Jurnal Kajian Veteriner
ISSN: 2356-4113
E-ISSN: 2528-6021
Vol. 9 No. 1:62-71 (2021)
DOI:https://doi.org/10.35508/jkv.v9i1.8426

INTENSITY LEVEL AND PREVALENCE OF Anisakis sp IN *Epinephelus sp.* AND *Rastrelliger sp* IN EAST INDONESIA

Maxs Sanam[1*], Annytha Detha[1], Diana Wuri[1], Susana Dangga[2]
[1]Laboratory of Animal Disease and Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Nusa Cendana, Kupang, Indonesia
[2]Faculty of Veterinary Medicine, Universitas Nusa Cendana, Kupang, Indonesia
*Corespondence e-mail: maxi_sanam@gmail.com

ABSTRAK

Anisakiasis adalah penyebab parasit zoonosis yang disebabkan oleh larva Anisakis sp. Manusia terinfeksi karena menelan ikan atau kerang mentah atau setengah matang yang mengandung Anisakis sp. Tujuan penelitian ini adalah untuk mengidentifikasi tingkat intensitas dan derajat kejadian infeksi Anisakis sp pada *Rastrelliger* sp dan *Epinephelus* sp di perairan timur Indonesia. Sebanyak 190 spesimen ikan diacak (random sampling) pada 95 *Rastrelliger* sp dan 95 *Epinephelus* sp dari tempat penjualan ikan di berbagai wilayah kota Kupang. Larva yang diperoleh dikumpulkan dan difiksasi dengan alkohol 70%, dan diwarnai menggunakan larutan semichen acetic carmine. Hasil ini memberikan informasi intensitas bahwa intensitas Anisakis sp pada *Epinephelus* sp dan *Rastrelliger* sp berturut-turut adalah 98% dan 3,15%. Derajat infeksi Anisakis sp. pada *Epinephelus* sp secara keseluruhan sebesar 7,80 termasuk kategori sedang, dan pada *Rastrelliger* sp sebesar 1,33 termasuk kategori rendah. Organ predileksi yang dominan pada ikan *Epinephelus* sp adalah organ otot, sedangkan pada ikan *Rastrelliger* sp predileksi yang dominan adalah usus. Hasil penelitian juga menegaskan bahwa infeksi Anisakis sp menyiratkan risiko tinggi spesies ikan *Epinephelus* sp sehingga diperlukan identifikasi lebih lanjut di tingkat molekuler.

Keywords: *Anisakis sp*, fish, foodborne diseases, zoonoses

INTRODUCTION

Anisakiasis is a zoonotic causa parasite caused by the larvae of Anisakis sp, the larvae of the third-stage nematode worms, which attach to the walls of the esophagus, stomach, or intestines (Mattiucci *et al*., 2018; Rahmati *et al*., 2020). Humans become infected through ingestion of raw or undercooked prepared fish or shellfish containing Anisakis sp . larvae (Bao *et al*., 2019; Baptista-Fernandes *et al*., 2017). Anisakiasis is most commonly found in areas where the habit of eating raw fish is common, such as Japan. In addition, the habit of consuming traditional culinary or dishes that use raw fish ingredients, such as Italy, has been reported to be a potential source of infection. However, as eating undercooked fish is now becoming more common, cases have also been reported in the United States, Europe, South America and other regions of

the world (Buchmann & Mehrdana, 2016; Caldeira *et al*., 2021). In the incidence of anisakiasis, humans are the unintentional hosts and become infected after consuming fish contaminated with third-stage larvae as infective larvae (Ivanovic *et al*., 2015; Ivanović *et al*., 2017).

In Indonesian marine waters there are various types of infected fish and all of them are types of fish commonly consumed by the Indonesian people, some of which have high economic value (Bobsaid *et al*., 2021; *EBSCOhost | 155850041 | Anisakis Infection of Belanger's Croaker (Johnius Belangerii Cuvier 1830) at The Indian Ocean Coast of Yogyakarta, Indonesia.*, n.d.). In Java, the prevalence of Anisakis sp was reported in Lamongan, Malang, Cilacap, Jogakarta, and in Muara Angke Jakarta in Katsuwonus pelamis, Trichiurus spp, Terapon jarbua, Lutjanus malabaricus fishes of 60%, 53.5%, 66.6% and 80% respectively (Bobsaid *et al*., 2021; *Presence of Anisakis Nematode Larvae in Indian Mackerel (Rastrelliger Spp.) along the Indian Ocean Southern Coast of East Java, Indonesia*, n.d.; Qurota Ayun *et al*., 2021; Siagian & Maryanti, 2021). In the Bali region, the prevalence of Anisakis sp has been reported in Selar crumenophthalmus fish with a prevalence of 80%, as well as in North Sumatra and Aceh in Euthynnus affinis and Echinorhynchus sp fish with a prevalence of 100% (Siagian & Maryanti, 2021). In the Makassar area, the prevalence of Anisakis sp in Auxis rochei fish is 43% (Anshary *et al*., 2014). In Eastern Indonesia, it was reported that Anisakis was present in Katsuwonus pelamis and Auxis thazard with prevalence of 16% and 20% respectively (Hibur *et al*., 2016). The prevalence of Anisakis sp was also reported in *Epinephelus* sp fish of 22% (Detha *et al*., 2018). Until recently there have been no reported cases of Anisakis sp in *Rastrelliger* sp and the latest progress of anisakis incidence in *Epinephelus* sp. This study aims to identify the prevalence of the incidence of anisakis in *Rastrelliger* sp, and the latest progress of the incidence of Anisakis sp in *Epinephelus* sp in eastern waters of Indonesia.

METHODS

Sampling Technique

A total of 190 fish specimens from 2 different fish species were collected from fish selling points in various regions in Kupang City, East Nusa Tenggara, Indonesia. Sampling was conducted in the 2020 period. The samples consisted of two fish specimens, *Epinephelus* sp and *Rastrelliger* sp. Sampling was carried out by random sampling on 95 *Rastrelliger* sp and 95 *Epinephelus* sp. The sampling period for *Rastrelliger* sp and *Epinephelus* sp was each carried out in stages, one

Vol. 9 No. 1:62-71 (2021)
DOI:https://doi.org/10.35508/jkv.v9i1.8426

week four times, so that a total of 190 fish would be examined.

Fish Inspection

The fish were collected randomly and put in a coolbox at 4 until they arrived at the laboratory to inhibit the spoilage of the samples. Fish samples that have been taken are placed on a tray and then the length is measured. Subsequently, surgery with a scalpel was performed on the ventral part of the fish. The incision starts from the cloaca towards the anterior to the operculum to take the innards of the fish to be examined. The innards or internal organs of the fish to be examined are collected from internal organs (liver, intestines, stomach, and muscles). The internal organs that have been taken are placed in a petri dish and given a physiological NaCl solution to keep the fish organs wet. The next step is to examine the presence of Anisakis sp. parasite infection visually as well as to calculate the number of parasites that contaminate fish offal and organs.

Identification Technique

The parasites obtained were collected in petri dishes and cleaned of debris that was still attached and then fixed with 70% alcohol. Larvae staining was carried out using Semichen acetic carmine solution which was dripped on the Anisakis sp parasite with gradual dehydration for 5 minutes with 70%, 85%, and 95% alcohol, respectively. The next step is to identify the parasite under a stereo microscope (Detha *et al*., 2018). The data collection consists of the number of detected larvae and their location. The larvae obtained were washed with saline solution for morphological identification at the genus level. Morphological identification of Anisakis sp larvae by observing the shape of the ventriculus, boring tooth, and mucron on a stereo microscope (Haryadi *et al*., 2019; Quiazon *et al*., 2008; Roca-Geronès *et al*., 2020; van Hien *et al*., 2021). Larvae are grouped into Anisakis sp. type I and type II based on the results of previous studies (Suryani *et al*., 2021; van Hien *et al*., 2021), and calculating the prevalence and degree of infection (Debenedetti *et al*., 2019; Gomes *et al*., 2020; Ozuni *et al*., 2021)

Data analysis

The number of fish containing larvae, the number of larvae for each fish was then calculated using standard infection parameters to measure prevalence, and the intensity or density of parasitic infections in fish populations. Calculation of prevalence was identified as the number of hosts infected with Anisakis divided by the number of hosts examined for that parasite species. Intensity measurement by counting the number of Anisakis larvae in one infected host.

RESULT AND DISCUSSION

Intensity Levels of Anisakis sp

The results showed that Anisakis sp. distributed in the digestive organs and muscles of fish (Table 1). Results on *Epinephelus* sp, Anisakis sp worms. more commonly found in the intestine as many as 290 Anisakis sp. from 74 fish, muscle as much as 252 Anisakis sp. from 49 fish, gonads of 87 Anisakis sp. of 22 fish, liver as many as 62 Anisakis sp. of 19 fish, stomach as many as 43 Anisakis sp. of 15 fish. The results showed that the degree of infection of Anisakis sp. on *Epinephelus* sp overall is 7.80 including moderate category (Faizal Ulkhaq *et al*., n.d.). Based on the results, it was found that 3 positive *Rastrelliger* sp. Anisakis sp. And found 4 larvae of Anisakis sp on the surface of the stomach and 3 others found in the intestine so that the total degree of infection of Anisakis sp. on *Rastrelliger* sp of 1.33 is in the low category (Abou-Rahma *et al*., 2016; Ajeng Nastiti *et al*., 2021).

Table 1. Epidemiological parameters and categories of Anisakis sp in *Epinephelus* sp and *Rastrelliger* sp

Epidemiological parameters	*Epinephelus sp*	*Rastrelliger sp*	Categories of *Epinephelus* sp	Categories of *Rastrelliger sp*
Intensity	7,80	1,33	Medium	Low
Prevalence	98%	3,15%	Almost always	Occasionally
Number of anisakis larvae	734	4		
Number of anisakis larvae in muscle	252	0		
Number of anisakis larvae in visceral organs	482	4		

Figure 2. Distribution of Anisakis sp larvae on visceral organs in *Epinephelus* sp and *Rastrelliger* sp

Vol. 9 No. 1:62-71 (2021)
DOI:https://doi.org/10.35508/jkv.v9i1.8426

Results of Calculation of the Prevalence of Anisakis sp.

Based on the results of the study, the prevalence of Anisakis sp. in *Epinephelus* sp, which is 98% where 94 of the 95 *Epinephelus* sp tested positive for Anisakis sp. *Epinephelus* sp positive Anisakis sp was found in fish with body length >19 cm. The prevalence of Anisakis sp. in *Epinephelus* sp, 98% is categorized almost always (Almost always), which means the infection is very severe. Similar results were also reported in Brondong Lamongan, East Java, obtaining a prevalence value of 100%, where a total sample of 30 *Epinephelus* sp was positive for Anisakis sp (Ajeng Nastiti *et al*., 2021). Another study also reported the prevalence of Anisakis sp. which is quite high is also reported at 80%, it is known that 8 out of 10 *Epinephelus* sp are positive for Anisakis sp larvae (Abou-Rahma *et al*., 2016).

The results of the calculation of the prevalence of Anisakis sp in *Rastrelliger* sp of 3.15% obtained from 3 *Rastrelliger* sp were positive for Anisakis sp. The larvae found have a body length of 24-27 cm. The prevalence of Anisakis sp. in *Rastrelliger* sp, 3.15% is categorized as occasional infection (*occasionally*) (Ajeng Nastiti *et al*., 2021). This result is similar to several studies which mention the prevalence of Anisakis sp. in *Rastrelliger* sp which is lower than *Epinephelus* sp (Detha *et al*., 2018; Faizal Ulkhaq *et al*., n.d.)

The prevalence of Anisakis sp in *Epinephelus* sp in Kupang waters has been previously studied (Ina Rohi Detha *et al*., 2018). From the results of previous studies, the prevalence of Anisakis sp in *Epinephelus* sp is 22% (Detha *et al*., 2018). Another study obtained a higher prevalence of 76.67%, so it can be said that in the last 3 years there has been a drastic increase in Anisakis sp infection with *Epinephelus* sp (Paremme *et al*., 2018). The prevalence and degree of infection of *Rastrelliger* sp when compared with *Epinephelus* sp is highly inversely proportional. The prevalence and degree of infection of *Rastrelliger* sp is lower than *Epinephelus* sp, presumably because *Rastrelliger* sp is a pelagic fish and *Epinephelus* sp is a demersal fish (Condini *et al*., 2014; Roca-Geronès *et al*., 2020).

Epinephelus sp is known as a demersal fish where this fish lives closer to the substrate or food residue deposits which are a factor in the emergence of disease so that immunity is reduced and is more easily attacked by parasites, while *Rastrelliger* sp is a pelagic fish that lives not close to the substrate or sedimentary debris. feed residue which is one of the factors causing parasite invasion (Faizal Ulkhaq *et al*., n.d.; Osman *et al*., 2021). Types of food *Epinephelus* sp is fish, squid, and shrimp measuring 10-25% body size where the food is an intermediate host of the larvae of Anisakis sp. so this can be a factor in the high

prevalence of Anisakis sp in *Epinephelus* sp (Raharjo *et al*., 2017).

The low prevalence and degree of infection of *Rastrelliger* sp is also thought to be due to the type of food of the two fish. It is known that *Epinephelus* sp is a type of carnivorous fish and *Rastrelliger* sp is a type of omnivorous fish. The results of observations on the eating habits of *Rastrelliger* sp showed that the largest composition of the stomach contents of *Rastrelliger* sp was Rhizosolenia which was 67.16%, Paracalanus Acartia 24.72%, anchovy 15.5%, and fish scales 10.33% (Salsabila *et al*., n.d.). Rhizosolenia is a phytoplankton and not an intermediate host of larvae of Anisakis sp. The largest composition of the stomach contents of *Rastrelliger* sp is phytoplankton, so it is suspected that the eating habits of *Rastrelliger* sp. This is one of the factors for the low prevalence of Anisakis sp in *Rastrelliger* sp (Shojaei, 2020).

Another factor suspected to be the cause of the low prevalence of Anisakis sp. in *Rastrelliger* sp is the age of the fish. The lifespan of *Rastrelliger* sp is shorter than that of *Epinephelus* sp. Studies show that the age of one *Epinephelus* sp can reach 40 years with a maximum length of 120 cm (Condini *et al*., 2014; Tadjuddah *et al*., 2013). However, *Rastrelliger* sp takes 48 months or 2 years to reach its maximum length of 36.8 cm (Nasution *et al*., 2015).

The risk of the presence of Anisakis sp type 1 in fishery products can have an impact on public health problems. The incidence of anisakiasis can also have an economic impact, especially on fish species that are often consumed. Danger of Anisakis based on the results obtained, namely the potential for exposure to Anisakis sp larvae and allergens after ingestion of fish containing larvae triggers the effect of hypersensitivity. In addition, it should be noted that the predilection organ of Anisakis can be found in large numbers in the muscles, the part of which is consumed by the public. Therefore, it is necessary to implement standardized processing safety measures.

CONCLUSION

The prevalence and intensity of Anisakis sp. *Epinephelus* sp fish are 98% and 7.80 which are included in the Almost always and Medium categories. The prevalence and intensity of Anisakis sp. on *Rastrelliger* sp fish by 3.15% and 1.33 which are included in the Occasional and Low categories.

Vol. 9 No. 1:62-71 (2021)
DOI:https://doi.org/10.35508/jkv.v9i1.8426

REFERENCES

Abou-Rahma, Y., Abdel-Gaber, R., & Kamal Ahmed, A. (2016). First Record of Anisakis simplex Third-Stage Larvae (Nematoda, Anisakidae) in European Hake Merluccius merluccius lessepsianus in Egyptian Water. *Journal of Parasitology Research*, *2016*. https://doi.org/10.1155/2016/9609752

Ajeng Nastiti, A., Gde, P., Julyantoro, S., Ayu, D., Pebriani, A., & Suryaningtyas, W. (2021). Diterima (received) 20 Mei 2021; disetujui (accepted) 11 Juni 2021; tersedia secara online (available online). *Curr.Trends Aq. Sci. IV*, *2*, 199–204.

Anshary, H., Sriwulan, Freeman, M. A., & Ogawa, K. (2014). Occurrence and Molecular Identification of Anisakis Dujardin, 1845 from Marine Fish in Southern Makassar Strait, Indonesia. *The Korean Journal of Parasitology*, *52*(1), 9. https://doi.org/10.3347/KJP.2014.52.1.9

Bao, M., Pierce, G. J., Strachan, N. J. C., Pascual, S., González-Muñoz, M., & Levsen, A. (2019). Human health, legislative and socioeconomic issues caused by the fish-borne zoonotic parasite Anisakis: Challenges in risk assessment. *Trends in Food Science and Technology*, *86*, 298–310. https://doi.org/10.1016/J.TIFS.2019.02.013

Baptista-Fernandes, T., Rodrigues, M., Castro, I., Paixão, P., Pinto-Marques, P., Roque, L., Belo, S., Ferreira, P. M., Mansinho, K., & Toscano, C. (2017). Human gastric hyperinfection by Anisakis simplex: A severe and unusual presentation and a brief review. *International Journal of Infectious Diseases*, *64*, 38–41. https://doi.org/10.1016/j.ijid.2017.08.012

Bobsaid, R., Sari, P. D. W., & Subekti, S. (2021). Occurance of Anisakis of mackarel tuna (Euthynnus affinis) from Sendangbiru fishing auction place, East Java, Indonesia. *IOP Conference Series: Earth and Environmental Science*, *679*(1). https://doi.org/10.1088/1755-1315/679/1/012060

Buchmann, K., & Mehrdana, F. (2016). Effects of anisakid nematodes Anisakis simplex (s.l.), Pseudoterranova decipiens (s.l.) and Contracaecum osculatum (s.l.) on fish and consumer health. In *Food and Waterborne Parasitology* (Vol. 4, pp. 13–22). Elsevier Inc. https://doi.org/10.1016/j.fawpar.2016.07.003

Caldeira, A. J. R., Pereira Alves, C. P., & Santos, M. J. (2021). Anisakis notification in fish: An assessment of the cases reported in the European Union rapid alert system for food and feed (RASFF) database. *Food Control*, *124*, 107913. https://doi.org/10.1016/J.FOODCONT.2021.107913

Condini, M. v., Albuquerque, C. Q., & Garcia, A. M. (2014). Age and growth of dusky grouper (*Epinephelus* marginatus) (Perciformes: Epinephelidae) in the southwestern Atlantic, with a size comparison of offshore and littoral habitats. *Fishery Bulletin*, *112*(4), 311–322. https://doi.org/10.7755/FB.112.4.7

Debenedetti, Á. L., Madrid, E., Trelis, M., Codes, F. J., Gil-Gómez, F., Sáez-Durán, S., & Fuentes, M. v. (2019). Prevalence and risk of anisakid larvae in fresh fish frequently consumed in Spain: An overview. *Fishes*, *4*(1). https://doi.org/10.3390/FISHES4010013

Detha, A. I. R., Wuri, D. A., Almet, J., Riwu, Y., & Melky, C. (2018). First report of Anisakis sp. in *Epinephelus* sp. in East Indonesia. *Journal of Advanced Veterinary and Animal Research*, *5*(1), 88–92. https://doi.org/10.5455/JAVAR.2018.E241

Faizal Ulkhaq, M., Setia Budi, D., Kenconojati, H., & Hanif Azhar, M. (n.d.). *Insidensi dan Derajat Infeksi Anisakiasis pada Ikan Hasil Tangkapan di Pelabuhan Perikanan Pantai Muncar, Banyuwangi, Jawa Timur (INCIDENCE AND DEGREE OF ANISAKIASIS INFECTION IN FISH CATCHES AT THE FISHERY PORT OF MUNCAR BEACH, BANYUWANGI, EAST JAVA)*. https://doi.org/10.19087/jveteriner.2019.20.1.101

Gomes, T. L., Quiazon, K. M. A., Kotake, M., Itoh, N., & Yoshinaga, T. (2020). Anisakis spp. in fishery products from Japanese waters: Updated insights on host prevalence and human infection risk factors. *Parasitology International*, *78*. https://doi.org/10.1016/j.parint.2020.102137

Haryadi, L., Suprayitno, E., Aulanni'am, A., & Hariati, A. M. (2019). Immune response evaluation in Balb/c mice after crude extract of Anisakis typica sensitization. *Veterinary World*, *12*(10), 1529–1534. https://doi.org/10.14202/VETWORLD.2019.1529-1534

Hibur, O. S., Detha, A. I. R., Almet, J., & . I. (2016). TINGKAT KEJADIAN PARASIT Anisakis sp. PADA IKAN CAKALANG (Katsuwonus pelamis) DAN IKAN TONGKOL (Auxis thazard) YANG DIJUAL DI TEMPAT PENJUALAN IKAN PASIR PANJANG KOTA KUPANG. *JURNAL KAJIAN VETERINER*, *4*(2), 40–51. https://doi.org/10.35508/JKV.V4I2.1019

Ina Rohi Detha, A., Agustiani Wuri, D., Almet, J., Riwu, Y., & Melky, C. (2018). *Epinephelus* sp. in East Indonesia. *Journal of Advanced Veterinary and Animal Research*, *5*(1), 88–92. https://doi.org/10.5455/javar.2018.e241

Ivanović, J., Baltić, M., Bošković, M., Kilibarda, N., Dokmanović, M., Marković, R., Janjić, J., & Baltić, B. (2017). Anisakis allergy in human. *Trends in Food Science & Technology*, *59*, 25–29. https://doi.org/10.1016/J.TIFS.2016.11.006

Ivanovic, J., Baltic, M. Z., Boskovic, M., Kilibarda, N., Dokmanovic, M., Markovic, R., Janjic, J., & Baltic, B. (2015). Anisakis Infection and Allergy in Humans. *Procedia Food Science*, *5*, 101–104. https://doi.org/10.1016/J.PROFOO.2015.09.028

Mattiucci, S., Cipriani, P., Levsen, A., Paoletti, M., & Nascetti, G. (2018). Molecular Epidemiology of Anisakis and Anisakiasis: An Ecological and Evolutionary Road Map. *Advances in Parasitology*, *99*, 93–263. https://doi.org/10.1016/BS.APAR.2017.12.001

Vol. 9 No. 1:62-71 (2021)
DOI:https://doi.org/10.35508/jkv.v9i1.8426

Nasution, M. A., Kamal, M. M., & Azis, K. A. (2015). PERTUMBUHAN DAN REPRODUKSI IKAN KEMBUNG LELAKI (*Rastrelliger* kanagurta Cuvier 1817) YANG DIDARATKAN DI PPN PALABUHAN RATU. *JURNAL PERIKANAN TROPIS*, *2*(1). https://doi.org/10.35308/JPT.V2I1.20

Osman, Y. A. A., Samy-Kamal, M., & El-Mahdy, S. M. (2021). Age, growth and mortality of Indian mackerel *Rastrelliger* kanagurta (Teleostei: Scombridae) in the Egyptian Red Sea coast. *Iranian Journal of Ichthyology*, *8*(3), 236–249. https://doi.org/10.22034/IJI.V8I3.593

Ozuni, E., Vodica, A., Castrica, M., Brecchia, G., Curone, G., Agradi, S., Miraglia, D., Menchetti, L., Balzaretti, C. M., & Andoni, E. (2021). *Prevalence of Anisakis Larvae in Different Fish Species in Southern Albania: Five-Year Monitoring (2016-2020)*. https://doi.org/10.3390/app112311528

Paremme, A., Paremme, A. M., Salosso, Y., & * S. (2018). IDENTIFIKASI PARASIT Anisakis sp PADA IKAN KAKAP PUTIH (Lates calcarifer), KAKAP MERAH (Lutjanus sanguineus), DAN KERAPU (*Epinephelus* sp) YANG DIPEROLEH DI PERAIRAN TELUK KUPANG. *Grouper: Jurnal Ilmiah Fakultas Perikanan Universitas Islam Lamongan*, *9*(2), 19–25. https://doi.org/10.30736/grouper.v9i2.40

Presence of Anisakis nematode larvae in Indian mackerel (Rastrelliger spp.) along the Indian Ocean southern coast of East Java, Indonesia. (n.d.). https://doi.org/10.13057/biodiv/d200136

Quiazon, K. M. A., Yoshinaga, T., Ogawa, K., & Yukami, R. (2008). Morphological differences between larvae and in vitro-cultured adults of Anisakis simplex (sensu stricto) and Anisakis pegreffii (Nematoda: Anisakidae). *Parasitology International*, *57*(4), 483–489. https://doi.org/10.1016/J.PARINT.2008.06.003

Qurota Ayun, N., Septiana Dewi, L., & Setyobudi, E. (2021). *The occurrence of Anisakis larvae on hairtail, Trichiurus lepturus caught from the Pangandaran Waters, West Java, Indonesia*. *22*(3). https://doi.org/10.13057/biodiv/d220339

Raharjo, H. M., Koesdarto, S., Soelih Estoepangestie, A. T., & Wardhani, K. (2017). *Preservation Effect of Grouper (Epinephelus sp) Fillet Against Survival of Anisakidae*. https://doi.org/10.18502/kls.v3i6.1101

Rahmati, A. R., Kiani, B., Afshari, A., Moghaddas, E., Williams, M., & Shamsi, S. (2020). World-wide prevalence of Anisakis larvae in fish and its relationship to human allergic anisakiasis: a systematic review. *Parasitology Research 2020 119:11*, *119*(11), 3585–3594. https://doi.org/10.1007/S00436-020-06892-0

Roca-Geronès, X., Segovia, M., Godínez-González, C., Fisa, R., & Montoliu, I. (2020). Anisakis and Hysterothylacium species in Mediterranean and North-East Atlantic fishes commonly consumed in Spain: Epidemiological, molecular and morphometric discriminant analysis. *International Journal of Food Microbiology*, *325*. https://doi.org/10.1016/j.ijfoodmicro.2020.108642

Salsabila, S., Affandi, R., Manajemen, D., Perairan, S., Perikanan, F., Kelautan, D. I., Pertanian, I., Agatis, B. J., Kampus, I., Dramaga, J., Barat, I., Author, K., & Pertanian Bogor, I. (n.d.). *Preferensi Makanan Ikan Kembung Lelaki (Rastrelliger kanagurta Cuvier, 1816) Terhadap Klorofil-A*.

Shojaei, G. M. (2020). Food and feeding habits of Indian mackerel (*Rastrelliger* kanagurta) in the southern part of Qeshm Island, Persian Gulf. *Iranian Journal of Fisheries Sciences*, *19*(2), 2020. https://doi.org/10.22092/IJFS.2018.120058

Siagian, F. E., & Maryanti, E. (2021). Anisakiasis Pada Ikan Laut Di Indonesia: Prevalensi, Sebaran Dan Potensi Patogenitasnya Pada Manusia. *Jurnal Ilmu Kedokteran (Journal of Medical Science)*, *14*(1), 11–23. https://doi.org/10.26891/JIK.V14I1.2020.11-23

Suryani, N., Subekti, S., Koesdarto, S., & Amiin, M. K. (2021). Morphological profile of L2 Anisakis typica on Indian Mackerel (*Rastrelliger* kanagurta) from Sedati Fish Auction, Sidoarjo-East Java, Indonesia using Scanning Electron Microscope (SEM). *IOP Conference Series: Earth and Environmental Science*, *679*(1). https://doi.org/10.1088/1755-1315/679/1/012059

Tadjuddah, M., Wiryawan, B., Purbayanto, A., & Wiyono, E. S. (2013). *PARAMETER BIOLOGI IKAN KERAPU (Epinephelus sp.) HASIL TANGKAPAN DI PERAIRAN TAMAN NASIONAL WAKATOBI, SULAWESI TENGGARA INDONESIA (Biological Parameters of Grouper (Epinephelus sp) Caught in Wakatobi National Park, Southeast Sulawesi, Indonesia) Oleh*. *4*(1), 11–21.

van Hien, H., Dung, B. T., Ngo, H. D., & Doanh, P. N. (2021). First morphological and molecular identification of third-stage larvae of Anisakis typica (Nematoda: Anisakidae) from marine fishes in Vietnamese water. *Journal of Nematology*, *53*, 2021–2031. https://doi.org/10.21307/JOFNEM-2021-010