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By Rumondang

DEPIK
Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan
Journal homepage: www.jurnal.unsyiah.ac.id/depik

Identification of grouper parasites (*Epinephelus coioides*) in Talawi District, Coal Regency

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ARTICLE INFO

Keywords:
Endoparasite
Parasites
Grouper

DOI: 10.13170/depik.11.3.27131

ABSTRACT

Batu Bara Regency is a region with abundant natural resources, one of which is the cultivation of grouper fish. Grouper is a member of the Serranidae family of coral reef fish. Many groupers are raised in ponds, which makes them susceptible to disease due to environmental and dietary factors that are not adapted to grouper fish, which is a native species. Parasites are one of the grouper-infecting diseases that, if left untreated, can result in mass mortality and harm farmers. The aim of this study was to identify parasites in ponds in Mesjid Lama Village, Talawi District, so that it would be easier to determine the type of parasite, its level of attack, and subsequent efforts to eradicate it. This research employs a sampling technique, specifically a random sampling of orange-spotted grouper. The results of the performed analysis include analyses of both ectoparasites and endoparasites. Several types of ectoparasites were identified, including *Cryptocaryon irritans*, *Pseudorhabdosynochus sp*, *Benedenia epinepheli*, and *Caligus sp*, while *Prosorynchus sp* was identified as the endoparasite. The most prevalent parasite found in the fish is *Cryptocaryon irritans*.

Introduction

Batu Bara Regency is one of the regions with the potential for natural resources in fish farming, including grouper farming. Grouper is an economically valuable coral reef fish belonging to the *Serranidae* family. In the tropics and subtropics, various species of groupers are dispersed. The orange-spotted grouper (*Epinephelus coioides*) is a species that is frequently encountered in the waters of Batu Bara (Rumondang *et al.*, 2019b). Orange-spotted grouper is a naturally occurring species that can be cultivated in ponds and floating net cages (KJA) (Annur *et al.*, 2021). Environment is the most important factor for grouper cultivation in ponds or KJA (Rumondang *et al.*, 2020); (Annur *et al.*, 2021). The growth of grouper is relatively rapid; consequently, both the local market and the export market can be satisfied (Aslianti and Setyadi, 2015).

Fish disease is a major issue in fish farming because it causes mass mortality and significant financial losses (Lafferty *et al.*, 2015); (Leaño, 2019). (Pasaribu, 2021). A pathogenic infection is the cause of grouper culture failure. Fish-infecting pathogens include parasites, viruses, and bacteria (Harikrishnan *et al.*, 2011); (Pasaribu, 2021). Pathogens that cause disease can be treated with several preventive measures such as vaccinating fish (Gudding and Muiswinkel, 2013); (Haryanto *et al.*, 2019); (Desrina *et*

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p-ISSN 2089-7790; e-ISSN 2502-6194
Received 27 July 2022; Received in revised from 03 September 2022; Accepted 05 September 2022
Available online 20 December 2022

Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan
Volume 11, Number 3, Page 476-482

al., 2011)., ; (Pasaribu, 2021) utilizing an immunomodulator derived from natural ingredients (Galina *et al.*, 2009); (Ridlo and Pramesti, 2009) and administering antibiotic chemotherapy (Defoirdt *et al.*, 2011), (Yulvizar *et al.*, 2014).

Parasites are organisms whose existence is dependent on other organisms (Sauyai *et al.*, 2014). According to Hendra et al. (2013) *in* (Annur *et al.*, 2021), a variety of parasites, including *Benedenia epinephenlis, Caligus sp., Cryptocaryon irritans, Pseudorhabdorhabdosynochus sp., and Trichordina*, can attack grouper. Handayani and Bambang (1999) in (Fidyandini *et al.*, 2012), state that diseases caused by parasites have a higher tolerance level than diseases caused by viruses and bacteria. However, this can not be assumed as parasitic infections can cause primary infections. Primary infection is the initial stage of a disease that can weaken the body's immune system and make it easier for other microorganisms to enter the body, resulting in numerous symptoms and death. Infectious diseases are transmissible from one individual to another (Wirawan *et al.*, 2018). The symptoms of parasitic infection that occur in fish result in a decrease in production value and quality. Parasite attacks also cause deformed fish bodies and fish can endanger the health of humans who consume them. So it is necessary to do research to identify the parasites that exist in mud grouper in Mesjid Lama Village, Talawi District, Batu Bara Regency.

Materials and Methods

Location and time of research

The samples were collected from several ponds in Mesjid Lama Village, Talawi District, Batu Bara Regency. In May of 2022, The identification of parasites was carried out at the Aquaculture Laboratory, Faculty of Agriculture, Asahan University. The research location is illustrated in Figure 1.

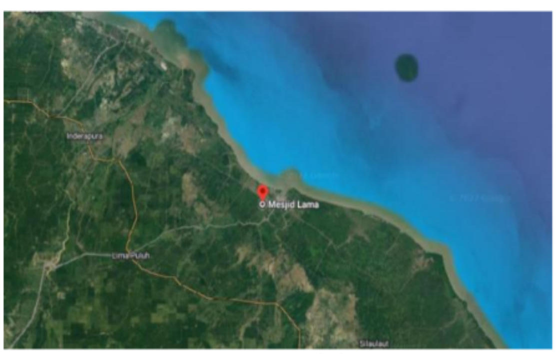

Figure 1. Research location.

Research Implementation

a. Preparation of tools and materials

In this study, the following instruments were utilized: object glass, cover glass, petri dish, microscope, scissors, tweezers, scalpel, ruler, dropper, beaker glass, camera for documentation, stationery, thermometer, pH meter, and DO meter (dissolved oxygen). While the used materials included live orange-spotted grouper samples, alcohol, distilled water, and tissue.

b. Research methods

In this study, orange-spotted grouper was sampled using a random sampling technique. Each member of the population has an equal chance of being selected as a sample. The random sample serves as the foundation for the development of more complex sampling techniques (Arieska and Herdiani, 2018).

c. Sampling

The sample of orange-spotted grouper consisted of 90 fish in four replications. Each sample is collected once per week. Each fish pond had as many as 10 fish sampled, with a total of 4 ponds used and a total of 40 fish sampled at one time. In accordance with clinical symptoms, fish samples were collected and placed in plastic bags containing water and oxygen. The fish were then transported to the Aquaculture Laboratory, Faculty of Agriculture, Asahan University. The Pocket Book of Fish Pest and Disease Control (Directorate of Areas and Fish Health, 2018) and Parasites and Disease of Fish were utilized for identification.

d. Sample inspection

The research was initiated by piercing the nerves of living fish with a needle and a scalpel. Mucus, fins, and gills are first examined on the exterior of the fish. At 4x and 10x magnification, the inside of the fish was examined macroscopically and microscopically with a microscope.

The examination of ectoparasites was conducted in accordance with the specified method (Fernando *et al.*, 1972).

- Fish scales, gills, and fins were examined for the presence of ectoparasites. Previously, the part of the body to be observed was scraped and then placed on a glass object. Observations were conducted with the aid of a microscope.
- Endoparasites are examined in order to determine the type of parasite that attacks the body's internal organs. The examination was carried out on the digestive tract, namely the stomach and intestines of fish by dissecting the

fish's body. All organs were extracted from the body, treated with a 1 ml of 0.9% NaCl solution, and observed under a microscope. Organs suspected of being infected with the parasite exhibit symptoms such as black spots. Separated infected organs were then examined under a microscope with a 100x magnification.

e. Main Parameters

The prevalence of parasites found in orange-spotted grouper was the primary variable studied. Disease prevalence refers to the number of organisms in a population that are infected with a disease under specific conditions and at a particular time (Shofiyah *et al*., 2022). Prevalence calculation used the formula of (Hadiroseyani *et al*., 2006). The research data will be presented in figures and tables.

$$\text{Prevalence} = \frac{\text{Number of samples infected with parasites}}{\text{Number of all samples examined}} \times 100\ \%$$

Prevalence rates refer to William and Bunkley (1996):
- Always: 99%-100%: Extremely severe infection
- Almost Always: 90%-98%: Severe infection
- Usually: 70%-89%: Moderate infection
- Frequently: 50%-69%: Extremely frequent infection
- Commonly: 30%-49%: Common infections
- Often: 10%-29%: Frequent infections
- Occasional: 1%-9%: Sometimes infection
- Rarely: <0.1% - 1%: Rare infection
- Very rarely: <0.01% - 0.1%: Extremely rare infection
- Almost never: <0.01%: Never Infection

The parasite intensity value for each pond is calculated by the following formula:

$$\text{Intensity} = \frac{\text{Number of infected parasite A}}{\text{Number of samples infected with parasite A}}$$

According to (Zelmer *et al*., 1998) the classification of intensity refers to:

Table 1. Parasite intensity criteria.

Infection Category	Intensity (ind/fish)
Extremely low	< 1
low	1 – 5
Moderate	6 – 55
Critical	56 – 100
Awfully	> 100
Virulent infection	> 1000

f. Water quality parameters

Observations of water quality included DO, temperature, salinity, and pH (acidity level) in this study. Every time a sample of orange-spotted grouper was taken, water quality measurements were performed.

Data analysis

A descriptive data analysis was performed on the results of parasite identification in orange-spotted grouper. The research data will be presented in the form of figures and tables, which serve to describe a situation, about what and how, how much, to what extent is being researched, and facts so that it is simple to draw conclusions.

Results

On the orange-spotted grouper (*Epinephelus coioides*), both ectoparasites and endoparasites were analyzed. Parasites that infect orange-spotted grouper (*Epinephelus coioides*) were discovered in this study, as shown in the table below:

Table 2. Types of existing parasites.

Parasite type	Infection host	Number of parasites
Ectoparasites		
Cryptocaryon irritans	Skin	56
Pseudorhabdosynochus sp.	Gill	29
Benedenia epinepheli	Gill	22
Caligus sp.	Skin	23
Endoparacite		
Prosorynchus sp.	Intestines	6

Table 3. Intensity and prevalence of parasites.

Parasite type	Intensity (Ind/Fish)	Prevalence (%)
Ectoparasites		
Cryptocaryon irritans	7	20
Pseudorhabdosynochus sp.	5	15
Benedenia epinepheli	4	15
Caligus sp.	6	10
Endoparasite		
Prosorhynchus sp.	3	5

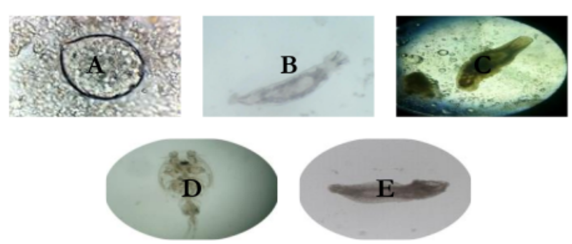

Figure 2. Parasite types: A. *Cryptocaryon irritans*; B. *Pseudorhabdosynochus* sp.; C. *Benedenia epinepheli*; D. *Caligus* sp.; E. *Prosorhynchus* sp.

From the four ponds where sampling was conducted, the following water quality parameters were determined:

Table 4. Water quality parameters.

Variables	Results
Water depth	32
DO	8,00 – 9,00
Brightness	9,5
Salinity %	30 – 32
Temperature °C	30
Current Speed	0,05
pH	7,5 - 8
Nitrate	0,98
Phosphate	0.92

Discussion

Based on the results of calculating the prevalence and intensity of ectoparasites for each species, *Cryptocaryon irritans* was determined to have the highest intensity. This parasite is responsible for fish's white spots. According to the table, the parasites discovered were endoparasites and ectoparasites. Infected endoparasites and ectoparasites are typically capable of causing damage to the fish's body tissues. Fish mortality can be caused by the presence of these parasites, but infectious agents, particularly the stigma of infection caused by endoparasites and ectoparasites, can also be a factor. According to Handajani and Samsundari (2005) *in* (Subekti *et al*., 2013) states that the growth and development of parasitic species outside the host's body, survival as a vector, intermediate horpes are strongly influenced by climate and weather (environment).

Characteristics of Existing Parasite Types

Cryptocaryon irritans

According to the study's findings, the parasite *Cryptocaryon irritant* (Figure 2) is frequently found in skin organs. The prevalence of *Cryptocaryon irritants* in Mesjid Lama Village, Talawi District, Batubara Regency, is 20 percent and 7 ind/fish. This parasite is highly contagious and can quickly cause mass death. *Cryptocaryon iritans* is a member of the Oligohymenophora class, the Ichthyophthiridae family, and the *Cryptocaryon* genus.

Cryptocaryon irritans is a pathogenic protozoan parasite belonging to the Ciliata genus that can cause white spot disease in infected fish (Haryanto *et al*., 2018). In captivity, *Cryptocaryon irritans* can cause mass mortality of groupers. In the host, parasitic cells are spherical with vibrating hairs and range in diameter from 0.3 to 0.5 mm.

This condition is commonly referred to as *Cryptocaryoniosis*. The characteristics of, among others, resemble those of a pear equipped with cilia on its body that actively move beneath the skin and gill epithelium. The adult *Cryptocaryon irritans* parasite will leave the host and swim freely for several hours before transforming into a cyst that remains at the bottom of the pond to grow and develop further, while 35 µm adult cells swim freely and seek new hosts. The immature phase is known as a pear-shaped trophon, while the adult is round and forms a cyst known as a toman, which after 6-8 days will develop into a young parasite capable of living without a host in less than 24 hours (Haryanto *et al*., 2018).

Clinical symptoms include a decrease in appetite (Haryanto *et al*., 2018) and lethargy in fish, as well as dark eyes and loose scales, white spots on the gills and body surface, and an increase in mucus. This disease is easily transmissible and causes mass mortality in a relatively short period of time. Heavy infected fish exhibit rapid respiration, excessive mucus, and rubbing against objects. Epithelial cells of the mucosa and skin become hyperplastic and further harmed (Anshary, 2008). The damaged skin will be more prone to secondary infection. This parasitic infection is typically influenced by poor water quality and conditions of water exchange, weakened host conditions, and high stocking densities.

This parasite can be treated during the Tomont and Theront phases if control is less effective during the cyst phase (Haryanto *et al*., 2018). Fish that have been treated are relocated because the parasitic cyst may still be present in their previous habitat. Five to seven days of treatment are administered until the fish are free of parasites (Slamet *et al*., 2008). Alternately, this parasite can be eliminated by soaking infected fish in fresh water for about 5 minutes This immersion lasted for three consecutive days. If there are wounds on the fish's body, 5 to 10 mg/L of acriflavin solution is added to the immersion.

Pseudorhabdosynochus sp.

According to the findings of the study, the parasite *Pseudorhadosynochus* sp (Figure 2) was frequently found in the gill organs. The prevalence of *Pseudorhadosynochus* sp in Mesjid Lama Village, Talawi District, Batubara Regency, was 15 percent and 5 ind/fish. This parasite can wreak havoc on a fish's respiratory system.

In warm marine environments, *Pseudorabdosynochus* sp. have been reported as parasitic on grouper (Serranidae; Epinephelinae). *Pseudorabdosynochus* sp belongs to the Platyhelminthes phylum, the

Monogenea class, and the *Pseudorabdosynochus* genus. This parasite can infect a fish's gills, making it difficult to observe and determine whether the fish is infected or not. The clinical symtomps of this condition are decreased appetite, abnormal swimming, a pale body color, and thinning and death of the fish. This parasite has a direct life cycle, which means it can attach to the gills and develop into adults without an intermediate host (Sumino *et al.*, 2017).

Pseudorabdosynochus sp., also known as gill parasites, are typically transmitted through direct contact. This parasite's life cycle does not require an intermediate host to reproduce. Adult parasites have two sexes (hermaphrodites). Its life cycle can directly lead to a population explosion in aquaculture that causes clinical disease (Reed et al., 2012) in (Putri *et al.*, 2016). The oval-shaped eggs of gill parasites are attached to the gill lamella with a filament. A pair of eye spots in the egg is formed during embryogenesis. The hamulus (skin-shaped protrusion) of young parasites is useful for attaching to the host. The parasite will mature into an adult parasite and grow larger. According to (Mahardika *et al.*, 2018), a single adult parasite can lay between 10 and 22 eggs per day. At the end of the egg's 0.021-0.120 mm-diameter oval shape is a spiral filament. At a temperature of 30°C and a salinity of 30 ppt, eggs can hatch into free-swimming larvae in two to six days. This parasite's life cycle lasts between 13 and 20 days.

Benedenia epinepheli

According to the study's findings, the parasite *Benedenia epinepheli* (Figure 2) is frequently found in the gills. The prevalence of *Benedenia epinepheli* in Mesjid Lama Village, Talawi District, Batubara Regency, was 15 percent and 4 ind/fish. This parasite can stunt the development of fish. *Benedenia epinepheli* belongs to the Platyhelminthes phylum, the Trematoda Monogenea class, and the *Benedenia* genus.

Benedenia spp., also known as skin worms, are commonly found attached to the skin of fish, where they cause irritation and secondary infections. It has an oval, flattened shape with a pair of antennae at the front and a haptor at the back. This parasite is easily observable when fish are immersed in fresh water. This parasite will separate and transform into a white color (Ode, 2013).

Benedenia sp. causes decreased appetite and decreased swimming activity in fish. At a severe level of infection, it causes sores on the fish's skin and allows bacteria to enter. If this parasite attaches to a fish's eye, it will result in blindness (Novriadi, 2014).

***Caligus* sp.**

The results of the study revealed that the parasite *Caligus* sp. (Figure 2) is frequently found on the fins' skin. In the village of Mesjid Lama, Talawi District, Batubara Regency, *Caligus* sp. is prevalent at a rate of 7.5% and 6 ind/fish. This parasite can cause slowed growth and deteriorating health in fish, leading to death. *Caligus* sp. is a member of the class Copepoda, the family Caligidae, and the genus *Caligus*.

Caligus sp. is a parasite with a sucking device known as a lanule that is located on the front plate and has four pairs of swimming legs. The swimming legs are used to get food. Food *Caligus* sp. refers to the fluid found in fish tissue after the mandible has reached the fish's skin. The mandible is extracted from the sucked organ. The mandible is awl- or lancer-shaped and is equipped with sharp, saw-like teeth (Musyaffak *et al.*, 2010). This parasite's clinical symptomps include lesions and bleeding on the skin of infected fish (Woo *et al.*, 2002) in (Fidyandini *et al.*, 2012).

Caligus sp. frequently attack on the skin and gills of marine fish. The female *Caligus* sp. has a long, unified head and thorax (cephalothorax), a soft, membrane-lined back, and a dorsal shell (Hardi, 2016). This parasite's life cycle begins with an egg, which hatches into a free-swimming larva, which then molts into an infective second larva. Infected fish manifest clinical symptoms and an organ volume increase. Infected fish will also swim erratically, and their skin will have wounds that cause bleeding and lesions (Woo *et al.*, 2002) in (Fidyandini *et al.*, 2012). Low water temperature is primarily responsible for this parasite's attacks (Hardi, 2016).

***Prosorhynchus* sp.**

The study revealed that the parasite *Prosorhynchus* sp. (Figure 2) is frequently found in the intestinal organs. The prevalence of *Prosorrynchus* spp. in the region of Mesjid Lama Village, Talawi District, Batubara Regency is 5% and 3 ind/fish. This parasite has a low rate of infection in orange-spotted grouper. *Prosorhynchus* sp. is a member of the class Trematoda, the family Prosorhynchus, and the genus Prosorhynchus.

According to Kabata (1985), the *Prosorhynchus* sp. has an elongated body with blunt ends on both sides. On the ovary, the cuticle is covered with spines, and its body is broad. This parasite has a testicle on its right side and a genital pore on its back (posterior). This parasite inhabits the intestines, abdominal cavity, and muscles of the host.

This parasite's life cycle begins with eggs that freely inhabit water, which then hatch and transform into *miracidium*. *Miracidium* can penetrate the skin of intermediate hosts such as snails. The parasites' bodies will transform into *cercaria* and they will be released into the water in search of a second intermediate host, such as fish and crustaceans. In the bodies of fish and crustaceans, parasites manifest as metacercaria. If fish or crustaceans are consumed raw or undercooked by other organisms or humans, it will cause intestinal worms, where metacercaria will mature into adults within the body of the definitive host (Cuomo et al., 2014). The clinical symptoms are not particularly damaging to the intestines, but a high parasite count will result in infection.

Water Quality

The relationship between growth and environmental conditions varies in grouper cultivation in ponds (Langkosono, 2005). Grouper requires a temperature range of 25 to 32 degrees Celsius, a salinity range of 20 to 32 ppt, a dissolved oxygen concentration of 4 to 8 ppm, and a pH range of 7.5 to 8.25, according to research conducted by an anonymous author in 2001. In addition, Akbar and Sudaryanto (2001) in (Langkosono, 2005) state that the optimal water quality for grouper growth is 27-29°C temperature, 30-33ppt salinity, DO > 5ppm, and pH between 8.0 and 8.2. Several ponds' water quality has been measured, and the resulting temperature is between 30 and 31 degrees Celsius. This is consistent with the claim (Rumondang *et al.*, 2019b) that the water temperature tolerated by orange-spotted grouper is between 25 and 32 degrees Celsius. Salinity ranges between 30 and 32 ppt; if fish's oxygen consumption rate is affected by salinity, weight gain and length are affected as well (Khalil *et al.*, 2015). Salinity is a factor that has direct effects on the metabolism of fish, including the osmoregulation procedure. Osmotic pressure and oxygen demand are two aspects of fish physiology that are affected by salinity. Variations in salinity of the water can affect the oxygen consumption rate of fish (Khalil *et al.*, 2015).

The pH measurement results for each pond indicated a pH range of 7.5-8. This is consistent with the assertion (Rumondang *et al.*, 2019a) that orange-spotted grouper requires relatively alkaline water pH (pH>7) in order to properly grow and reproduce. If there is a change in the pH value (acid or alkaline) in ponds or waters, the ecological balance will be disturbed, and the pH affects the fertility of waters where microorganisms from the photosynthesis process use CO_2 during the process (Valentino *et al.*, 2018).

The results of temperature measurements in each pond indicated a temperature range between 30 and 31 degrees. These findings provide support for grouper cultivation. According to Effendi (2003) *in* (Valentino *et al.*, 2018), the optimal temperature for tiger grouper cultivation is 30°C. According to, the optimal temperature for grouper culture development is between 27 and 30,9 degrees Celsius. The presence of solar radiation, air, weather, and location all influence the temperature of the water.

Conclusion

Based on the findings of the study, it can be concluded that *Cryptocaryon irritans, Pseudorhabdosynochus sp, Benedenia epinepheli, Caligus sp, and Prosorynchus sp* are the parasites that attack grouper fish in ponds in the Mesjid Lama Village. The *Cryptocaryon irritans* is the most frequent parasite found in fish.

Recommendation

Based on the research that has been performed, it is necessary to conduct additional research and a more in-depth study of parasites in orange-spotted grouper in the village of Mesjid Lama, Batubara Regency, to discover additional parasites that were not observed in this study. To learn how to treat and prevent grouper parasites, further research is required.

Acknowledgments

The authors would like to thank the implementers of the Scientific Research Program, the Directorate General of Higher Education, Research, and Technology with Universities that are recipients of the 2021 Scientific Research Program grants, with the designation number for the Village Research Fund recipients being 4025/E4/AK.04/2021 and the Education Fund Management Institute (LPDP).

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How to cite this paper:
Rumondang, R., Harmayani, H., Manurung, H. P., Putri, A. Sari, I. 2022. Identification of grouper parasites (*Epinephelus coioides*) in Talawi District, Coal Regency. Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan, 11(3): 476-482.

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