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## DISEASE DYNAMICS OF BARRAMUNDI (*Lates calcarifer*) IN FLOATING NET CAGES OF AMBON BAY, MALUKU

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### ABSTRACT

This study aimed to evaluate the potential infection of bacterial *Vibrio* spp. and parasites in cultured barramundi (*Lates calcarifer*) and wild fish inhabiting Inner Ambon Bay. The research was conducted for six months (August–December 2024) using purposive sampling, with five cultured barramundi and five wild individuals collected each month. Bacteria were isolated from liver and kidney tissues using SWC agar and TCBS agar, and identified with the API 20 NE kit. Parasite identification was performed microscopically on gill, skin, and intestinal samples. The results revealed that *Vibrio* spp. were detected at relatively high abundance, dominated by *V. harveyi*, *V. alginolyticus*, and *V. vulnificus*. Identified parasites included *Trichodina* sp., *Cryptocaryon irritans*, *Benedenia* sp., and nematodes. Parasite infection prevalence in cultured barramundi ranged from 40–70%, whereas prevalence in wild fish reached up to 60%. These findings indicate the potential for cross-transmission of diseases between cultured barramundi and wild fish populations in Inner Ambon Bay.

**KEYWORDS:** Inner Ambon Bay; Floating Net Cages; *Lates calcarifer*; *Vibrio* spp.; Parasites

### ABSTRAK

Penelitian ini bertujuan mengevaluasi potensi infeksi bakteri *Vibrio* spp. dan parasit pada ikan kakap putih (*Lates calcarifer*) budidaya dan ikan liar di perairan Teluk Ambon Dalam. Penelitian dilaksanakan selama enam bulan (Agustus–Desember 2024) dengan metode purposive sampling, masing-masing lima ekor ikan kakap putih budidaya dan lima ekor ikan liar setiap bulan. Isolasi bakteri dilakukan dari organ hati dan ginjal menggunakan media SWC agar dan TCBS agar, sedangkan identifikasi bakteri menggunakan kit API 20 NE. Identifikasi parasit dilakukan melalui pemeriksaan mikroskopis pada insang, kulit, dan usus. Hasil penelitian menunjukkan bahwa *Vibrio* spp. ditemukan dengan kelimpahan relatif tinggi, didominasi oleh *V. harveyi*, *V. alginolyticus*, dan *V. vulnificus*. Parasit yang teridentifikasi meliputi *Trichodina* sp., *Cryptocaryon irritans*, *Benedenia* sp., dan nematoda. Prevalensi infeksi parasit pada ikan budidaya berkisar 40–70%, sedangkan pada ikan liar mencapai 60%. Hasil penelitian ini mengindikasikan adanya potensi penularan silang penyakit antara ikan budidaya dan ikan liar di Teluk Ambon Dalam.

**KATA KUNCI:** Teluk Ambon Dalam, KJA, Kakap Putih, *Vibrio* spp., Parasit

## 15 INTRODUCTION

Barramundi (*Lates calcarifer*), also known as Asian sea bass, is a major aquaculture commodity in the Asia–Pacific region, including Indonesia. This species has high economic value, rapid growth, and broad tolerance to environmental conditions, making it widely cultivated in floating net cage (KJA) systems in coastal waters and bays (Williams et al., 2017; FAO, 2022). Inner Ambon Bay is a strategic location for aquaculture development because of its relatively calm waters and favorable environmental conditions (Manoppo et al., 2017).

However, intensification of aquaculture in open systems increases vulnerability to infectious diseases. Diseases are a major limiting factor in marine aquaculture and can cause significant economic losses (Subasinghe et al., 2001; Samsing et al., 2023). Among bacterial pathogens, the genus *Vibrio* represents opportunistic bacteria commonly associated with vibriosis in barramundi. Several species, such as *V. alginolyticus*, *V. harveyi*, *V. parahaemolyticus*, and *V. vulnificus*, are known to cause systemic infections and internal organ damage (Austin & Zhang, 2006; Subramaniam et al., 2020). The incidence of vibriosis generally increases under conditions of high temperature, poor water quality, and high stocking density, all of which are common in intensive farming (Defoirdt, 2016).

External parasites also represent a serious threat. Species such as *Trichodina* sp., *Cryptocaryon irritans*, and *Neobenedenia* sp. have been reported to cause irritation, epithelial tissue damage, chronic stress, and increased susceptibility to secondary infections (Gibson-Kueh et al., 2012; Hutson et al., 2018). Parasite prevalence is relatively high in tropical and subtropical waters, particularly in aquaculture systems with high stocking density (Whittington et al., 2018).

Another important issue is the potential cross-transmission of diseases between cultured and wild fish. Wild fish around cages may serve as reservoirs for pathogens, and both direct and indirect interactions (through water, feed, or feces) can facilitate transmission (Wang et al.,

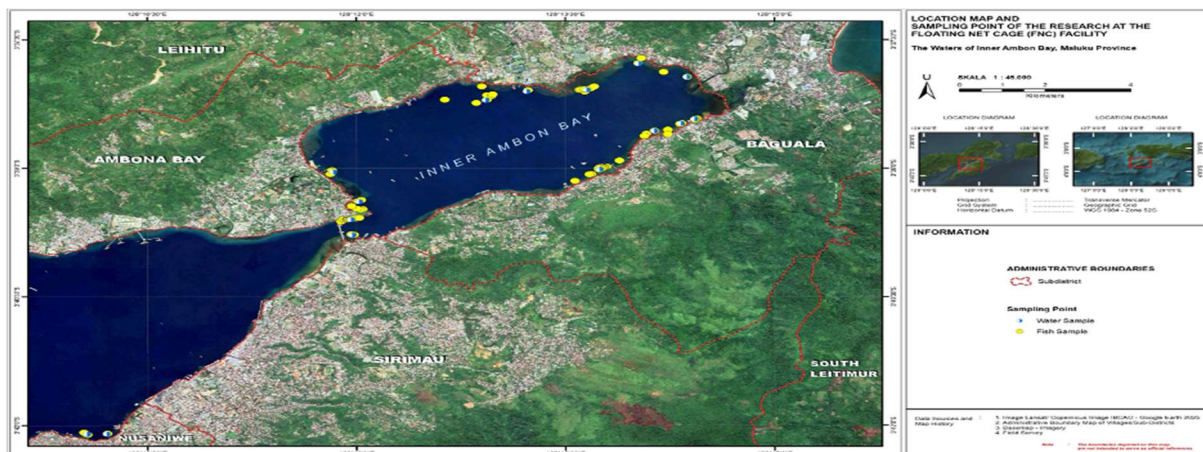
2019; Samsing et al., 2023). The risk of this process increases when biosecurity practices are not well implemented.

To date, studies on the disease dynamics of *L. calcarifer* that include both cultured and wild fish populations in Inner Ambon Bay are still limited. Therefore, this study aimed to evaluate the presence and prevalence of *Vibrio* spp. and parasite infections in cultured and wild barramundi in the region. The results are expected to serve as a scientific basis for developing more effective prevention and control strategies for diseases in open marine aquaculture systems.

## MATERIALS AND METHODS

### Study Period and Location

The research was conducted in Inner Ambon Bay, Indonesia, a major center for marine fish farming using floating net cages (KJA). Sample analyses were performed at the Aquaculture Laboratory, Faculty of Fisheries and Marine Science, Universitas Pattimura Ambon, and at the Fish Health and Disease Laboratory, Marine Aquaculture Development Center (BBPL) Waiheru–Ambon. The study lasted five months, from August to December 2024. Sampling sites for fish collection and in situ water quality measurement are shown in Figure 1.



## Research Design and Sampling Technique

A purposive sampling method was applied to obtain representative barramundi (*Lates calcarifer*) from two populations: cultured fish maintained in KJA and wild fish captured around the cages. Each month, five cultured fish and five wild fish were collected, resulting in a total of 50 specimens.

## Water Quality Measurement

Water quality parameters were measured in situ at KJA sites and wild fish capture locations. Parameters included temperature (°C), salinity (ppt), pH, dissolved oxygen (DO, mg/L), and ammonia (NH<sub>3</sub>-N, mg/L). Measurements were performed using a portable YSI multiparameter device and an ammonia test kit according to standard procedures.

## Clinical and Morphological Observation

Captured fish were examined visually to identify clinical signs of disease, including changes in body color, skin lesions or ulcers, abdominal swelling, and abnormal behavior (e.g., slow swimming or surface aggregation). Morphological parameters, such as total length and body weight, were also recorded.

## Bacterial Isolation and Identification

Liver and kidney tissues were sampled. Organ surfaces were sterilized, and tissues were aseptically excised. Samples were inoculated on SWC agar and TCBS agar, and incubated at 28–30 °C for 24–48 h. Colonies were identified using the API 20 NE system (bioMérieux) to determine *Vibrio* species based on biochemical profiles (Austin & Austin, 2016).

## Parasitological Examination

Gill, skin, and intestinal tissues were examined for parasites. Wet mounts were prepared in physiological saline and observed under a light microscope. Parasites were identified morphologically with reference to tropical fish parasite identification keys (Woo, 2006; Gibson-Kueh et al., 2012).

## Data Analysis

The total bacterial count and total *Vibrio* count were calculated using the spread plate method with the formula:

$$\sum \text{bacteria} = N / (\sum \text{spread}) \times 1/F$$

where:

$\Sigma$  bacteria = number of bacterial cells (CFU/g)

N = number of colonies

F = dilution factor

Total plate count (TPC) for bacteria and *Vibrio* spp. was analyzed quantitatively. Data were subjected to analysis of variance (ANOVA) at a 95% confidence level using SPSS version 25.0 (IBM Corp.). When significant differences ( $p < 0.05$ ) were detected, Tukey's HSD test was applied to determine significantly different groups. Identification of *Vibrio* species from biochemical and API 20 NE results was analyzed descriptively.

For parasites, prevalence and intensity of infection were calculated using the following formulas:

$$\text{Prevalence (\%)} = (\text{Number of infected fish} / \text{Number of examined fish}) \times 100$$

$$\text{Intensity} = (\text{Total number of parasites in infected fish} / \text{Number of infected fish})$$

The results were presented in tables and descriptive graphs to illustrate infection levels in cultured and wild fish throughout the study period.

## RESULTS AND DISCUSSION

### RESULT

Table 1. Mean Water Quality Parameters in Inner Ambon Bay during the Study Period (August–December 2024).

Parameters	Month					Normal Range	References
	Aug	Sept	Oct	Nov	Dec		
Temperature (°C)	28.5	29.0	29.8	30.2	30.5	26–32	Boyd & Tucker (2012); KepMen LH No. 51/2004
Salinity (ppt)	32.0	32.5	33.0	33.5	34.0	28–34	MMAF (2016); Effendi (2003)
pH	7.6	7.8	7.9	8.0	8.2	7.5–8.5	Effendi (2003); Ministry of Environment No. 51/2004
Dissolved Oxygen (mg/L)	5.2	4.8	4.0	3.5	3.0	> 5	Boyd & Tucker (2012); KKP (2016)
Ammonia (mg/L)	0.12	0.15	0.20	0.26	0.32	< 0.1	Effendi (2003); Boyd & Tucker (2012); KepMen LH No. 51/2004

Note: Primary data from field measurements, 2024.

Based on Table 1 above, it is observed that temperature and salinity values gradually increased from August to December 2024. The pH values remained within the normal range for tropical marine waters, although minor fluctuations were noted, still within acceptable limits. A significant decrease in dissolved oxygen (DO) levels occurred, dropping from 5.2 mg/L in August to only 3.0 mg/L in December. Ammonia levels also showed an increasing trend, approaching toxic thresholds (>0.3 mg/L) by the end of the study. These conditions indicate a degradation in water quality, especially during the transitional season toward the rainy period, potentially causing physiological stress in fish and increasing the risk of pathogen infections. The decline in DO and rise in ammonia were found to correlate with a spike in disease prevalence in barramundi (Asian sea bass) from October to December. Field observations revealed differences in clinical symptoms between farmed and wild fish. These symptoms are presented in Table 2.

Table 2. Clinical Signs of Cultured (*Lates calcarifer*) and Wild

No	Clinical Symptoms	Description	Farmed	Wild Fish
1	Lesion/Ulcer	Red lesions or ulcers on skin and fins	+	-
2	Pale gills	Gill discoloration, indicative of anemia/stress	+	+
3	Swollen abdomen	Indication of systemic infection or fluid accumulation	±	-
4	Excess mucus production	Thick mucus layer on body surface, response to irritation	+	±
5	Slow movement	Fish appear weak, swim passively or near the surface	+	±
6	Skin hemorrhage	Red spots on skin due to local bleeding	±	-
7	Damaged/eroded fins	Fin edges torn or eroded	±	-

Note: (+) frequently observed; (±) occasionally observed; (-) not observed (Primary data, 2024).

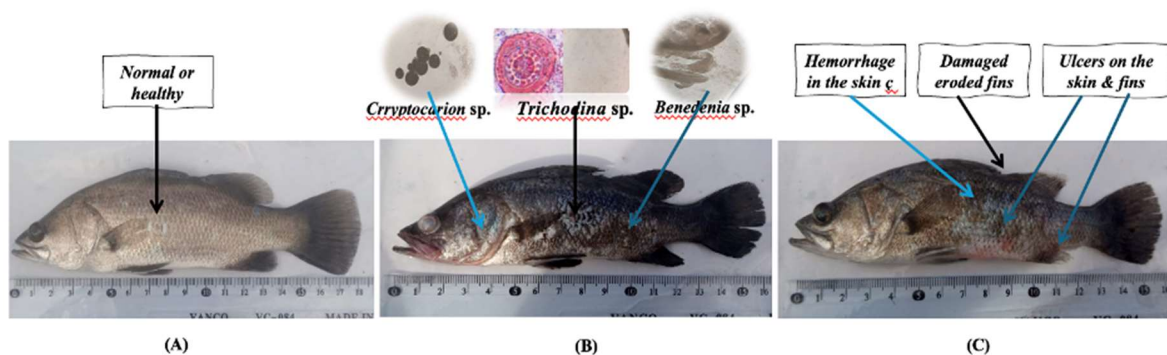


Figure 2. Clinical conditions of barramundi (*Lates calcarifer*): (A) Normal, healthy fish showing bright body coloration and intact fins without lesions; (B) Fish exposed to parasites (*Cryptocaryon* sp., *Trichodina* sp., and *Benedenia* sp.) exhibiting skin hemorrhage and external irritation; (C) Fish exposed to *Vibrio* spp. showing hemorrhages on the skin, eroded fins, and ulcerative lesions consistent with vibriosis symptoms

Bacterial isolation was conducted from the liver and kidney organs of barramundi (*Lates calcarifer*) from both farmed and wild populations. Bacterial cultures were grown on TCBS (Thiosulfate-Citrate-Bile-Sucrose) and SWC agar media. The colonies that developed exhibited morphological characteristics typical of the *Vibrio* genus, indicated by yellow and green coloration on TCBS agar. Further identification using the API 20 NE system revealed three dominant *Vibrio* species: *V. harveyi*, *V. alginolyticus*, and *V. vulnificus*. The occurrence rates of each species in the respective fish populations are presented in Table 3 below.

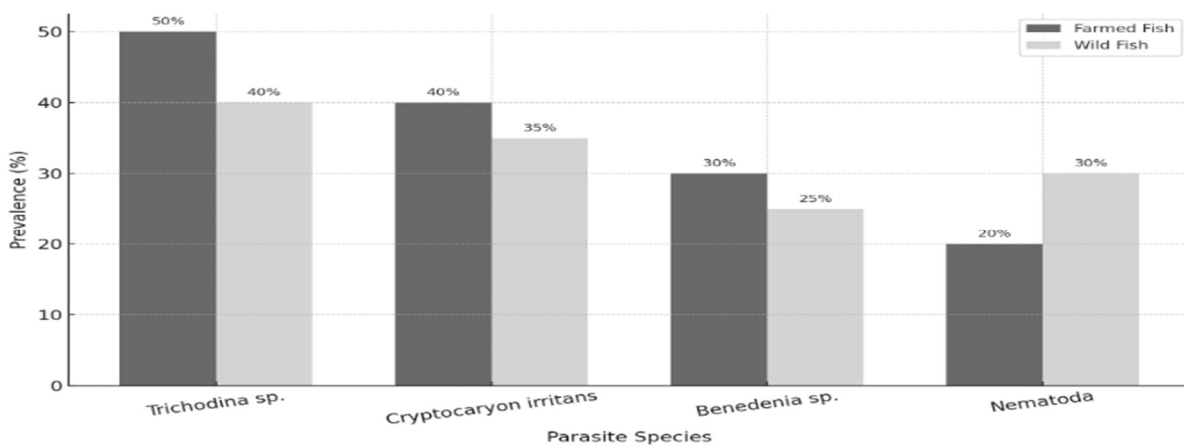
**Table 3. Prevalence of *Vibrio* spp. bacteria in farmed barramundi (*Lates calcarifer*) and wild fish in the Inner Ambon Bay**

No	Bacterial Species	Farmed Barramundi (%)	Wild Fish (%)
1	<i>Vibrio harveyi</i>	60	45
2	<i>Vibrio alginolyticus</i>	50	30
3	<i>Vibrio vulnificus</i>	40	25

Microscopic examination of the gill, skin, and intestinal tissues revealed the presence of four main types of parasites: *Trichodina* sp., *Cryptocaryon irritans*, *Benedenia* sp., and nematodes (not yet identified to the genus level). Most of the parasites were found infecting the gills and body surface, while nematodes were detected in the digestive tract.

**Table 4. Prevalence of parasites in farmed barramundi (*Lates calcarifer*) and wild fish in the Inner Ambon Bay**

No	Parasite Species	Infection Site	Farmed Barramundi (%)	Wild Fish (%)
1	<i>Trichodina</i> sp.	Insang, kulit Gills, skin	50	40
2	<i>Cryptocaryon irritans</i>	Insang Gills	40	35
3	<i>Benedenia</i> sp.	Kulit, sirip Skin, fins	30	25
4	Nematoda (unspecified)	Usus Intestine	20	30



**Figure 3. Prevalence (%) of Parasites in Cultured (*Lates calcarifer*) and Wild**

Infection intensity refers to the average number of parasites infecting each infected fish. Based on the observations (Table 5 and Figure 4), the infection intensity in farmed barramundi tends to be higher than in wild fish for most of the identified parasite species (Table 5).

**Table 5.** Intensity of parasitic infections in farmed barramundi (*Lates calcarifer*) and wild fish in the Inner Ambon Bay

No	Parasite Species	Infection Site	Intensity in Farmed Fish (mean parasites/individual)	Intensity in Wild Fish (mean parasites/individual)
1	<i>Trichodina</i> sp.	Gills, skin	15	10
2	<i>Cryptocaryon irritans</i>	Gills	12	9
3	<i>Benedenia</i> sp.	Skin, fins	9	7
4	Nematoda (unspecified)	Intestine	6	8

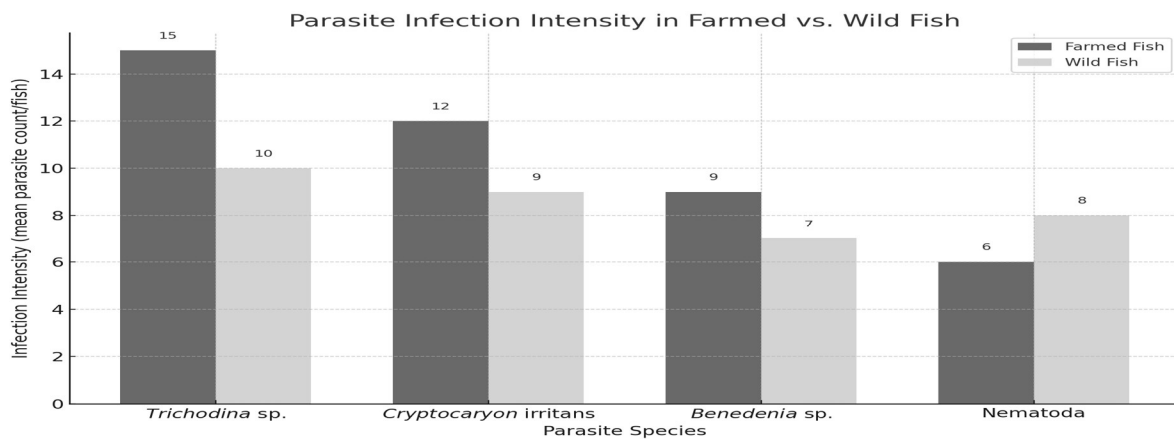


Figure 4. Mean Intensity of Different Parasitic Infections in Cultured (*Lates calcarifer*) and Wild

The results of the study showed that the abundance of *Vibrio* spp. in barramundi varied across months, with a general increasing trend from August to December 2024. In farmed fish, *V. harveyi* was identified as the dominant species with the highest abundance, reaching 6.0 log CFU/g in December. This value was higher than that of *V. alginolyticus* (5.3 log CFU/g) and *V. vulnificus* (4.8 log CFU/g). In contrast, the abundance of *Vibrio* spp. in wild fish was relatively lower and more uniform, although it also showed an increasing trend toward the end of the study period.

Statistical analysis using ANOVA showed a significant difference in farmed fish ( $p < 0.05$ ). Further Tukey post-hoc tests revealed that the main differences occurred between *V. harveyi* and the other two species. Meanwhile, no significant differences were found among *Vibrio* spp. species in wild fish.

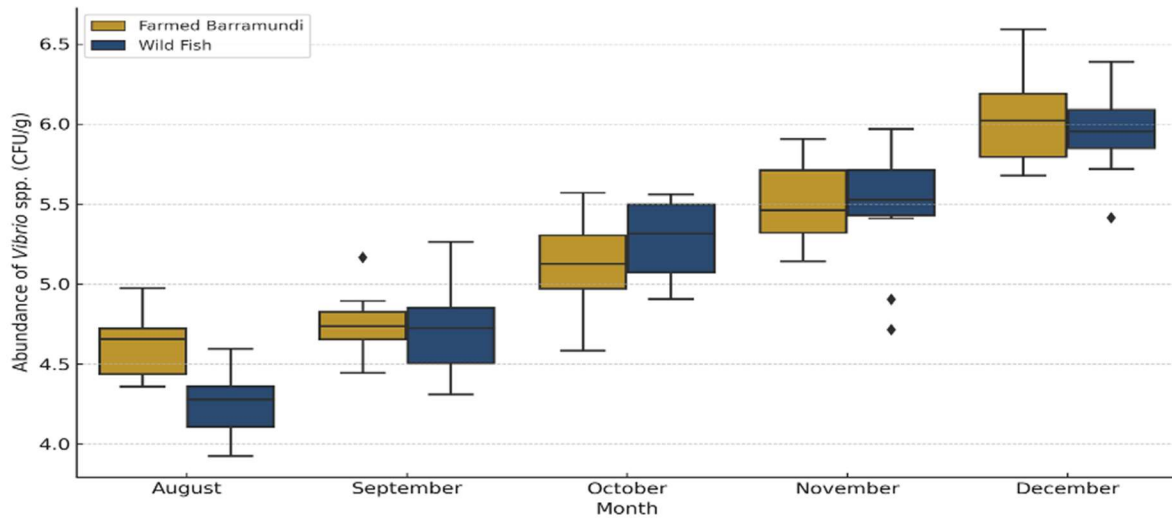


Figure 5. Distribution of *Vibrio* spp. Abundance in Cultured and Wild Barramundi (*Lates calcarifer*) from August to December 2024

## DISCUSSION

The results of the study showed that water quality in Inner Ambon Bay fluctuated, particularly with a decrease in dissolved oxygen (DO) levels and an increase in ammonia concentration during November–December 2024. These conditions may suppress fish physiology and increase susceptibility to disease. This phenomenon is consistent with the findings of Le et al. (2021), who stated that environmental degradation, especially low DO and high ammonia, is a key factor triggering the emergence of diseases in tropical marine fish.

Farmed barramundi exhibited several clinical symptoms, such as pale body, skin lesions, fin damage, abdominal distension, and gill hemorrhage. These symptoms are consistent with the manifestations of vibriosis in tropical marine fish caused by *Vibrio* spp., particularly *V. harveyi* (Dong et al., 2020). Morphological changes in organs, including swollen liver, pale kidney, and gill lamella hyperplasia, indicate physiological responses to environmental stress and pathogen infection. Recent studies have also confirmed that histopathological changes in target organs can serve as early indicators of infectious diseases in marine farmed fish (Dong et al., 2020).

These morphological alterations reinforce the hypothesis that environmental stress due to declining water quality contributes to increased fish susceptibility to parasitic infections. The interaction between tissue damage caused by parasites and colonization by opportunistic bacteria, especially *V. harveyi*, is suspected to be the main trigger of vibriosis clinical

3 symptoms in farmed fish. This finding is consistent with Luo et al. (2020), who reported that parasite–bacteria co-infections play a major role in the disease dynamics of tropical marine aquaculture.

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22  
5 Bacterial identification using the API 20 NE kit confirmed the presence of three dominant species: *V. harveyi*, *V. alginolyticus*, and *V. vulnificus*. The abundance of *V. harveyi* in farmed fish increased significantly, reaching 6.0 log CFU/g in December, which was significantly higher compared to the other two species (ANOVA,  $p < 0.05$ ; Tukey,  $p < 0.05$ ). These results align with the report of Tan et al. (2022), which identified *V. harveyi* as the most virulent species in tropical marine fish aquaculture. In contrast, *V. alginolyticus* and *V. vulnificus* were found at lower abundances, consistent with their role as opportunistic flora that can become pathogenic when fish are subjected to environmental stress (Chen et al., 2021).

21 In addition to bacteria, parasitological examination revealed the presence of *Trichodina* sp., *Cryptocaryon irritans*, *Benedenia* sp., and nematodes. Infection prevalence in farmed fish reached 40–70%, while in wild fish it was about 60%. The highest infection intensity was recorded for *Trichodina* sp., particularly in November–December. The high prevalence and intensity of these ectoparasites may cause damage to gill and skin tissues, thereby increasing fish susceptibility to secondary infections by *Vibrio* spp. (Luo et al., 2020). The findings indicate that disease dynamics in barramundi are influenced by a complex interaction between environmental factors, bacteria, and parasites (Rahman et al., 2020).

Overall, water quality deterioration plays a major role in increasing disease prevalence in barramundi. Clinical symptoms, organ morphological changes, dominance of *V. harveyi*, and the high infection rates of *Trichodina* sp. and *Cryptocaryon irritans* confirm that disease dynamics in open-sea farming systems in Inner Ambon Bay result from multifactorial interactions. Wild fish, although harboring lower pathogen abundance, still act as natural reservoirs and may serve as sources of transmission to farmed populations.

## CONCLUSION

30 Based on the results of this study on disease dynamics in barramundi (*Lates calcarifer*) in Inner Ambon Bay, it can be concluded that the decline in water quality—particularly low dissolved oxygen (DO) and increased ammonia concentrations at the end of the observation period—is closely associated with the rise in disease prevalence. Clinical symptoms and organ

anatomical changes indicate the presence of complex pathogenic infections. Among bacteria, *Vibrio harveyi* was identified as the dominant and most virulent species, significantly different from *V. alginolyticus* and *V. vulnificus* in farmed fish. Meanwhile, the parasite *Trichodina* sp. showed the highest prevalence and infection intensity, thereby increasing the risk of co-infection with *Vibrio* spp. In addition, wild fish act as pathogen reservoirs, with the potential to transmit diseases to farmed fish populations around floating net cages.

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