Endoparacite investigation in some fish species with high economic value obtained from Canakkale, Turkey

ABSTRACT

The study is an endoparasitic examination of chub mackerel (*Scomber japonicus* Houttuyn 1782), bogue (*Boops boops* Linnaeus 1758), horse mackerel (*Trachurus trachurus* Linnaeus 1758), sardine (*Sardina pilchardus* Walbaum 1792), and anchovy (*Engraulis encrasicolus* Linnaeus 1758) fish taken from Çanakkale fish state as an annual sampling between 01 September 2019- 01 September 2020. One thousand three hundred thirty-seven fishes were sampled. The external morphological examination of the fish brought to the laboratory was made, then the conditions of their visceral organs and their visceral structures were examined by dissection method. Nematodes collected alive from many parts of the body were kept in absolute (99.9%) ethanol. Parasites in ethanol were kept in glycerin for a few days before being examined under the microscope. Nematodes were first examined morphologically on a stereomicroscope and then under a light microscope for easy species identification. Classical detection methods have been used for species determination. As a result, among the individuals belonging to the Anisakidae family, *Anisakis* spp. L3 larval stage type 1 and type 2, *Anisakis physeteris* larval stage, *Contracaecum* larval stage, *Hysterothylacium aduncum* L3 larval stage were found. The density, abundance, and prevalence rates of the parasites in the infected fish are determined as a percentage.

Keywords: Anisakidae, bogue, chub mackerel, horse mackerel, nematoda

INTRODUCTION

Food safety can ensure that foodstuffs do not adversely affect consumer health by protecting them from chemical, biological, and physical hazards in the food chain (Official Gazette, 2008; Tayar, 2010; 2020). Increasing concerns about food safety with the changes in consumer habits cause both organizational and functional developments in the private sector and public sector. The state must adopt standards that provide a new perspective on food safety to ensure that the private sector gains an advantage in the competitive environment while preparing legislation and making controls on these issues. In addition, the existence of standards and structures to ensure food safety in every country raises compatibility problems with each other. It negatively affects the trade between countries producing similar products. For this reason, many companies and countries have come together and set standards for safe food supply and supply (FAO, 2011; Basaran, 2016). Since contamination can occur at every stage of the food production chain, public and regulatory authorities (European Commission, EFSA), International Organizations (FAO, WHO, OIE and CAC), producers and retailers of the food agriculture sector (BRC, IFS, GLOBALG.AP).

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Research Article

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During the production process from the farm to the end consumer, they have adopted that food safety and quality can be achieved with an integrated and multidisciplinary approach, considering the entire food chain. The increase in clinical cases related to food safety has encouraged the private retail sector to develop management systems that ensure integrity, traceability, safety, and quality in food production. There is a constantly increasing customer demand for safe food supply from production to consumption in the aquaculture sector. For this reason, quality must be reliably maintained during the processing, storage, and marketing of seafood, which is a portion of sweet food. Producing healthy and high-quality fishery products depends on compliance with hygiene rules at every stage from production to consumption (Erkan et al., 2008). Some types of fish, affluent in fatty acids essential for human health, have found more places in our tables. Especially in the waters of Turkey, sea fish such as anchovy, sardines, horse mackerel, bogue, and chub mackerel have become indispensable for the fish market. Regardless of the type of fish in Turkey, almost all these mentioned fish species are encountered every season.

We can find these fish species on almost every table in our country and find their place on the tables by using different methods. To maintain a healthy life, attention should be paid to hygienic conditions in fish processing, and care should be taken not to consume undercooked or raw. Many disease factors can be passed on to humans due to undercooked and raw consumption or non-compliance with hygienic conditions (Zhang et al., 2018). One of these factors is endoparasites. Endoparasites are creatures that start their life cycle freely in water and can display a different life cycle in each host it enters. They continue their life cycle by entering the fish from places such as the mouth and porter. While some species use fish as their final hosts, others become their last hosts by consumption of fish by mammals and birds. Endoparasites live in different parts of the body, from the blood to the organs in living things. They begin with oral ingestion and are mainly seen in the gastrointestinal region, including blood, brain, different parts of the cranium, and the heart. Unless severe symptoms are observed, humans can detect it too late or not (Chen et al., 2015; Casti et al., 2017).

Some of the endoparasites isolated from fish, such as chub mackerel, horse mackerel, anchovy, sardines, and bogue, are nematodes (Karl and Levsen, 2011; Ozer and Olguner, 2013). There are body structures between 50 mm -10 cm in length and 1-20 µm in diameter. They are cream-white. Their bodies are covered with a flexible, soft, and thick cuticle layer. Knowing the esophageal structure and its distance to the ventricular structure and its location with the intestinal structure are essential in determining the species (Elibuyuk Sahin, 2006; Tepe and Oğuz, 2013; Cavallero et al., 2015; Roca et al., 2018). These differences in the ventricular structure-small intestine junction are important taxonomic features for identifying species in the Anisakidae family. The nervous system structure is in the form of a ring called a nerve ring or a nerve collar around the esophagus. The excretory system ends with the discharge opening. The structure of the excretory opening guides the separation of nematode species, with different shapes opening out from the base of the sub ventral lips or the nerve ring. Different types of L2 and L3 forms from only larval forms of nematodes are observed in fish. Nematode species that use fish or mollusks as final hosts do not pass on to humans or other mammals, being non-zoonotic. In fish and marine mammals that are the last host; with L3, L4, they turn into 5th stage larvae (L5) and then become mature (Pekmezci et al., 2014; Kroff et al., 2017; Ozturk, and Yesil, 2018). Humans are caught by consuming the carrier intermediate hosts, which contain L3 in their tissues, and the fish, which are the second

intermediate hosts containing L4, in raw or undercooked form.

In the Canakkale region, the most affordable and preferred fish types hunted and observed at the fish market stalls are chub mackerel, horse mackerel, bogue, anchovies, and sardines. The parasites found in these fish cause problems in the local economy and the fish's visual and meat quality. Consumption of fish with parasites is not preferred. Our study was carried out by purchasing these fish from the fish market with monthly samples all year round. It was aimed to determine the effects of endoparasites, especially nematodes, seasonal and monthly infection formation, fish height and weight, and the prevalence, density, and abundance of the parasites in fish by examining the fish purchased from the fish market.

MATERIAL and METHOD

Fishes were sampled monthly from the Çanakkale Fish Market for 12 months between September 2019 and 2020. In the research, a total of 1337 fish, including 89 chub mackerel, 171 bogue, 350 horse mackerel, 277 anchovy, and 450 sardines, were examined in terms of endoparasites. As a result of the observations made, generally, free-moving nematode parasite individuals were found buried on the internal organ surfaces of the fish. All parasites in each fish were carefully collected and taken into 99.9% absolute ethanol for microscopic examinations. Parasite individuals taken in ethanol were softened by taking them in glycerin during the examination and made suitable for photography by allowing the alcohol to evaporate. The parasites were first examined under a stereomicroscope, then taken between the coverslip and taken to a more detailed examination under the light microscope and photographed. Species of the parasites were determined by considering the anatomical and morphological features of the parasite, such as the size, general appearance, features of the head and tail structures, esophagus, and secum lengths. As the type designation key; Elibuyuk Sahin (2006) Setyobudi et al., (2011); Mehlhorn, (2012); Sahin, (2014); Pozio, (2015); Morsy et al., (2017); Simsek et al. (2018) benefited from the studies.

RESULTS

In the fish samples taken into the study, 98 nematode individuals were isolated from chub mackerel, 2 in bogue, 27 nematodes in horse mackerel, 10 nematode individuals in anchovy, and no endoparasites were found in sardines. It was observed that the isolated nematodes mostly wandered on the organs in the body cavity. However, some of the nematodes taken from the chub mackerel fish in January were on the liver (Figure 1).

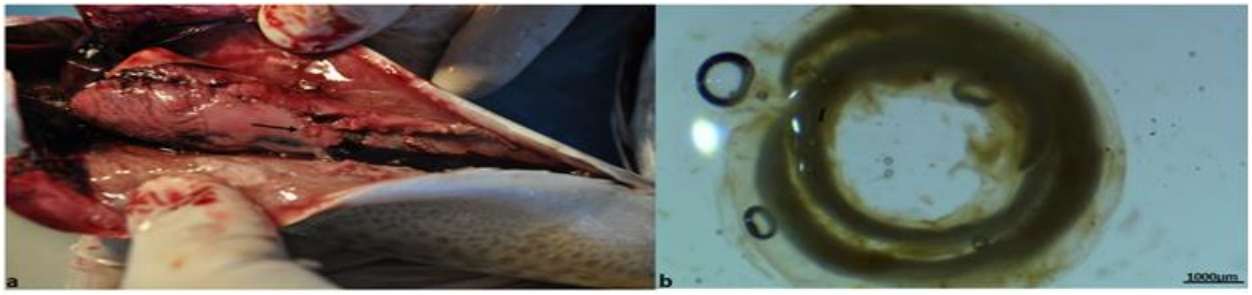

Figure 1. a) Nematode with cyst (arrow) buried in chub mackerel's liver, b) Nematode in cyst.

In the March sampling of the horse mackerel, it was observed that they were embedded in the cysts on the gonads of the female fish individual. It was determined that the

nematodes taken from fish whose parasitological examinations were completed, Ansisakidae family members, *Anisakis typica* L3 Type 2 larva (Figure 2), *Contracaecum pelagicum* L3 larva (Figure 3), *Hysterothylacium* spp. L3 larvae non-mucous stage (synonymous: *Contracaecum* spp.) L3 larva (Figure 4), *Anisakis physeteris* L3 larvae (Figure 5) *Anisakis* spp. L3 type 2, 3 (Figure 8), *Contracaecum* spp. L3 larva (Figure 7), *Hysterothylacium* spp., *Anisakis simplex* L3 larvae (Figure 6) larva has been identified.

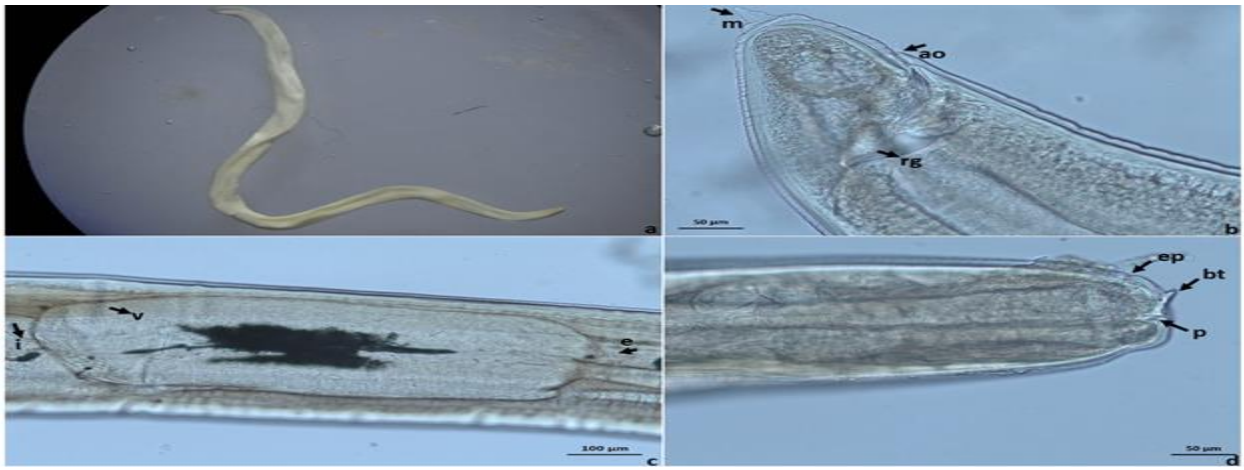

Figure 2. Sampling from chub mackerel (*Scomber japonicus*) *Anisakis typica.* L3 Type 2; a) External Morphology, b) Posterior Part (m: mucron, ao: anus opening, rg: rectal gland), c) Ventricular Part (v: ventriculus, e: esophagus, i: intestine), d: Anterior Part (bt: tooth, ep:, p: papilla).

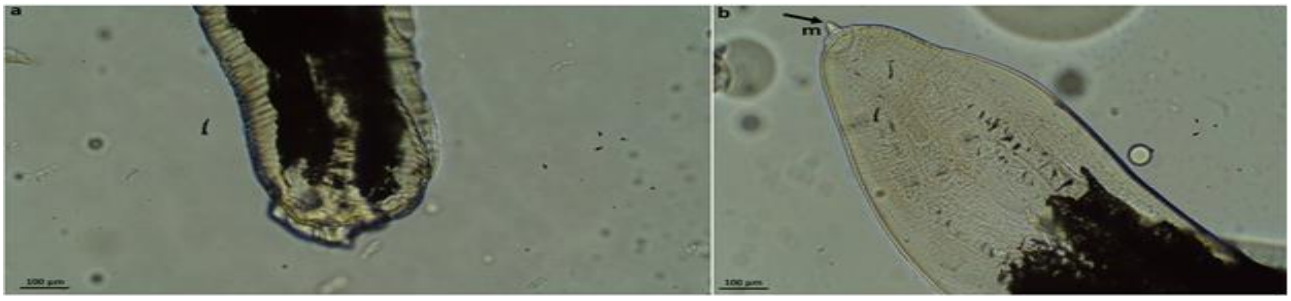

Figure 3. Sampling from chub mackerel (*Scomber japonicus*) Larva of *Contracaecum pelagicum* L3 a) Anterior part (cephalic end), b) Posterior part (m: mucron).

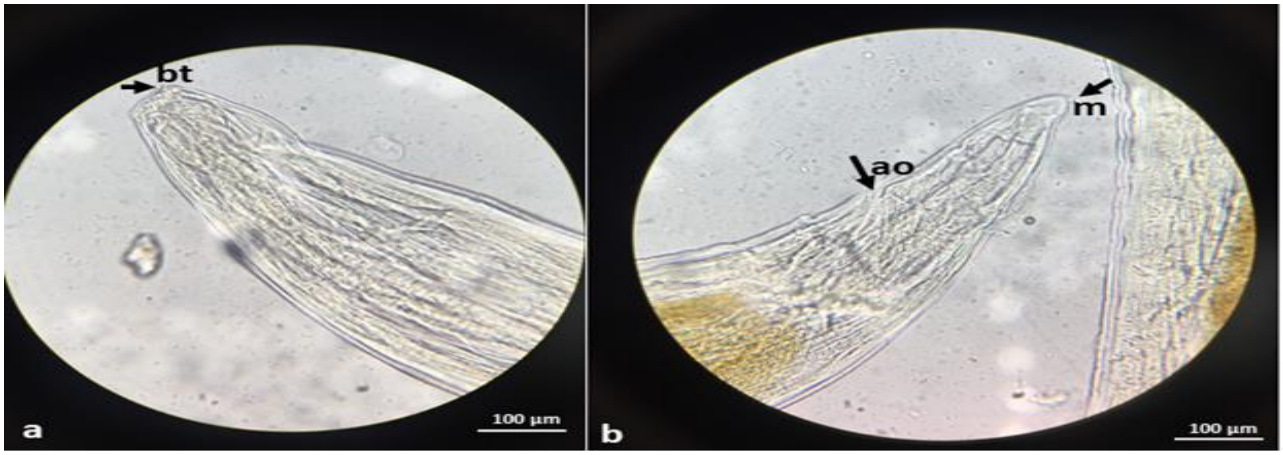

Figure 4. Sampling from horse mackerel (*Trachurus trachurus*) (*Contracaecum* spp., Synonymous) *Hysterothylacium* spp. L3 form larva a) anterior part cephalic Son (bt: tooth); b) posterior part (m: mucoron, ao: anus opening).

While nematodes occur mainly in the autumn season, the fish taken in the summer and spring periods show high, respectively. Annual nematode prevalence is 4.26%. Of the 137 nematodes, 98 (72%) were isolated from chub mackerel, 27 (20%) from horse mackerel, 10

(7.3%) from Anchovy, and 2 (1%) from bogue. There is no nematode isolated from sardines. Parasitic nematodes were primarily observed in March. The annual prevalence of the parasite is 43.82%, its density is 2.51, and its abundance is 1.10. The seasonal dominance percentage of nematodes, which are primarily observed in chub mackerel throughout the year.

The total number of nematodes observed in 7 of the horse mackerel is 27 (19.71%) and it is 2% prevalent in annual sampling. Its density is 3.86 and abundance is 0.08. The annual prevalence level of nematodes in bogue found to be 0.58%, the average density as 2, and the average abundance as 0.012. The annual prevalence level of nematodes in anchovy is 3.63%, density one, and abundance 0.04.

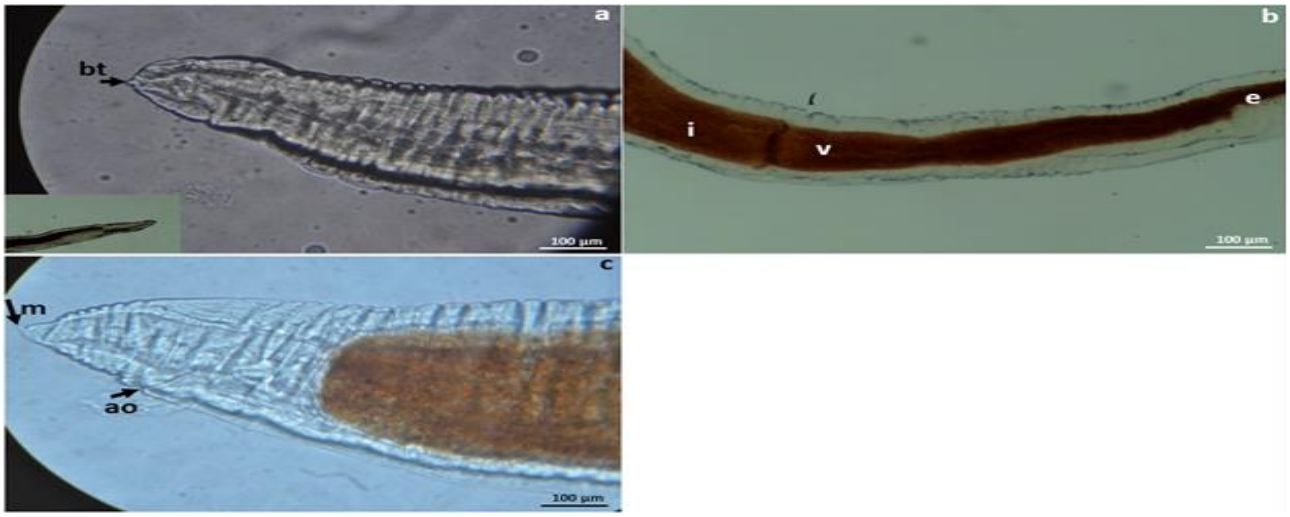

Figure 5. *Anisakis physeteris* L3 larval form in horse mackerel (*Trachurus trachurus*). a) Anterior Part (bt: tooth), b) Venricular Part (i: intestine, v: ventriculus, e: esophagus), c: Posterior Part (m: mucoron, ao: anus opening).

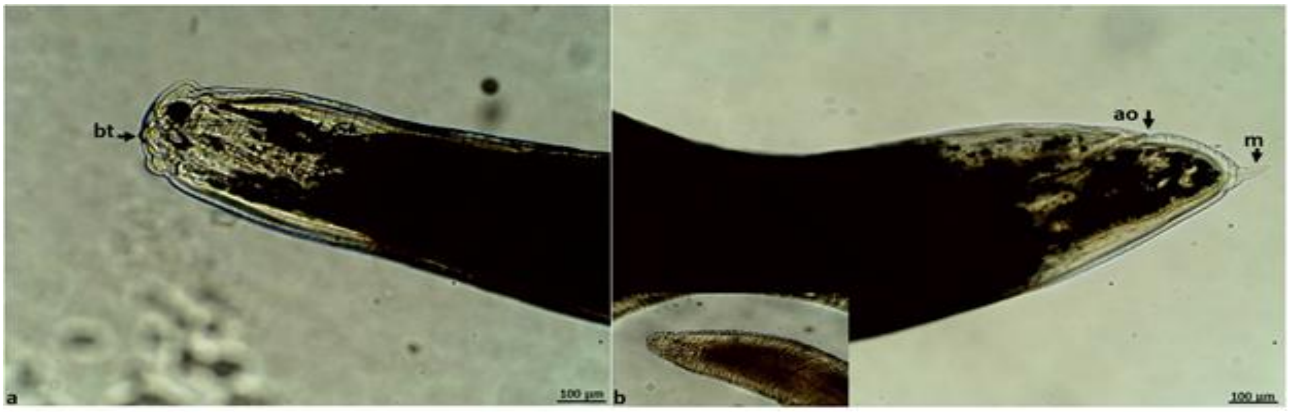

Figure 6. *Anisakis simplex* L3 larval form in anchovy (*Engraulis encrasicolus*). a) Anterior Part (bt: tooth), b) Posterior Part (ao: anus opening, m: mucron).

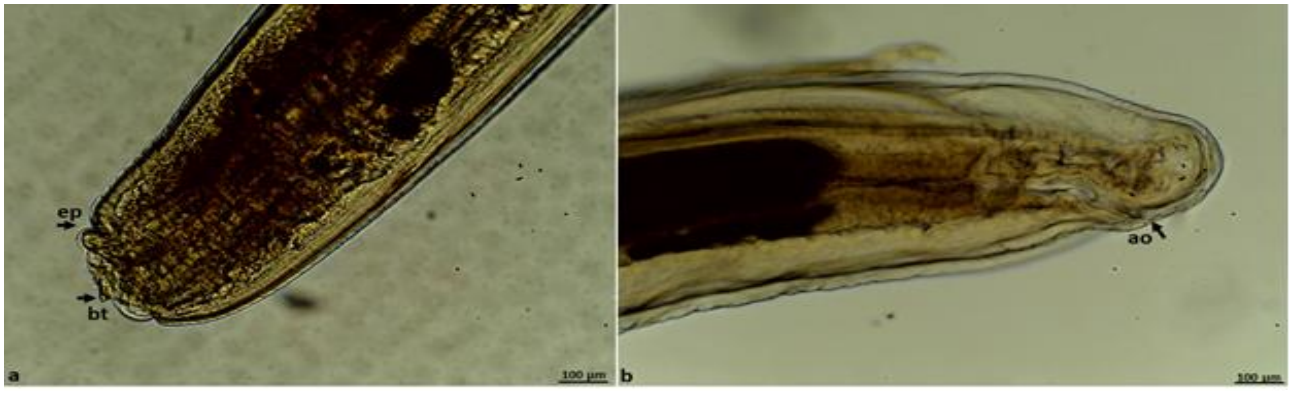

Figure 7. *Contracaecum* sp. in bogue (*Boops boops*). L3 Larva a) Anterior part (ep: excretory opening, bt: tooth), b) Posterior part, (without mucous, ao: anus opening).

In March, parasitism was observed in chub mackerel at a minimum height of 19.7 cm and a weight of 61.32 gr. Parasitism in horse mackerel was detected in March at a minimum length of 15.2 cm and a weight of 28.87 g. In May, when parasitism was observed, the average height of bogue is a minimum of 26.16 cm, and weight is 177.3 gr. During the months (January and August), when nematodes were found in 10 of the 350 anchovies, the minimum fish length and weight are 14.90 cm, respectively: 14.01 cm and 19.74 g 16.50 gr.

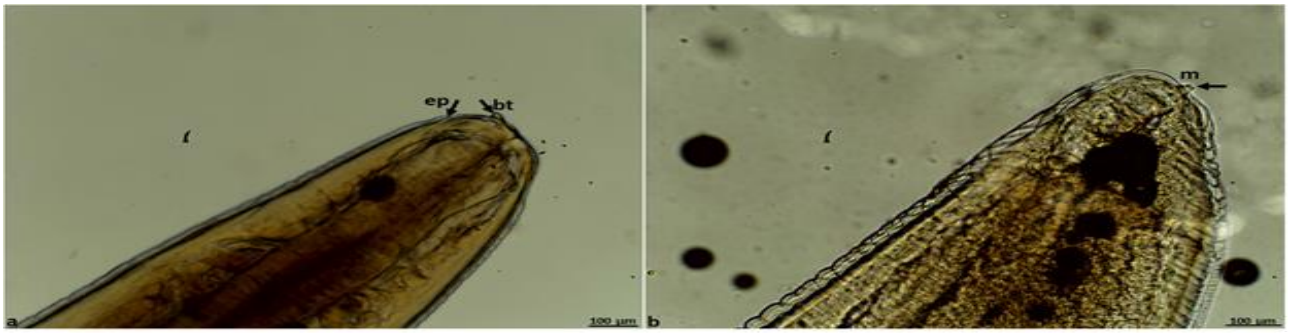

Figure 8. *Anisakis* spp. in bogue (*Boops boops*). L3 larva a) Anterior part (ep: discharge opening, bt: tooth), b) Posterior part (m: mucron).

As a result of the study, the variation in the prevalence, average density, and abundance of nematodes without differentiating fish species by months; the highest prevalence of 47 parasites (10.68%) was calculated in March, the lowest recorded as 1 (0.76%) in April.

DISCUSSION

In this study, horse mackerel, chub mackerel, bogue, sardines, and anchovies caught from the North Aegean Sea, Canakkale region, and frequently consumed by the local people, were examined for zoonotic fish parasites. As a result of the investigation, the locations of parasites, infected fish rates, number of parasites, abundance, density, prevalence values, and the prevalence (%) by height, weight, and species were calculated. As a result of the parasitological examination of the fish, *Anisakis typica* L3 Type 2 larva, *Contracaecum pelagicum* L3 larva, *Hysterothylacium* spp. (synonymous: *Contracaecum* spp.) L3 larva, *Anisakis physeteris* L3 larval have been identified. Molina et al. (2000) parasitologically analyzed fish consisting of 18 different species, including haddock, from fish markets in the Northern part of Cordoba State. Individuals belonging to the Anisakidae family identified in the body cavity and muscles of fish. The study reported that these parasites are at their peak seasonally, with 42% in haddock fish, in the spring months. In our study, the most intense parasitization in collegial fish was observed in March with 72%. This showed us that the prevalence value and seasonal density in both studies coincided with the spring months. In addition, studies have shown that nematode species were higher in common chub mackerel and horse mackerel fish compared to other fishes studied between January and June, and the rate of parasitization with nematodes decreased in the period from July to December. No parasitism was observed in sardines throughout the year. Tuncel and Akmırza (2006) encountered *Contracaecum aduncum* and *Anisakis simplex* nematodes in their parasitological examination on anchovy fish caught from the coast of Sinop and the Marmara Sea. It stated that in 21% of the anchovies examined, *Contracaecum aduncum* found in 98.8%, and *Anisakis simplex* in 0.17%. Our study revealed that nematode individuals

isolated from anchovies from fish form belong to the L3 larval form of *Anisakis simplex* parasite. It was observed that the nematode density of anchovy fish was low in annual sampling. Nematodes belonging to *Anisakis* and *Contracaecum* species were determined by parasitological examinations of the horse mackerel in a study (Akmırza 2001) conducted in the Aegean Sea and South Marmara offshore. He reported that parasites are localized, especially in fish intestines, and their prevalence was 8.77% and 38%, respectively. They stated that in year-round sampling, the parasites reached their highest level in March and their lowest in September. Our study observed that the highest parasitization intensity was observed in horse mackerel and mackerel fish in March, while parasitism was observed only in chub mackerel fish in September.

Keser et al. (2007) conducted parasitological examinations in *Trachurus trachurus*, *Engraulis encrasicolus*, *Sardinella aurita*, *Liza saliens*, *Belone belone*, *Sparus aurata*, *Solae solea*, *Scomber scombrus* and *Pomatomus saltatrix* fish species in a study they conducted in the Dardanelles. As a result of the study, they detected *Hysterothylacium aduncum* and *Anisakis simplex* nematodes. They did not find any nematode parasites in *Sardinella aurita*. In light of the findings of our study, parasitism was observed in different densities and abundances on an annual basis in horse mackerel fish, and no parasitism was found in sardine fish (*Sardina pilchardus* Walbaum 1792). Our study supports the results of Keser et al.

Hysterothylacium adumcum Rudolphi, 1802 species were identified in the parasitological examination of sardines (*Sardina pilchardus* Walbaum 1792) fishes caught from the Eastern and Southern coasts of Spain. This parasite reported a 25.21% prevalence, a 2.10 density, and a 0.52 abundance level (Rello et al., 2008). No parasites were found during the annual parasitological examination on sardines taken from the Canakkale fish state in our study.

In April, two nematodes (1.46%) were found in the bogue studied. The annual prevalence level is 0.58%, the average density is 2, and the average abundance is 0.012. *Hysterothylacium* sp. L3 larvae non-mucous stage and *Anisakis* spp. L3 Type 2 larvae have been identified. Of these species, only Anisakis can be found Atlantic (MacKenzie et al., 2008), Mediterranean (Mattiucci et al., 2008), Marmara and Black Sea (Tepe, 2011). In his study in 2019, Köksal isolated nematodes from thirty different fish species from the intestine, pyloric cecum, gall bladder, and body cavity. All nematodes we isolated in our study observed in similar organs, and two nematodes were detected in the sac in the liver of the fish.

Especially in Turkey, which has an important place in terms of aquaculture, laws and regulations and healthcare professionals responsible for the protection and control of fishery products should examine the issue of zoonoses because these creatures are also crucial in terms of public health. Humans are essential hosts, especially for their aquatic consumption habits and zoonotic creatures to continue their life cycle. To prevent Anisakidosis, which is frequently seen in the Far East and Asian countries, it is recommended to avoid the consumption of raw and undercooked seafood, especially fish, and to pay attention to food and hygiene conditions in places where fishery products sold.

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