Prevalence, Intensity and Histopathology of Copepodic Parasite Infection on the Groupers in Batam Waters, Riau Islands, Indonesia

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Abstract

Endang Wijayanti, Hendrianto, Indah Istiqomah, Murwantoko. 2021. Prevalence, Intensity and Histopathology of Copepodic Parasite Infection on the Groupers in Batam Waters, Riau Islands, Indonesia. Aquacultura Indonesiana. 22(2): 1-9. The parasite infection in cultured fish can be dangerous and cannot be treated immediately. Parasitic copepods are the most frequently encountered taxa parasitizing fish and cause significant economic loss in mariculture. The objective of this study were to determine the intensity and prevalence of copepodic parasite on cantang hybrid grouper (\textit{Ephinephelus} sp.) and tiger grouper (\textit{Ephinephelus fuscoguttatus}), and to determine the histological change caused by parasite infection. This study was conducted from five fish farmers at Batam waters, Riau Islands composed of seven cantang hybrid grouper populations, and two tiger grouper populations. For parasit observation, at least 36 fishes were randomly sampled from each grouper population. The fish showing symptoms of red nodules in the oral cavity was designed as positive infected fish. The counting number of parasites was carried out by observation using a binocular microscope. The infected and uninfected tissues were preserved on neutral buffered formalin and processed for histology. The copepodic parasite was found both in cantang hybrid grouper (\textit{Ephinephelus} sp.) and tiger grouper (\textit{E. fuscoguttatus}). The parasite was found at high prevalence on cantang grouper with average prevalence of 62% with highest prevalence at 87%. The intensity copepodic parasite on cantang hybrid grouper is at low level with an average intensity of five parasites/fish. The infection on as tiger grouper with an average intensity of 4.5 and prevalence 40.25%. The prevalence and intensity of copepodic parasite on cantang grouper tend to increase with increasing fish size. Copepodic parasite infection in fish tissue caused congestion, polymorphonuclear, haemorrhage, hyperplasia and mucus proliferation.

Key words : Copepode, \textit{Ephinephelus}, Prevalence, Intensity, Histopathology

Introduction

Grouper is an important food fish due to its commercial value. Based on national production data provided, almost 155,000 tonnes of grouper were produced in 2015 with a total value of USD 630 million (FAO, 2017). Several species of grouper have been intensively cultured in Indonesia and served as an important fish commodity in Indonesia. The tiger grouper (\textit{Ephinephelus fuscoguttatus}) and mouse grouper (\textit{Cromileptes altivelis}) seeds have been produced from hatchery to fulfill grouper demands. The hybrid groupers have also been produced in hatcheries by breeding different species of grouper. The cantang hybrid grouper (\textit{Epinephelus} sp.) is the result of crossbreeding between female tiger grouper and male giant grouper (\textit{E. lanceolatus}). The crossbreed between the female tiger grouper and the male brown-marbled grouper (\textit{E. microdon}), named the cantik grouper (Ismi \textit{et al.}, 2013). Grouper cantang culture has developed well from rearing of fry to consumption size (Ismi et al,}
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2012), fast grow in cages (Sutarmat and Yudha, 2013).

The emergence of diseases is one of the main problems in aquaculture. Emerging diseases of epizootics frequently cause substantial and often explosive losses in fish populations, resulting in significant economic losses in commercial aquaculture and causing threats to valuable stocks of wild aquatic animals (Walker and Winton, 2010). In net cage, fishes are likely to be infected by parasites from wild fishes. Since they are rather concentrated in their net cages, they can become a source of parasite propagation and dispersal (Nowak, 2007). Koesharyani et al., 2001) have compiled the viral, bacterial, parasitic, and non-infectious diseases in grouper. Infestation of parasites on fishes may cause secondary infections of cultured marine fishes and led to economic losses in marine aquaculture (Johnson et al., 2004; Costello, 2009).

One of the most common groups of marine ectoparasites of fish are crustaceans belonging to the order Copepoda. Parasitic copepods are some of the most frequently encountered taxa parasitizing fish and cause significant economic loss in marine culture. Of the 30 families of copepods that parasitize marine fish, members of Caligidae (Copepoda: Siphonostomatoida) are the most common and known as ‘sea lice’ (Boxshall, 2015). Parasitic crustaceans of the family Caligidae, often referred to as sea lice among the aquaculturists, are responsible for many infectious diseases outbreaking in marine aquaculture, especially in intensive aquaculture (Johnson et al., 2004).

The copepod infections on fish have been reported at different prevalence and intensity. The Lepeophtheirus sp was reported as one of the main parasite on hybrid grouper cultured in floating net cages at Kaping Bay, Gerokguk, Buleleng, at the prevalence of 26.67% (Zafran et al., 2019). The Epinephelus coioides in Segara Anakan lagoon, Central Java has been infected by Caligus cf. Epinepheli at prevalence 4.8%, Pennellidae at prevalence at 71.4%. The parasite Pennellidae was found at the intensity of 30.1 indiv/fish (Yuniar et al., 2007). The wahoo (Acanthocybium solandri) collected from Parangipettai, southeast coast of India was infected by Lernaeenicus seeri at mean prevalence of 42.29% with highest prevalence of 62.82 % and with mean and maximum intensity at 3.22 and 33 parasite/fish, respectively (Raja et al., 2014). The L. seeri was a mesoparasite, since the head and neck are inserted and attached to the muscle by making a wound/hole on the body and the rest of the parasite body with the egg sacs hanging outside. Prevalence was recorded (Raja et al., 2014). The infection Pseudocaligus uniartus on Siganids (Siganus sp) degraded fins and skins and caused mortality within 2 days. The histological observation showed the epidermal layer of skin was deeply eroded, the body skin was infiltrated by inflammatory (Anshary et al., 2013) Batam waters, Riau Islands is one of the important mariculture areas in Indonesia. Here we reported the copepodic parasite infection on grouper fishes in Batam waters.

Materials and Methods

The study was conducted in Batam waters, Riau Islands, involving five farms with following Global Position System location, Farm_A N0.96549 ° E 104.04590 °, Farm_B N0.98110 ° E 104.02938 °, Farm_C N0.96128 ° E 104.07394 °, Farm_D N0.95948 ° E 104.07306 ° and Farm_E N0.94685 ° E 104.07771°. From those location the seven populations of cantang hybrid (Epinephelus sp.) population were existed composed by 1, 2, and 3 of small, mid, and big size populations respectively, and two populations of tiger grouper

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(Epinephelus fuscoguttatus) of mid and big size populations.

For parasite observation, at least 36 fishes were sampled randomly from each population to achieve a 95% confidence level. The sampling was conducted at freshwater treatment time in July – August 2019. The fishes were immersed in fresh water and observed in the oral cavity. Fish showing the red nodule in the oral cavity was identified as fish positive (+) infected and collected for intensity parasit observation in the laboratory. The fish was anesthetized using cold water. The nodules were scraped off, put in 0.9% NaCl on a glass slide. The number of parasites was observed under a light microscope (Olympus BX5). The calculation of prevalence and mean intensity of infection refers to Bush et al., 1997. Interpretation of prevalence and intensity results refers to Williams & Bunkley (1996). For histology, the normal and infected tissues were preserved on neutral buffered formalin. Decalcification or tissue softening was done by immersing in decalcifier solution for three days. Fixation was carried out by immersion the tissue in 4% phosphate buffered formalin for 24 hours. The dehydration was carried out using TP 1020 tissue processor (Leica), in gradually increased alcohol (70%, 80%, and 90%, absolute 1, absolute 2, absolute 3) for 60 minutes each. The clearing was carried out after dehydration using xylol 1 and xylol 2. The tissue was then infiltrated with liquid paraffin, followed by blocking. The section was conducted to produce a 5-7 microns section using RV-240 rotary microtome (Yamato). The Hematoxylin Eosin (HE) staining was applied to the tissue sections. The tissue was observed under a microscope.

Result

The observation of the nodules under microscope showed that crustacean parasites were found. The parasite was characterized by paddle like swimming legs, body comprises with a cephalosome, a postcephalic trunk, and the anal somite which represent with telson. The cephalosome consist of 5 cephalic somites and the thoracic somite which bears the maxillipeds (Figure 1). Based on Huys & Boxshall (1991), this parasite belonged to the subclass copepod. This parasite has been found both in cantang hybrid grouper (Epinephelus sp.) and tiger grouper (Epinephelus fuscoguttatus).

Figure 1. The copepodic parasite found in nodule characterized by segmented animal with cephalosome, trunk, and anal somite in the present of claws

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In this study, the cantang grouper were cultured in five farms, whereas the tiger grouper only cultured in one farm. The copepodic parasites that attack cantang grouper fishes on cages in Batam waters vary in intensity and prevalence (Fig. 2). The average prevalence is 62%, the highest prevalence is in Farm_B with a prevalence of 87%, while the lowest is in Farm_E with a prevalence of 25%. The average intensity of seven parasites/fish with the highest intensity was 12 parasites/fish in Farm_D and the lowest in Farm_C with an intensity of three parasites/fish. Those results indicated that the location and culture management seem affected to the prevalence and intensity of parasites.

Several fish sizes were observed in this study. We classified the fish with size 10-100 g into small size, fish with size 100 - 300 g into mid size, and fish more than 300 g into big size. The prevalence of copepodic parasite on cantang grouper in Batam waters increased with increasing fish size, the prevalence on big size was 76%, whereas in small size was only 22%. The intensity also showed the intensity of copepodic parasite was increased with the increasing size of cantang grouper (Figure 3).
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Figure 3. The intensity and prevalence of copepodic parasite on different sizes of cantang hybrid grouper (*Ephinephelus* sp.) cultured in Batam waters

The prevalence of copepodic parasite which infects tiger grouper at mid sizes was 50% with an intensity of three parasites/fish, while at big size it has a prevalence at 30.5% with an intensity of five parasites/fish. In Figure 4 it is seen that the bigger the fish, the more parasites that infect tiger grouper, but the lower the prevalence.

Figure 4. The prevalence and intensity of copepodic parasite on different sizes of tiger grouper (*Ephinephelus fuscoguttatus*) cultured in Batam waters

Histological observation showed the normal fish has healthy primary lamellae with thin secondary lamellae (Fig 5a), parasite-infested gill lamellae showed congestion, mucus proliferation, hiperplasia, secondary lamella erosion, and parasitic pieces (Fig 5b).
Figure 5. a. Normal fish gill (lp: primary lamella, ls: secondary lamella) b. gill lamellae infested with copepodic parasite. (els: secondary lamella erosion, pm: epitelium proliferation, k: congestion, pp: parasitic pieces) (H&E, 400x)

Epithelial cells in healthy lip tissue can be seen in Figure 5a. In infected fish, there is an inflammatory cell infiltration in the dermis (polymorphonuclear cells), congestion, increased number of goblet cells (Fig 5b).

Figure 6. a. Healthy lip tissue with distinct healthy epithelium, b. Lip tissue infested with copepodic parasite (sg: goblet cells; sp: polymorphonuclear cells; k: congestion; v: vacuoles (H&E, 200x)

Discussion

The presence of parasites in cultured grouper in Indonesia have been reported by several authors. The Pseudorhabdosynochus sp., Trichodina sp., dan Haliotrema sp. have been reported infected on groupers from cages in Pesawaran Lampung (Ningsih et al., 2016). Pseudorhabdosynochus sp., Benedenia sp., Neobenedenia sp. (Subekti et al., 2012), Zeylanicobdella sp. and Neobenedenia sp. (Mahasri et al., 2019) have been reported from mouse grouper (Cromileptes altivelis) from Situbondo waters. The serial observation for five months in Buleleng waters showed that marine leech (Zeylanicobdella arugamensis), skin fluke (Benedenia sp.), Trichodina sp., gill fluke, Lepeophtheirus sp., and Cryptocaryon irritans were infected groupers (Zafran et al., 2019). The gill fluke (Pseudorhabdosynochus sp.) and sea leeches (Zeylanicobdella arugamensis) have been judged as cause agent of mortality of cantang and cantik hybrid groupers during 2016 in Buleleng water (Mahardika et al., 2019).

The report on infection of crustacean parasites on grouper in Indonesia is limited such as Lepeophtheirus sp. (Caligidae) in Buleleng waters (Zafran et al., 1997, Zafran et al., 2019). This
Lepeophtheirus sp. was only found once with low prevalence on cantang and cantik hybrid groupers and was noted as non significance parasite (Zafran et al., 2019). In this study we found the copepodic parasite infected mouse grouper (Epinephelus fuscoguttatus) and cantang hybrid grouper (Epinephelus sp.) at Batam waters at high prevalence reach to 87%, and caused significant morbidity as can be seen as the presence of red nodules in the oral cavity. The Lepeophtheirus sp had anterior part/head at a relatively big (Zafran et al., 1997). In this study, the size of anterior part/head of the parasite is not to big, and the claws are present, so that not belong to the Lepeophtheirus. Those results indicated that in this study we found a new copepodic parasite infection on grouper.

The prevalence and intensity of copepodic parasite on cantang hybrid grouper from the five farms were varied in value. The highest prevalence was in Farm_B with a prevalence of 87%, while the lowest was in Farm_E with a prevalence of 25%. Farm_B is located near settlements and restaurants, while Farm_E is located far from residential areas, this is one of the causes of the high prevalence in Farm_B. Tobing (2000) stated that the prevalence in a habitat in waters is influenced by the components of the habitat such as temperature, pressure, oxygen content and others. The level of leeches (ectoparasite) infestation depends on the location and cultivation management (Murwantoko et al., 2018).

In this study, the longer the grouper is cultured in cage, was followed by the higher the prevalence and intensity values of parasite infection. This high prevalence and intensity values might due to copepodic parasite able to adapt to the environment so that they can reproduce or infect other fish. Alifuddin et al., 2003 also showed that the older fish has higher prevalence and parasite intensity, the wider area of the fish body gave change for the parasite to increase colonization. However in tiger grouper, the bigger grouper has higher intensity but lower prevalence. This data may be caused due to the limiting sample as in this study the data only generated form one population of mid and big size of tiger grouper.

Histological observation in this study showed the congestion, hiperplasia/epitelium proliferation onsecondary lamella and erosion in parasite-infested gill lamellae, inflammatory cell infiltration in the dermis (polymorphonuclear cells), congestion, increased number of goblet cells in parasite-infested lip. The proliferation of mucus cells is stimulated by the attachment of parasites to the gills to protect gills that are digested by the parasites by producing a lot of mucus on the surface of the gills (Hossain et al., 2007). Necrosis caused by hypersecretion of mucus causes a perforation in the blood vessels so that oxygen and nutrients cannot reach the location of the hyperplastic epithelial cells. If it continues, it will cause tissue death. The increase in mucus secretion by excessive mucus cells can interfere with the respiratory process in fish because the oxygen osmosis process does not occur completely in the gills which are covered with mucus secretions and also due to the presence of necrotic lamella epithelial cells (Roberts, 2012).

Congestion that occurs in the lip tissue is a passive process caused by decreased venous blood flow. Congestion will show a red discoloration, depending on the degree of oxygenation of the blood. Congestion is also the first pathological symptom of tissue damage and an increase in the amount of blood in the blood vessels so that the blood capillaries appear to be widened and the sinusoids are filled with lots of erythrocytes (Ratnawati et al., 2013).
Polymorphonuclear are white blood cells with multilobed nuclei and cytoplasmic granules. There are three types of polymorphonuclear namely neutrophils, eosinophils, and basophils. The increase in the number of neutrophils is a result of the immune mechanism acting as an immune response. This is related to the main function of neutrophils, namely the destruction of foreign material through the phagocytosis process. The increase in the number of neutrophil cells indicates an increase in the activity of macrophages that destroy foreign particles (Rahma, 2016). The attachment process of copepodic parasite caused inflammation so that polymorphonuclear cells are produced more and collected in the inflamed area so that the eosin Hematoxylin staining will appear blue.

Infection copepodic parasite is local and not systemic, however if not addressed it will cause death in fish. Respiratory failure, secondary infection from bacteria or viruses can occur due to weak resistance of the infected host. The host will have a reduced appetite due to parasitic disorders in the oral cavity. Parasitic infections can reduce or impair the ability of the host to forage (Weinstein and Heck, 1977). So that the method to control this Copepodic parasite infection should be addressed in future.

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