Occurance of Anisakis of mackarel tuna (*Euthynnus affinis*) from Sendangbiru fishing auction place, East Java, Indonesia

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Abstract. This study aims to determine the prevalence and the intensity of *Anisakis* from Mackarel Tuna (*Euthynnus affinis*). A total number of 180 of mackarel tuna were collected from Sendangbiru Fishing Auction Place, East Java, Indonesia. Fish were examined and observed for the *Anisakis* using a stereo microscope and a light microscope. The morphology of *Anisakis* were observed after colored by Semichen-acetic carmine and drawn by camera lucida. *Anisakis simplex* L3 were found in all of the fish sample with the predilection in the gastrointestinal and mesenterium of Mackarel Tuna. The prevalence of *A. simplex* is 7.5%, while the intensity is 1.7.

1. Introduction

Mackerel tuna (*Euthynus affinis*) is a fishery commodity that has a high economic value in Indonesia. The distribution of mackerel tuna includes South Java, Banda Sea, Maluku Sea, Flores Sea, Sulawesi Sea, Indian Sea, Halmahera Sea, North Aceh waters, West Sumatra, North Sulawesi, Tomini Bay, Cendrawasih Bay, and Arafuru Sea [1]. Sendangbiru is the biggest contributor to the amount of TTC production in the Malang Regency [2]. In 2018, tuna, skipjack, and mackarel tuna (TCT) contributed foreign exchange of US $ 713.9 million or 14.69% of the total exports of fishery products. Meanwhile, in terms of volume, in 2018 Indonesia's TCT exports amounted to 1,033,211 tons/year or 14.69% of the total exports of fishery products [3].

This study focuses on the prevalence and intensity of nematode endoparasitic worms in mackarel tuna. In Indonesia, research on zoonotic nematode worms in tuna still needs to be improved because mackarel tuna is a fish that has high economic value, and contains nutrients that are needed, and beneficial to the human body.

This study aims to find out more about the prevalence, and intensity of nematode endoparasitic worms in mackarel tuna (*E. affinis*) in the Fish Auction Place (TPI), Sendangbiru, Malang. So that it can add information to the public regarding the prevalence, and intensity of nematode worms in Mackarel tuna (*E. affinis*) at the Fish Auction Place (TPI), Sendangbiru, Malang.
2. Material and methods

2.1. Material

This research was conducted out in November 2018 - February 2019. This study used a survey method. Sampling was done by a random sampling method as many as 180 mackarel tuna obtained from traders at the fish auction place (TPI) Sendangbiru Malang.

The equipment used for endoparasitic worm identification and prevalence calculation is a sectio set (PrimaMed-B14, Germany), a tissue pipette (NescoLab, Indonesia), a ruler (Butterfly, Indonesia) digital scales (SF-400, Beijing), hand gloves (Sensi, Indonesia) masks (Sensi, Indonesia), label paper (Fox-121, Indonesia), microtube (GP-005A, China), binocular microscope equipped with a Lucida camera (Olympus-CX21, China).

The main material used in this study were 180 mackarel tuna (E. affinis). Materials used for the identification of endoparasitic worms, and their staining were 70% alcohol, 85% alcohol, 95% alcohol (Merck, Germany), acid alcohol (Alcohol 70% + HCl (Merck, Germany), base alcohol (Alcohol 70% + NaHCO3 (Merck, Germany)), PZ (Physiological NaCl) (Otsu-Ns, Germany), aquades sterile (Otsu-Wi, Germany), entellan (Merck, Germany), glycerin alcohol 5% (Merck, Germany), and Semichoen-Acetic Carmine (Sigma, Germany).

2.2. Method

2.2.1. Work procedures

2.2.1.1. Preparation of tools and materials

Prepare a sectio set (scalpel, surgical scissors, tweezers, object-glass, and cover glass) and a tray. Next, prepare mackerel tuna samples to be observed.

2.2.1.2. Sampling

Sampling was carried out with as many as 180 tuna fish obtained from traders at the Fish Auction Place (TPI) Sendangbiru Malang following what was stated by Cameron [4] as many as three times.

2.2.1.3. Endoparasite worms examination and identification

The fish that are in the tub is taken, and placed on the tray, then their body length is measured, weighed then performed surgery, and the digestive tract is taken from the fish for examination. The fish surgery starts from the anal part, and then leads to the anterior part of the body to the posterior part of the operculum, then it is cut dorsally to the lateral line, and cut towards the anal.

Fish that have been dissected are natively observed by directly examining the liver, muscles, spleen, body cavity, pylorus and gonads, besides that, it is also seen in the digestive tract and fish feces.

2.2.1.4. Endoparasite worms staining

The staining of endoparasite worms was carried out by the Semichoen Acetic Carmine method which refers to the modified Kuhlmann [5], namely by washing the worms that have been stored in 5% glycerin alcohol with a PZ solution then fixing them between two glass objects, and tying both ends with threads then inserted in 5% glycerin alcohol for 24 hours. In the next step, the worms are put in 70% alcohol for five minutes. After that, the glass object was transferred in a carmine solution that was diluted with 70% alcohol with a ratio of 1: 2, for 2 hours, then the worms were removed from the object glass, then moved the worms in an acidic alcohol solution (70% alcohol + HCL) for two minutes. When finished, the worms were transferred in an alkaline alcohol solution (70% alcohol + NaHCO3) for 20 minutes. Furthermore, graded dehydration was carried out using alcohol 70%, 85%, and 95% for five minutes, respectively. Then the mounting is done by taking the worm, and placing it on the object-glass, then dripping it with the entellan solution, and closed with a cover-glass.

2.2.1.5. Endoparasitic worms identification

Endoparasite worms identification was carried out after staining the endoparasitic worm samples.
Observations were made under a binocular microscope with a magnification of 100x, and 400x, then the endoparasite worm specimens were depicted using a Lucida camera. The key to identifying endoparasites used in this study is based on Arai and Smith [6].

### 2.2.1.6 Data analysis

The data on the identification results of endoparasitic worms that infect tuna are presented descriptively in the form of images for the identification of endoparasites, and tabular form for prevalence and intensity.

### 3. Result and discussion

#### 3.1. Result

The results showed that the presence of *A. simplex* (type III) larvae attached to the gastro-intestinal, and the stomach of the mackarel tuna. The results of *A. simplex* staining with Semichon Acetic Carmine observed by using a binocular microscope, and Lucida camera can be seen in Figure 1, 2 and 3.

![Figure 1](image1.png)

**Figure 1.** Anterior body of *A. simplex* third larvae stage. Description: A. Image of staining based on binocular microscope (400x magnification), B. Image with a binocular microscope equipped with a Lucida camera. (Bt) Booring tooth, (Ep) Esophagus, (Ve) Ventriculus.

The *A. simplex* larvae were found as an elongated cylindrical body shape with a booring tooth, the esophagus is round, and has a ventriculus. The results showed that the prevalence rate of endoparasitic worms in mackerel tuna caught at the Fish Auction Place (TPI) Sendangbiru, Malang tract was 5.0%. The prevalence value of *A. simplex* parasites on mackerel tuna in Sendangbiru TPI is occasional classified.

![Figure 2](image2.png)

**Figure 2.** Mid-body of *A. simplex* third larvae stage. Description: A. Image of staining based on binocular microscope with 400x magnification, B. Image with binocular microscope equipped with a Lucida camera. (Kl) Cuticle, (I) Intestine.
Figure 3. Posterior body of A. simplex third larvae stage.

Description: A. Image of staining based on binocular microscope with 400x magnification, B. Image with binocular microscope equipped with a Lucida camera. (Ep) Excretory pore, (Mc) Mucron.

The data from the calculation of the prevalence of tuna infected with the endoparasitic worm A. simplex obtained from the catch at the Fish Auction Place (TPI) Sendangbiru, Malang can be seen in Table 1.

Table 1. The prevalence of mackerel tuna infected with third stage larvae of A. simplex at Fish Auction Place (TPI) Sendangbiru, Malang

| Total of Mackarel Tuna Examined (fish) | Total of Mackarel Tuna Infected (fish) | Prevalence(%) |
|--------------------------------------|---------------------------------------|---------------|
| 180                                  | 9                                     | 5.0           |

The Intensity level of mackerel tuna (E. affinis) infected with third stage larvae of A. simplex obtained from the Fish Auction Place (TPI) Sendangbiru, Malang can be seen in Table 2.

Table 2. The intensity of mackerel tuna (E. affinis) infected with third stage larvae of Anisakis simplex at the Fish Auction Place (TPI) Sendangbiru, Malang

| Types of Worm Species | Total of Mackarel Tuna Infected (fish) | Total of Endoparasitic Worms | Intensity |
|-----------------------|---------------------------------------|-----------------------------|-----------|
| Anisakis simplex      | 9                                     | 16                          | 1.8       |

Table 3. Morphometer of third stage larvae of A. simplex infecting mackerel tuna (E. affinis) at Fish Auction Place (TPI) Sendangbiru, Malang

| Parameter          | Measurement (mm) |
|--------------------|------------------|
|                    | Range            | Average          |
| Body length        | 2.80 – 5.20      | 4.20             |
| Body width         | 0.17 – 0.41      | 0.29             |
| Esophagus          | 0.61 – 0.98 x 0.11 – 0.38* | 0.79 x 0.24* |
| Ventriculus        | 0.17 – 0.41 x 0.02 – 0.23* | 0.28 x 0.11* |
| Mucron             | 0.03 – 0.06 x 0.01 – 0.03* | 0.05 x 0.02* |
| Boring tooth       | 0.02 – 0.09 x 0.02 – 0.06* | 0.06 x 0.04* |

3.2. Discussion

The third larva stage of A. simplex is found in this study. This is consistent with Suryani [7] who found Anisakis in mackerel tuna Fish Auction Place (TPI) Sedati, Sidoarjo. Goffredo et al. [8] also stated that the discovery the third larva stage of A. simplex larvae of tuna fish in Italian waters. Aibinu et al. [9] stated that the second larva stage of Anisakis that had hatched from eggs infected small crustaceans which were then eaten by the fish as the second intermediate host, and developed into third larva stage in the fish bodies.
The A. simplex larvae found in this study have a total length of 4.20 mm, and a width of 0.29 mm, the esophagus has a length of 0.79 mm and a width of 0.24 mm, and the ventriculus has a length of 0.28 mm, and a width of 0.11 mm. This is consistent with Sonko et al. [10] who found A. simplex larvae in the digestive tract of mackerel (Scomber australasicus), and (Trichiurus lepturus) in Taiwanese waters with a length of 2-5 mm. Anisakis infection was also found in several commercial fish in the Aberdeen market with a total length of 4-8 mm [11].

Based on the prevalence and intensity data above, according to Williams and Williams [12] the prevalence value between 1-9% is included in the occasional category, while the intensity value between 1-5 is in the light category. This illustrates that the A. simplex occasional infects mackerel tuna with light intensity levels. The fish can be infected with A. simplex influenced by some factors, including the condition of the water environment. Dirty environmental conditions also cause the fish to become stressed which ultimately lowers the fish's immune system [9]. According to Gaglio et al. [13] the factors that trigger stress in fish are the imbalance of the interaction of biotic, and abiotic factors which can cause the fish's body power to decrease so that it is prone to disease.

4. Conclusion
Based on this analysis, the endoparasite worms that infect mackerel tuna (E. affinis) is A. simplex with a prevalence rate of 5.0% and is included in the occasional category. The intensity level is 1.8 parasites/fish and is included in the category of light parasites.

5. References
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6. Acknowledgement
This research was supported independently.