepheli* and *Neobenedenia girellae* were encountered on the body surface of the red snappers, while *Haliotrema epinepheli* were found in the gills. The present findings are similar to Kritsky's (2012) review in the Persian Gulf, the eastern, Indo-west Pacific Ocean, and Mexico gulfs snappers.

The helminth ectoparasite *Benedenia epinepheli*, which infested the body surface of the red snappers as stated previously by Ravi and Yahaya (2016), had a predilection, especially on the dorsal and anal fins and in the skin area. When examining the ectoparasitic worms in the red snappers, *Benedenia epinepheli* was found to infest the dorsal fin area of 4 out of 30 sample fish, and infestation on the anal fin was rare.

The present research also discovered an infestation of the ectoparasitic worm *Neobenedenia girellae*. During the investigation, *Neobenedenia girellae* was found on the body surface, especially in the head area, in 13 out of 30 sample red snappers. It can be said that the worms had the highest prevalence on the body surface and rarely showed up on the

dorsal fin. *Neobenedenia girellae* had a predilection for the skin around the head and would also cause blindness, but was not found on the dorsal fin and the remaining body parts such as the gills and the internal organs.

The ectoparasitic worm *Haliotrema epinepheli* was also encountered in the present research. The predilection of the worm was exclusively for the gills. All sample fish infested by *Haliotrema epinepheli* demonstrated clinical symptoms of excessive mucus and swimming on the water surface. A severe infection of *Haliotrema epinepheli* could damage the filaments of the gills and cause the infected fish to suffer respiratory disorders or, in the worst case, die.

Based on the visual results, all sampled fish were infested by *Haliotrema epinepheli* in the gills. The *Benedenia epinepheli* infestation was present on the dorsal and anal fins, while the *Neobenedenia girellae* infestation occurred on the body surface, head, and dorsal fin, suggesting that *Haliotrema epinepheli* predominantly infested fish gills, eliminating competition between worm species in the areas. The fish infested by the helminth ectoparasite *Haliotrema epinepheli* rarely suffered wounds in their gill lamellae.

Although many of the red snappers (*Lutjanus argentimaculatus*) cultivated in the floating net cages at BBPBL Lampung were infested by *Haliotrema epinepheli* in the gills, none of the fish died. *Benedenia epinepheli* and *Neobenedenia girellae* did not leave any serious impact on the red snappers' health in small numbers. However, if *Benedenia epinepheli* and *Neobenedenia girellae* were present in significant numbers, they could be fatal to the fish (Melianawati and Aryati, 2012).

Based on the results of the examination on the 30 sample red snappers, 100% of the fish were positively infested with helminth ectoparasites, suggesting that the infestation intensity in floating net cages was very high. The infestation with helminth ectoparasites in the cultivation of floating net cages occurred due to the poor fish rearing and uncontrolled water quality since the cultivation was weather-dependent. Another factor presumed to decrease the red snappers' physical endurance was the stress resulted from the flow of water and net cages spoilage from the attachment of parasites and other microorganisms which blocked water circulation, which was mentioned by Cooper (2015). In addition, stress could also be caused by high density, which allowed parasites to develop rapidly, with high density triggered competition for space, food, and oxygen. The mortality rate from infestation with helminth ectoparasites at this cultivation location (East Java) was categorized as so high that the red snapper population in these floating net cages was smaller compared to other populations.

CONCLUSION

All of the fish samples were positively infected by helminth ectoparasites, including *Neobenedenia girellae*, *Haliotrema epinepheli*, and *Benedenia epinepheli*. Therefore, in the East Java region, Ocean fish are most likely to be infected with these parasites. In fish inspections, parasite-related infections should be considered cautiously, especially in marine fish like Red Snapper (*Lutjanus argentimaculatus*).

DECLARATIONS

Authors' contribution

All the authors contributed equally to this study.

Competing interests

The authors have not declared any conflict of interest.

Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

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GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

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RUBRIC: 6TH-8TH SCIENCE ARGUMENT (CER)

CLAIM

Take an arguable position on the scientific topic and develop the essay around that stance.

ADVANCED	The essay introduces a precise, qualitative and/or quantitative claim based on the scientific topic or text(s), regarding the relationship between dependent and independent variables. The essay develops the claim and counterclaim fairly, distinguishing the claim from alternate or opposing claims.
PROFICIENT	The essay introduces a clear, qualitative and/or quantitative claim based on the scientific topic or text(s), regarding the relationship between dependent and independent variables. The essay effectively acknowledges and distinguishes the claim from alternate or opposing claims.
DEVELOPING	The essay attempts to introduce a qualitative and/or quantitative claim, based on the scientific topic or text(s), but it may be somewhat unclear or not maintained throughout the essay. The essay may not clearly acknowledge or distinguish the claim from alternate or opposing claims.
EMERGING	The essay does not clearly make a claim based on the scientific topic or text(s), or the claim is overly simplistic or vague. The essay does not acknowledge or distinguish counterclaims.

EVIDENCE

Include relevant facts, definitions, and examples to back up the claim.

ADVANCED	The essay supplies sufficient relevant, accurate qualitative and/or quantitative data and evidence related to the scientific topic or text(s) to support its claim and counterclaim.
PROFICIENT	The essay supplies relevant, accurate qualitative and/or quantitative data and evidence related to the scientific topic or text(s) to support its claim and counterclaim.
DEVELOPING	The essay supplies some qualitative and/or quantitative data and evidence, but it may not be closely related to the scientific topic or text(s), or the support that is offered relies mostly on summary of the source(s), thereby not effectively supporting the essay's claim and counterclaim.
EMERGING	The essay supplies very little or no data and evidence to support its claim and counterclaim, or the evidence that is provided is not clear or relevant.

REASONING

Explain how or why each piece of evidence supports the claim.

ADVANCED	The essay effectively applies scientific ideas and principles in order to explain how or why the cited evidence supports the claim. The essay demonstrates consistently logical reasoning and understanding of the scientific topic and/or text(s). The essay's explanations anticipate the audience's knowledge level and concerns about this scientific topic.
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PROFICIENT	The essay applies scientific reasoning in order to explain how or why the cited evidence supports the claim. The essay demonstrates logical reasoning and understanding of the scientific topic and/or text(s). The essay's explanations attempt to anticipate the audience's knowledge level and concerns about this scientific topic.
DEVELOPING	The essay includes some reasoning and understanding of the scientific topic and/or text(s), but it does not effectively apply scientific ideas or principles to explain how or why the evidence supports the claim.
EMERGING	The essay does not demonstrate clear or relevant reasoning to support the claim or to demonstrate an understanding of the scientific topic and/or text(s).

FOCUS

Focus your writing on the prompt and task.

ADVANCED	The essay maintains strong focus on the purpose and task, using the whole essay to support and develop the claim and counterclaims evenly while thoroughly addressing the demands of the prompt.
PROFICIENT	The essay addresses the demands of the prompt and is mostly focused on the purpose and task. The essay may not acknowledge the claim and counterclaims evenly throughout.
DEVELOPING	The essay may not fully address the demands of the prompt or stay focused on the purpose and task. The writing may stray significantly off topic at times, and introduce the writer's bias occasionally, making it difficult to follow the central claim at times.
EMERGING	The essay does not maintain focus on purpose or task.

ORGANIZATION

Organize your writing in a logical sequence.

ADVANCED	The essay incorporates an organizational structure throughout that establishes clear relationships among the claim(s), counterclaims, reasons, and evidence. Effective transitional words and phrases are included to clarify the relationships between and among ideas (i.e. claim and reasons, reasons and evidence, claim and counterclaim) in a way that strengthens the argument. The essay includes an introduction and conclusion that effectively follows from and supports the argument presented.
PROFICIENT	The essay incorporates an organizational structure with clear transitional words and phrases that show the relationship between and among ideas. The essay includes a progression of ideas from beginning to end, including an introduction and concluding statement or section that follows from and supports the argument presented.
DEVELOPING	The essay uses a basic organizational structure and minimal transitional words and phrases, though relationships between and among ideas are not consistently

clear. The essay moves from beginning to end; however, an introduction and/or conclusion may not be clearly evident.

EMERGING

The essay does not have an organizational structure and may simply offer a series of ideas without any clear transitions or connections. An introduction and conclusion are not evident.

LANGUAGE

Pay close attention to your tone, style, word choice, and sentence structure when writing.

ADVANCED

The essay effectively establishes and maintains a formal style and objective tone and incorporates language that anticipates the reader's knowledge level and concerns. The essay consistently demonstrates a clear command of conventions, while also employing discipline-specific word choices and varied sentence structure.

PROFICIENT

The essay generally establishes and maintains a formal style with few possible exceptions and incorporates language that anticipates the reader's knowledge level and concerns. The essay demonstrates a general command of conventions, while also employing discipline-specific word choices and some variety in sentence structure.

DEVELOPING

The essay does not maintain a formal style consistently and incorporates language that may not show an awareness of the reader's knowledge or concerns. The essay may contain errors in conventions that interfere with meaning. Some attempts at discipline-specific word choices are made, and sentence structure may not vary often.

EMERGING

The essay employs language that is inappropriate for the audience and is not formal in style. The essay may contain pervasive errors in conventions that interfere with meaning, word choice is not discipline-specific, and sentence structures are simplistic and unvaried.