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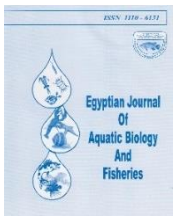

Parasitic and Bacterial Pathogens in Milkfish (*Chanos chanos*) from South Sulawesi Aquaculture Sites

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ABSTRACT

Milkfish (*Chanos chanos* (Forsskål, 1775)) is a primary aquaculture commodity in Southeast Asia, particularly in Indonesia. The intensification of aquaculture systems increases the risk of bacterial and parasitic infections, which may lead to significant production loss. This study aimed to identify bacterial and parasitic pathogens in milkfish from two aquaculture sites in South Sulawesi: the Milkfish Pond Installation (ITP) Marana, Maros, and community ponds in Pangkep. Gill histopathological examination, parasite identification, and bacterial isolation on tryptic soy agar (TSA) and thiosulfate-citrate-bile salts sucrose (TCBS) media, followed by identification using VITEK matrix-assisted laser desorption/ionization, showed marked differences between sites. At ITP Marana, Maros, parasite prevalence was low (3.3%), with only *Zoothamnium* sp. being detected. In contrast, in the Pangkep community ponds, the prevalence was very high (66.7%), dominated by *Trichodina* sp. and *Apiosoma* sp., with an intensity of 51 parasites per fish. Bacterial profiles also differed, with Maros samples dominated by *Staphylococcus* spp. and opportunistic bacteria, whereas Pangkep samples showed dominance of *Vibrio* spp. and the more virulent *Photobacterium damselae*. Gill histopathology revealed hyperplasia, haemorrhage, and congestion, indicating interactions between tissue damage caused by parasites and bacterial colonisation. These findings provide baseline epidemiological data on multi-pathogen infections in milkfish aquaculture and emphasize the need for integrated biosecurity strategies.

INTRODUCTION

Aquaculture is a key sector of the global food supply chain. According to the latest FAO report, global aquaculture production in 2022 reached 130.9 million tons, surpassing marine capture fisheries for the first time, with Asia contributing 91.4% of the total production **(FAO, 2024)**. The updated FishStat dataset further highlights Indonesia as one of the world's largest producers, along with China and India, contributing to approximately

7% of the global production. Milkfish (Chanos chanos (Forsskål, 1775)) is a major aquaculture species. Global milkfish production in 2022 was approximately 1.2–1.3 million tons, with more than 70% of it originating from Indonesia and the Philippines **(FAO, 2025)**. In Indonesia, milkfish farming has been practiced for centuries and has developed from traditional to intensive systems **(Riany *et al*., 2023)**. By 2022, annual milkfish production exceeded 600 thousand tons, positioning it as a strategic national commodity of high economic value, an affordable protein source, and a vital contributor to local livelihoods **(Jose & Divya, 2022)**.

However, as aquaculture intensification increases, milkfish become increasingly vulnerable to various pathogens. Different ecological factors in aquaculture environments are likely to affect the frequency, distribution, and intensity of parasite infestations **(Zamri *et al*., 2025)**. Milkfish aquaculture faces multiple challenges, among which the most critical limiting factor is production loss due to infectious diseases caused by parasites, bacteria, fungi, and viruses (**Assefa & Abunna, 2018)**. Bacterial infections, particularly those caused by *Vibrio* spp., are the most common diseases in aquaculture systems and often lead to substantial economic losses **(Mohamad *et al*., 2019b; Deng *et al*., 2020; Ngasotter *et al*., 2021)**. Recent reports have documented fin rot in milkfish fingerlings associated with *Vibrio harveyi,* which was proven to be pathogenic through challenge tests, reinforcing the role of *V. harveyi* as a significant pathogen in tropical aquaculture (**Superio *et al*., 2021; Zin *et al*., 2025)**. Moreover, contemporary surveys show that *Vibrio parahaemolyticus* from aquaculture often carries antimicrobial resistance and virulence genes **(Lee *et al*., 2025)**. Additionally, aquaculture environments are increasingly recognized as reservoirs of resistance determinants **(Deng *et al*., 2025).**

Parasitic infestations are also a serious constraint. A study from East Java found gill parasites *Dactylogyrus* and *Capillaria* with prevalence ranging from 2.25–5% and intensities of 1–2 parasites per fish **(Atsnani & Farikhah, 2023)**. Another study documented infestations of *Dactylogyrus vastator* in nursery and grow-out ponds, with prevalences of 5.33 and 2.0%, respectively **(Hidayatullah *et al*., 2020)**. Although the prevalence is not high, parasites can reduce growth productivity, decrease resistance to diseases and increase the risk of secondary infections **(Buchmann & Lindenstrøm, 2002)**.

In this study, we made initial observations on milkfish and the pathology of the gills, including lamellar hyperplasia, haemorrhage, and congestion. The gills are the primary organs for respiration and osmoregulation and are a major immune barrier against aquatic pathogens; therefore, they are highly sensitive to environmental changes **(Poleksić & Mitrović-Tutundžić, 1994; Roberts 2012)**. Histopathological gill analysis has been successful in illustrating early responses to environmental stress and infection, as evident from early responses before the appearance of clinical symptoms **(Mallatt, 1985; Bernet *et al*., 1999)**. The first part of this study, the histology of the gill, is both valid and methodological and provides a scientific foundation for subsequent work: the identification of parasites and bacteria in milkfish samples.

Considerable improvements have been made in methods for identifying pathogens. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is widely utilized in aquaculture microbiological laboratories, providing a method for quick, accurate, and low-cost identification compared with traditional methods **(Piamsomboon *et al.*, 2020)**. This procedure identifies the masses of bacterial proteins/peptides and matches them to reference libraries and is 99.9% accurate at the species level **(Çağatay, 2024; Lee *et al.*, 2024)**. Meanwhile, the identification of fish parasites is still based largely on morphological investigation with stereomicroscopy or compound microscopy, which makes it much easier to determine characteristic features such as the denticles of *Trichodina* spp.

Pathogen detection is essential for disease prevention and proper treatment of fish **(Sufardin *et al.*, 2021a)**. However, studies on multipathogen profiles in milkfish raised in Indonesia are scarce, especially those involving early histopathological evidence of parasite and bacterial identification. Most previous research has focused on single pathogens or isolated case reports **(Cruz- Lacierda *et al.*, 2011)**. There are no available baseline data regarding the overall diversity of both parasitic and bacterial pathogens in milkfish originating from the main aquaculture sites in South Sulawesi. This open space is significant because this period is an important phase in the production process of crucial susceptibility to environmental stress and secondary infections. The absence of such baseline data creates a knowledge gap that may hinder the development of biosecurity strategies and disease control programs based on local epidemiological evidence.

Therefore, this study aimed to identify bacterial and parasitic agents in milkfish (*Chanos-chanos*) collected from two aquaculture sites in South Sulawesi, Indonesia. Specifically, this study aimed to (i) examine gill histopathology in milkfish larvae as an initial step in assessing fish health, (ii) identify the dominant bacterial and ectoparasitic pathogens in samples from both sites, and (iii) describe the prevalence, intensity, and abundance of milkfish parasites in the samples. The results are expected to provide baseline epidemiological data that can support further research, strengthen fish health monitoring systems, and serve as a reference for developing more effective and sustainable milkfish health management strategies at the regional level.

MATERIALS AND METHODS

Fish sampling

This study was conducted in South Sulawesi at two distinct locations from March to August 2025. The first location was the Milkfish Pond Installation (ITP) Marana in Maros Regency, while the second was the community ponds in Pangkep Regency, South Sulawesi. Fish sampling was performed randomly using small hand nets. At the first location, the milkfish seed source was local fish, and the sampled fish were categorized into three stages: fry, nursery, and grow-out (referred to as fry, nursery, and grow-out stages), with a total of 150 fish. At the second location, 120 fish originating from Bali

Regency were sampled four times during the fry and nursery stages. The fish were transported alive in plastic bags filled with seawater and oxygen to the Fish Parasites and Diseases Laboratory for parasite and bacterial isolation and identification.

Parasite examination

Milkfish (n = 150 at site 1 and n = 120 at site 2) were examined immediately after arrival at the laboratory. The weight and length of the fish were measured, and they were placed on a glass slide with a few drops of saline water for observation under a compound microscope (CX23, Olympus, Germany) and stereomicroscope. The parasites were photographed using a BX53 Olympus microscope equipped with an Olympus EP50 camera. The parasites were counted, isolated, and preserved in 70% ethanol for further morphological identification. Parasite identification was conducted using standard taxonomic keys **(Kabata, 1985; Lom & Dyková, 1992)** and further verified using the descriptions in Fish Parasites: Pathobiology and Protection **(Woo & Buchmann, 2012)**.

Histopathological examination

Histopathological analysis was conducted as described by **Espinosa *et al.* (2019)**. Infected organs were selected and preserved in 10% buffered formalin and rinsed with a physiological solution. The samples were dehydrated using a graded series of alcohol concentrations (70, 80, 90, 96, and 100%), cleared in xylene, and subsequently embedded in paraffin wax. The tissue was sectioned to a thickness of 3µm, mounted on glass slides, dewaxed, rehydrated, and stained with haematoxylin and eosin. Specimens were examined under a light microscope, and pathological changes in the gills were described following conventional histopathological procedures developed for fish pathology studies **(Bernet *et al.*, 1999)**.

Bacterial isolation

Histopathological analysis was conducted as described by **Espinosa *et al.* (2019)**. Infected organs were selected and preserved in 10% buffered formalin and rinsed with a physiological solution. The samples were dehydrated using a graded series of alcohol concentrations (70, 80, 90, 96, and 100%), cleared in xylene, and subsequently embedded in paraffin wax. The tissue was sectioned to a thickness of 3µm, mounted on glass slides, dewaxed, rehydrated, and stained with haematoxylin and eosin. Specimens were examined under a light microscope, and pathological changes in the gills were described following conventional histopathological procedures developed for fish pathology studies **(Bernet *et al.*, 1999)**.

Morphological and biochemical characterization

Bacterial colonies that were morphologically characterized based on color, shape, transparency, elevation, and margin structure, were further identified using the VITEK MALDI-TOF MS system (bioMérieux, software v1.00.46). Single colonies growing on TSA/TCBS plates were smeared onto the target plates using a sterile loop. Each spot was

overlaid with 1µL of α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution and air-dried for 1– 3min before analysis. For some Gram-positive or thick-walled isolates, on-plate extraction with 70% formic acid was performed to enhance protein signal detection. Spectral acquisition was automated and compared with the VITEK MS reference database. The identification results were expressed as confidence values, with scores ≥99% considered valid at the species level. Isolates scoring <99% or yielding low discrimination were re-tested with replicate spots or subcultured for re-analysis **(Patel, 2013; Piamsomboon *et al.*, 2020; Sivanesan *et al.*, 2023; Çağatay, 2024)**.

Data analysis

Raw data were recorded in Microsoft Excel and analyzed using SPSS (version 25). Parasite prevalence was calculated as the percentage of infected fish, and mean intensity was calculated as the average number of parasites per infected fish. As parasite count data often do not follow a normal distribution, comparisons between sites or fish size groups were analyzed using the Kruskal–Wallis test. Statistical significance was set at $\alpha = 0.05$ and applied when normality and homogeneity assumptions were not satisfied.

Prevalence was calculated as the proportion of infected hosts to the total number of hosts examined.

$$Prevalence\ (\%) = \frac{Number\ of\ infected\ fish}{Total\ number\ of\ fish\ examined} x100$$

Intensity was calculated as the number of parasites found divided by the number of infected fish.

$$Intensity = \frac{Number\ of\ parasites\ found}{Total\ number\ of\ infected\ fish}$$

Abudance was calculated as the number of parasite found divided by the number of fish examined

$$Abundance = \frac{Number\ of\ parasites\ found}{Total\ number\ of\ fish\ examined}$$

Bacterial Density :

$$CFU/mL = (Bacteria\ number\ x \frac{1}{Dilution\ factor})x10$$

RESULTS

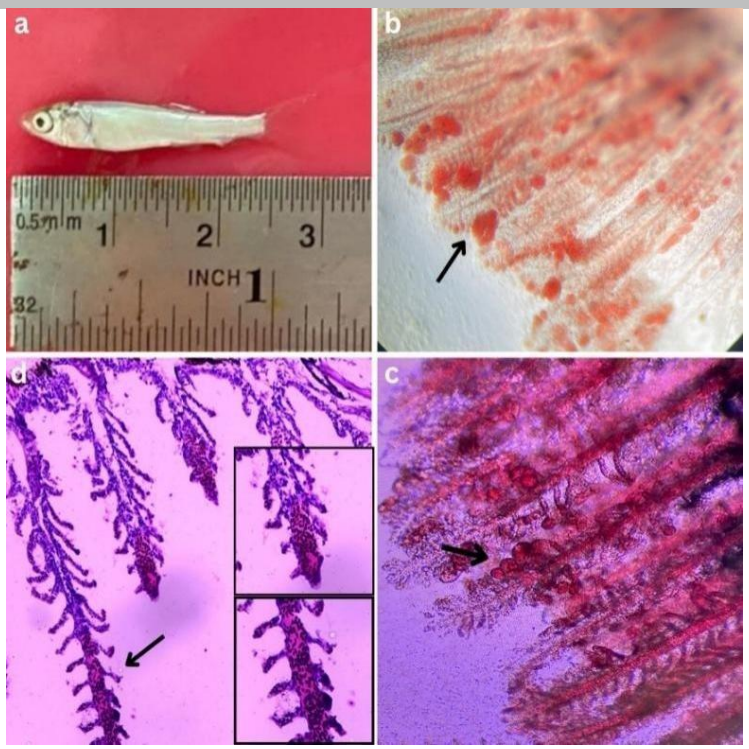

Fig. 1. Sample of milkfish (*Chanos chanos* (Forsskål, 1775)). (a) Milkfish used in this study. (b) Macroscopic view of the gills showing hemorrhagic lesions (arrow). (c) Light microscopy showing lamellar hyperplasia and tissue alteration (arrow; inset indicates tip deformation). (d) Histological section of gills stained with H&E demonstrating hemorrhage and blood congestion (arrow) (b,c, dan d with 20x magnification)

Fig. (1) shows milkfish (*Chanos chanos* (Forsskål, 1775)) larvae (~3 cm) and their gills. Macroscopic observation under a stereo microscope (Fig. 1b) revealed reddish lesions and haemorrhagic areas (black arrow), suggesting blood vessel congestion in the gill filament. Light microscopy (Fig. 1c) revealed haemorrhagic regions (accumulation of erythrocytes) in the distal part of the gill lamellae, suggesting tissue damage due to physiological stress response or pathogen infection. Histological sections (Fig. 1d) revealed alterations in the gills, including secondary lamellar hyperplasia and tissue thickening (black arrows). The inset shows tears at the tips of the lamellae, indicating severe pathological alterations.

Parasite infestation

At Site 1 (ITP Marana, Maros), only one out of 150 samples was infected at the nursery stage, harboring *Zoothamnium* sp. with 15 individuals (prevalence 3.33%, intensity 15, abundance 0.4) (Table 1). In contrast, the infection rate was much higher at Site 2 (Community ponds, Labakkang, Pangkep), with 80 of the 120 larvae (66.7%) infected with 2260 parasites, dominated by the trichodinids *Trichodina* sp. and *Apiosoma* sp.

The nursery stage showed consistent infection, with a prevalence ranging from 8 to 80% across samplings, whereas at the fry stage (from Bali stock), the prevalence reached as high as 96.7%. The maximum infection levels were observed at this fry stage, with an intensity of 51.27 and abundance of 49.57 (Table 2). These results demonstrate that parasite burden was stage- and location-dependent, and fry and nursery stages in community ponds were particularly vulnerable to ponds, with only a single nursery-stage infection of *Zoothamnium* sp. recorded. Parasite organ distribution varied greatly among the sites (Table 3). At Site 1, *Zoothamnium* sp. was detected only in the gills (15 individuals), without infecting the fins or tails (overall prevalence 1%, abundance 0.4). At Site 2, *Trichodina* sp. was most commonly associated with the tail (782 individuals). In contrast, *Apiosoma* sp. was more abundant in the fins (839 ind.) and tail (593 ind.) samples. Gill infections were relatively low (4–10 fish per tank). The prevalence of *Trichodina* sp. was 50.83% with an intensity of 12.9, whereas *Apiosoma* sp. had a prevalence of 24.17% with a maximum intensity of 50. Despite these differences, statistical analysis showed no significant differences between parasite species within each site ($P > 0.05$) in prevalence (H=1.500; $P= 0.221$), abundance (H= 1.500; $P = 0.221$), or intensity (H= 0.000; $P = 1000$) between sites.

Analysis of parasite distribution according to size (Table 4) showed significant differences ($P< 0.05$) in prevalence ($P = 0.6$), intensity ($P = 0.012$), and abundance ($P = 0.6$) across size groups. At Site 1, infection was detected only in the 2.1– 3cm group, with 15 *Zoothamnium* sp. (prevalence, 3.22%; intensity, 15; abundance, 0.4). No parasites were found in the other size groups. At Site 2, the infection was more widespread. The 1– 2cm size group exhibited the highest infection level, with 1,238 parasites (318 *Trichodina* sp. and 920 *Apiosoma* sp.). The prevalence ranged from 26 to 31%, with an intensity of 10.25–24.86. Larger larvae (≥4 cm) showed markedly lower prevalence (8–9%) and abundance (<1).

Table 1. Milkfish installation pond (ITP Marana, Maros)

Location 1	Total sample	Rearing stage	Total Infected fish	Total Parasites	Parasites species	Prevalence (%)	Intensity	Abundance
Sampling 1	30	Fry	0	0	0	0	0	0
Sampling 2	30	Fry	0	0	0	0	0	0
Sampling 3	30	Nursery	1	15	*Zoothamnium* sp	3.33	15	0.4
Sampling 4	30	Nursery	0		0	0	0	0
Sampling 5	15	Grow-out	0	0	0	0	0	0
Sampling 6	15	Grow-out	0	0	0	0	0	0
Total Sample	150		1	15	1 species			

Location 2	Total sample	Rearing stage	Total Infected fish	Total Parasites	Parasites species	Prevalence (%)	Intensity	Abundance
Sampling 1	30	Nursery	23	350	*Trichodina* sp	77	15,21	11,66
Sampling 2	30	Nursery	24	373	*Trichodina* sp	80	15,54	12,43
Sampling 3	30	Nursery	4	50	*Trichodina* sp.	8	12,5	1,67
Sampling 4	30	Fry (from Bali)	29	1487	*Trichodina* sp. *Apiosoma* sp.	96,67	51,27	49,57
Total	120		80	2260				

Table 2. Community pond (Labakkang Pangkep)

Location 1 Milkfish Installation Pond (ITP Marana,Maros)								
		Organ infected						
Parasite species	Total infected fish	Tail	Fin	Gills	Total parasite	Prevalence (%)	Intensity	Abundance
Zoothamnium sp.	1	0	0	15	15	1	15	0,4
Location 2 Community Pond, Labakkang Pangkep								
	Total	Organ infected			Total	Prevalence		Abundance
Parasites species	infected fish	Tail	Fin	gills	parasite	(%)	Intensity	
Trichodina sp.	61	782	4	4	790	50,83333	12,9	6,58
Apiosomoa sp.	29	593	839	10	1442	24,16667	50	12,01

Table 3. Prevalence and mean intensity of parasites species

Table 4. Identification of parasites based on length intervals at different sampling locations

Location 1 Milkfish Installation Pond (ITP Marana,Maros)								
Length (cm)	Sample	Total sample	Parasite species	total parasites	Infected fish	Prevalence (%)	Intensity	Abundance
1-2cm	14	150	0	0	0	0%	0	0
2,1- 3cm	31		*zoothamnium*	15	1	3.22%	12	0,4
3,1-4 cm	30		0	0	0	0	0	0
4,1-5 cm	45		0	0	0	0	0	0

5,1-10 cm	15		0	0	0	0	0	0
10,1-19	15		0	0	0	0	0	0

Location 2 Community Pond, Labakkang Pangkep

Length (cm)	Sample	Total sample	Parasite species	Total parasites	Infected fish	Prevalence (%)	Intensity	Abundance
1-2 cm	47	120	*Trichodina* sp	318	31	26%	10,25	2,65
			Apiostoma sp	920	37	31%	24,86	7,66
2,1- 3cm	25		*Trichodina* sp	200	15	13%	13,33	1,66
			Apiostoma sp	316	13	11%	24,30	2,63
3,1-4cm	28		*Trichodina* sp	183	12	10%	15,25	1,525
			Apiostoma sp	206	10	8%	20,6	1,716666667
4,1-5cm	20		*Trichodina* sp	117	11	9%	10,63	0,975
			Apiostoma sp	0	0	0%	0	0

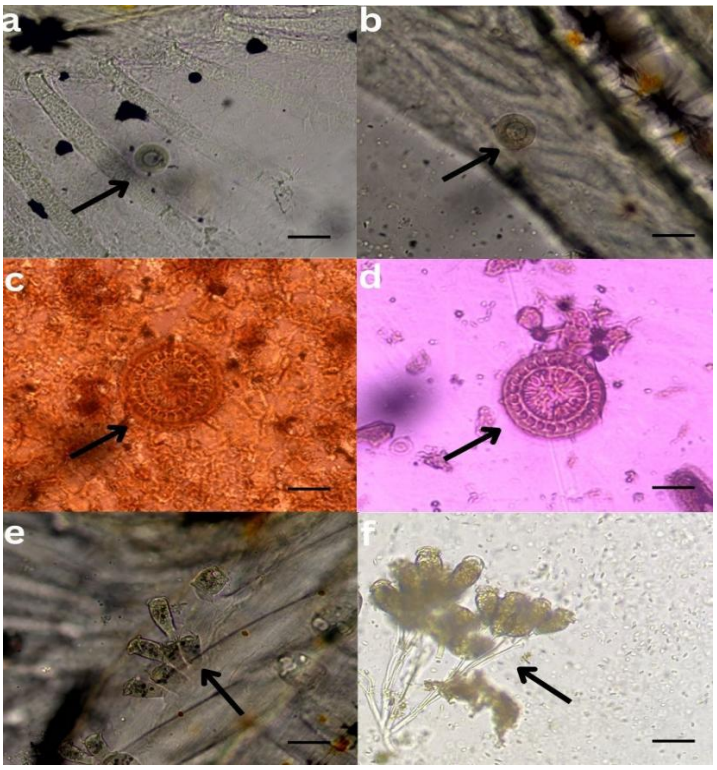

Fig. 2. Ectoparasites observed on the milkfish. Arrows indicate (a) *Trichodina* sp. on fins (b*) Trichodina sp* on tails (c and d) *Trichodina sp*. stained with AgNO3 2%. (e) *Apiosoma* sp. (f) *Zoothamnium* sp. (a,b,e, and f 20x magnification). (c dan d 40x magnification)

Microscopic observations of ectoparasites confirmed the presence of several protozoan species (Fig. 2). *Trichodina* sp. attached to the fins and tails (Fig. 2a,b) displayed disc-shaped bodies with characteristic denticle rings, which were further highlighted after staining (Fig. 2c,d). *Apiosoma* sp. appeared as elongated, vase-shaped ciliates attached to the fins (Fig. 2e). *Zoothamnium* sp. formed branched colonial stalks, which was consistent with the morphology of sessile peritrich ciliates (Fig. 2f).

Bacteria Infestation

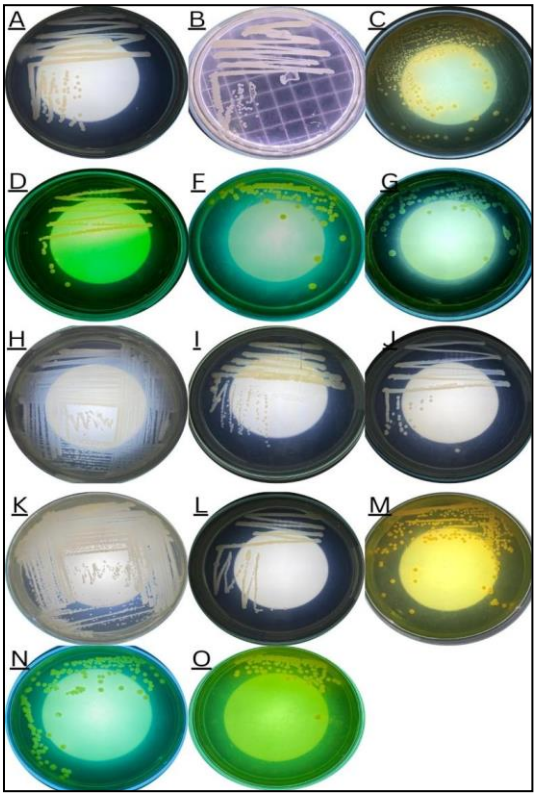

Fig. 3 Isolate of bacteria on TSA and TCBSa. A.M1 (TSA), B. M2 (TSA), C. M3 (TCBSA), D. M4 (TCBSA), E. M5 (TCBSA), F. M6 (TCBSA), G. M7 (TSA), H. M8 (TSA), I. P1 (TSA), J. P2 (TSA), K. P3 (TSA), L. P4 (TCBS), M. P4 (TCBS), N. P5 (TCBS), O. P6 (TCBS)

Table 5. Morphological character of bacterial

NO	Isolat code	Color	Form	Elevation	Margin	Surface texture	Size
1	M1 (TSA)	white, yelolwish	circular	low convex	entire	smooth	large
2	M2 (TSA)	white	circular	convex	entire	smooth	small
3	M3 (TCBSA)	green	circular	convex	entire	smooth	small
4	M4 (TCBSA)	green	circular	low convex	entire	smooth	large
5	M5 (TCBSA)	green	circular	convex	entire	smooth	Large
6	M6 (TCBSA)	green	circular	low convex	entire	smooth	Large
7	M7 (TSA)	white	circular	convex	entire	smooth	small

8	M8 (TSA	white	circular	convex	entire	smooth	small
9	P1 (TSA)	white	circular	low convex	entire	smooth	large
10	P2 (TSA)	white	circular	convex	entire	smooth	small
11	P3 (TSA)	white	circular	convex	entire	smooth	small
12	P4 (TCBS)	yellow	circular	convex	entire	smooth	Large
13	P5 (TCBS)	green	circular	convex	entire	smooth	Large
14	P6 (TCBS)	green	circular	convex	entire	smooth	Large

Bacterial isolation and colony counts revealed differences in bacterial species and numbers between the treatment and control areas of the study. At Site 1 (ITP Marana, Maros), the highest colony counts were associated with *Staphylococcus arlattae* (3.40×10^5 CFU/mL), followed by *Staphylococcus sciuri* (2.30×10^5 CFU/mL), *Exiguobacterium aurantiacum* (2.35×10^5 CFU/mL), *Vibrio vulnificus* (2.00×10^5 CFU/mL), and *Vibrio parahaemolyticus* (2.54×10^5 CFU/mL). Other identified bacteria included *Grimontia hollisae* (1.50×10^5 CFU/mL), *Shewanella algae* (2.5×10^4 CFU/mL), and *Staphylococcus cohnii* ssp. *urealyticus* (1.8×10^4 CFU/mL).

At Site 2 (community ponds, Pangkep), dominant isolates included *Photobacterium damselae* (2.29×10^5 CFU/mL), *Vibrio campbellii* (2.29×10^5 CFU/mL), *Vibrio parahaemolyticus* (2.46×10^5 CFU/mL), and *Vibrio alginolyticus* (2.15×10^5 CFU/mL). Overall, pathogenic *Vibrio* spp. were more abundant and diverse at Site 2 than at Site 1, and Site 1 had a higher proportion of Gram-positive opportunistic bacteria (*Staphylococcus* spp.) and environmental taxa such as *Exiguobacterium*. Validation with VITEK MALDI-TOF MS (bioMérieux, software v1.00.46) confirmed 12 isolates with 99.9% confidence, including *Vibrio*, *Staphylococcus*, *Exiguobacterium*, *Photobacterium*, and *Shewanella*. Two isolates remained unidentified, suggesting the possibility of novel strains or database limitations.

Table 6. Location, bacterial species, and bacterial density (CFU/mL)

Location	Rearing Stage	Sampling No.	Bacteria Species	CFU/mL
ITP Marana, Maros	Fry	1	*Staphylococcus scuiri*	$2.3\text{x}10^5$
			Staphylococcus arlattae	$3.4\text{x}10^5$
ITP Marana, Maros	Fry	2	*Exigoubacterium aurantiacum*	$2.3\text{x}10^5$
ITP Marana, Maros	Nursery	3	*Vibrio vulnificus*	$2.0\text{x}10^5$
			Vibrio parahaemolyticus	$2.5\text{x}10^5$
ITP Marana, Maros	Nursery	4	*Grimontia hollisae*	$1.5\text{x}10^5$
ITP Marana, Maros	Grow-out	5	*Shwanella algae*	$2.5\text{x}10^4$
ITP Marana, Maros	Grow-out	6	*Staphylococcus cohnii ssp urealyticus*	$1.8\text{x}10^4$
Tambak Masyarakat, Pangkep	Nursery	1	No Identification	$1.1\text{x}10^2$
			Photobacterium damselae	$2.2\text{x}10^5$

Tambak Masyarakat, Pangkep	Nursery	2	*No identification* *Vibrio alginotycus*	$1.7x10^4$ $2.1x10^5$
Tambak Masyarakat, Pangkep	Nursery	3	*Vibrio parahaemolyticus*	$2.4x10^5$
Tambak Masyarakat, Pangkep	Fry (from Bali)	4	*Vibrio campbelii*	$2.2x10^5$

The Kruskal–Wallis test showed no significant differences in bacterial density between the two sites (H = 0.601–0.608; P = 0.436–0.438; $P > 0.05$). Nevertheless, the descriptive analysis indicated wide variability in CFU/mL values across the rearing stages. At site 1 (ITP Marana, Maros), bacterial counts ranged from $1.8x10^4$ CFU/mL in the grow-out stage (*Staphylococcus cohnii ssp. urealyticus)* to $3.40x10^5$ CFU/mL in the fry stage (*Staphylococcus arlattae).* At site 2 (Community ponds, Labakkang, Pangkep), values ranged from very low in the nursery stage (1.1×10^2 CFU/mL unidentified isolate) to very high in the nursery and fry stages (2.46×10^5 CFU/mL *Vibrio parahaemolyticus;* $2.29x10^5$ *Vibrio campbellii*.). This indicates that, although they were not statistically different, ecological and management differences between sites could contribute to the pathogen composition and potential infection risk of the pathogen.

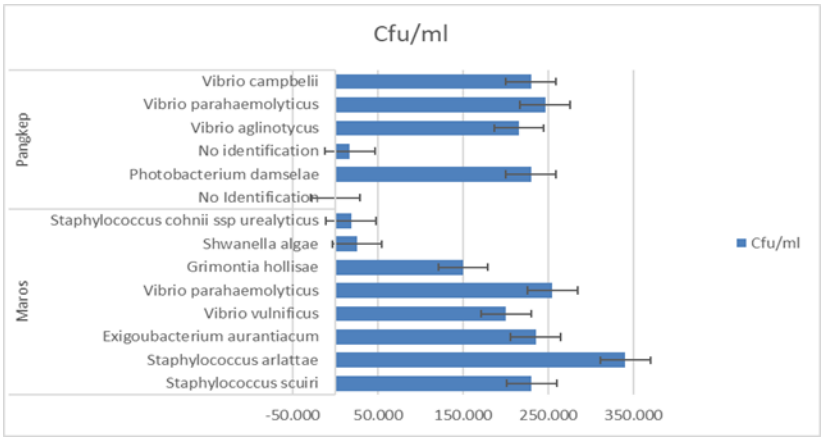

Fig. 4. Bacterial isolates (CFU/ml) identified from milkfish (*Chanos chanos* (Forsskål, 1775))

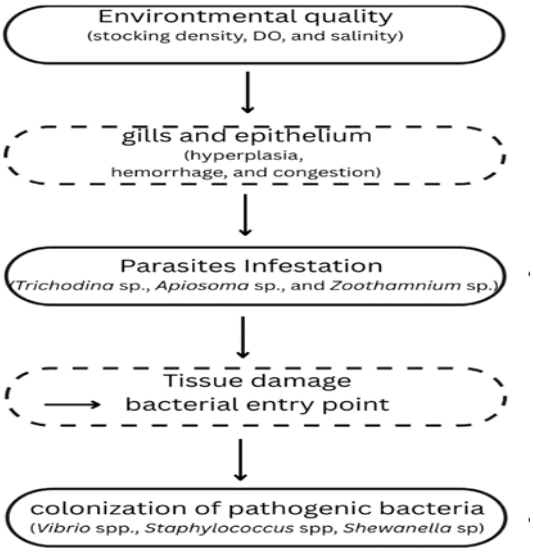

Fig. 5. Proposed schematic pathway of parasite–bacteria interactions in milkfish under aquaculture conditions

Table 7. Bacterial identification with Vitek MS system

No	Isolat code	Species of bacteria	confidence	Isolate report
1	M1 (TSA)	*Staphylococcus scuiri*	99.9%	25009133-1
2	M2 (TSA)	*Staphylococcus arlattae*	99.9%	25009134-1
3	M3 (TCBSA)	*Exigoubacterium aurantiacum*	99.9%	25009131-2
4	M4 (TCBSA)	*Grimontia hollisae*	99.9%	25009132-1
5	M5 (TCBSA)	*Vibrio parahaemolyticus*	99.9%	25014586-1
6	M6 (TCBSA)	*Vibrio vulnificus*	99.9%	25014587-1
7	M7 (TSA)	*Shwanella algae*	99.9%	25014588-1
8	M8 (TSA	*Staphylococcus cohnii ssp urealyticus*	99.9%	25014589-1
9	P1 (TSA)	*No identification*	0%	25019869-2
10	P2 (TSA)	*Photobacterium damselae*	99.9%	25019395-1
11	P3 (TSA)	*No identification*	0%	25019394-1
12	P4 (TCBSA)	*Vibrio alginotycus*	99.9%	25020834-1
13	P5 (TCBSA)	*Vibrio parahaemolyticus*	99.9%	25020833-1
14	P6 (TCBSA)	*Vibrio campbelii*	99.9%	25021418-1

DISCUSSION

Infestation with *Trichodina* sp., *Apiosoma* sp., and *Zoothamnium* sp., which were occasionally observed at low prevalence, caused damage to the gill epithelium, which was associated with hyperplasia of the primary and secondary lamellae and the release of excess mucus. These pathologies interfere with respiration and provide gaps for opportunistic

bacteria, including *Vibrio* spp., resulting in possible mass mortality **(FAO, 2006; Yanong *et al.,* 2025)**. Gill tissue in *Chanos chanos* (Fig. 1) demonstrated secondary lamellar hyperplasia, haemorrhage, and vascular congestion. These structural arrangements create a conducive environment for parasitic infections and support opportunistic bacterial invasion, particularly by *Vibrio* spp. **(Pramanik *et al.,* 2024; Li *et al.,* 2025)**. The same observation was noted by **Sufardin *et al.* (2021a, b)**, who observed lamellar damage in gills co-infected with *Trichodina* sp. and *Aeromonas* spp.

The addition of changes in the gill microbiota and high stocking density as environmental parameters in stressed tissue also enhances the synergism between parasites and bacteria, causing increased damage to the host **(Mahieddine *et al.,* 2025)**. Gill tissue is typically considered the most sensitive tissue in response to aquaculture-associated stress **(Bernet *et al.,* 1999; Roberts, 2012)**. In this study, we also found dominant parasites on the dorsal fins and tails. Biologically, colonization of these non-mucosal organs is often of medical significance because parasites may be transmitted from the body surface to these organs. Colonization of these areas leads to disruption of epithelial integrity, enhanced mucus production, and tissue damage, promoting secondary infections from opportunistic bacterial pathogens, such as *Vibrio* and *Aeromonas* **(Rigos & Katharios, 2010)**. Consequently, while gill observation is important, examination of fins and tails is scientifically justified because it supports the idea of multipathogen interaction in *Chanos chanos* **(Takashima & Hibiya, 1995)**.

Parasite identification in milkfish

The large differences in parasite abundance between the two sites suggest that aquaculture management and environmental quality are key factors driving infection. The lower prevalence at Site 1 (ITP Marana, Maros) (Table 1) represents a controlled water regimen, low stocking density, and regular monitoring of the environmental conditions. In contrast, the high prevalence at Site 2 (community ponds, Pangkep) (Table 2) may be caused by the high stocking density and sedimentary source of organic matter on the pond bottom, which is more beneficial for hyperinfection of parasitic protozoa, such as *Trichodina* in milkfish **(Palma, 2015; Hidayatullah *et al.,* 2020; Atsnani *et al.,* 2023)**.

The differences in target organs (Table 3) suggest partial organ specificity for different parasite species. *Zoothamnium* sp., a sessile ectocommensal ciliate, preferentially infects gills with lamellar structures suitable for attachment **(Lom & Dykova, 1992; Anshary, 2014)**. Gill infections are relatively infrequent but still present significant hazards by obstructing respiration **(Buchmann & Lindenstrøm, 2002)**. In comparison, motile *Trichodina* sp. was relatively more abundant in the tails, as expected for fish-to-fish transmission through direct contact with other fish. *Apiosoma* sp. Dominance in fins and tails can be attributed to mucous-enriched habitats and continuous contact with external substrata. This is in accordance with the findings that ectoparasites preferentially attach to organs with a thick mucosal layer **(Woo & Buchmann, 2012)**. **Kabata (1985)** emphasized

that the external surfaces of fish are protected by mucus, which also acts as a substrate and food source for ectoparasites.

Smaller milkfish were more vulnerable to parasitic infections (Table 4) because of their underdeveloped immune system. In the early stages of development, adaptive immunity has not yet developed; therefore, fish are more prone to infection **(Magnadóttir, 2018)**. In addition, smaller larvae have a larger ratio of surface area to body mass, which provides more opportunities for colonization **(Buchmann & Lindenstrøm, 2002)**. These results are consistent with previous reports from Indonesian hatcheries, where *Trichodina* was the most common protozoan parasite infecting milkfish larvae, with a prevalence of 40–60% **(Ismi *et al.*, 2022)**. In this study, the prevalence of *Trichodina* in Pangkep ponds reached 50.8%, and the highest prevalence occurred in 1– 2cm larvae (26%), which decreased with increasing host size. This reduction indicates improved immune resistance and epithelial integrity in larger fish than in smaller fish. Similar trends have been reported in other farmed species, where larvae or juveniles appear to be more susceptible to infection than adults **(Sitjà-Bobadilla, 2008; Woo & Buchmann, 2012)**. Furthermore, studies conducted in Nigeria have revealed that gastrointestinal parasites are more prevalent among smaller fish than among their larger counterparts, implying that immature life stages are more susceptible to parasitic infections **(Nwani, 2023)**.

Severe *Trichodina* infestations have been reported to cause severe destruction of fish skin and gill epithelia, allowing secondary infections by bacteria and fungi to cause greater stress and higher mortality **(Valladão *et al.*, 2016)**. This parasite is commonly considered an opportunistic ectoparasite that prevails under low water quality and high stocking density, which is usually practiced in traditional pond systems **(Ismi *et al.*, 2022; Sharp, 2025)**. Infections found at Site 2 were of moderate prevalence (24.17%) but with very high intensity (50 parasites per fish), with the fins and tails being the main sites of colonization. These organs are more exposed to the environment and are considered appropriate substrates for attachment **(Lom & Dyková, 1992; Woo & Buchmann, 2012)**. The highest prevalence was found in 1–2 cm larvae (31%) and decreased with fish size, once again demonstrating the higher susceptibility of younger life stages **(Magnadóttir 2018)**.

Although *Zoothamnium* sp. was found to have moderate prevalence at Site 1, its existence is consistent with previous findings **(Yanong *et al.*, 2021; Sri *et al.*, 2022)**, demonstrating that colonial ciliates can still cause severe respiratory epizootics if not controlled. In extreme cases, Zoothamnium sp. infestations have been associated with impaired respiration and high mortality.

In conclusion, the parasite identification results underscore the importance of protozoan ectoparasites as a threat to milkfish during the hatchery and nursery phases of their life cycles. The variation in infection levels among sites and size groups calls for the establishment of random water quality control, suitable stocking densities, and regular parasitological monitoring in hatcheries and grow-out ponds. Such actions are not only

necessary for minimizing mortality but are also essential for the sustainability of milkfish aquaculture in Indonesia.

Bacterial identification

Bacterial isolation and identification revealed site-specific microbial compositions in C. *chanos* larvae (Table 6) in the present study. At Site 1 (ITP Marana, Maros), *Staphylococcus* spp. (*S. sciuri*, *S. arlattae*, and *S. cohnii* ssp. *urealyticus*) were predominant, with relatively high abundance. They are sticky opportunistic bacteria that generally inhabit the skin or mucosa and can provoke secondary infections when fish become stressed or when tissues are injured, for example, due to gill lesions **(Zhang *et al.*, 2022; Wu *et al.*, 2023)**. This finding is consistent with the histopathology of the gills found in this study, in which haemorrhage and hyperplasia were observed, which may have acted as a portal of entry for opportunistic pathogens.

Site 2 (community ponds, Pangkep) contained pathogenic *Vibrio* spp. (*V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*, *V. campbellii*) and *Photobacterium damselae* were dominant. These pathogens are long-standing in marine aquaculture and cause vibriosis and septicaemia, which are frequently accompanied by tissue necrosis and osmoregulatory dysfunction **(Austin & Zhang, 2006; De Schryver & Vadstein, 2014)**. *Photobacterium damselae* is associated with severe septicaemia and significant mortality in marine fish **(Osorio *et al.*, 2018)**.

Grimontia hollisae and *Shewanella algae* were also interestingly isolated at Site 1 (Table 6) in lower numbers. Although not commonly documented in aquaculture, *G. hollisae* has recognised enteropathogenic potential in humans, and *S. algae* has been associated with necrotic lesions in marine fish **(Janda & Abbott, 2012)**. Their identification complicates the microbial community structure and implies their potential function as opportunistic pathogens in stressed habitats. Site differences in bacterial community composition probably represent the different aquaculture management strategies employed. The former, from ponds in the Maros area, were dominated by less pathogenic opportunistic species/taxa, whereas the latter, from ponds in Pangkep, where traditional practices are applied, exhibited heightened virulence patterns for *Vibrio* spp. These trends are consistent with those of previous studies that defined the effects of environmental quality and stocking density on microbial community composition and infection risk **(Hai, 2015)**.

Haemorrhage and vascular congestion in the observed histopathological lesions suggest that gill epithelial damage may predispose larvae to bacterial colonization. A similar crosstalk involving parasitic ciliates and bacterial pathogens has been well described in aquaculture, where disruption of the epithelium, in combination with bacterial toxins, worsens endothelial damage **(Woo & Buchmann, 2012; Sitjà-Bobadilla, 2022; Li *et al.*, 2025)**. The effects of parasitic infections are not limited to physical tissue damage but also include changes in the gut and gill microbiota, which facilitate colonization by

opportunistic bacteria with higher virulence and resistance potential. **Kumar *et al.* (2024)** reported that isolates from parasitically infected fish exhibited strong haemolytic activity, biofilm-forming ability, and resistance to multiple drugs, highlighting the risk of synergistic pathogen interactions in aquaculture systems.

The integration of histopathological and microbiological information not only confirms the disease but also provides insight into the biological aspects of parasite–bacteria- environment interactions in larval milkfish health. These results imply the need for disease control measures based on microbial ecology. Supplementation with probiotics, biofloc technology, and RAS has been proposed to subdue the dominance of pathogenic *Vibrio* spp. by increasing competition with non-pathogenic microorganisms **(Kesarcodi-Watson *et al.*, 2008; De Schryver & Vadstein, 2014)**. The adoption of these measures would present feasible options for farmers to minimize the risk of diseases and enhance the survival of milkfish in the larviculture stage.

Occurrence of parasites and bacteria on rearing stage

The present study showed that the rearing stage significantly affected the dynamics of parasite and bacterial infections in milkfish. The fry and nursery stages had the highest rates and severity of protozoan parasites, particularly *Trichodina* sp. and *Apiosoma* sp. This aligns with the underdeveloped immune response in smaller fish and their higher surface area-to-body mass ratio, which makes it easier for ectoparasites to attach **(Buchmann & Lindenstrøm, 2002; Magnadóttir, 2018)**.

Bacterial patterns followed a similar trend based on the rearing stage. At Site 1 (Maros), parasitic infections were almost absent, with only one nursery-stage case of *Zoothamnium* sp. with a prevalence of 3.3%. However, bacterial isolates were consistently found at all fish stages. The fry stage had high counts of *Staphylococcus arlattae* (3.40×10^5 CFU/mL) and *S. sciuri* (2.30×10^5 CFU/mL), whereas the nursery stage featured *Vibrio vulnificus* and *V. parahaemolyticus*. Even grow-out fish harboured opportunistic bacteria such as *Staphylococcus cohnii* and *Shewanella algae,* despite being free of parasites. This indicates that bacterial colonization can occur in controlled ponds with low parasite pressure and may represent a hidden health risk **(Zhang *et al.*, 2022; Wu *et al.*, 2023)**.

In contrast, Site 2 (Pangkep) fry from Bali exhibited the highest parasite burden (96.7% prevalence; intensity 51.27) and high bacterial counts, dominated by pathogenic *Vibrio campbellii* (2.29×10^5 CFU/mL) and *V. parahaemolyticus* (2.46×10^5 CFU/mL). This double burden illustrates how damage to epithelial disruption caused by parasites in the early stages allows colonization by virulent bacteria. The nursery stage further confirmed this convergence, with parasite prevalence ranging from 8% to 80%, with co-detection of *Photobacterium damselae* and *V. alginolyticus*, both of which are linked to septicaemia in marine fish **(Austin & Zhang, 2006; Osorio *et al.*, 2018)**.

In addition, the introduction of fish fry from external sources, such as Bali, increases the risk of pathogen transmission and disrupts the health of larvae due to transport stress and a lack of prior adaptation to local pond conditions. Previous studies major route for the spread of parasites and bacteria, thereby increasing susceptibility to disease in the early stages **(Arthur & Subasinghe, 2002; Bondad-Reantaso *et al*., 2005)**

Table 8. Comparison of milkfish rearing conditions between ITP Marana, Maros and Labakkang, Pangkep

Aspect	Site 1 (ITP Marana, Maros)	Site 2 (Labakkang, Pangkep)
Seed source	Local (reared in experimental facility)	Fry imported from Bali
Culture system	Controlled ponds, low stocking density, regular water exchange, and routine monitoring.	Traditional ponds, high stocking density, organic matter accumulation
Parasitic infection	Negligible (only 1 case of *Zoothamnium* sp., 3.3% nursery stage)	Very high at fry (96.7%, intensity > 50) and moderate to high at nursery (8-80%) with *Trichodina* sp. and *Apiosoma* sp.
Bacterial infection	Opportunistic bacteria (*Staphylococcus* spp., *Exiguobacterium*, *Shewanella*); present even in absence of significant parasites	Pathogenic bacteria (*Vibrio campbellii*, *V. parahaemolyticus*, *V. alginolyticus*, *Photobacterium damselae*) co-occurring with heavy parasite infestation
Stage specific vulnerability	Fry and grow-out free of major parasites, but opportunistic bacterial colonisation	Fry highly vulnerable (double burden: parasites+bacteria); nursery also affected by multiple pathogens

Taken together, these results demonstrate that the fry and nursery stages are crucial not only for protozoan parasite infestations but also for bacterial colonization. At Site 1, parasites were rare, but bacterial opportunists were present. In contrast, Site 2 had high parasite loads, which increased bacterial invasion. This interaction between parasites and bacteria in the early stages emphasises the need for integrated biosecurity, microbial

monitoring, and strict quarantine of seed stocks, especially when sourced from locations outside Bali during milkfish farming.

CONCLUSION

This study demonstrated distinct patterns of parasitic and bacterial infections in milkfish (*Chanos chanos*) from two aquaculture sites in South Sulawesi, Indonesia. At Site 1 (Milkfish Installation ponds, Maros), parasite prevalence was low, but bacterial diversity was high and was dominated by *Staphylococcus* spp., *Exiguobacterium*, and several *Vibrio species*. Conversely, Site 2 (community ponds, Pangkep) harbored a high prevalence of parasites, especially *Trichodina* sp. and *Apiosoma* sp., and an increase in the severity of pathogenic bacteria, such as *Photobacterium damselae* and *Vibrio* spp. This study also highlighted that fry and nursery stages were the most vulnerable, whereas the grow-out stages were less affected.

These results support the evidence that parasitic infections may reduce gill epithelial integrity, allowing easy entry of bacteria and enhancing the mortality risk in milkfish. This study provides useful baseline information on multi-pathogen relationships in milkfish aquaculture and highlights the pressing demand for the implementation of an integrated biosecurity and aquaculture health system.

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Author contributions

Mutiyah Amalia Rachmat collected and processed fish samples, analyzed data, and prepared the manuscript. Hilal Anshary designed and conceptualized the research project and contributed to data interpretation and revisions during manuscript preparation. Sriwulan also contributed to data interpretation and revisions during manuscript preparation. All authors have read and approved the final manuscript.

Ethical Statement

This study was conducted with formal approval from the Health Research Ethics Committee of Hasanuddin University in accordance with the relevant protocol for the use of experimental animals (Protocol No. 14725092164).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this study.

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