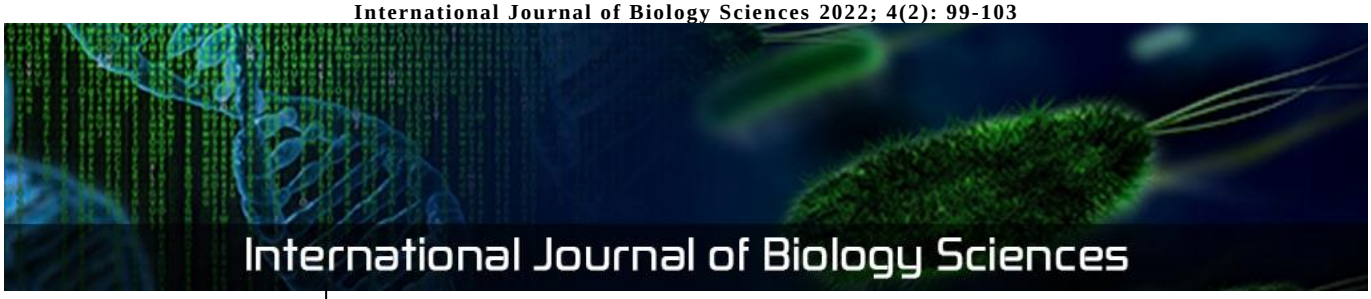

ISSN Print: 2664-9926
ISSN Online: 2664-9934
Impact Factor: RJIF 5.45
IJBS 2022; 4(2): 99-103
www.biologyjournal.net
Received: 20-05-2022
Accepted: 21-06-2022

Ririn Agustiya
Aquaculture Study Program, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, Indonesia

Gunanti Mahasri
Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, Indonesia

Sri Subekti
Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, Indonesia

Corresponding Author:
Sri Subekti
Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, Indonesia

Identification of morphometric and intensity of marine leech *Zeylanicobdella* infestation on cantang grouper from Brondong district, Lamongan and Mandangin Island, Madura

Ririn Agustiya, Gunanti Mahasri and Sri Subekti

DOI: https://doi.org/10.33545/26649926.2022.v4.i2b.83

Abstract
Cantang grouper (*Epinephelus fuscoguttatus lanceolatus*) is the result of hybridization between female tiger grouper and male grouper fish which is widely cultivated because it has fast growth, easy to breed, and lower cannibalism. Cultivation of cantang grouper can be carried out in semi-intensive ponds and floating net cages (FNC) which have the potential for grouper nursery and rearing activities. One of the problems in the cultivation of cantang grouper is the presence of disease attacks caused by marine leeches *Zeylanicobdella* with a prevalence of up to 100% in grouper culture in floating net cages.
This study aims to determine differences in species and intensity of marine leech *Zeylanicobdella* infestation in cantang grouper (*Epinephelus fuscoguttatus lanceolatus*) from semi-intensive ponds in Brondong District, Lamongan and floating net cages (FNC) on Mandangin Island, Madura. The method used in this research is a survey and sampling is done by purposive sampling method. Cantang groupers measuring 15-20 cm from semi-intensive ponds and 20-25 cm from floating net cages each were taken as many as 35 individuals. Identification of *Zeylanicobdella* marine leeches using a binocular microscope and making sketches of ectoparasites with the help of a binocular microscope equipped with a lucida camera.
Marine leech species found to infest the cantang grouper (*Epinephelus fuscoguttatus lanceolatus*) is *Zeylanicobdella arugamensis* which was found on the body surface, fins, gills, and eyes. The results of statistical analysis using the *Mann-Whitney U test* showed that there was no significant difference ($p >$ 0,05) between species and intensity of *Zeylanicobdella arugamensis* in cantang grouper reared in semi-intensive ponds and floating net cages. The intensity of marine leeches *Zeylanicobdella arugamensis* in semi-intensive ponds was in the medium category (14,74 individuals/head) and in floating net cages was in the medium category (27,74 individuals/head). The highest intensity of infestation marine leeches *Zeylanicobdella arugamensis* in floating net cages of 27,74 individuals/head. There was no significant difference in the intensity of *Zeylanicobdella arugamensis* marine leech infestation in cantang grouper both from semi-intensive ponds and floating net cages (FNC) which were included in the medium category.

Keywords: Cantang grouper, marine leeches *Zeylanicobdella*, morphometric identification, intensity, semi-intensive ponds, floating net cages (FNC)

Introduction
Grouper is one of the fishery commodities that have opportunities both in the domestic and international markets, besides that the selling value is also quite high (Paruntu, 2015) [15]. One type of grouper that is in demand for cultivation is the cantang grouper (*Epinephelus fuscoguttatus lanceolatus*) which is the result of hybridization between the female tiger grouper (*Epinephelus fuscoguttatus*) and the male kertang grouper (*Epinephelus lanceolatus*). Along with the development of grouper cultivation, both those reared in semi-intensive ponds with a soil base in Brondong District, Lamongan Regency and floating net cages (FNC) on Mandangin Island, Madura, causing various problems in aquaculture activities. According to WWF (2015) [20], poor cultivation processes, such as drastic changes in water quality parameters, accumulation of organic matter from leftover feed, and unpredictable weather factors can cause disease in fish, one of which is caused by infection with pathogens such as parasites, bacteria and viruses. One of the types of diseases that often attack grouper cultivation is caused by parasitic attacks. Parasites are organisms that use other organisms of different types for shelter and to get food (Diani *et al*., 2004) [4].

One type of ectoparasite that attacks the cultivation of cantang grouper is the marine leech infestation of *Zeylanicobdella* which is a parasite of the phylum Annelida and sub class Hirudinea (Zafran *et al*., 2019) [21].

The morphometric identification of ectoparasites is useful for knowing the structure and morphology of a parasite which is included in the morphometric characteristics (body length, body width, organ diameter) so that it can be used to determine the genus or species of the parasite (Nurrochmah *et al*., 2016) [14]. There were two previously reported *Zeylanicobdella* species, namely *Zeylanicobdella arugamensis* De Silva, 1963 [3] and *Zeylanicobdella stellata* Moore, 1958. The species *Zeylanicobdella arugamensis* was first discovered in grouper culture as a major host in the Philippines and has been observed attacking a number of brackish water and marine fish in Sri Lanka, the Malaya Peninsula in Singapore, and India (Cruz-Lacierda, 2000) [2]. *Zeylanicobdella stellata* was first discovered in the toby fish (*Tetraodontidae*) from South Africa (Utevsky, 2007) [18]. Intensity is the average number of parasites that infest each fish (Mahasri *et al*., 2019) [10]. Factors that can affect the level of parasite infestation in aquatic biota are genetics which plays an important role in the composition of the components of the fish immune system, unbalanced feed nutrition, type and age of fish, aquatic environment, and fish activity or movement (Hardi, 2015) [6].

Methodology

This research was carried out in October 2020-January 2021. Sampling of cantang grouper was carried out in semi-intensive ponds in Brondong District, Lamongan as the first location and floating net cages (FNC) on Mandangin Island, Madura as the second location. Inspection, coloring, and identification of *Zeylanicobdella* marine leeches were carried out at the Anatomy and Aquaculture Laboratory and Microbiology Laboratory, Faculty of Fisheries and Marine, Airlangga University. The sample of cantang grouper used is 15-20 cm in size from Brondong District, Lamongan and 20-25 cm in size from floating net cages on Mandangin Island, Madura by using purposive sampling method. Each sampling location of cantang grouper fish was taken as many as 35 tails. The main parameters in this study were the type and intensity of the *Zeylanicobdella* marine leech. The supporting parameters in this research are water quality which includes (salinity, temperature, DO, pH, ammonia, nitrite and nitrate).

Zeylanicobdella Marine Leech Examination

Examination of *Zeylanicobdella* marine leeches is carried out by taking or removing *Zeylanicobdella* attached to the body surface of the sample fish and counting the number of *Zeylanicobdella* infesting in each fish, then put and stored in a sample pot containing 5% glycerin alcohol for the purposes of the coloring process and identification of marine leeches *Zeylanicobdella* more. *Zeylanicobdella* marine leech staining was carried out using the *Semichen-Acetic Carmine* method (Kuhlmann, 2006) [8].

Zeylanicobdella marine leech staining using the *Semichen-Acetic Carmine* method, namely by *Zeylanicobdella* which has been stored in 5% glycerin alcohol and then put in 70% alcohol for 5 minutes, then fixed between two glass objects and tied at both ends with thread (raffia). The object glass and the *Zeylanicobdella* sample were put into the *Carmine* solution and soaked for ± 8 hours depending on the thickness of the cuticle of the sample, then the *Zeylanicobdella* sample could be removed from the object glass fixation. The next step is to transfer the *Zeylanicobdella* sample in an acidic alcohol solution for 2 minutes, then transfer it to an alkaline alcohol solution for 2 minutes. Then, dehydration was carried out in stages with 70% alcohol for 5 minutes, 85% alcohol for 5 minutes, and 95% alcohol for 5 minutes. Furthermore, mounting in Hung's I solution for 20 minutes. *Zeylanicobdella* samples from Hung's I solution were taken and placed on a clean object glass and dripped with sufficient Hung's II solution on the specimen, then covered with a cover glass. The results of the staining can already be used as preparations for *Zeylanicobdella* marine leeches whose body parts will be observed using a binocular microscope equipped with a *Lucida* camera with 40x and 100x magnifications.

Identification of *Zeylanicobdella* Marine Leeches

Observations were made under a binocular microscope with a magnification of 40x and 100x, then the *Zeylanicobdella* marine leech specimen was depicted using a binocular microscope equipped with a *Lucida* camera with a magnification of 40x and 100x. The identification of *Zeylanicobdella* marine leeches was carried out by observing morphological characteristics and morphometric measurements of body parts then compared with identification keys from books or journals. The body parts used as the key to morphometric identification of *Zeylanicobdella* marine leeches are by measuring body length, body width, diameter of the oral sucker and diameter of the caudal sucker (Murwantoko *et al*., 2018) [13]. The identification key in this study was based on Chandra (1991) [1].

Calculation of *Zeylanicobdella* Marine Leech Intensity

Intensity is the average number of individual parasites per infested fish. Calculation of the intensity in this study using a formula based on Mamani *et al*. (2004) [11] and the determination of parasite intensity categories based on Williams and Williams (1996) [19] are presented in Table 1.

$$\text{Intensity (individual/tail)} = \frac{\text{Number of parasites found}}{\text{Number of fish infested}}$$

Table 1: Parasite Intensity Category

Intensity	Category
0	Normal
1-5	Light
6-50	Medium
51-100	Heavy
>100	Very heavy
>1000	Super infection

Source: Williams and Williams (1996) [19].

Results

The results of the identification of marine leeches in cantang grouper (*Epinephelus fuscoguttatus lanceolatus*) from 35 samples from semi-intensive ponds in Brondong District, Lamongan and 35 samples from floating net cages on Mandangin Island, Madura based on the identification key according to Chandra (1991) [1] found species of marine leeches, namely *Zeylanicobdella arugamensis* in both maintenance systems. Based on the results of morphometric measurements of 70 samples of ectoparasite *Zeylanicobdella arugamensis* that infested cantang grouper in semi-intensive

ponds and floating net cages with size comparison based on Murwantoko *et al*. (2018) [13] can be seen in Table 2.

Table 2: Results of morphometric measurements of *Zeylanicobdella arugamensis* infesting can tang grouper

Parameters	Size (mm)		
	Semi-intensive ponds	Floating Net Cages (FNC)	Murwantoko *et al*. (2018) [13]
Body lenght	4.12-6.95	5.23-8.95	8-18
Body width	0.30-1.27	0.43-1.89	0.5-2.0
Oral sucker diameter	0.15-0.30	0.19-0.39	0.3-0.5
Caudal sucker diameter	1.11-1.29	1.24-1.67	1.0-1.8

The morphology of *Zeylanicobdella arugamensis* after staining with *Semichen-Acetic Carmine* which was observed using a binocular microscope can be seen in Figure 1.

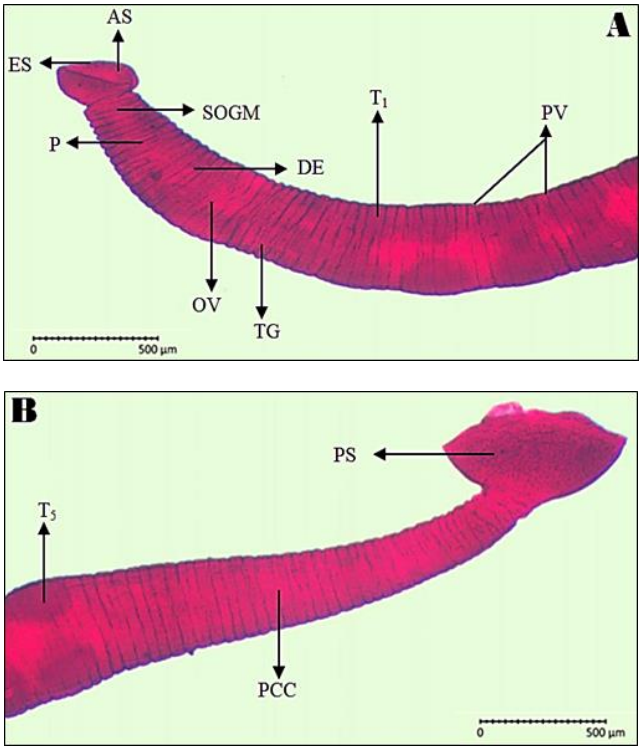

Fig 1: *Zeylanicobdella arugamensis* with *Semichen-Acetic Carmine* staining was observed using a 40x magnification binocular microscope

Description: (A) Anterior, AS: Anterior *sucker*, ES: *Eye spot*, SOGM: *Suboesophageal ganglionic mass*, P: *Proboscis*, DE: *Ductus ejaculatorious*, OV: *Ovary*, TG: *Testicular ganglion*, T: Testis, PV: *Pulsatile vesicles*. (B) Posterior, PS: Posterior *sucker*, PCC: *Posterior crop caecum*. Scale bar: 500 µm.

Marine leech *Zeylanicobdella arugamensis* drawn using a binocular microscope equipped with a *lucida* camera can be seen in Figure 2.

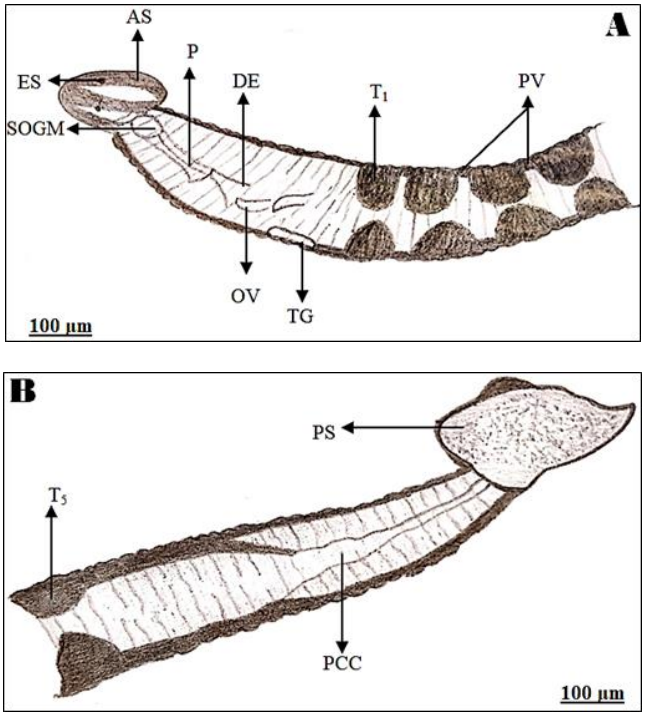

Fig 2: *Zeylanicobdella arugamensis* observed with a binocular microscope equipped with a *lucida* camera with 100x magnification

Description: (A) Anterior, AS: Anterior *sucker*, ES: *Eye spot*, SOGM: *Suboesophageal ganglionic mass*, P: *Proboscis*, DE: *Ductus ejaculatorious*, OV: *Ovary*, TG: *Testicular ganglion*, T: Testis, PV: *Pulsatile vesicles*. (B) Posterior, PS: Posterior *sucker*, PCC: *Posterior crop caecum*. Scale bar: 500 µm.

The determination of the intensity *Zeylanicobdella arugamensis* marine leech in this study was based on the number of *Zeylanicobdella arugamensis* infesting cantang grouper divided by the number of fish infested in each rearing system and then categorized according to the references from Williams and Williams (1996) [19]. Based on the results of statistical analysis using the *Mann-Whitney U test*, it showed that there was no significant difference ($p > 0.05$) between the intensity of *Zeylanicobdella arugamensis* in cantang grouper reared in semi-intensive ponds and floating net cages. The results calculation intensity of *Zeylanicobdella arugamensis* in cantang grouper fish raised in different maintenance systems can be seen in Table 3.

Table 3: The results calculation intensity of *Zeylanicobdella arugamensis* that infested cantang grouper

Maintenance system	Number of samples (+)	Number of parasites	Infestation Degree (individuals/head)	Category Williams and Williams (1996) [19]
Semi-intensive ponds	35	516	14.74	Medium
Floating Net Cages (FNC)	35	971	27.74	Medium

Note: the results of statistical analysis using the *Mann-Whitney U test* Superscript are different in the column and there is no significant difference ($p > 0.05$).

Measurement of water quality at each cultivation location aims to determine the condition of the waters in the cantang grouper rearing system. Measurement of the quality of aquaculture water was carried out before sampling cantang grouper at each location, namely semi-intensive ponds and floating net cages (FNC). Water quality parameters measured included temperature, DO (dissolved oxygen), pH, salinity, ammonia, nitrite and nitrate. Water quality as supporting data in this study is a very important factor in the cultivation of cantang grouper. Data on the results of water

quality measurements in semi-intensive ponds and floating net cages (FNC) with comparisons based on the BSN (2014) can be seen in Table 4.

Table 4: The results of measuring water quality in semi-intensive ponds and FNC with a comparison of ideal water quality according to BSN (2014)

Location	Temperature (ºC)	DO (mg/L)	pH	Salinity (ppt)	Ammonia (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)
Semi-intensive ponds	28	4	8	35	0.5	0.25	10
Floating Net Cages (FNC)	30-31	6-7	7-8	34	3	0.5	25
BSN (2014)	28-32	≥ 4	7.5-8.5	24-33	≤ 0.01	≤ 1	≤ 1.5

Discussion

This marine leech species in cantang grouper is found attached to the entire body surface, fins (dorsal, ventral, pectoral, and caudal), operculum and eyelets. *Zeylanicobdella arugamensis* found in cantang grouper in semi-intensive ponds has a body length of 4.12-6.95 mm, a body width of 0.30-1.27 mm, an anterior sucker diameter of 0.15-0.30 mm, and a diameter posterior suckers 1.11-1.29. While the floating net cages have a body length of 5.23-8.95 mm, a body width of 0.43-1.89 mm, an anterior sucker diameter of 0.19-0.39 mm, and a posterior sucker diameter of 1.24-1.67. Based on Murwantoko *et al*. (2018) [13] *Zeylanicobdella arugamensis* adult stadia species that infest cantang grouper have a body length of 8-18 mm, a body width of 0.5-2.0 mm, an anterior diameter of the sucker 0.3-0.5 mm, and a posterior diameter of sucker 1.0-1.8. The difference in the size of *Zeylanicobdella arugamensis* in the two rearing media was due to the different rearing environment and sampling locations that could affect the body and organ size of the marine leech.

According to Kua *et al*. (2010) [7] stated that the adult stage *Zeylanicobdella arugamensis* has a body length ranging from 4.5-14 mm. In addition, based on Ravi and Yahaya (2017) [16] found the sea leech *Zeylanicobdella arugamensis* on Jerejak Island, Penang, Malaysia with a body length of 20-25 mm, body width 0.6-2.3 mm, anterior sucker diameter 0.3-0.5 mm and the diameter of the posterior sucker is 1.6-1.8 mm. This shows that *Zeylanicobdella arugamensis* has a wide range of body and organ sizes.

Based on the results of *Zeylanicobdella arugamensis* morphometric measurements, it can be seen that the size of *Zeylanicobdella arugamensis* in floating net cages has body length, body width, anterior sucker diameter, and posterior sucker diameter which are larger than in semi-intensive ponds. This is because the environmental conditions of cultivation in floating net cages are more supportive of *Zeylanicobdella arugamensis* habitat in the development of its life cycle. Kua *et al*. (2010) [7] explained that calm water conditions are known to have an impact on the longer life cycle of *Zeylanicobdella arugamensis* in experiencing growth.

Based on the morphology in its classification, *Zeylanicobdella arugamensis* is included in the phylum Annelida because its body is cylindrical in shape which is arranged lengthwise like a pile of rings (Chandra, 1991) [1]. *Zeylanicobdella arugamensis* belongs to the class Clitellata because in the anterior part of this ectoparasite worm there is a *Cupuliform* oral sucker and on the posterior it is larger than the anterior (De Silva, 1963 in Chandra, 1991) [3, 1]. *Zeylanicobdella arugamensis* is usually called a marine leech which belongs to the subclass Hirudinea because it has two suckers consisting of an oral sucker on the anterior and a caudal sucker on the posterior. *Zeylanicobdella arugamensis* belongs to the order Rhynchobdellida and the family Piscicolidae because this species does not have jaws but has a sucking tool to take nutrients from the host called a *Proboscis*. This marine leech is included in the genus *Zeylanicobdella* because it has fourteen or more *annuli* and has a cylindrical body shape and a pair of eye spots on the lower third of the anterior sucker so that this ectoparasite worm species belongs to the *Zeylanicobdella arugamensis* species (Chandra, 1991) [1].

The two locations of cantang grouper cultivation, namely semi-intensive ponds and floating net cages, did not find any differences in the species of *Zeylanicobdella arugamensis*, this was because at the two cultivation locations the main media for *Zeylanicobdella arugamensis* was the source of water from sea or brackish water which had sufficient salinity value tall. Based on Mahardika *et al*. (2020) [9] explained that marine leeches and their cocoons can live and hatch in saltwater to brackish water (5-30 ppt). In addition, according to Zafran *et al*. (2020) [22] *Zeylanicobdella arugamensis* marine leeches are always present and infest grouper throughout the year, especially grouper cultured in hatcheries and ponds that use sea water directly and grouper cultured in floating net cages (FNC).

The intensity of *Zeylanicobdella arugamensis* in both rearing systems was in the medium category. Factors supporting *Zeylanicobdella* infestation include the turbidity of the incoming water is too high, but it is also influenced by salinity and environmental conditions of the waters. This species, both egg and adult stages, is capable of hatching and living in saltwater to brackish water with a salinity of 5-30 ppt (Mahasri *et al*., 2019) [10]. Parasite infestation is related to the greater the size or weight of the host, the higher the parasite infestation to the host. This is because the length of maintenance of the host in a waters results in the longer the time the host has for contact with the parasite (Riko *et al*., 2012) [17].

The intensity of *Zeylanicobdella arugamensis* at the two locations of cantang grouper cultivation did not show any difference, this was because semi-intensive ponds and floating net cages could be used by marine leeches as a medium for attaching their eggs. According to Kua *et al*. (2010) [7] *Zeylanicobdella arugamensis* eggs which have a sticky, slimy ring layer on the surface of the egg serves to stick to the substrate. Zafran *et al*. (2019) [21] added that Hirudinea eggs can attach to various solid objects in the pond environment and marine cages, for example (pond walls and nets) and can hatch at any time. In addition, the quality of cultivated water can also affect the life cycle of *Zeylanicobdella arugamensis*, one of which is the salinity factor. According to Kua *et al*. (2010) [7] explained that *Zeylanicobdella arugamensis* can reproduce optimally at 30 ppt salinity and at salinity below 30 ppt it can still reproduce but more slowly.

Based on the results of Fadlullah's research (2020) explains that high and low salinity can affect the life cycle of

Zeylanicobdella arugamensis. Cantang grouper reared in ponds with a salinity of 33-40 ppt were infested with *Zeylanicobdella arugamensis* in the rare to moderately high category. *Zeylanicobdella arugamensis* is a marine leech that has high physiological plasticity so that it easily adapts to extreme environmental changes by forming air bubbles on the dorsoventral part of its body. This is a factor that *Zeylanicobdella arugamensis* can survive in a wide salinity range.

Conclusion

There was no difference in the species of marine leech *Zeylanicobdella arugamensis* infesting cantang grouper (*Epinephelus fuscoguttatus lanceolatus*) from semi-intensive ponds in Brondong District, Lamongan and floating net cages (FNC) on Mandangin Island, Madura. There was no difference in the intensity of the *Zeylanicobdella arugamensis* marine leech infesting cantang grouper (*Epinephelus fuscoguttatus lanceolatus*) from semi-intensive ponds in Brondong District, Lamongan and floating net cages (FNC) on Mandangin Island, Madura.

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