Zeylanicobdella arugamensis intensity and histopathology of hybrid grouper skin from soil and concrete ponds

M Nisa1, G Mahasri2,4, S Subekti3

1Magister Program of Fisheries Science, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, Indonesia
2Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya 60115 Indonesia
3Department of Marine, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya 60115 Indonesia
4Corresponding author: mahasriot@gmail.com

Abstract. Z. arugamensis was a parasite that often infested hybrid grouper (E. fuscoguttatus x E. lanceolatus). This study aimed to differentiate the Z. arugamensis intensity and the skin histopathology of hybrid grouper from concrete and soil ponds. The research method was a survey and data analysis using the IBM SPSS 21 Mann Whitney U Test. This study used 50 of hybrid grouper each from soil and concrete ponds. The intensity of Z. arugamensis from soil ponds was significantly different with concrete ponds. Z. arugamensis infestation from soil ponds were 13 individuals including the intensity of Z. arugamensis in the medium category of 6.69 individuals/fish. Z. arugamensis infestation from concrete ponds were 10 individuals, including the intensity of Z. arugamensis in the light category was 2.9 individuals/fish. The results of histopathological scoring of hybrid grouper skin from soil ponds were significantly different with concrete ponds. The results of skin histopathological scoring of hybrid grouper from soil ponds were 1.1 for 13 fish while from concrete ponds it was 0.5 for 10 fish. This study showed that there was a significant difference in the intensity of Z. arugamensis and skin histopathology of hybrid grouper from concrete and soil ponds.

1. Introduction
The production of hybrid grouper increases every year due to high interest from foreign consumers [1]. Grouper exports have increased every year reaching 30.75% [2]. The hybrid grouper was the result of hybridization between the female Epinephelus fuscoguttatus and the male Epinephelus lanceolatus [3]. Rearing of hybrid grouper in ponds with a soil base increased the effect of organic matter accumulation on the pond bottom and interactions with soil microorganisms [4]. Fish rearing with a concrete pond would make it easier for the substrate to spread on the pond wall so that it could increase the population of ectoparasites [5]. This would result in a decrease in water quality and stress for fish. Factors that influence
the emergence of parasites were generally triggered by poor water quality conditions, weak host conditions, and high stocking densities [6].

The decreasing of water quality would presence ectoparasites in the fish body and morphological damage [7]. The impact of ectoparasite infestation could be seen from the diagnosis through skin histopathology. Histopathological damage due to parasites was hyperplasia, fusion, telangiectasia and necrosis [8]. This study aims to distinguish the intensity of *Z. arugamensis* and the skin histopathology of hybrid grouper from concrete ponds and soil ponds. The results of the study were expected to be used to determine the appropriate prevention to reduce losses due to ectoparasite attacked on hybrid grouper.

2. Materials and methods

The research was conducted in August 2019. The research method used was a survey. Sampling used purposive sampling method with a total sample of 50 hybrid groupers taken from each pond. The hybrid groupers used were 10-15 cm for 50 fish taken from each soil pond in Lamongan and concrete pond in Situbondo.

Examination of the intensity of *Z. arugamensis* in hybrid grouper at the Laboratory of Anatomy and Culture, Faculty of Fisheries and Marine, Airlangga University, Surabaya. Examination of the parasite *Z. arugamensis* in hybrid grouper was appropriate [9] regarding the morphology of *Z. arugamensis*. The intensity of *Z. arugamensis* according to [10], could be determined by the following formula:

\[
\text{Intensity} = \frac{\text{Number of infested } Z. \text{ arugamensis}}{\text{Number of fish infested with } Z. \text{ arugamensis}}
\]

The results of the *Z. arugamensis* intensity then categorized according to [11]. Histopathological observations of hybrid grouper skin at the Microbiology Laboratory, Faculty of Fisheries and Marine, Airlangga University, Surabaya. The histopathological preparation procedure according to [12]. Histopathological examination of the skin of hybrid grouper with a 400x magnification light microscope 6 times and scoring the changes that occurred to determine the type and level of tissue damage, with values of 0, 1, 2, 3, 4 [13]. *Z. arugamensis* intensity data and skin histopathology of hybrid grouper were analyzed using the IBM SPSS 21 Mann Whitney U Test.

3. Results and discussion

3.1. Intensity of *Z. arugamensis* In Hybrid Grouper

Hybrid grouper infested with *Z. arugamensis* were 13 fish from soil ponds and 10 fish from concrete ponds. Hybrid grouper infested with *Z. arugamensis* was then calculated according to [13] intensity. The results of the intensity of *Z. arugamensis* could be seen in table 1 below.

| Sampling Point   | Total fish examined (fish) | Fish infested with *Z. arugamensis* | Total *Z. arugamensis* | *Z. arugamensis* Intensity (individual/fish) | Williams and Williams (1996) Category |
|------------------|----------------------------|-------------------------------------|------------------------|---------------------------------------------|--------------------------------------|
| Soil Pond        | 50                         | 13 fish                             | 87                     | 6.69                                        | Medium                               |
| Concrete Pond    | 50                         | 10 fish                             | 29                     | 2.9                                         | Light                                |
Analysis using IBM SPSS 21 Man Whitney U Test showed the intensity of *Z. arugamensis* from soil ponds was significantly different with a significance of 0.009 (p≤0.05) with concrete ponds. Hybrid grouper infested with *Z. arugamensis* from soil ponds were 6.69 individuals/fish was in the medium category. Hybrid grouper infested with *Z. arugamensis* from concrete ponds was categorized as light, namely 2.9 individuals/fish.

3.2. Skin Histopathology Of Hybrid Grouper

The skin of hybrid grouper that was infested with the *Z. arugamensis* parasite was then taken to be used as histopathological samples. The histopathological assessment of hybrid grouper skin could be seen in table 2 below.

| Sampling Point | *Z. arugamensis* intensity (individual/fish) | Histopathological Scoring | Damage       |
|----------------|--------------------------------------------|---------------------------|--------------|
| Soil Pond      | 6.69                                       | 1.08                      | Damage ≤25%  |
| Concrete Pond  | 2.9                                        | 0.5                       | No damage    |

Analysis using the IBM SPSS 21 Man Whitney U Test showed that the histopathological score of hybrid grouper skin from soil ponds was significantly different with a significance of 0.036 (p≤0.05) with concrete ponds. The histopathological scoring results of hybrid grouper skin from soil ponds was 1.08 with ≤25% histopathological scoring consisting of erosion, inflammation and congestion. Hybrid grouper from concrete ponds had a histopathological score of 0.5 which means that there was no skin tissue damage. The skin tissue of hybrid grouper from soil and concrete ponds could be seen in Figure 1 below.
Figure 1. Skin histopathology of hybrid grouper infested with *Z. arugamensis*: A. there is no tissue damage from concrete ponds with light intensity; B. Tissue damage ≤25% were inflammation (i) and congestion (k) from the soil pond with medium intensity.

3.3. Water Quality
Water quality measurements were carried out during surveys in ponds using a thermometer, refractometer, pH paper, dissolved oxygen and ammonia test kits. The results of water quality measurements in ponds could be seen in table 3 below.

| Water Quality | Temperature °C | DO ppm | pH  | Salinity g/L | Ammonia (NH₃) ppm |
|---------------|----------------|--------|-----|--------------|------------------|
| Soil Pond     | 29             | 5      | 7   | 31           | 1,2              |
| Concrete Pond | 31             | 7      | 7,5 | 31           | 0,5              |
| SNI8036.2:2014| 28-32          | min 4  | 7,5-8,5 | 24-33       | <0,01            |

Standard [14] was the recommended water quality for hybrid grouper rearing so that the fish could grow and develop in optimal conditions. Water quality in soil pond had abnormal values according to standards [14], namely ammonia 1.2 and 0.5 from concrete ponds.

The Water quality from soil pond showed a higher organic matter content, which could be seen from the value of ammonia (1.2 ppm) compared to concrete ponds (0.5 ppm). Ammonia (NH₃) levels in the waters were the result of fish metabolism in the form of feces, urine, and leftover feed [15]. High organic matter content could cause the pH of the water to decrease. This could be seen from the pH levels of the soil ponds, namely 7 and 7.5 from the concrete ponds. An increase in pH could increase the toxicity of ammonia so that
fish became stressed [16]. Decreased water quality was one of the factors causing the emergence of infectious agents such as parasites or other pathogens that caused disease in fish [17]. The decreasing in water quality supported parasite infestation in fish.

The intensity of *Z. arugamensis* from soil ponds was significantly different \( p \leq 0.05 \) with concrete ponds. The intensity of *Z. arugamensis* from soil ponds was 6.69 individuals/fish in the medium category while from concrete ponds was 2.9 individuals/fish for light category. The higher intensity of *Z. arugamensis* from soil ponds could be due to the higher organic matter content. *Z. arugamensis* have liked waters with high water turbidity [18]. According to [19] *Z. arugamensis* included leeches that obtain food by sucking blood from their host, did not have jaws but had a nutrient sucking device on the host called proboscyst [19]. *Z. arugamensis* infestation results in pale skin, redness and swelling [20]. The infestation would result in skin tissue damage.

According to [21] fish infested with leech did not cause death but were chronic so that it would result in stunted growth, decreased host fecundity, and could cause tissue damage to organs. Histopathological examination of fish organs was carried out to see tissue changes that occurred due to pathogen infestations that allowed for tissue abnormalities [22].

The results of histopathological scoring of hybrid grouper skin from soil ponds were significantly different \( p \leq 0.05 \) with concrete ponds. The skin of hybrid grouper from soil ponds showed \( \leq 25\% \) tissue damage while there was no skin tissue damage from concrete ponds. Diseases could occur in waters due to an imbalance between the environment, fish, and pathogens [23]. Tissue damage that occurs were erosion, congestion and inflammation. Erosion could occur when the probosyst attached to and taken nutrients through attachment to the epidermal layer. Epithelial erosion of the epidermis was the erosion of the epidermal layer [24]. *Z. arugamensis* attached to the skin using 2 suckers then injected histamine into the blood vessels and sucked nutrients using a probocyst. Blood coagulation at the attachment site could be inhibited by injecting saliva so that the host's blood coagulation enzyme (thrombin) could be inhibited [25].

Inflammation was the body's first defense response due to pathogen attack. Inflammation was a natural process to maintain the homeostasis of the body due to the entry of foreign agents or compounds [26]. Congestion was marked by a red color in the cells, this occurred due to an increase in blood in the blood vessels. Congestion was accompanied by an increase in the number of eosinophil granule cells and could cause hemorrhage if blood leaks out of the vessel [24]. The discharge of blood from the blood vessels was due to the rupture of the blood vessels.

4. Conclusion

The intensity of *Z. arugamensis* in hybrid grouper from soil ponds was significantly different \( p \leq 0.05 \) with concrete ponds. The intensity of *Z. arugamensis* in hybrid grouper from soil ponds was 6.69 individuals/fish, including the medium category, while from concrete ponds, 2.9 individuals/fish was included in the light category. The results of histopathological scoring of hybrid grouper skin from soil ponds were significantly different \( p \leq 0.05 \) with concrete ponds. The results of histopathological scoring of the skin of hybrid grouper from soil ponds were 1.1 for 13 fish while from concrete ponds it was 0.5 for 10 fish. This study showed that there was a significant difference in the intensity of *Z. arugamensis* and the histopathological scoring results of hybrid grouper skin from concrete ponds and soil ponds.

5. References

[1] Lopez, V. G. dan F. C. Orvay. 2003. Preliminary Data on the Culture of Juveniles of the Dusky Grouper, *Epinephelus marginatus* (Lowe, 1834). Hidrobiologica, 13(4) : 321-327.

[2] Kementerian Kelautan dan Perikanan. 2018. Satu Data Internet. https://kkp.go.id/djp/artikel/304-kkp-tegaskan-kinerja-neracaperdagangan-ikan-kerapu-positif. Diakses pada tanggal 28 April 2021.
[3] Nurhayati, K., S. Endang dan E. Rahayu. 2014. Identifikasi dan Prevalensi Ektoparasit Pada Ikan Kerapu Hybrid (Ephinephelus fuscoguttatus-lanceolatus) Hasil Budidaya Keramba Jaring Apung (Kja) Di BPBAP Situbondo dan Gundil Situbondo. Universitas Negeri Malang. Malang. 2 hal.

[4] Rahayu, A. 2017. DAYA DUKUNG LAHAN TAMBAK BUDIDAYA IKAN KERA PU (Ephinephelus spp) DI KECAMATAN BRONDONG KABUPATEN LAMONDON. Jurnal Grouper. Vol 8 (1): 13-19.

[5] Hendrajat, E. M dan H. Suryanto. 2007. Budidaya Udang Vannamei Pola Tradisional Plus di Kabupaten Maros Sulawesi Selatan. Jurnal Media Akuakultur, 2 (2) : 67-70.

[6] Nofasari, N., T. Raza’i., R. Wusolari. 2019. Identifikasi dan Prevalensi Ektoparasit pada Ikan Air Tawar dan Laut Dilokasi Budidaya Perikanan Bintang Kepulauan Riau. Jurnal Intek Akuakultur. 3(1): 92-104.

[7] Misganaw, K., & Getu. A.2016. Review on major parasitic crustacean in fish. Fisheries and Aquaculture Journal, 7(3), 13-17.

[8] Sudaryatma, P dan N. Y Eriawati. 2017. DAYA DUKUNG LAHAN TAMBAK BUDIDAYA IKAN KERA PU (Ephinephelus spp) DI KECAMATAN BRONDONG KABUPATEN LAMONDON. Jurnal Grouper. Vol 8 (1): 13-19.

[9] Chandra. M. 1983. Zeylanicobdella arugamensis. Rec. Zool. Surv. India. (1)80 :273.

[10] Barton, D. P. and S. J. Richard. 1996. Helminth infracomunities in Litoria genimaculata (Amphibia: anura) from Birthday Creek, an Unsoil rainforest stream in Northern Queensland, Australia. International Journal for.
infestations degree. The 1st International Conference on Biotechnology and Food Sciences. 1(1): 1-8.

[21] Saputra, L. 2011. Deteki Morfologi dan molecular parasite anisakis spp pada ikan tongkol (Auxis thazard). Skripsi. Budidaya Perairan. Fakultas Ilmu Kelautan dna Perikanan. Universitas Hasanuddin. Makassar. 56 hal.

[22] Safratilofa.2017. Histopatologi Hati dan Ginjal Ikan patin (Pangasionodonhypopthalmus) yang diinjeksi Bakteri Aeromonas hydrophila. JurnalAkuakultur Sungai dan Danau, 2(2):83-88 Standar Nasional Indonesia 8036. 2014. Ikan kerapu hybrid (Epinephelus fuscoguttatus, Forsskal 1775 >< Epinephelus lanceolatus, Bloch 1790) Bagian 2: Produksi Benih Hibrida. BSN. hal 9-10.

[23] Kriswijayanti, B., K. Kismiyati., dan K. Kusnoto. 2013. IDENTIFIKASI DAN DERAJAT INFESTASI Lernaea PADA IKAN MASKOKI (Carassius auratus) DI KABUPATEN TULUNGAGUNG, JAWA TIMUR. Journal of Aquaculture and Fish Health. 3(1):1-7.

[24] Sari, R., W. Tjahjaningsih dan K. Kismiyati.2018. Perubahan Histopatologi Jaringan Kulit Ikan Komet (Carassius auratus) akibat infestasi Argulusjaponicas. Jurnal Ilmiah Perikanan dan Kelautan, 10(1):1-11.

[25] Lucas, J. S., P. C. Southgate dan C. S. Tucker. 2012. Aquaculture : Farming Aquatic Animals and Plants. Brisbane, Australia. University of Queenssoil.p 240- 241.

[26] Ikawati. Z. 2011.Farmakoterapi Penyakit Sistem Saraf Pusat. Bursa Ilmu. Yogyakarta. hal 334.

6. Acknowledgements
The authors highly appreciate to BPBAP Situbondo for permitting us conduct the research in their farms and also to Faculty of Fisheries and Marine, Airlangga University for the facilities provided during the research.