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ORIGINAL ARTICLE

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Prevalence, intensity and histopathology of *Zeylanicobdella arugamensis* infestation on groupers reared on different aquaculture systems

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Abstract

The marine leech, *Zeylanicobdella arugamensis*, is a major threat to aquaculture in grouper-producing countries including Indonesia. This study aimed at investigating prevalence, intensity and histopathology of the ectoparasite in humpback and hybrid groupers cultured in different rearing systems. A total of 260 groupers (60 humpback groupers and 200 hybrid groupers) were used for samples. The marine leech was observed on skin, fins, gills and mouth, followed by histopathological assay on the skin tissue. The results showed that prevalence of the leech in both groupers was higher when they were cultured in the floating net cages compared with the hatchery, $p < .05$. Furthermore, humpback grouper had a higher prevalence than hybrid grouper when they were cultured in a similar system, $p < .05$. Meanwhile, there was no significant difference in intensity between the two groupers, $p > .05$. Within the hybrid groupers, the highest prevalence was obtained from hybrid groupers reared in the earthen ponds. Histopathological studies showed that the infected groupers exhibited inflammation, congestion and erosion of the epidermis layer. Hybrid grouper had more severe histopathological lesions in the skin tissues. These results suggested that species and type of aquaculture system had significantly determined the prevalence, intensity and severity of lesion in *Z. arugamensis* infestation.

KEYWORDS
fish disease, grouper, histopathology, intensity, prevalence

1 | INTRODUCTION

Indonesia is the third largest grouper-producing country in the world after China and Taiwan (Rimmer & Glamuzina, 2019; Session, 2017). The total Indonesian production of groupers in 2015 was recorded at 8,972 tons, 15,089 tons in 2016 and increased significantly to 605.132 tons in 2018 (BKIPM, 2018). The most common species of groupers which are cultured by Indonesian farmers are humpback grouper (*Cromileptes altivelis*) and hybrid "Cantang" grouper (*Epinephelus* sp.). Among the cultured species, humpback grouper is the most expensive, ~IDR 450,000/kg or ~US$ 45, much higher than the hybrid grouper, ~IDR 85,000/kg or ~US$ 8. However, even though cheaper, the hybrid grouper is cultured by many fish farmers due to faster growth, required 8 months to reach marketable size (~400–500 g) compared with humpback grouper which needs at least 20 months (De, Ghaffar, & Das, 2014). However, the development of Indonesian grouper aquaculture has nowadays been hampered by diseases including *Zeylanicobdella arugamensis* infestation.

Zeylanicobdella arugamensis is a marine leech which belongs to the phylum Annelida and Family of Piscicolidae (De Silva, 1963).

The pathogenicity of *Z. arugamensis* has been previously reported in several studies. Sommerville (1998), for instance, reported that a severe infestation intensity of ectoparasites in grouper resulted in acute death. Similarly, a study by Kua (2008) confirmed that fish infested by the ectoparasite died within 3 days following infestation due to secondary infections with pathogenic bacteria such as *Vibrio algnolyticus*. According to Cruz-Lacierda, Toledo, Tan-Fermin, and Burreson (2000), the parasite has been commonly found in Indonesia. However, the first case of *Z. arugamensis* infestation was reported in detail in 2008 by Slamet et al. (2008) in several species including brown-marbled grouper (*Epinephelus fuscoguttatus*), green grouper (*Epinephelus suillus*), humpback grouper (*C. altivelis*), coral grouper (*Epinephelus corallicola*), seabass (*Lates calcarifer*) and snapper (*Lates argentinaculatus*). The next study was reported by Mahardika, Mastuti, Sudewi, and Zafran (2018) infecting hybrid grouper (*Epinephelus fuscogutattus*♀ × *Epinephelus lanceolatus*♂) in Gondol, Bali Island (Mahasri, Wulansari, & Imani, 2019), in Situbondo, East Java, Indonesia. The above studies did not provide data on differences in pathogenicity in the different species of groupers, nor did they examine the influence of rearing conditions on the prevalence and intensity of infestation. Thus, more studies that cover broader topics are still required.

This present study was conducted to investigate the prevalence and infestation intensity of *Z. arugamensis* on the two most common groupers species cultured in Indonesia, hybrid grouper and humpback grouper. In addition, the effect of different aquaculture systems (hatchery, floating net cage, concrete pond and earthen pond) was also studied. Furthermore, histopathology of *Z. arugamensis* in skin tissues of infested groupers was further analysed.

2 | MATERIALS AND METHODS

2.1 | Study area

The present study was carried out at the brackish water Aquaculture Development Center (BBAP), Situbondo, East Java, Indonesia (Figure 1). Four aquaculture systems were chosen for collecting samples which were hatchery, floating net cage, concrete pond and earthen pond. Prevalence and intensity were examined in situ while the characterization and histopathology assay were carried out at the Laboratory of Anatomy and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga Surabaya, East Java, Indonesia, from September to December 2019.

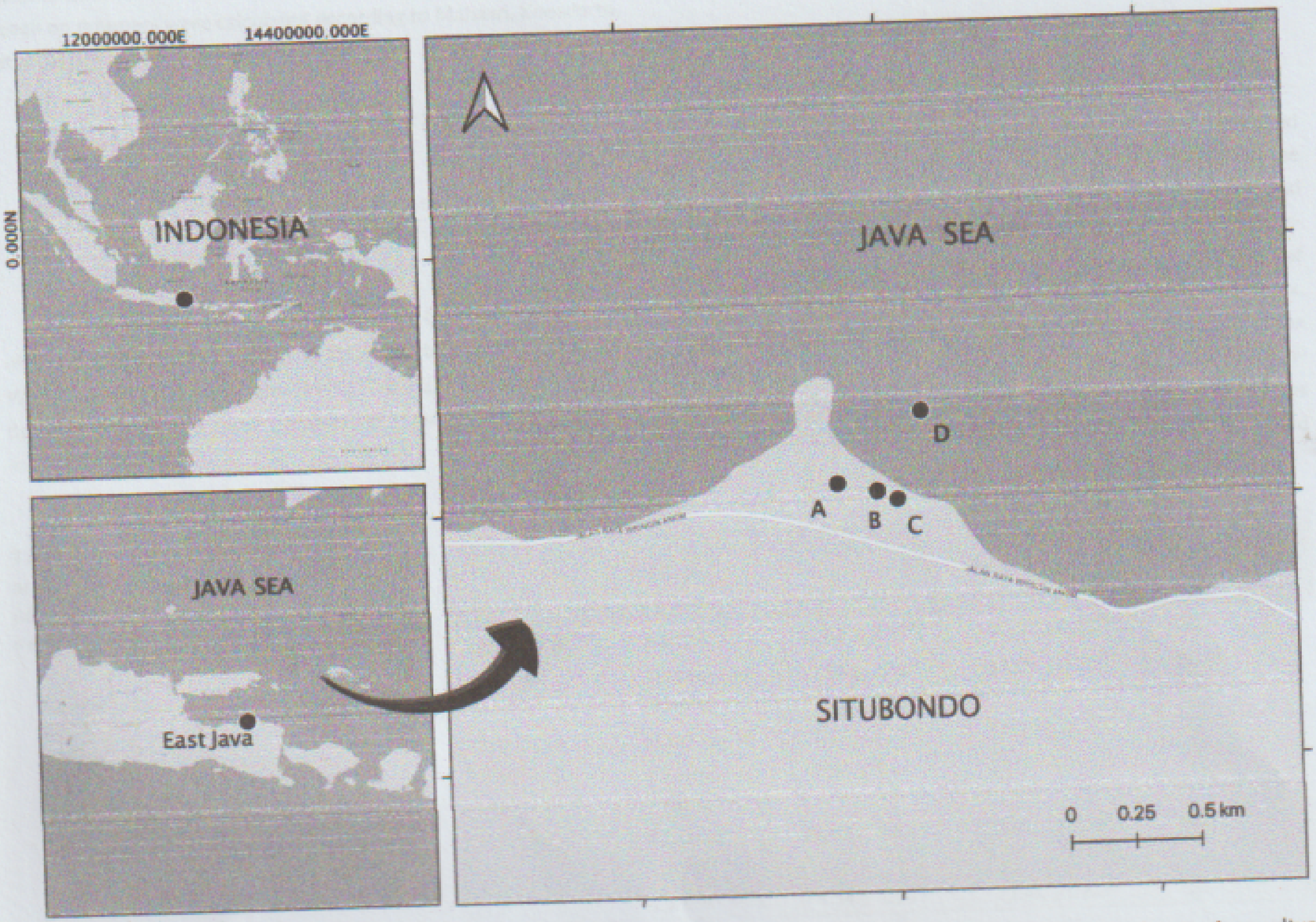

FIGURE 1 Sampling locations: (a) Earthen pond, (b) concrete pond, (c) hatchery and (d) floating net cage in the Brackishwater Aquaculture Development Center, Situbondo, East Java, Indonesia

2.2 | Fish samples

A total of 260 groupers consisting of 60 humpback groupers and 200 hybrid groupers (*Epinephelus* sp.) with 17–23 g wet weight or 10–13 cm length were collected from four locations at Brackishwater Aquaculture Development Center Situbondo, East Java, Indonesia (Table 1). Each fish was collected randomly using a scope net and placed on a bucket containing sea water and brought to the laboratory for examination.

2.3 | Examination of marine leech

The examination of marine leech on the fish samples was performed by a native method according to Noga (2010). In brief, marine leech was examined on skin, fins, gills and mouth directly and followed by scraping by gently scraping along the side of the body, fins with a scalpel, while gills were clipped. The result of scraping was placed on an object glass and added a drop of physiological buffer solution (PBS). The scraping results were observed using a binocular microscope with a magnification of 40x and 100x. Marine leeches found in this stage were collected, counted and preserved in 5% glycerine alcohol until further uses. Prevalence and intensity of the marine leech on groupers were calculated according to Mahasri, Koesdarto, et al. (2019) with the following formula:

$$\text{Prevalence} = \frac{\sum \text{infected fish}}{\sum \text{fish samples}} \times 100\%,$$

$$\text{Intensity} = \frac{\sum \text{marine leeches}}{\sum \text{infected fish}}.$$

In addition, the present study determined the level of infestation of the marine parasite in each fish according to a category made by Williams and Bunkley-Williams (1996) (Table 2). The level of infestation was defined as the average number of marine leeches found in every fish sample.

2.4 | Staining procedure and identification

The marine leeches were stained using Semichon's acetic carmine according to the protocol of Ngamniyom, Manaboon, and Panyarachun (2012). Briefly, the marine leech in 5% glycerine alcohol was washed with piperazine (PZ) solution. Then, the leech was tied and flattened between two slides, followed by immersion in 70% alcohol for 2 min. After that, the leech was immersed in a carmine solution for 8 hr. The next step was transferring the leech into the acidic alcohol solution (70% alcohol + HCl) for 2 min and moved into an alkaline alcohol solution (alcohol + $NaHCO_3$) for 20 min; thereafter, gradual dehydration was performed by putting in serial alcohol solutions: 70% alcohol for 5 min, 85% alcohol for 5 min and 95% alcohol for 5 min. Then, the leech was mounted in Hung's I solution for 20 min and placed on a clean glass object and drops of Hung's II solution and covered by a glass cover. The leech was afterwards observed under a binocular microscope equipped with a Lucida camera for identification purposes.

The identification of marine leech was performed based on its morphology and morphometric measurement results which were later compared with the parasitic identification key book and journal by Kabata (1985).

2.5 | Histopathology

The histopathological assay was carried out according to a protocol of Muntiha (2001), with slight modifications. In brief, 5 infected fish from each grouper and rearing system were killed and tissue specimens of skin were cut into small pieces of 3 mm thickness and fixed in 10% buffered neutral formalin (BNF) for 24 hr. The fixed tissues were rinsed in tap water, dehydrated through a graded series of ethanol, infiltrated with xylene and then embedded in paraffin wax. Five-micron-thick sections were cut in a rotary microtome from the tissue blocks and mounted on glass slides. The sections were deparaffinized in xylene, rehydrated through decreasing concentrations of ethanol, stained with haematoxylin and eosin (HE) and examined

TABLE 1 Fish size (total wet weight and total length) and total samples collected from four different aquaculture systems

Species	Rearing system	Fish size: WW ± SD(g)	Fish size: TL ± SD (cm)	Number of samples (fish)
Humpback grouper	Hatchery	17.64 ± 3.49^{a}	$11.63 \pm 0.85^{A,B}$	30
	Floating net cages	17.84 ± 2.66^{a}	11.29 ± 0.77^{A}	30
Hybrid grouper (Cantang)	Hatchery	17.29 ± 2.59^{a}	11.09 ± 0.67^{A}	50
	Floating net cages	17.34 ± 1.64^{a}	11.38 ± 0.43^{A}	50
	Concrete pond	21.86 ± 3.64^{b}	12.33 ± 0.41^{B}	50
	Earthen pond	$18.54 \pm 1.94^{a,b}$	11.41 ± 0.63^{A}	50

Abbreviations: SD, standard deviation; TL, total length; WW, wet weight.
Different superscripts indicate that the values are significantly different at $p < .05$.

TABLE 2 Level of infestation

Level of infestation (parasites)	Categories
<1	Very light
1–5	Light
6–10	Moderate
>10–50	Medium
51–100	Heavy
>100–999	Very heavy
>1,000	Superinfection

Note: Sumber: Williams and Bunkley-Williams (1996)

under a light microscope. Histopathological changes including types of lesions such as erosion of epidermis layer, inflammation and congestion were examined from the fish samples, and then, the severity of lesion was determined as light, medium and heavy. The severity of lesion was considered "light" when the type of lesion was found in less than 30% of infected fish, moderate 30%–70% of infected fish and heavy when the type of lesion was found in more than 70% of occurrence.

2.6 | Water quality measurements

Water quality measurements were carried out every week for three weeks at the hatchery, floating cage net, concrete pond and earthen ponds. The water quality parameters were measured using a thermometer for temperature, DO meter (Lutron DO-55509) to measure dissolved oxygen, a pH meter (pHhep, HANNA) to measure pH, a digital refractometer (Benchtop) to measure salinity and an ammonia test kit (Redwood Aquatic, API) to measure ammonia level.

2.7 | Data analysis

Data on the prevalence and infestation intensity of *Z. arugamensis* were compared between species and aquaculture systems using multivariate analysis of variance (MANOVA) with statistical software (SPSS version 22) after three assumptions (normal distribution, equal variance and independent) were fulfilled. However, histopathological data were analysed descriptively by comparing the type and severity of lesions on the infected skin sections as well as the percentage of fish affected by similar types of lesions.

3 | RESULTS

3.1 | Prevalence and intensity

The present study consisted of two studies which were (a) a comparison of prevalence and intensity of marine leech in two-grouper species (humpback grouper and hybrid grouper) which were cultured in similar rearing system (hatchery and floating net cages), and (b) a comparison of prevalence and intensity of marine leech within hybrid grouper cultured in four different rearing systems (hatchery, floating net cage, concrete pond and earthen pond).

The first result showed that the prevalence of marine leech in the humpback grouper was significantly higher than that in the hybrid grouper when they were cultured in similar types of aquaculture rearing systems (floating net cage or hatchery) ($F = 5.75$, df 1,14, $p = .03$). Cultured in hatcheries, the mean prevalence of the marine leech was 16.67% for the humpback grouper and zero prevalence for hybrid grouper. While cultured in floating net cages, the prevalence was 53.33% for humpback grouper and 10% for the hybrid grouper (Table 3). Furthermore, the prevalences of the marine leech were significantly higher when both fish were cultured in the floating net cages than when the fish were cultured in the hatchery ($t = 2.851$, df 10, $p = .017$). The second comparison indicated that hybrid groupers reared in the earthen pond had the highest prevalence followed by those hybrid groupers reared in concrete ponds and floating net cage, and the lowest was in the hatchery ($F = 346.41$, df 3,8, $p < .01$). As presented in Table 3, the prevalence was 100% in the earthen ponds, 31.11% in the concrete ponds, 10% in the floating net cages and 0% in the hatchery. The result also indicated that the marine leeches were mostly attached to pectoral, ventral, anal and caudal fins in both farms (Figure 2). Large numbers of the leeches were also found in the skin folds behind the lower jaw, under the operculum, eyes and inside the mouth.

A similar pattern was obtained in the infestation intensity. As presented in Table 3, the infestation intensity was not significantly different between the two-grouper species when they were cultured in

TABLE 3 Prevalence and intensity of marine leech, *Zeylanicobdella arugamensis*, on humpback grouper and hybrid grouper reared in the hatchery, floating net cages, concrete ponds and earthen ponds

Fish species	Aquaculture systems	Prevalence (%)	Intensity (leeches/fish)
Humpback grouper	Hatchery	$16.67 \pm 5.77^{a,b}$	1.67 ± 0.29^{B}
	Floating net cage	$53.33 \pm 25.82^{a,b}$	5.87 ± 4.37^{B}
Hybrid grouper (Cantang)	Hatchery	0.00 ± 0.00^{a}	0.00 ± 0.00^{A}
	Floating net cage	10.00 ± 3.33^{a}	4.83 ± 1.61^{B}
	Concrete pond	$31.11 \pm 7.69^{a,b}$	3.79 ± 0.51^{B}
	Earthen pond	100.00 ± 0.00^{c}	14.05 ± 1.71^{C}

Note: $^{(a, b, c), (A,B,C)}$Superscript in common represents that the values are no significantly different, $p > .05$.

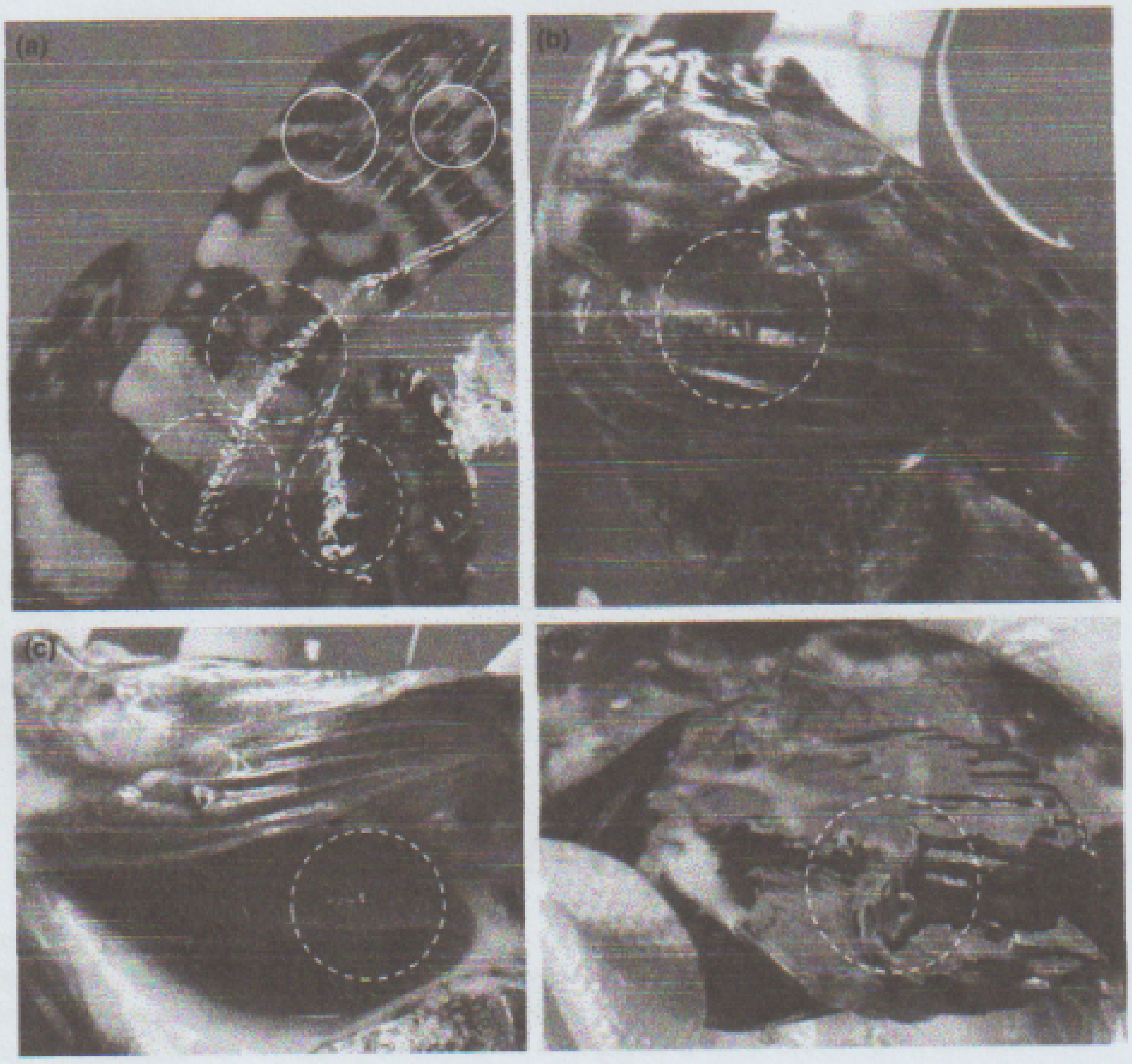

FIGURE 2 Marine leech, *Zeylanicobdella arugamensis*, attached to caudal fin (a), gill (b, c) and pectoral fin (d)

a similar type of rearing system ($F = 3.11$, df 3, 11, $p = .71$). However, infestation intensity was significantly higher in those hybrid groupers when the culture is in the earthen pond compared with the hatchery, floating net cage or concrete ponds ($F = 73.98$, df 3, 8, $p < .01$). The highest infestation rate was 14, 4, 5 and 0 for the earthen pond, concrete pond, floating net cage and hatchery, respectively. For humpback grouper, the values ranged from 1 to 2 (hatchery) and 3 to 11 leeches/fish in the floating net cages. While hybrid grouper ranged from 5 to 6 (floating net cage), 1 to 6 (concrete ponds) and 3 to 25 in earthen ponds.

The morphological characteristics of the marine leech viewed under a light microscope with 400x magnification were as follows:

- Colour: appeared to dark-brown colour
- Body form: elongated and cylindrical body narrowing at both ends with oral and caudal suckers, lines with lighter colours on the dorsal and deep black on the ventral.
- Body size: Total length ranged from ~6.6 mm including both anterior and posterior suckers, the area and body width 3.6 mm, the oral sucker up to 1.9 mm in diameter and having a pair of eyes located on the dorsal surface the anterior or oral sucker. While posterior sucker was generally wider than the oral sucker, up to 2.85 mm in diameter

Based on these morphological characteristics, the marine leech was identified as *Z. arugamensis*. The morphological appearance of *Z. arugamensis* live and preserved specimens isolated from the present study is presented in Figure 3.

The infected fish were also showed several abnormalities including in behaviour or anatomical features. The infected fish for instance were swimming slowly, mostly found on the near of aeration, less appetite and often rubbing the body on the surface of pond walls or the net in the floating net cage. The fish skin also appeared to be darker especially around the attachment area of the Zeylanicobdella. Other results showed that the attachment and feeding sites of the *Z. arugamensis* exhibited, haemorrhages, frayed fins and swelling around both anterior and posterior sucker areas on the host's skin (Figure 4). Lesions characterized by the presence of white ulcers on the skin surface were observed on the attachment site of Zeylanicobdella.

3.2 | Histopathology

There were several histopathological changes in the skin tissues of infected fish including erosion of the epidermal layer, inflammation and congestion. Among the lesion types, the epidermis layer was the worse type being considered as heavy due to more than 70% occurrence (Table 4). Upon excision, skin tissues of infected fish showed that the infected fish was partial loss of the epithelium and disintegrate due to the ectoparasite attachment. While the two other lesion types (inflammation and congestion) were considered moderate and light due to the occurrence of <70% and 30%, respectively. The inflammation or inflammatory cells in the skin tissue due to ectoparasitic infestation were indicated by dilation of local blood vessels resulting in excessive local blood flow marked by leucocytes or white blood cells. Furthermore, the other

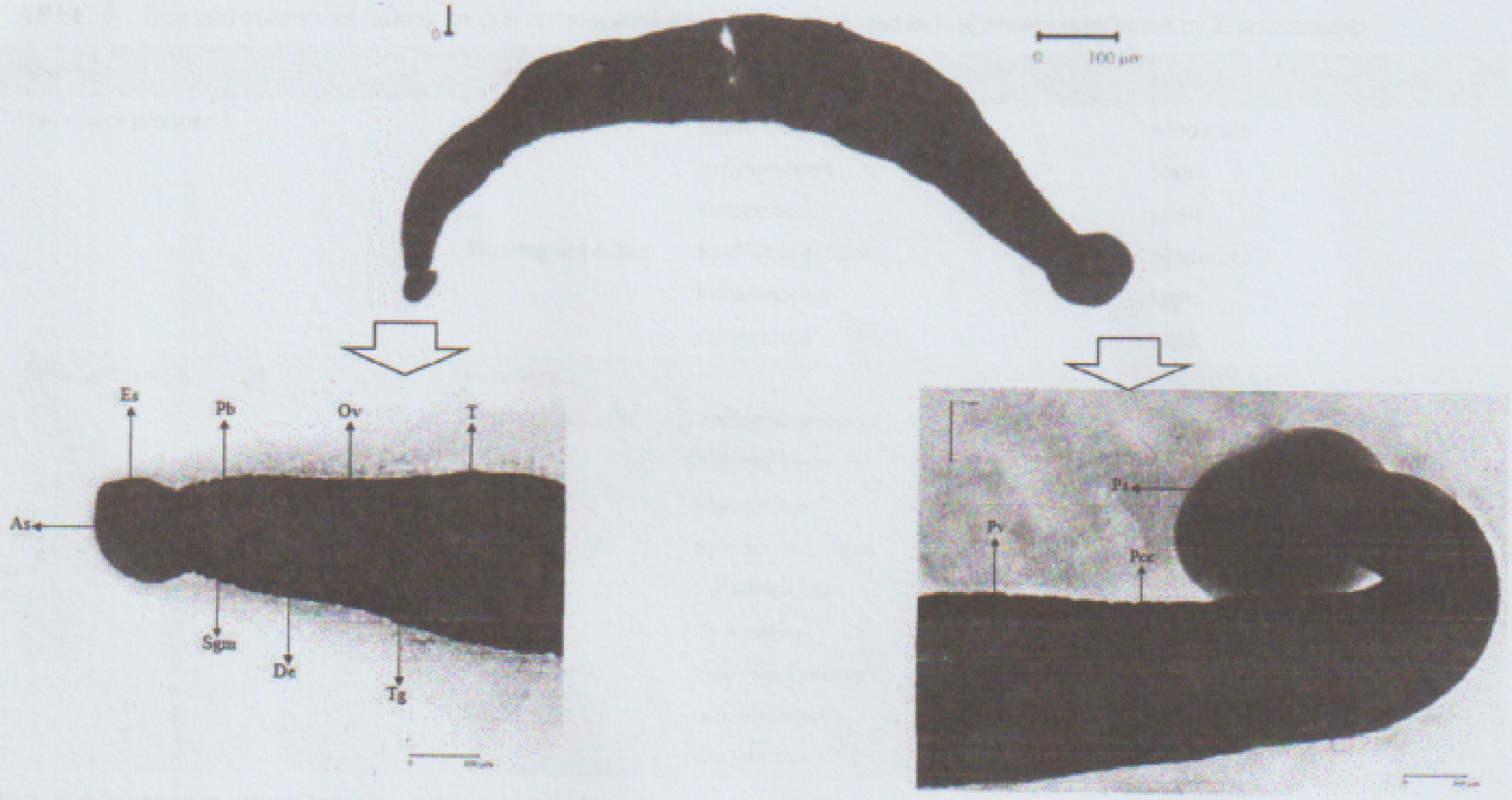

FIGURE 3 Morphology of *Zeylanicobdella arugamensis* viewed under a light microscope with 40× magnification after staining with carmine. Description: (1) anterior sucker, (2) posterior sucker. Anterior and posterior parts viewed under a binocular microscope with 100× magnification. (a) Anterior part. Anterior sucker (As), eyespot (Es), suboesophageal ganglionic mass (Sgm), proboscyst (Pb), testis (T), ductus jaculatorius (De), testicular ganglion (Tg) and ovary (Ov). (b) Posterior part. Pulsatile vesicle (Pv), posterior crop caecum (Pcc) and posterior sucker (Ps)

abnormality on the skin section of infested fish was congestion, indicated by the increasing number of eosinophil granule cells due to blood comes out of blood vessels. The histopathological data also showed that the level of skin tissue damages in infected fish showed that the infestation of *Z. arugamensis* had a more severe histopathological impact on epidermis erosion and congestion on the hybrid grouper compared with the humpback grouper. In addition, the most severe effects of the *Z. arugamensis* infestation were observed from skin tissue of fish reared in the earthen and concrete ponds (Table 4).

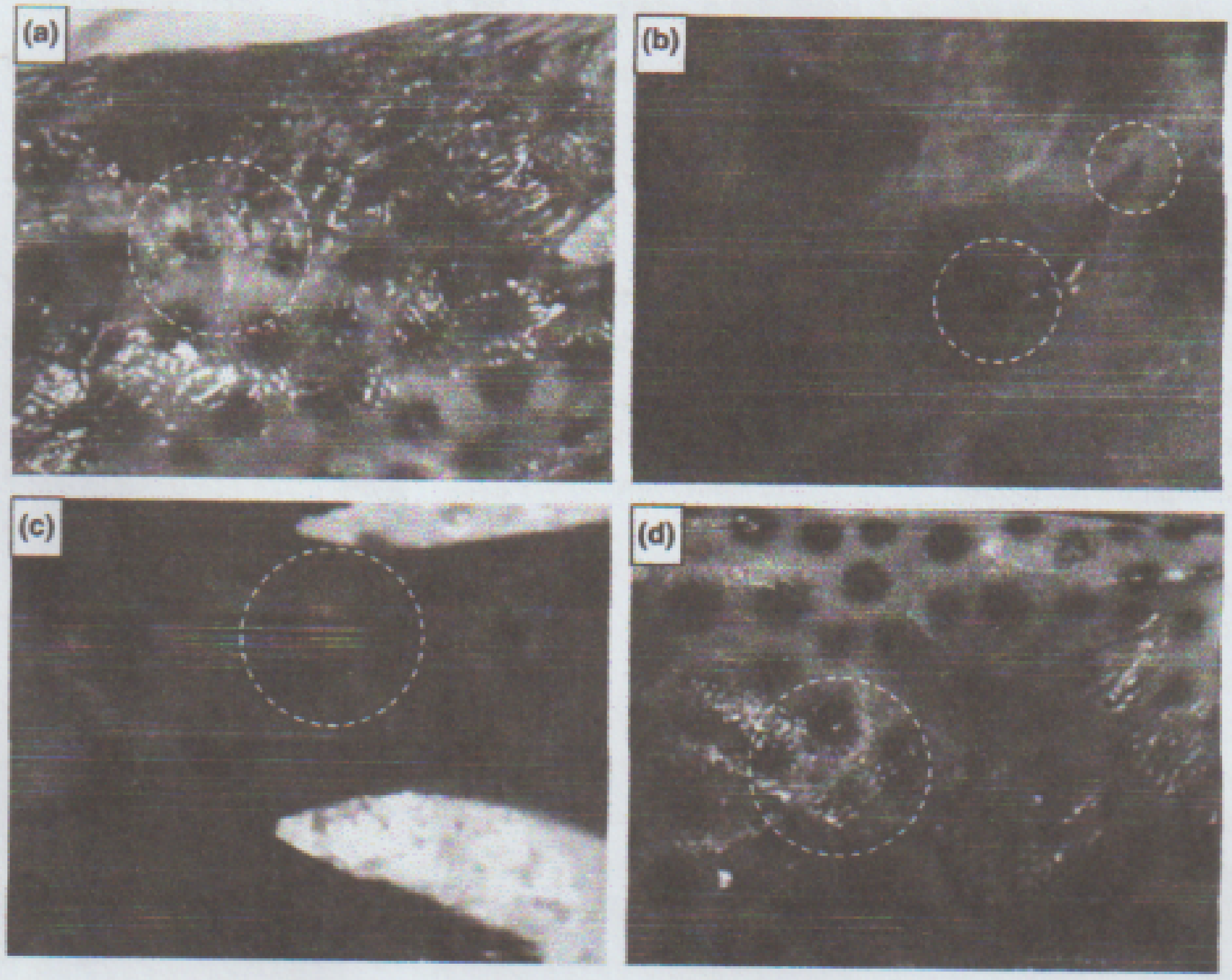

FIGURE 4 Frayed fins, haemorrhages and swollen areas of fish skin infected by *Z. arugamensis*. (a) haemorrhages of fin base; (b) pale colour and haemorrhages; (c) swelling and reddish; and (d) swelling and pace colour on fish skin

TABLE 4 Type and severity of lesions on skin histology section of humpback and hybrid groupers infected by *Z. arugamensis*

Species	Rearing system	Type of lesions	Severity
Humpback grouper	Hatchery	Epidermis erosion	Moderate
		Inflammation	Light
		Congestion	Light
	Floating net cages	Epidermis erosion,	Moderate
		Inflammation	Light
		Congestion	Light
Hybrid grouper (Cantang)	Hatchery	-	-
	Floating net cages	Epidermis erosion	Heavy
		Inflammation	Light
		Congestion	Moderate
	Concrete pond	Epidermis erosion	Heavy
		Inflammation	Light
		Congestion	Moderate
	Earthen pond	Epidermis erosion	Heavy
		Inflammation	Light
		Congestion	Moderate

Note: Five fish samples were taken from each fish and rearing groups. Light: <30% occurrence in fish samples, moderate: 30%–70% occurrence and heavy: >70% occurrence.

TABLE 5 Temperature, salinity, pH dissolved oxygen (DO) and ammonia levels in rearing water of groupers reared in the hatchery, concrete ponds, earthen pond and floating net cages

	Humpback grouper		Hybrid grouper			
Parameters	Hatchery	Floating net cage	Hatchery	Floating net cage	Concrete pond	Earthen pond
Temperature (°C)	27.07 ± 3.72^a	32.00 ± 1.00^a	27.67 ± 3.06^a	29.93 ± 1.20^a	28.67 ± 0.58^a	29.90 ± 1.15^a
Salinity (ppt)	31.33 ± 1.53^A	30.67 ± 0.58^A	32.33 ± 2.52^A	33.67 ± 1.53^A	32.00 ± 2.65^A	31.67 ± 2.52^A
pH	7.43 ± 0.40^x	8.00 ± 0.20^x	7.57 ± 0.51^x	7.97 ± 0.15^x	7.53 ± 0.45^x	7.07 ± 0.12^x
DO (mg/L)	5.65 ± 1.55^X	4.73 ± 0.31^X	5.62 ± 0.46^X	4.59 ± 1.47^X	3.83 ± 0.46^X	5.13 ± 1.94^X
Ammonia (mg/L)	2.75 ± 1.39^z	1.50 ± 0.50^z	2.58 ± 1.66^z	0.58 ± 0.14^z	2.00 ± 1.32^z	0.77 ± 0.12^z

Note: Values are mean with three measurement replicates. Common superscripts indicate that the mean values are not significantly different, $p > .05$.

3.3 | Water quality

Water quality measurements showed that temperature, dissolved oxygen (DO), pH, level of ammonia and salinity were not significantly different among the rearing waters, $p_{values} > .05$. In general, the measurement had 27°C–32°C water temperature, 30–33 ppt salinity, 7–8 pH, 3–6 mg/L DO, 0.5–3 mg/L ammonia (Table 5).

4 | DISCUSSIONS

The present study reported prevalence, intensity and histopathology of *Z. arugamensis* infestation in humpback and hybrid groupers cultured in a hatchery, floating net cages, concrete ponds and earthen ponds in Situbondo, East Java, Indonesia. Based on its morphological characteristics, the marine leech was identified as *Z. arugamensis* (Cruz-Lacierda et al., 2000; Mahardika, Mastuti, Sudewi, et al., 2018; Murwantoko, Negoro, Isnansetyo, & Zafran, 2018; Nagasawa & Uyeno, 2009; Palm, Yulianto, Theisen, Rueckert, & Kleinertz, 2015). In Indonesia, the similar marine leech had been identified using a molecular method based on nucleotides sequence of mitochondrial cytochrome oxidase subunit I (COI) and confirmed that the leech as *Z. arugamensis* (Murwantoko et al., 2018). Based on these identification results, we could also confirm that the marine leech infecting groupers in the present study was *Z. arugamensis*. The marine ectoparasite was mostly found attached to the body surface, eyes, mouth, ventral fins and dorsal fins. These results were very similar with previous studies in the same species (Azmey, Taruna, Taha, & Arai, 2020; Jamaris, Roza, & Mahardika, 2019; Mahardika, Mastuti, Sudewi, et al., 2018; Murwantoko et al., 2018) or different fish species (Azuar, Raza'i, & Miranti, 2019; Nagasawa, Shimadzu, & Ikeda, 2012; Nagasawa & Uyeno, 2009; Ravi & Shariman Yahaya, 2017). Accordingly, a study by Cruz-Lacierda et al. (2000) also showed that *Z. arugamensis* mostly found attached on fins (dorsal, which then causes leucocyte cell migration. In addition, another damage of skin tissue due to the ectoparasite infestation was in the form of congestion, the occurrence of blood damages caused by circulatory disorders which can result in a lack of oxygen and nutrients (Nabib & Maidie, 1987). Congestion is generally preceded by a swelling of the cell where the cell will enlarge so that the blood flow is disrupted; this causes blood damages in several places. According to Flik and Wiegertjes (2005), congestion is an abundance of blood in the area of certain blood vessels. Congestion is generally followed by an increase in the number of eosinophil granule cells. Ressang (1984) added that congestion is the blocking of blood caused by circulatory disorders which can result in a lack of oxygen and nutrients. The cell swelling might be caused by an increase in cell permeability, where cells are unable to maintain ionic homeostasis and fluid resulting in the transfer of extracellular fluid into the cell (Rizgalla et al., 2016).

Many papers have described a strong correlation between the quality parameters of rearing water and parasite infestation in aquaculture industries including temperature (Jansen & Bakke, 1991; Karvonen et al., 2013; Möller, 1978; Scott & Nokes, 2009; Strepparava et al., 2018), salinity (Cheung, Nigrelli, & Ruggieri, 1979; Ernst, Whittington, Corneillie, & Talbot, 2005; Möller, 1978; Rogowski & Stockwell, 2006; Soleng & Bakke, 1997), dissolved oxygen (Mikheev, Pasternak, Valtonen, & Taskinen, 2014; Molnar, 1994; Yoshinaga, 2001), pH and ammonia (Banu, Khan, & Thulin, 2004). However, to the authors' knowledge, publications concerning the correlation of *Z. arugamensis* infestation on groupers are still very limited, only a correlation with temperature (Kua et al., 2010, 2014; Mastuti & Zarman, 2018). Thus, many studies are still required to describe deeper on *Z. arugamensis* infestation on groupers.

The five parameters of water quality in the sampling locations fell within the desirable and acceptable limits for the culture of groupers except for temperature and ammonia (Table 7). The water temperature in hybrid grouper fluctuated within a minimum of 25°C and a maximum of 27°C, which is slightly lower than the optimal temperature for the fish, 28°C–32°C (Mursitorini & Ramdhani, 2013; SNI, 2011). At the temperature of 28°C–32°C, *Z. arugamensis* took 2 weeks to grow to a mature adult and start producing cocoons (Kua et al., 2010). Furthermore, Kua et al. (2014) reported that the best temperature for the cocoons to hatch was 27°C, and having the highest survival rate in juvenile and adult stages was recorded at 25°C. This factor might explain why the prevalence and intensity of *Z. arugamensis* were higher in hybrid grouper cultured in the hatchery and earthen ponds, with a water temperature of 25°C–27°C.

While ammonia level obtained in the present study ranged from 0.5 to 5 mg/L, and according to (SNI, 2011), the value was higher than tolerance levels for grouper, 0.01 mg/L. These water conditions might trigger the emergence of ectoparasite helminth (Fauzi, Mokoginta, & Yaniharto, 2008). To the authors' knowledge, there were no papers that documented the effect of ammonia concentration in raring water on the prevalence of infestation of *Z. arugamensis*. However, the high level of ammonia could suppress the immune system of groupers. A study by Schuwerack, Lewis, Hoole, and Morley (2002) confirmed that several types of leucocytes were decreased when the fish was exposed to ammonia at a concentration of 0.5 mg/L. Accordingly, Ojwala, Otachi, and Kitaka (2018) concluded that the exposure of fish to a high level of ammonia (>0.05) induced cellular and immunological changes in fish which later highly determined their vulnerability to parasite infestation. Thus, better water quality management is highly recommended for decreasing parasite infestation in grouper cultures.

5 | CONCLUSIONS

The present study demonstrated the infestation of humpback and hybrid groupers cultured in floating net cages, concrete pond, earthen pond and hatchery in Situbondo, East Java, Indonesia. The prevalence and infestation intensity of *Z. arugamensis* on humpback grouper and hybrid grouper were higher when they were cultured in floating net cages than in hatcheries. Another result showed also that hybrid groupers reared in the earthen pond had the highest prevalence and intensity of *Z. arugamensis* compared with those groupers reared in the hatchery, floating net cage or concrete ponds. Most of the marine leech was abundantly distributed in fins, gills and mouth. The marine leech was mostly found attached to fins, gills and mouth. These results may suggest hybrid grouper was more vulnerable to the marine leech infestation, especially when they were cultured in earthen ponds.

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CONFLICT OF INTEREST

The authors declare that there was no conflict of interest.

TABLE 7 Temperature, salinity, pH dissolved oxygen (DO) and ammonia levels in rearing water of groupers reared in the hatchery, ponds and floating net cages

References	Temperature (°C)	Salinity (ppt)	pH	DO (mg/L)	Ammonia (mg/L)
SNI (2011)	28–32	28–33	7.5–8.5	≥5	≤0.01
Mursitorini and Ramdhani (2013)	27–31	≥5	7.8–8.2	30–33	≤1.0

Abbreviations: CP, concrete ponds; EP, earthen pond; FNC, floating net cage; HbG, hybrid grouper; Hc, hatchery; HG, humpback grouper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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