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Stress Response and Pathology of Hybrid Groupers (*Epinephelus lanceolatus* × *Epinephelus fuscoguttatus*) Infested *Trichodina* sp. Maintained in Hatchery from Bali, Indonesia

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ABSTRACT

The demand for grouper fish continues to increase, with the Ministry of Fisheries' annual production target of 9,000 tons. However, intensive cultivation systems pose risks due to poor water quality, making grouper fish susceptible to disease and health problems. This study investigated the impact of *Trichodina* sp. infection on the blood glucose levels of hybrid grouper fish. The study was conducted in May 2024 by observing 30 fish and measuring blood glucose. The results showed that 20 fish showed glucose levels above the normal range (> 57.6 mg/dL), ranging from 61 to 114 mg/dL. In contrast, nine fish had glucose levels in the normal range (28.8 - 57.5 mg/dL), and one fish had low glucose levels of 26.7 mg/dL. High glucose levels in most fish indicate stress or metabolic disorders, possibly related to *Trichodina* sp. infestation. This parasitic infection can compromise the fish's immune system, making the fish more susceptible to disease. The results emphasize the need for effective parasite management and optimal water quality in intensive culture systems. To maintain grouper health and productivity, it is important to monitor water quality, control ectoparasites, and conduct further research on the long-term effects of these infections on blood glucose and overall health.

Keyword: anatomical pathology, blood glucose levels, *Epinephelus* sp., *Trichodina*

INTRODUCTION

Grouper fish is Indonesia's leading non-oil and gas export commodity, alongside seaweed, shrimp, and tuna fish. Indonesia is the world's largest exporter of grouper, especially live grouper (Sirait *et al*., 2022). Grouper (*Epinephelus* sp.) is a fishery commodity with high economic value in the domestic and export markets (Suhesti, 2021; Rumondang *et al*., 2023). The Ministry of Maritime Affairs and Fisheries targets grouper production of 9,000 tons annually. Grouper production in 2022 – 2024 continues to increase by 3,954 tons (KKP, 2024). The problem with intensive grouper cultivation is the decline in water quality, which causes attacks of various diseases and death (Li *et al*., 2025). These diseases can come from viruses, bacteria, parasites, or non-infectious diseases such as malnutrition and deformity (Roza, 2017). Okon *et al*. (2023) emphasized that poor water quality in cultivation can cause stress in hybrid grouper fish, leading to a weakened immune system and making fish more susceptible to

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pathogenic infections, including bacterial and parasitic diseases. Parasitic diseases can be dangerous because they trigger the emergence of other pathogenic organisms and cause infections (Avia & Ulkhaq, 2019). Decreased water quality causes ectoparasite attacks, one of which is *Trichodina* sp. (Pramanik, 2022). According to Mahasri *et al*. (2020), *Trichodina* sp. attacks the body's surface, such as skin, gills, and fins (Mahasri *et al*., 2020). *Trichodina* sp. infestation causes a disease known as trichodiniasis, which causes skin inflammation, irritation, and physiological stress in fish, ultimately affecting growth and overall health. Jamaris *et al*. (2019) reported that the parasite *Trichodina* sp. attacks 20-40% of hybrid grouper fish in floating net cages in Gondol, Bali. In another case, Wiradana *et al*. (2021) stated that there were ectoparasites of *Trichodina* sp. species with a prevalence of 10%.

Diseases caused by *Trichodina* sp. infestation can weaken the immune system and induce stress in fish. Stressed fish require additional energy to maintain vital functions, leading to physiological strain (Overstreet, 2021). One indicator of stress in fish is glucose levels (Jiang *et al*., 2017). Suboptimal glucose levels indicate that fish are stressed during cultivation and transportation. Stress can cause immunosuppression in fish, making them more susceptible to disease (Duan *et al*., 2025). Therefore, this study aims to examine blood glucose levels and determine *Trichodina* sp. infections in hybrid grouper fish.

MATERIALS AND METHODS

The study was conducted in May 2024 using a survey method. Anatomical pathology examination and blood glucose levels were conducted at the Anatomy and Cultivation Laboratory, Faculty of Fisheries and Marine Sciences, Airlangga University.

Sampling Method

The research procedure begins with a location survey. The continued Sampling of canting grouper fish seeds was carried out by purposive Sampling. Cameron's (2002), Sampling of 30 hybrid grouper fish infested by *Trichodina* sp. has characteristics of slow swimming, pale body, and moving to the pool with a size of 9-10 cm.

Ectoparasite Examination

The ectoparasite examination was carried out in vitro (direct observation) using a microscope at 40×, 100×, and 1000× magnification. Previously, the test fish were prepared and then scraped on the surface of the body, gills, and fins of the fish. The scraping results were rinsed with distilled water and then ready to be observed under a microscope (Ridhwan *et al*., 2018). Furthermore, the determination of the infestation degree category was based on Mahasri (2007).

0 – 4: normal category
5 – 25: light category
26 – 50: Medium category
51 – 80: heavy category
> 80: Very heavy category

Blood Glucose Measurement

Glucose measurement is done by taking 0.1 ml of fish blood using a syringe and adding 10% EDTA. EDTA does not change the shape of blood cells, inhibits platelet aggregation, and maintains blood cell morphology. The blood taken is dripped onto the blood glucose test strip attached to the blood glucose meter, which uses the Easy Touch brand. Then, wait until the blood glucose meter displays the blood glucose value (Eames *et al*., 2010).

Pathological and anatomical examination

The method of anatomical, pathological examination is by macroscopic examination;

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the fish's overall body condition is observed, including skin, fins, eyes, gills, colour, lesions, swelling, or deformation. The scoring method refers to (Roberts, 2012).
Score 0: No inflammation
Score 1: Mild inflammation
Score 2: Moderate inflammation and swelling
Score 3: Severe inflammation, damage body
Score 4: Severe inflammation with necrosis

Water Quality Measurement

All water quality parameters in this research were measurement using conventional tools, including a thermometer, DO meter, refractometer, and pH using PHT-026 6 in 1 and Ginkioi AP test kit for (ammonia, nitrate, nitrite).

Data Analysis

The data obtained and collected was analyzed descriptively with a case study. The data is presented in images and tables that show *Trichodina* sp infestation, anatomical pathology, and blood glucose levels in grouper fish.

RESULTS AND DISCUSSIONS

Result

The examination of *Trichodina* sp. ectoparasites in grouper revealed that all 30 fish sampled were infested with *Trichodina sp*. This parasite commonly infests external body surfaces, including the skin, fins, and gills, and is frequently found in juvenile fish. Infested fish typically exhibit symptoms such as pale body coloration, reduced appetite, restlessness, lethargy, and body-rubbing behavior. The results of the *Trichodina* sp. ectoparasite examination are presented in Figure 1.

Table 1. Ectoparasite Examination of Hybrid Grouper

Number of fish examined	Infestation degree category (Mahasri, 2007)	Results of observations of the anatomical pathology of the hybrid grouper	Scoring of results of observation of anatomical pathology (Roberts, 2012)	Observation statement
30	Normal (0 – 4)	3	Score 0 : No inflammation	There are no signs of injury on the fish's body surface, and its gills are normal and bright.
	Light (5 – 25)	5	Score 1 : Mild inflammation	Pale body surface, the fish rubs its body on the side of the aquarium; the fish is quite weak.
		9	Score 2: Moderate inflammation and swelling	Pale body surface, the fish rubs its body on the side of the aquarium. It is weak, its gills and eyes protrude, and the gills are pale.
	Medium (26 – 50)	13	Score 3 : Severe inflammation, damaged body	Pale body surface, weak, protruding eyes, pale gills, wounds on the gill side, and body rejuvenation (dorsal fin, pectoral fin, and caudal fin).

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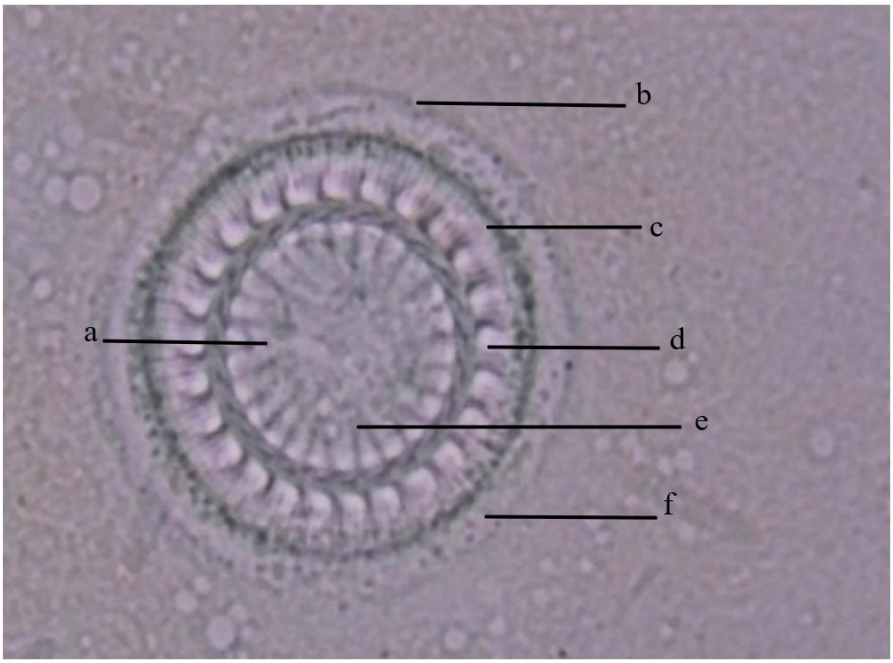

Figure 1. *Trichodina* sp. 1000×. (a: Thorn. b: Cilia. c: Border Membranae. D: Blade. E: Adhesive disk. f: Radial pin)

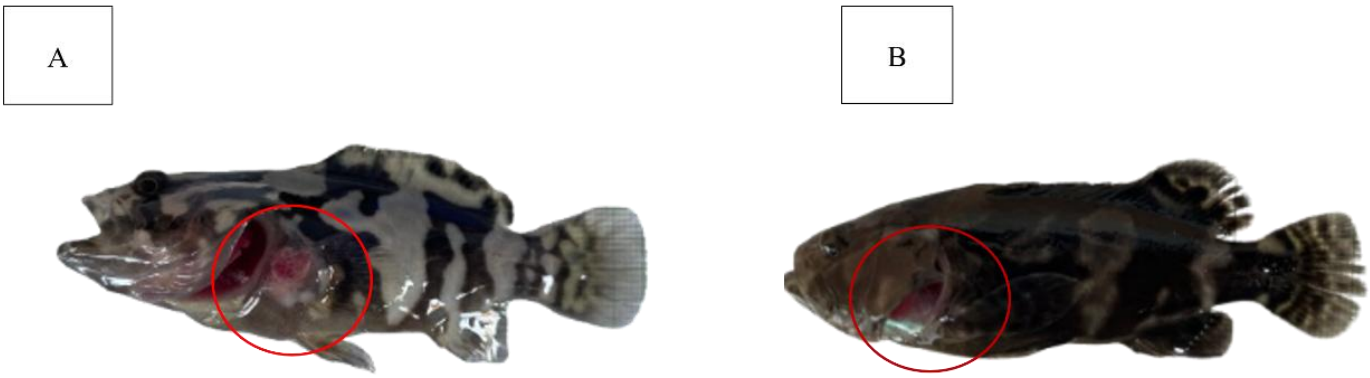

Figure 2. (A) The anatomical pathology of the hybrid grouper fish has ulcers on the pectoral fins. (B) The gills of the grouper fish are pale and slightly injured.

The level of *Trichodina* sp. infestation in grouper has been determined and presented in (Table 1). The level of infestation is categorized: normal, mild, and moderate, (William & William, 1996). The results showed that three fish were in normal condition, 14 had mild infestation, and 13 had moderate infestation. Meanwhile, the results of anatomical pathology observations showed that three fish had no inflammation, five had mild inflammation, nine had moderate inflammation, and 13 had severe inflammation. Each infestation condition has a different and critical impact on health conditions. Fish show subtle symptoms in mild infestation, such as mild irritation, increased mucus production, and mild gill inflammation. Under moderate conditions, fish are more susceptible to other pathogens, which can result in suboptimal health and performance. Severe parasite infestations can cause major damage to gill tissue, interfere with respiration, and cause hypoxia. Number of Fish Examined Based on Infestation Level Category (Mahasri, 2007).

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Table 2. Blood glucose levels of hybrid grouper

Number of fish examined (tails)	Results of measuring blood glucose levels		Category of blood glucose levels of hybrid grouper (Mahasri *et al.*, 2020)	Category
	Number of fish in one category (tail)	Blood glucose level values		
30	1	26,7 mg/dL	0 – 28,7 mg/dL	Below normal
	9	27 – 54 mg/dL	28,8 – 57,5 mg/dL	Normal
	20	61 – 114 mg/dL	> 57,6 mg/dL	Above normal

The blood glucose levels of *Trichodina* sp. infestation in grouper have been determined and are presented in Table 2. Based on the results of the study, there was one fish with glucose levels below normal, namely <28.7 mg/dl, nine fish with normal glucose levels, namely between 27 - 54 mg/dl, and 20 fish with glucose values above normal, with a range between 61 -114 mg/dl.

Discussion

The pathological and anatomical observations on hybrid grouper fish showed that three fish were in normal condition and had no inflammation, five had mild inflammation, nine had moderate inflammation, and 13 had severe inflammation. *Trichodina* sp. infestation in mild to severe conditions causes wounds on the body and gills of the fish. This statement is supported by Yunus & Wijaya (2023), who stated that *Trichodina* sp. attacks can cause wounds on the body and damage to the gills, namely necrosis and hypertrophy, hyperplasia, secondary lamella fusion, and blood congestion at the base of the primary lamella. In addition, *Trichodina* sp. infestation can cause secondary infections caused by bacteria with clinical symptoms, namely wounds on the surface of the body and gills (Anshary, 2021).

The wound healing process in fish occurs in three phases: inflammation, proliferation, and maturation (Hanchapola *et al.*, 2024). The inflammatory response occurs when the body's immune system fights infection and prevents further damage (Mokhtar *et al.*, 2023). White blood cells are responsible for attacking and cleaning dead tissue and starting the healing process. After the inflammation phase, the fish body begins cell proliferation to facilitate wound healing. Fibroblasts and collagen are important in forming new tissue to cover wounds. During maturation, the newly formed tissue strengthens and develops into stronger connective tissue (Yanuhar *et al.*, 2022). Several factors that influence the wound healing process in grouper include optimal environmental conditions, a strong immune system that accelerates recovery, and sufficient nutritional intake to support tissue regeneration (Gunawan & Suprayitno, 2021).

Glucose levels are one of the indicators of stress in fish (Jiang *et al.*, 2017). Based on the study's results, 20 out of 30 fish were in a state of glucose levels above normal, and one was below normal. Grouper fish with glucose levels above normal indicate that they are by the stress response. High blood glucose levels are suspected of fish experiencing stress and requiring more energy to survive, which causes several metabolic dysfunctions, especially in several organs such as the liver, bones, and overall energy supply through the body (Li *et al.*, 2022). *Trichodina* sp. infestation in fish causes stress, thereby increasing glucocorticoid levels, which causes an increase in glucose levels, which function as an energy source while fish are under stress (Rachmawati *et al.*, 2010). The body's ability to utilize glucose for stress responses depends on efficient glucose uptake into cells. Once in the

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cells, glucose is rapidly metabolized to meet physiological and energy needs. When the glucose supply is sufficient, it stimulates glycogenesis and lipogenesis, facilitating energy storage (Mahasri *et al*., 2022). In addition, based on the study results, nine fish infested with *Trichodina* sp. had glucose levels below normal, indicating that the fish were experiencing energy deficiency, which caused insufficient energy supply to the tissues. Low blood glucose levels cause impaired movement, lack of coordination, and reduced ability to respond to environmental stimuli, all because glucose is an important key factor for many primary energy sources of tissues (Liu *et al*., 2023). Blood glucose levels are also influenced by several factors, including feed, liver glycogen storage status, and developmental stage (Saputra *et al*., 2023).

CONCLUSION

From observing 30 hybrid grouper fish kept in a hatchery in Bali, Indonesia, all were infected by *Trichodina* sp. with various infestation levels. As many as 13 of them were moderately infected. This causes an increase in stress levels which are then proven by glucose level tests, there are 20 out of 30 fish whose glucose levels are above normal.

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AUTHORS' CONTRIBUTIONS

The contribution of each author is as follows, PGEM; collected the data, drafted the manuscript, and designed the table as well as the graph. GM and LS; devised the main conceptual ideas and conducted a critical revision of the article. All authors discussed the results and contributed to the final manuscript

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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