Marine Leech *Zeylanicobdella arugamensis* Infestation As A Predisposing Factor For *Vibrio alginolyticus* Infection On The Hybrid Grouper “Cantang” (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*) From Traditional Ponds In The Kampung Kerapu Lamongan East Java Indonesia

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Abstract. Marine leech *Zeylanicobdella arugamensis* is one of the ectoparasites that can infest grouper fish, causing wounds on the place where it is attached, so that it can act as a predisposing factor for secondary infection by pathogenic bacteria such as *Vibrio alginolyticus*. The purpose of this study was to determine *Z.arugamensis* marine leech infestation as a predisposing factor for *V.alginolyticus* infection on the Cantang grouper from traditional ponds in the kampung kerapu lamongan. This research is a transversal survey and data analysis using Pearson correlation. Observations were made in the Laboratory of Anatomy and Fish Culture, and the examination of bacteria was made by conventional methods at BKIPM Juanda Surabaya. Based on the results obtained, 17 of 30 Groupers were known to be infested marine leech. Meanwhile, 5 infested fish were positive for *V.alginolyticus* with a TPC calculation of 0.12-2.13 × 10⁴ CFU/ml. This moment indicates that Infestation of the parasite *Z.arugamensis* can act as a predisposing factor for the occurrence of *V.alginolyticus* bacterial infection because there is a positive correlation, that is, the higher the amount of *Z.arugamensis* infestation followed by the TPC *Vibrio* sp.

1. Introduction

Grouper is a fisheries commodity that is widely distributed in tropical and subtropical marine waters which is now starting to develop and is cultivated in several countries in Southeast Asia because of its high economic value [1]. One of these countries is Indonesia, which is the third-largest grouper producer in the world after China and Taiwan [2]. The total production of grouper that has been successfully exported in 2018 reached 4.41 million tons and 3.06 in 2019 [3]. Several species of grouper have been successfully cultivated in Indonesia, one of which is the Hybrid Grouper “Cantang”. Hybrid Grouper was first produced by the Borneo Marine Research Institute in Sabah (Malaysia) from the cross-breeding (hybridization) of female tiger grouper (*Epinephelus fuscoguttatus*) and male grouper (*Epinephelus lanceolatus*) [4]. Hybrid Grouper has several advantages, namely growth rates, high survival rates and relatively easy hatchery processes, resistance to disease, and tolerance to environmental changes [5,6].
One of the areas in Indonesia that is a centre for grouper cultivation is Lamongan, which is located in the Kerapu Village, Kentong, Labuhan, Brondong with a cultivation system that uses a traditional pond. Grouper production in Labuhan village is marketed both to meet domestic consumption (local) and some that meet quality standards are sent abroad such as Singapore, Malaysia, Taiwan, Laos, China, and Japan [7]. However, in the process of grouper cultivation, the problem that is often faced is the presence of parasitic diseases due to infestation of Piscicolidae such as marine leech Z.serugamensis which is a parasitic class in marine fish [8]. Ectoparasite infestation is the number of parasites that infect fish which can inhibit growth and become an entry point for other diseases. Z. arugamensis can live by sucking blood. The pathogenicity of this parasite is the low but severe infection can cause skin lesions so that it can act as a predisposing factor for secondary infection by pathogenic bacteria such as V.alginolyticus [9,10]. V. alginolyticus is a species of bacteria that causes vibriosis in groupers [11]. Basically, Vibrio can act as a predisposing factor because these bacteria are included in opportunistic bodies so that the Vibriosis outbreak can occur due to environmental stress which makes the fish weak or is induced by a primary infection caused by parasites, causing tissue damage in fish and becoming a profitable site for the fish Vibrio spp to initiate infection [12]. The existence of secondary infection can worsen the condition of the fish to cause death.

The purpose of this study was to determine the infestation of marine leeches Zeylanicobdella rugamensis as a predisposing factor for Vibrio alginolyticus infection in Hybrid Grouper “Cantang” (Epinephelus fuscoguttatus x Epinephelus lanceolatus) from traditional ponds in the Lamongan grouper village.

2. Materials and methods
The study was conducted in July - August 2020. This study was a transverse survey with a cross-sectional study design [13]. Sampling in this study using a purposive sampling method, where samples are taken according to the requirements required in the study [14]. The number of samples taken was 30 Hybrid Grouper "Cantang" (17-21 cm) obtained from traditional ponds in Kampung Kerapu, Kentong, Labuhan, Brondong, Lamongan, East Java. According to [15], the number of samples that can be taken in survey research is 27-30 individuals at one location. The samples were then observed at the Anatomy and Fish Culture Laboratory, Faculty of Fisheries and Marine Airlangga University, Surabaya.

The method of examining marine leeches on fish samples was carried out using the native method according to [16]. Examination of marine leeches covers the surface of the body and fins by scraping slowly and taking it directly by pulling it out using tweezers then taking the gills directly using tweezers on the part that is manifested by marine leeches. Marine leeches that have been found will be collected and counted to determine the infestation, then preserved with 5% glycerin alcohol [17]. The number of marine leeches in positive infested samples was counted per head, then grouped according to the degree of infestation according to the [18] category. The infestation rate was defined as the average number of leeches found in each fish sample.

The examination is then carried out the isolation of bacteria contained in the scar due to the presence of marine leeches in fish samples to determine the presence of bacterial infection. The bacterial examination was carried out at BKIPM Juanda Surabaya using conventional methods. Conventional detection of pathogenic bacteria uses an enrichment broth followed by colony isolation on selective media, biochemical identification, and TPC calculation [19,20].

Data that has been obtained between the amount of marine leech infestation and TPC of Vibrio sp. infection in Hybrid Grouper were analyzed by using Pearson correlation test.

3. Results and discussion
3.1 Results
The results of the ectoparasite examination on Hybrid Grouper were 17 fish infested with Z.arugamensis from the total of 30 fish treated. From the fish infested with marine leeches, 5 fish samples were isolated from the bacteria and TPC was calculated. The examination data are shown in table 1.
Table 1. The results of the amount of marine leechs infestation and the TPC calculation of *Vibrio* sp.

| Sample | Σ Infested Marine Leechs | Category of Level Infestation | TPC of *Vibrio* sp. (×10⁴ CFU/ml) |
|--------|--------------------------|-------------------------------|----------------------------------|
| 1.     | 11                       | Medium                        | 1.92                             |
| 2.     | 4                        | Light                         | 0.12                             |
| 3.     | 9                        | Moderate                       | 0.87                             |
| 4.     | 11                       | Medium                        | 2.13                             |
| 5.     | 3                        | Light                         | 0.32                             |

Based on the results of the research in table 1. The data was processed using the Pearson correlation test which showed that there was a positive correlation with a correlation coefficient of \( r=0.929 \) (\( p<0.05 \)) namely there was a very close correlation between the amount of marine leeches infestation and the TPC calculation of the *Vibrio* sp. bacteria on the Hybrid grouper. The graph of the correlation between the amount of marine leech infestation with the TPC of *Vibrio* sp. the Hybrid Grouper can be seen in Figure 1.

**Figure 1.** Correlation graph between the amount of marine leechs infestation and the TPC of *Vibrio* sp. on Hybrid Grouper.

Based on the observations that have been carried out, many marine leeches were found attached to the surface of the body and fins with the condition of the body surface causing bleeding and redness on the skin which triggered secondary infections so that when the vibrio
bacteria were isolated, the *V. alginolyticus* bacteria were obtained positive results. The figure of fish samples infested with *Z. arugamensis* and positive *V. alginolyticus* are shown in Figure 2.

![Figure 2](image_url)

**Figure 2.** Infestation, haemorrhages and swollen areas of fish skin infected by *Z. arugamensis*, A: *Z. arugamensis* infestation on fins and body surface and B: Haemorrhages and swelling on the body surface.

### 3.2 Discussion

In this study, reports the relationship between *Z. arugamensis* infestation which can act as a predisposing factor for *V. alginolyticus* bacterial infection. Based on the observations, it was shown that the ectoparasite infested the Hybrid grouper was *Z. arugamensis*. *Z. arugamensis* is a marine leech which belongs to the phylum Annelida and Family of Piscicolidae [21]. Most of the marine leeches are found attached to the surface of the body, eyes, mouth, and fins. This is also consistent with previous research on the same fish species [8,22,23,24,25].

Marine leeches are very firmly attached to their host's body. Adult marine leeches will leave their host and then lay their eggs attached to the tub or maintenance equipment with a life cycle from laying eggs to hatching takes time. The egg life cycle to hatching and becoming an adult leech takes 17-22 days [26,24,27]. Marine leeches are not deadly directly but can inhibit the growth of infected fish. This is related to the clinical symptoms of ectoparasite infested fish, namely increased stress levels, loss of appetite, showing slow movement and swimming on the water surface[11,28,29]. In addition, these marine leeches can cause swelling and haemorrhage of the skin until the fins become thinner [30]. The pathogenicity of this parasite is low, but the heavily infected host animal will suffer from chronic anemia, the fish becomes weak and allows secondary infection by fungi or bacteria, especially in the wound area where the parasite is attached [31,32].

Parasitic infections cause tissue damage and increase the fish's susceptibility to secondary fungal or bacterial infections, and erosion at the site of attachment can create a possible pathway for secondary pathogens to enter the body [33]. This statement is in line with the research results that have been obtained, it shows that marine leechs infestation in Hybrid Grouper can be a predisposing factor for secondary infection by bacteria such as *V. alginolyticus*. This is because *Z. arugamensis* attaches to host with a sucker where the sucker sticks right into the blood vessel and the marks of attachment will then cause wounds and haemorrhages [25]. This was in agreement with [33] who stated that parasites often induce tissue or organ damage in fish due to their presence, attachment and feeding. Similar results have also been reported by [31,10] that *Z. arugamensis* infestation can cause secondary infection by bacteria such as *V. alginolyticus*. Basically, Vibrio is an opportunistic body. *Vibriosis* outbreaks can occur due to environmental stress which makes the fish weak or is induced by a primary infection caused by parasites, causing tissue damage in fish and a favourable site for vibrio bacteria to initiate infection [12].

The initial clinical symptoms of fish infected with this disease are anorexia or loss of appetite accompanied by blackened body-colour, haemorrhage of the lower jaw and the whole body, and a swollen stomach. Fish that are infected will also experience loss of balance and display abnormal swimming behaviour. Vibrio bacteria that infect marine fish at the juvenile stage are not only weak and black in color, but will also stimulate excessive mucus production [34]. In addition, the correlation test
shows that there is a positive correlation between the high number of marine leechs infestations followed by the high TPC value of *Vibrio* sp. From the TPC value of *Vibrio* sp. isolated from the Hybrid Grouper the highest value showed $1.92 \times 10^4$ CFU / ml. This value is still within normal limits so that it does not cause death. According to [35] reported that the pathogenicity test of *V.alginolyticus* infection in rat grouper *Cromileptes altivelis* (size 4-5 cm) caused death in test fish with an LD50 value of $4.5 \times 10^6$ CFU/fish.

4. **Conclusion**

The infestation of the parasite *Zeylanicobdella arugamensis* can act as a predisposing factor for the occurrence of *V.alginolyticus* bacterial infection because there is a positive correlation, namely the higher the number of *Zeylanicobdella arugamensis* infestations followed by the TPC *Vibrio* sp.

5. **References**

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