Food Control

Five-years management of an emerging parasite risk (Eustrongylides spp., Nematoda) in a fishery supply chain located on Trasimeno Lake (Italy)

--Manuscript Draft--

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Abstract:	In the last few years, the widespread diffusion of potentially zoonotic parasitic nematodes of the genus Eustrongylides in the Trasimeno Lake, Central Italy, prompted FBOs operating in the freshwater fish supply chain to define preventive measures to reduce or eliminate this new hazard from fishery products. The results of the self-checks for parasite risk management of a fishermen's cooperative over a five-year period (January 2016-April 2021) are presented. Nine freshwater commercial species differently processed (filleted, whole gutted or whole ungutted) were investigated: perch (Perca fluviatilis), largemouth black bass (Micropterus salmoides), big-scale sand smelt (Atherina boyeri), eel (Anguilla anguilla), black bullhead (Ictalurus melas), carp (Cyprinus carpio), tench (Tinca tinca), goldfish (Carassius auratus), and pumpkinseed sunfish (Lepomis gibbosus). The presence of visible parasites was assessed by visual inspection during processing and recorded. Eustrongylides spp. were found in all species examined except for goldfish. Eustrongylides sp. occurrence was negligible in large mouth black bass, eel, carp, and tench, while increasing prevalence rates over the years were observed in fillets of perch <400g (from 4.2% in 2016 to 68% in 2021), batches of sand smelt (<1% to 40%) and pumpkinseed sunfish (6% to 99%). Still low but slightly increasing prevalence rates were also observed for black bull head. The rising of the infection in perch, sand smelt, and pumpkinseed sunfish lead to a progressive implementation of preventive measures including the definition of a sampling plan for the visual inspection followed by trimming or removal of the parasites from the muscle or the application of a threshold value to define the marketability of fish batches. This is the first study describing an approach for the management of the emerging risk posed by Eustrongylides spp. nematodes in a freshwater fishery supply chain. Besides providing an updated epidemiological scenario in Lake Trasimeno, where this parasite was described for the first time in Italy in 2015, it could support other FBOs in the implementation of preventive measures to safeguard consumers' health and trust.
Suggested Reviewers:	Pekmezci Gokmen Zafer Ondokuz Mayis University: Ondokuz Mayis Universitesi zpekmezci@omu.edu.tr He is one of the main expert on this topic. He published a very recent paper as regards the molecular identification of this new emerging parasite in Turkey. Pekmezci, Gokmen Zafer, and Cenk Soner Bolukbas. "Morphological and molecular characterization of Eustrongylides excisus larvae (Nematoda: Dioctophymatidae) in

	Sander lucioperca (L.) from Northern Turkey." Parasitology Research 120.6 (2021): 2269-2274.
	Monica Caffara Alma Mater Studiorum Universita di Bologna: Universita di Bologna monica.caffara@unibo.it She is an expert on this topic: Mazzone, A., Caffara, M., Gustinelli, A., Agnetti, F., Sgariglia, E., Lo Vaglio, G., ... & Fioravanti, M. L. (2019). Morphological and molecular characterization of larval and adult stages of Eustrongylides excisus (Nematoda: Dioctophymatoidea) with histopathological observations. Journal of Parasitology, 105(6), 882-889.
	Vasco Menconi Zooprophylactic Institute of Piemonte Liguria and Valle d'Aosta: Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta vasco.menconi@izsto.it He conducted one of the few study regarding this parasite in Italy: Menconi, V., Riina, M. V., Pastorino, P., Mugetti, D., Canola, S., Pizzul, E., ... & Prearo, M. (2020). First Occurrence of Eustrongylides spp.(Nematoda: Dioctophymatidae) in a Subalpine Lake in Northwest Italy: New Data on Distribution and Host Range. International journal of environmental research and public health, 17(11), 4171.

Pisa, 02nd October 2021

Dear Editor,

please find enclosed the manuscript entitled "**Five-years management of an emerging parasite risk (*Eustrongylides* spp., Nematoda) in a fishery supply chain of Trasimeno Lake (Italy)"** to be considered for publication in Food Control.

The consumption of fishery products, also raw or undercooked, has increased dramatically in the recent years. This habit can favour the transmission of zoonoses, including those of parasitic origin. A wide range of freshwater fish species may act as intermediate or paratenic hosts of the parasitic nematodes of the genus *Eustrongylides*, which may impact the quality and safety of fish products.

This study was carried out over a five-year period (January 2016-April 2021) in collaboration with a fishermen's cooperative of the Lake Trasimeno (Perugia, Italy), where a professional and recreational fishing activity exists and where the widespread diffusion of *Eustrongylides* spp. prompted the progressive implementation of control measures in the supply chain. Nine commercial species were involved: perch (*Perca fluviatilis*), largemouth black bass (*Micropterus salmoides*), big-scale sand smelt (*Atherina boyeri*), eel (*Anguilla anguilla*), black bullhead (*Ictalurus melas*), carp (*Cyprinus carpio*), tench (*Tinca tinca*), goldfish (*Carassius auratus*), and pumpkinseed sunfish (*Lepomis gibbosus*). The presence of visible parasites was assessed by visual inspection during processing and recorded using a specific check list. *Eustrongylides* spp. were found in all species examined with the exception of goldfish. Noticeably increasing prevalence rates over the examined period were observed in fillets of perch <400g (4.2% to 68%), batches of sand smelt (<1 to 40%) and pumpkinseed sunfish (6% to 99%), while still low but slightly increasing prevalence rates were observed for black bull head. *Eustrongylides* sp. occurrence was negligible in large mouth black bass, carp, tench and eel. As regards perch, the most infected species used for fillets production, preventive measures comprised belly flaps trimming or removal of the parasite when possible, while fish heavily contaminated were excluded from human consumption. From 2019 onwards, rising infection levels and the presence of the larvae in the anterior epaxial and posterior muscle section, other than in the belly flaps, lead to a further increase in processing time and in the percentage of rejection, causing a substantial economic loss for the FBO. Moreover, to manage the risk in sand smelt and pumpkinseed sunfish, the two other highly massively infected species sold in batches such as pumpkinseed and sand smelt, the FBO developed a sampling plan and in subsequently established a threshold value to define the marketability of fish batches

Data from this study provide an updated epidemiological scenario in Lake Trasimeno, where *Eustrongylides* was described for the first time in Italy in 2015, and could support the freshwater fish industry in the implementation of preventive measures to safeguard consumers' health and trust.

The manuscript has not been published elsewhere nor is it being considered for publication elsewhere. All authors have approved this manuscript, agree to the order in which their names are listed, declare that no conflict of interests exists and disclose any commercial affiliation.

Yours sincerely,
Andrea Armani and co-authors

Dear Editor, I’m sending you the revised version of the manuscript entitled “**Five-years management of an emerging parasite risk (*Eustrongylides* spp., Nematoda) in a fishery supply chain located on Trasimeno Lake (Italy)”** after the corrections you requested about the references.

I have checked the references presenting issues according ot the "Reference cheking results" of the journal, also considering to the authors guideline.

Out of the 26 not validated references highlighted by MaRs:

11 were already cited as they are, in previous manuscript published in Food Control or other International Journal.

6 were removed as not essential

2 were substituted with a link in the text

7 have been corrected following the authors guideline.

Details had been sent to you on your private e-mail.

Best regards

Andrea Armani

Highlights

1. *Eustrongylides* sp. nematodes are becoming widespread in the Trasimeno Lake, Italy (85)
2. The local freshwater fishery supply chain is increasingly affected by *Eustrongylides* sp. (91)
3. 5-years of self-checks for parasites of a fishermen's cooperative are presented (82)
4. Nine commercial species differently processed were investigated (65)
5. Prevalence rates in perch, sand smelt and pumpkinseed sunfish are noticeably increasing (90)

Declaration of competing interest

None

Five-years management of an emerging parasite risk (*Eustrongylides* spp., Nematoda) in a fishery supply chain located on Trasimeno Lake (Italy)

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ABSTRACT

In the last few years, the widespread diffusion of potentially zoonotic parasitic nematodes of the genus *Eustrongylides* in the Trasimeno Lake, Central Italy, prompted FBOs operating in the freshwater fish supply chain to define preventive measures to reduce or eliminate this new hazard from fishery products. The results of the self-checks for parasite risk management of a fishermen's cooperative over a five-year period (January 2016-April 2021) are presented. Nine freshwater commercial species differently processed (filleted, whole gutted or whole ungutted) were investigated: perch (*Perca fluviatilis*), largemouth black bass *(Micropterus salmoides*), big-scale sand smelt (*Atherina boyeri*), eel (*Anguilla anguilla*), black bullhead (*Ictalurus melas*), carp (*Cyprinus carpio*), tench (*Tinca tinca*), goldfish (*Carassius auratus*), and pumpkinseed sunfish (*Lepomis gibbosus*). The presence of visible parasites was assessed by visual inspection during processing and recorded. *Eustrongylides* spp. were found in all species examined except for goldfish. *Eustrongylides* sp. occurrence was negligible in large mouth black bass, eel, carp, and tench, while increasing prevalence rates over the years were observed in fillets of perch <400g (from 4.2% in 2016 to 68% in 2021), batches of sand smelt (<1% to 40%) and pumpkinseed sunfish (6% to 99%). Still low but slightly increasing prevalence rates were also observed for black bull head. The rising of the infection in perch, sand smelt, and pumpkinseed sunfish lead to a progressive implementation of preventive measures including the definition of a sampling plan for the visual inspection followed by trimming or removal of the parasites from the muscle or the application of a threshold value to define the marketability of fish batches. This is the first study describing an approach for the management of the emerging risk posed by *Eustrongylides* spp. nematodes in a freshwater fishery supply chain. Besides providing an updated epidemiological scenario in Lake Trasimeno, where this parasite was described for the first time in Italy in 2015, it could support other FBOs in the implementation of preventive measures to safeguard consumers' health and trust.

KEYWORDS: parasite hazard, freshwater fish, nematode larvae, *Eustrongylides* spp.

1. Introduction

In recent decades, the consumption of fishery products has increased dramatically around the world. However, despite their nutritional properties, fishery products can be responsible for the transmission of zoonoses, including those of parasitic origin (Dorny et al., 2009). Although great attention has been given to fish-borne parasites present in the marine environment, and to anisakid nematodes (EFSA, 2010), also those associated to freshwater ecosystems should not be disregarded. In fact, human food habits, such as the increasing consumption of raw or undercooked fish products, may influence the transmission of fish borne zoonosis (D'Amico et al., 2014; Pozio et al., 2013; Scaramozzino et al., 2018; Scholz et al., 2009).

Nematodes of the genus *Eustrongylides* (Family Dioctophymatidae) have a complex indirect life cycle in freshwater ecosystems, with fish-eating birds as definitive hosts (Measures, 1988; Spalding et al., 1993; Spalding & Forrester, 2008). Parasite eggs are shed through birds' faeces and ingested by aquatic oligochaete worms, the first intermediate hosts, while planktivorous and benthivorous fish act as second intermediate hosts (Moravec, 2013; Spalding & Forrester, 2008). Predatory fish, as well as amphibian and reptile species, can serve as paratenic or transport hosts (Bjelić-Čabrilo et al., 2013; Melo, 2016; EFSA, 2007).

A worldwide geographical distribution has been described for *Eustrongylides* spp. (Coyner et al., 2002; Haugen et al., 2008; Xiong et al., 2013; Kaur et al., 2013; Mazzone et al., 2019). In Italy, the first record occurred in 2015 in perch (*Perca fluviatilis*, Linnaeus) from the Trasimeno Lake (Umbria region, Central Italy) (Dezfuli et al., 2015). Since then, its presence in the same lake was confirmed in perch and detected in other commercially important fish species (Branciari et al., 2016; Mazzone et al., 2019). Recently, it has also been recorded in other lacustrine ecosystems in Central (Guardone et al., 2021) and Northern Italy (Menconi et al., 2021). Studies performing molecular characterization at species level of *Eustrongylides* sp. collected in Italy (Guardone et al., 2021; Mazzone et al., 2019) identified them as *E. excisus*, one of the three species described so far (together with *E. tubifex* and *E. ignotus*) (Measures, 1988b).

Eustrongylides spp. may have an impact on public health. In fact, humans may represent accidental hosts in case of consumption of infected raw or undercooked fish products (Food and Drug Administration, 2012; 2019). At least five cases of human infection have been described in the USA, due to the consumption of live minnows and raw fish (Centers for Disease Control, 1982; Eberhard et al., 1989; Narr et al., 1996; Wittner et al., 1989), and two other cases occurred in South Sudan more recently (Eberhard and Ruiz-Tiben, 2014). The location of *Eustrongylides* spp. larvae both in the viscera and the flesh of various fish species of commercial interest may increase the zoonotic risk (Food and Drug Administration, 2012).

Eustrongylides spp. larvae are clearly visible due to their dimension (larvae up to 59 mm long have been described by Eberhard et al., in 1989) and their pink-red color and perfectly fit with the definition of visible larvae: *"a parasite or a group of parasites which has a dimension, colour or texture which is clearly distinguishable from fish tissues*" (Commission decision EEC 140/1993). The presence of these visible parasites not only represents a public health issue, but also alters the commercial quality of fishery products (Reg. EC No 178/2002), making them unfit for the market. The European legislation provides that all Food Business Operators (FBOs) must conduct a non-destructive visual inspection of the fishery products for the detection of "*visible parasites*" ("*which in terms of size, colour or texture is clearly distinguishable in fish tissues*"), to avoid placing "*obviously contaminated products*" on the market (Commission Regulation EC 2074/2005). The same Regulation also establishes that "*during production the visual inspection shall be performed on a representative number of samples. The persons in charge of establishments shall determine the scale and frequency of the inspections by reference to the type of fishery products, their geographical origin and their use*". Therefore, FBOs are requested to establish correct preventive procedures in the self-control plan for managing the risk due to the presence of parasites in fishery products for discarding heavily infected fish that can have a direct impact on products' quality and on consumers' health (D'Amico et al., 2014).

In the Trasimeno Lake area, a professional and recreational fishing activity exists. In particular, the freshwater fish industry around the Lake is expanding into the market both by processing the most appreciated species that are considerably increasing their commercial value and by exploiting the great potential of undervalued or invasive fishes (e.g. goldfish) employed as ingredients in processed food (Branciari et al., 2017). Among the most exploited species, perch (*Perca fluviatilis*, Linnaeus), largemouth black bass (*Micropterus salmoides*, Lacépède) and eel (*Anguilla anguilla*, Linnaeus) have the highest economic importance, followed by tench (*Tinca tinca*, Linnaeus), black bullhead (*Ictalurus melas*, Rafinesque), sand smelt (*Atherina boyeri*, Risso) and carp (*Cyprinus carpio*, Linnaeus). Others, such as goldfish (*Carassius auratus*, Linnaeus) and pumpkinseed sunfish (*Lepomis gibbosus*, Linnaeus) represent less appreciated/valuable species (Branciari et al., 2017).

In the last years the widespread diffusion of *Eustrongylides* spp. in the Trasimeno Lake prompted FBOs to face a new risk and to adopt and continuously update preventive measures in their self-control plan to eliminate or reduce the presence of the parasite in the finished product. The present study, carried out in collaboration with a fishermen's cooperative (Perugia, Italy) over a five-year period (from January 2016 to April 2021), is the first study describing the management of the emerging risk posed by the presence of *Eustrongylides* spp. nematodes in a freshwater fishery supply chain in Italy, in all the nine fish species of commercial interest mentioned above. Thus, besides providing an updated epidemiological scenario on the spreading of this parasitic infection, data derived from this investigation could support the freshwater fish industry in the selection and implementation of the most appropriate preventive measures to safeguard public health and market reputation.

2. Materials and Methods

2.1 Lake environment and freshwater fishery supply chain description

This study was carried out in collaboration with a fishermen's cooperative located in the coastal area of the municipality of San Feliciano (PG), Italy. The cooperative is composed of about 40 professional fishermen, each with a private small fishing boat and operating mainly in the east coast of the lake Trasimeno (Fig. 1). Lake Trasimeno (Umbria, Italy; 43°08'N 12°06'E) is the fourth largest (about 128 km^2) width lake in Italy and the largest laminar one, as it is relatively shallow (average depth: 4.72 m; max. depth: 6.3 m). This lake hosts a large biodiversity, counting 19 fish species dominated by the Cyprinidae Family (Branciari et al., 2020). Fish are caught using different centimeter mesh sizes of gillnets and pots (gillnets of 24 mm, 28 mm, 70 mm, and 90 mm, respectively; pots of 8 mm for eel and 6 mm for big-scale sand smelt) following standardized fishing methods (Regional Regulation n. 2 of 15 February 2011 of Umbria Region). The cooperative owns two UE-approved plants for fish reception and processing (Fig. 1). After capture the caught fish is disembarked within maximum four hours (usually two) and immediately transferred to the reception plant. This is composed by a fish reception area where fishes are sorted according to the species, boxed, weighed, covered with ice and transferred to a temperature stabilization room. After 24 h (except for eel which remains in cold storage for 72 h) fish is transferred to the nearby processing plant, where fish are submitted to gutting, filleting, and skinning (or different combination of the mentioned steps). To avoid microbiological contamination of the products, filleting in particular is performed removing fish muscles without opening the visceral cavity (Video 1). All processing phases are conducted manually.

2.2 Fish collection

Fish were caught over a five-year period (from January 2016 to December 2020), and data were also collected in the first four months of 2021 (from January 2021 to April 2021), during daily catches throughout the fishing season of each species. The survey involved the following 9 commercial fish species: perch (*Perca fluviatilis*, Linnaeus), largemouth black bass *(Micropterus salmoides*, Lacépède), big-scale sand smelt (*Atherina boyeri*, Risso), eel (*Anguilla anguilla*, Linnaeus), black bullhead (*Ictalurus melas*, Rafinesque), carp (*Cyprinus carpio*, Linnaeus), tench (*Tinca tinca*, Linnaeus), goldfish (*Carassius auratus*, Linnaeus), and pumpkinseed sunfish (*Lepomis gibbosus*, Linnaeus). Fish were caught and processed as reported in section 2.1. The overall volume of fish catches and the number of samples examined by species are reported in Table 1.

2.3 Fish processing, data collection and management of the parasitological risk

Fishes were processed differently according to the species and their commercial destiny, to obtain different types of products, for which the preventive measures to control the parasitic risk varied (Table 2). The presence of visible parasites was assessed on a different number of specimens according to the species/type of products according to the Commission Regulation EC 2074/2005 and recorded by trained handlers using a specific check list defined by the FBO during the fish processing: in the case of perch <400g, largemouth black bass, black bullhead, goldfish, carp and tench 100% of the products (filleted or whole gutted and eventually deskinned) were examined, while a sampling plan was established for fishes sold as whole ungutted (sand smelt, perch ≥400 g, 10% of the eel, and pumpkin seed) (Table 2). Starting from 2020-2021, for sand smelt, usually caught in very large batches, and pumpkinseed sunfish the sampling plan included the visual inspection of ≥ 29 specimens for each batch, in agreement with the approach proposed for the detection of *Anisakis* sp. in anchovies (Circular Letter VS8/C790/94 of the Lombardy Region). The plan also included the criteria to accept or reject the batch of production based on the results of the inspection. For the other two species (eel, perch ≥400g), considering their commercial value the FBO performed a monthly sampling on approximatively 5% of the captured fish (Table 2).

For all species, a visual inspection was carried out in a continuous manner in good lighting conditions to detect visible parasites and avoid commercialization of "*obviously contaminated products*" (Commission Regulation EC No 2074/2005). In particular, the visual inspection was carried out on the external viscera surface, abdominal cavity, liver, and roe at the time of evisceration in the case of whole gutted products. In the case of filleted products, only fillets were visually inspected, as the whole viscera are discarded without opening the cavity, as mentioned in section 2.1 (Video 1). The number and localization of the visible parasites in the fillet (Anterior Ventral – AV, which is the anterior hypaxial muscle, almost all constituted by the belly flap; Anterior Dorsal – AD, anterior epaxial muscle; Posterior Ventral – PV, posterior hypaxial muscle; and Posterior Dorsal – PD, posterior epaxial muscle, as defined in Fig. 2) were recorded in the check list mentioned above. Visible nematodes were identified to the genus level, according to the macroscopic and microscopic characteristics described in Mazzone et al. (2019), Measures (1988a) and Panesar and Beaver (1979). Moreover, a subset (n=10) of parasites collected from perch fillets was submitted to molecular identification by amplifying the ITS region as described in Guardone et al., (2021).

In case of low infection levels, parasite removal was conducted by the FBOs. When heavier infections occurred the parasites removal was done if the number and distribution of the larvae as well as the size of the fish fillet allowed it, otherwise the products were excluded from human consumption. For some species sold as whole, a treatment for killing the larvae (freezing) was applied when possible.

In addition to the aforesaid procedures, for fish species commercialized as whole to local restaurants, the preventive measures also included providing the restaurateurs information on the possible presence of parasites in the fish flesh and the assurance that the fish would be processed in a way that would kill the parasites (e.g. by cooking or freezing). Restaurateurs were also asked to send information concerning the presence of parasites back to the cooperative.

2.4 Statistical analysis

The data obtained from the visual inspection of each fish species during the years were analysed by Quantitative Parasitology QP 3 (Rózsa et al., 2000) for statistical analyses. The following indices were calculated for each year of observation: prevalence (P = number of parasitized fish/number of total examined fish); mean intensity (MI = number of parasite individuals found in infected fish), and mean abundance (MA = mean number of parasites found in all fish) and the relative 95% confidence intervals (CIs). The comparison of the prevalence rates observed in the various species during the investigated years was performed using the chi-square test. For the MI and MA data, a bootstrap test was implemented. The level of significance was set at $p < 0.05$.

3. Results

Visible larvae were found in all the examined species, with the exception of goldfish (Table 1). Yearly volumes of catches, number of examined specimens and the results of the parasitological examinations (prevalence, mean abundance and mean intensity) of the filleted products are reported in Table 3. Perch was the most caught and processed species and consequently the highest number of fillets examined by the FBO belonged to this species. Also, the highest prevalence of *Eustrongylides* sp. larvae in fillets were found in this species. Prevalence rates increased noticeably, from the lowest values recorded in 2016 (4.2%) and 2017 (4.1%) to the highest (68%) in 2021 (Table 3). Lower prevalence rates were found in in largemouth black bass fillets since 2016. In this species, the parasite prevalence remained stable and was always below 1%. Infection in fillets of the black bullhead was found starting from 2018 onwards, and the prevalence recorded was very low until 2020, while a slightly higher prevalence (2.7%) was recorded in the first four months of 2021. A very low prevalence (<0.5%) of *Eustrongylides* sp. was recorded in both carp and tench, only starting from 2018 (Table 3).

In filleted products, the mean abundance was generally lower than 0.01 and the MI never exceeded 1 in fillets of all species except for perch, for which an increase in the MA was recorded, starting from values below 1 (0.04) in 2016 and 2017 and reaching a value of 6 in 2021. A similar trend was registered for the MI, reaching a value of 8.28 in 2021 (Table 3).

Regarding the larvae localization in the fillets, in perch the larvae were exclusively found in the AV (particularly in the belly flaps) from 2016 to 2018. Starting from 2019 the majority of the larvae were still located in the belly flaps (52.72, 52.77, 57% in 2019, 2020, 2021, respectively) but localization in the other portion of the anterior epaxial muscle section (33.28%, 29%, 21.88% in 2019, 2020, 2021, respectively) and posterior hypaxial muscle section (15%, 14%, 18% in 2019, 2020, 2021, respectively) occurred. Only in the last two years (4.23% and 3.12% in 2020, 2021, respectively) a small percentage of larvae were lodged in the posterior epaxial muscle sections . In largemouth black bass larvae were found only in the belly flap. In the remaining species different locations were observed (belly flap in black bullhead; anterior epaxial muscle in tench; AD, AV, and PV muscle quadrants in carp), although the number of positive specimens was very low (maximum 3 per year). Furthermore, in perch fillets, the nematodes were free in musculature or more rarely encapsulated (Fig. 3). Nematodes in fibrous capsule were also found in eel's flesh, but never in other species.

As regards fish sold whole and gutted, parasites were detected only in eel starting from 2019 and with very low prevalence values: 0.009% (0.00-0.03 95% CI) in 2019; 0.036% (0.00-0.078 95% CI) in 2020 and 0.19% (0.025-0.37 95% CI) in the first four months of 2021. Very low mean abundances (0.0001, 0.0004 and 0.002 in 2019, 2020 and 2021 respectively) were found and MI never exceeded 1. The larvae were found on the internal side of the abdominal cavity and, also detected by observing the external muscle surface.

As regards fish commercialized as whole ungutted (sand smelt, pumpkinseed sunfish, perch ≥ 400 g and 10% of eel), the volume of catches, number of examined specimens and the results of the parasitological examinations (prevalence, mean abundance and mean intensity) are reported in Table 4. In both sand smelt and pumpkinseed sunfish, an increase of prevalence rates over time was recorded. In sand smelt, the prevalence was below 1% until 2018, after which the prevalence rose considerably, reaching a value of about 40% in 2021. Similarly, an increasing trend was recorded for pumpkinseed sunfish, with even higher values, starting around 6% in 2016 and rising-up to 99% at the beginning of 2021. In pumpkinseed sunfish, a higher MA than in sand smelt was also recorded, and the larvae were lodged in the visceral cavity and also in the muscle. In sand smelt, the number of parasites per fish specimens never exceeded one and the larva was generally located in the coelomic cavity. For perch ≥ 400 g and 10% of eel, data on the infection level deriving from the FBO sampling plan were considered together with the restaurateurs' feedbacks. In perch ≥ 400 g, the prevalence registered was below 1% and did not increase over the years. A low prevalence was detected in eel, reaching a value of approximately 1% in 2021.

All the visible parasites recovered during the investigate period were morphologically identified as larvae belonging to the genus *Eustrongylides*. The ITS sequences (submitted to Genbank Accession Nrs XXX) obtained from the subset of molecularly identified larvae from perch muscle presented 100% identity with sequences of *E. excisus* by BLAST analysis on GenBank, confirming the presence of this species in the lake (Mazzone et al., 2019).

4. Discussion

Fish may act as definitive, intermediate or paratenic hosts of a variety of parasites, some of which can cause zoonotic infections in humans if ingested, while others (not zoonotic) may only represent a quality defect (Reg. EC 178/2002). Among fish parasites, nematodes of the family Anisakidae have attracted considerable scientific attention in the last 20 years, with an increasing number of epidemiological studies in several fish species, a rising number of human cases in Western countries and the improvement of diagnostic techniques (EFSA 2010; Guardone et al., 2018; Mattiucci 2013), as well as the development of a dedicated legislation body (D'Amico et al., 2014). While anisakid nematodes are typically associated with the marine environment, other parasites are present in freshwater habitats. Among these, fish-borne trematodes have a renowned public health impact, historically associated with the Asian countries (Sithithaworn et al., 2007; https://www.who.int/news-room/fact-sheets/detail/foodborne-trematode-infections). However, *Opistorchis felineus*, the only species circulating in the EU, has caused disease outbreaks also in Italy, where four individual cases and eight outbreaks occurred from 2003 to 2011, with 211 confirmed human infections. All cases derived from consumption of raw tench fillets fished from two lakes (Bolsena and Bracciano) in central Italy (Pozio et al., 2013).

4.1 Diffusion of Eustrongylides sp. in the Trasimeno Lake

Fish-borne nematodes associated to freshwater fish species have been less investigated compared to those in the marine environment, although the genus *Eustrongylides* has lately attracted increased attention due to zoonotic aspects (Mazzone et al., 2019) and pathogenic potential in definitive and intermediate hosts (Xiong et al., 2013). In fact, *Eustrongylides* spp. may induce mortality of nestling ardeids and other wading birds (Spalding & Forrester, 1993, 2008) and pathological as well as behavioural alterations, such as slower swimming, in fish (Coyner et al., 2002; personal observation). As mentioned, the presence of this parasite in Italy was reported for the first time in the Trasimeno Lake in 2015 (Dezfuli et al., 2015) and subsequently confirmed therein in the same as well as in other fish species and in definitive hosts (Branciari et al., 2016; Mazzone et al., 2019).

In this study, considering that data were collected during routine processing by FBOs and that the procedure adopted during filleting implies the immediate discard of the visceral cavity, it was not possible to provide visceral prevalence rates for filleted products. However, the presented results of FBOs self-checks for parasite risk management provide further epidemiological data on the spreading of *Eustrongylides* spp. in nine species of commercial interest for the lake community.

An increasing prevalence rate over the examined period was observed for perch <400g, sand smelt and pumpkinseed, while still low but slightly increasing prevalence rates were observed for black bull head, and very low prevalence values (<1%) were observed for large mouth black bass, eel, carp and tench. *Eustrongylides* sp. larvae were never found in goldfish, in agreement with previous observations from the Trasimeno Lake (Branciari et al., 2016) and with a recent study conducted in north Italy (Menconi et al., 2020).

Perch is a commonly reported host for this parasite (Moravec, 2013). In fact, its infection by *Eustrongylides* spp. with different prevalence rates was already described in Slovakia (P: 9.3%; Juhásová et al., 2019), Ukraine (P: 85.1-90%; Goncharov et al., 2018; Matvienko et al., 2015), Turkey (P: 74.2-94.1%; Soylu 2013; Yardimci et al., 2018) and Bulgaria (P: 13.9%; Shukerova et al., 2010).

In Italy, apart from the Trasimeno Lake, infection in perch was reported by a recent parasitological survey on several fish species in San Michele Lake (Piedmont, northwest Italy) (P: 10%) (Menconi et al., 2020) and by fishermen in Ceresio Lake and Montorfano Lake in north Italy (Lombardy region) (https://www.tio.ch/ticino/attualita/1397786/trovato-un-parassita-nel-pesce-persico-del-ceresio; https: //www.parcovallelambro.it/news/presenza-parassiti-nel-pesce-del-lago-montorfano). The prevalence rates found in the last period in the present study (~55% in 2020 and 68% in the first four months of 2021) appear to be quite high, especially considering that they only refer to the presence of larvae in the muscle, while higher prevalence rates in the literature (85.1-90% Goncharov et al., 2018; Matvienko et al., 2015; P: 74.2-94.1% Soylu 2013; Yardimci et al., 2018) are referred to both viscera and muscle. In fact, distribution of *Eustrongylides* in perch body cavity, on the liver surface, in the bladder and gonads was also described (Matvienko et al., 2015, Goncharov et al., 2018).

The common finding of *Eustrongylides* spp. larvae in perch muscle (Fig. 3) allows to hypothesize that their migration occurs both during the life of the host, and *post-mortem* (Branciari et al., 2016). The possible localization of the larvae in the muscle when the host is alive is supported by previous histological examinations of the perch muscle surrounding the larvae, which showed granulomas resulting from the intense host response, with a fibrous capsule (Branciari et al., 2016; Dezfuli et al., 2015), where newly formed microvessels were also observed (Branciari et al., 2016). A single, reddish, coiled larva was generally present inside the nodules. The parasites often appeared structurally intact, with a well-defined cuticle, only occasionally degenerated (Branciari et al., 2016). Similar observations were confirmed in this study (Fig. 3, b). However, the ratio between the number of larvae migrating during the host life and that of the larvae migrated *post-mortem* is unclear. Specific studies, such as those conducted on *A. pegreffii* in anchovies (Cipriani et al., 2016) will be carried out.

Furthermore, it must be noted that, while high infection levels (prevalence and mean intensity) were observed, particularly in the last years, these were only referred to perch <400 g; this may also be related to the inclusion of oligochaete in juvenile perch diet (Ceccuzzi et al., 2011; Goncarov et al., 2018). On the contrary the prevalence in larger specimens dropped dramatically, being always <1%, and a low intensity was also observed. This phenomenon appears to be opposite to the accumulation of larvae during lifetime which occurs for example for *Anisakis* spp. in some fish species (Bao et al., 2019). Further studies are also needed to better investigate this aspect.

Also, sand smelt, whose prevalence rates increased from 1% to 40% in the investigated five years, has already been reported as a host of *Eustrongylides* spp. in Turkey (P: 6%, Colak, 2013), while Italian reports besides the Trasimeno (Branciari et al., 2016; http://indice.spvet.it/archivio/numero-99/649.html) only include a recent description in the Massaciuccoli Lake in Tuscany (Central Italy) (Guardone et al., 2021).

As regards pumpkinseed sunfish, previous literature records in Italy are only available for the recent detection in lake San Michele (P: 18.3%, Menconi et al., 2020). Slightly higher rates (20-27%) were observed in the USA in two different species of the same genus (*Lepomis cyanellus* and *L. auritus*, McAllister et al., 2015 and Bauer and Whipps, 2013, respectively). Thus, for both sand smelt and pumpkinseed, beside an increased prevalence over the analyzed years, a higher degree of infection than that reported by other authors (Guardone et al., 2021; Menconi et al., 2020) was found. As perch, both species have been reported to include oligochaetes in their diet (Alessio et al., 2017; Ghetti et al., 2007).

It is interesting to note that, even with lower infection levels (prevalence rates always <3%), a significant increase in prevalence rates was found also in black bullhead. On the contrary, no infection was found in other *Ictalurus* species investigated in Italy (*I. punctatus*, Menconi et al., 2020) and in Mexico (*I. meridionalis*, Salgado-Maldonado et al., 2011).

In this study, the low prevalence of *Eustrongylides* observed in fillets of largemouth black bass (<1%) are in contrast with those obtained in specimens from north Italy, where a higher level of parasitized specimens (16.7%) was reported (Menconi et al., 2020). A possible explication could be the bigger average size and age of the fish sampled in this study respect to those studied by Menconi et al. (2020). Indeed, it has been demonstrated that the feeding behavior evolves with size and age, and the diet shifts from small invertebrates to a wide variety of fish (Münster et al., 2015). Prevalence rates varying from 6 to 10% have been reported for this species in the USA (Bauer and Whipps, 2015). Furthermore, in largemouth black bass specimens examined in this study fibrous capsules were never evident around the larvae, which appeared to be free in the musculature, in agreement with the observations of Menconi et al., (2020).

In eel, visual inspection highlighted the presence of *Eustrongylides* sp. with low prevalence rates, in both filleted and whole gutted products. This host was already found to be infected in Germany (P: 3.3%, Jakob et al., 2012). As mentioned, larvae in this fish species were detected in the visceral cavity not only free but also surrounded by fibrous tissues adjacent at the peritoneum. The encapsulated form, in the wall of the celomatic cavity, was already reported by other authors (Urdes et al., 2015).

Although a very low number of parasites were isolated from carp and tench fillets (while no parasites were found in the whole gutted presentation), this represents the first record of positivity for these species in Italy, in contrast with other studies focusing on the same lacustrine ecosystem (Branciari et al., 2016; Mazzone et al., 2019), and, for carp, also with studies in Kazakhstan, Iraq, Turkey, Mexico and Ethiopia (Abdybekova et al., 2020; Amare et al., 2014; García-López et al., 2016; Yardimci et al., 2018), in which this species was always negative. The low prevalence recorded suggests that the infection in these two fish species may have occurred accidentally, probably due to the ubiquitous presence of the parasite in Trasimeno Lake. The discrepancy with previous observations in the Trasimeno Lake (Branciari et al., 2016), may suggest that the parasite diffusion has grown, exerting a pressure on biota not usually involved in the life cycle of the nematode. In fact, due to their feeding habit, tench and carp are unlikely subjected to *Eustrongylides* infection (Alaş et al., 2010; Vasconi et al., 2015). On the contrary, predatory fish species such as perch have greater potential to become highly infected with multi-host parasites acquired during feeding (Williams et al., 2021).

Altogether, the strong rise in infection rates in perch <400g, sand smelt and pumpkinseed sunfish, as well as the slightly increasing prevalence rates in black bull head, and the occurrence of the parasite also in species not commonly reported as host, like the carp, suggests a widespread and increasing diffusion of nematodes of the genus *Eustrongylides*. The involved species is probably *E. excisus*, as suggested by the results of the subset of larvae molecularly identified and by the previous identification of this parasitic species in this lake by Mazzone et al., (2019) in the Lake. Recent findings from the study of Pekmezci et al., (2021) molecularly identified this species also in in the Derbent Dam Lake, Samsun City, (Turkey) further supporting the widespread diffusion of this species.

In the Trasimeno lake, the diffusion of the parasite might be influenced by the growth of the cormorants' population, which is concentrated around the lake, likely due to changing migratory patterns due to climate change, already documented in other areas (Gienapp & Bregnballe 2012; Frederiksen et al., 2018). A potential role of migratory cormorants in spreading parasites has already been described for *Contracaecum* sp. (Mattiucci et al., 2020). In addition, Goncharov et al. (2018) suggested that environmental factors favouring the growth of the oligochaete population may play a role in the high prevalence in fish. In fact, rising prevalence rates have already been described in other areas (Danube basin and the Caspian Sea), and possibly attributed to climate and anthropogenic changes (Urdes et al., 2015; Fallah et al., 2015).

4.2 Presence of Eustrongylides sp. in different types of products: management of the parasite risk by the Fishermen's cooperative

The aforesaid changing dynamics appear to be increasingly impacting the parasite risk and its management by the cooperative fishing in the Trasimeno Lake. Albeit a zoonotic potential has been attributed to *Eustrongylides* spp. and the parasite is widely distributed in freshwater ecosystems, to date only a few cases described in the USA and in South Sudan are reported in the literature (Centers for Disease Control, 1982; Eberhard et al., 1989; Eiras et al., 2018; Eberhard and Ruiz-Tiben, 2014; Narr et al., 1996; Wittner et al., 1989), and no human infection has been reported in Italy. In relation to the zoonotic aspects, we also need to consider that no molecular characterization of the parasites was conducted in the human cases. Further molecular data clarifying the species involved in human cases are needed to assess the zoonotic potential of each species (Guardone et al., 2021; Mazzone et al., 2019). Molecular studies will help in better describe the geographical distribution, the epidemiology, and the taxonomical classification of this genus (Abe, 2011; Pekmezci et al., 2021).

Furthermore, any parasitized fish, independently from the zoonotic potential of the parasite, is unfit for human consumption for reasons of contamination by extraneous matter. Specifically, Regulation EC 178/2002 states that food shall not be placed on the market if it is unsafe (i.e., injurious to health or unfit for human consumption). In fact, even if preventive measures such as freezing can be implemented, the repulsive appearance of heavily parasitized fishery products may cause consumers' rejection and may also damage the brand reputation (D'Amico et al., 2014; Branciari et al., 2016; Mazzone et al., 2019). In fact, "disgusting" larvae, which are particularly evident due to their color and dimension, may be clearly visible to the naked eye (Fig. 3).

Thus, the Fishermen's cooperative implemented a self-control plan with different preventive measures to control the parasitic risk according to the different processing applied to the nine processed species. The increasing infection levels in perch fillets prompted the cooperative to assess by visual inspection the 100% of the fillets. Even though visual inspection and candling were reported to have a low efficiency for Anisakidae detection; their performance depends on fillet thickness, size, texture, colour, and fish species, as well as on the training and skills of operators (Chalmers et al., 2021). Therefore, the procedure was adopted considering the clear colour of the processed fillets as well as their thinness, the colour and dimension of *Eustrongylides* sp. parasites and the high level of training of the operators. Subsequently, operators intervene with trimming or removing of the parasite when possible, considering the high commercial value of this product. On the contrary, fish heavily parasitized are excluded from human consumption and discarded as animal by-products (Category 1) according to the Regulation (EC) No 1069/2009. Trimming of the belly flap was initially chosen, as this procedure is recommended as an effective method to remove parasites by international authorities on food safety (https://www.fda.gov/media/80777/download; http://www.fao.org/3/x5951e/x5951e01.htm). A recent work by Bao et al., (2021) reported that trimming the belly flaps of highly parasitized cod may reduce the number of anisakids in stockfish by 74%. However, the differential distribution of the parasites in the fish muscle may affect the efficiency of the procedure (Levsen and Lunestad, 2010). In this study, in fact, trimming of perch fillets was feasible until 2018, then, starting from 2019, even though most of the larvae were in the belly flap, a localization in the anterior epaxial and posterior muscle portion also occurred. Therefore, removal of each single larva from the fillets became necessary (Fig. 4). However, the high prevalence rates and intensity levels registered in the first four months of 2021, caused an increase in processing time by the FBO and a 20% increase in the percentage of rejection, thus leading to a substantial economic loss. The presence of parasites in fishery product has already been highlighted as an important economic burden on the stakeholders (Abollo et al., 2001). Possible solutions for controlling the infection level in perch are not straightforward. Immediate refrigeration (on board of the fishing boat) could prevent the *post-mortem* larval migration, but the extent of *intra-vitam* migration is still unclear and this would not be affected by this measure.

As regards the other two species for which high levels of infection were observed, starting from 2020-2021 the FBO decided to establish a threshold value to define the fish marketability. Although the importance of foodborne parasites (FBP) is recognised by many sectors of the food industry, standardized analytical methods and validation procedures for testing food for FBP are lacking (Chalmers et al, 2020). The European Hygiene Package does not precisely define a maximum limit for parasites in fish batches, leaving some space to FBOs and/or Control Authorities to establish their own criteria. In fact, if EU regulations (Reg. EC No 852/2004, Reg (EC) No. 853/2004 and the related implementing measures) do not specify the sampling or analysis methods, FBOs may use appropriate methods contained in other Community or national legislation or, if they are not available, methods that allow to obtain results equivalent to those obtained using the reference method, provided that these methods are scientifically validated in accordance with internationally recognized standards or protocols (Reg. EC No 852/2004). This lack of standard settings regards the *quantum satis* concept, as no maximum limit of parasite in fish is defined. Therefore, in the first four months of 2021, a sampling plan was structured for sand smelt and pumpkinseed, based on a procedure proposed for managing *Anisakis* risk in anchovies (Guardone 2016, Circular n. 1 of 1997 Liguria Region, Circular Letter VS8/C790/94 Lombardy Region). The plan included the sample size and the criteria to accept or reject the batch of production based on the results of the inspection. To define fish marketability, the mean abundance (MA) (total number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of hosts of that species examined) (Bush et al., 1997) of each batch was calculated after visual inspection and used to set a threshold. The threshold value defined by the FBO was based on the protocol proposed by the Liguria Region in Circular n. 1 of 1997. Sampling size for each batch (daily capture) was constituted by 29 specimens. In fact, according to the Lombardy Region circular (Circular Letter VS8/C790/94), knowing the total weight of the fish lot, it is possible to calculate the total number of specimens and then, by means of conversion rates and using an appropriate table, the number of subjects to be examined in each case. In the case of fish species caught in large batches (>600 specimens), the number of subjects to collect is, at least, 29 (Guardone et al., 2016). The acceptable batches for pumpkinseed sunfish were those harboring a maximum number of three larvae per fish in a maximum of 10% of the sample examined (3 fish, corresponding to a total of 9 larvae) with the MA threshold of 0.3. For sand smelt, for which the MI is generally equal to one, the acceptable batches were considered those with a number of parasitized specimens up to 10% of the sample analyzed (3 fish, corresponding to a total of 3 larvae)

(Guardone et al., 2016; Guardone et al., 2017), thus giving a MA threshold of 0.1. Such criteria allowed to establish if each batch should be intended for free consumption, submitted to freezing for killing the parasite or was not marketable. The applied threshold brought in 2021 to judge the entire stock of pumpkinseed sunfish unfit for human consumption and not marketable, causing a stop in fishing this species and a consequent loss of income for the fishermen, while for sand smelt the protocol defined by the FBO requires a freezing treatment to assure the inactivation of parasites.

As regards sand smelt the catch was mainly commercialized as fresh until the season fishing 2019-2020 then the quota requiring a preventing freezing has highly increased determining an increment in the overall cost of management for the FBO. However, it has to be specified that no specific sanification measures using cold temperatures exist for this parasite, and European legislation parameters were only developed on anisakids. The FDA guidelines suggest the following: cook the fish to an internal temperature of 145ºF for 15 seconds; to 155ºF for comminuted fish, such as fish cakes, and 165ºF for stuffed fish (FDA, 2012). However, studies on the resistance of this emerging parasites during processing (freezing and cooking) are of utmost importance considering its entrance in the Italian and European fish supply chain.

Finally, consumers should be educated about the possibility that, despite FBOs, and all the personnel involved in the quality and safety assurance efforts, parasites might be present in wild fish products. Information on correctly managing such defects should always be sought, also in order to avoid excessive and unnecessary alarmism.

4. Conclusions

The present investigation is the first study describing the management of the emerging risk posed by *Eustrongylides* nematodes in a freshwater fish processing plant. The widespread diffusion of this parasite in the Trasimeno lake (Italy), testified by the presented epidemiological results on nine commercial species, has required appropriate implementation of the self-control plan to assure its elimination or reduction in the finished product. In fact, although the zoonotic potential of all species belonging to the genus *Eustrongylides* is to be ascertained, an aspect not to be underestimated is that the presence of this parasites in the flesh may affect the quality, making the fish repugnant to the consumers and causing substantial economic losses to FBOs. Considering that this nematode has lately been reported in several Italian lakes and that its presence is known to be widespread globally in a vast range of species, the results of this study provide a possible management of this new hazard in the freshwater fish industry to safeguard public health and products' quality.

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Figure 1

Geographical location of the Trasimeno Lake Fishermen's Cooperative and aerial map of the landing area, reception and processing plants, with examples of fish landing and reception of sand smelt (*Atherina boyeri*).

Figure 2

Perch fillet (used as example) with the quadrants used for registering the parasite location in the musculature: Anterior ventral (AV), Anterior dorsal (AD), Posterior ventral (VP), and Posterior Dorsal (DP) quadrants.

Figure 3

Presence of larvae of the genus *Eustrongylides* in perch (*Perca fluviatilis*) fillets (a) and detail of a fibrous capsule from which an intact *Eustrongylides* sp. larvae was extracted (b).

Figure 4

Removal of *Eustrongylides* sp. larvae from perch (*Perca fluviatilis*) fillets.

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Table 1. Presence of *Eustrongylides* spp. in fish. Total length (cm) and total weight (g) of fish.

Fish species	Body length	Weight	Overall Kg of processed fish (2016-2021)	Overall number of examined fish (2016-2021)	Presence of parasite
Perch (*Perca fluviatilis*)	18.92±2.49	158±10.80	46939	450609	+
	>24	>400	404	871	
Largemouth black bass (*Micropterus salmoides*)	22.43±1.52	160±25.18	31940	255515	+
Black bullhead (*Ictalurus melas*)	12.95±2.95	190±25.15	3064	8698	+
Carp (*Cyprinus carpio*)	73.80±11.60	4100±227	107845	25076	+
			71896	16717	
Tench (*Tinca tinca*)	38.08±2.28	780±61.01	15929	19809	+
			12653	15736	
Goldfish (*Carassius auratus*)	23.83±1.77	445±41.44	15375	34427	n.d.
Eel (*Anguilla anguilla*)	58±10.07	258±25	10682	42732	+
Big-scale sand smelt (*Atherina boyeri*)	8.00±1.92	10.52±2.12	157801	8661	+
Pumpkinseed sunfish (*Lepomis gibbosus*)	6.50±1.22	35±4.03	2850	5147	+

Table 2

Type of processing	Market presentation	Species	Management of the parasitological risk
Gutting, filleting and deskinning	Fillet	Perch <400g Largemouth black bass Black bullhead Goldfish Carp (60% of the production) Tench (~50% of the production)	Visual inspection of 100% of the fillets
Gutting (and skinning*)	Whole gutted fish (and deskinned*)	Carp (40% of the production) Tench (~50% of the production) Eel (90% of the production)	Visual inspection of viscera and abdominal cavity (and dorsal muscle*) of 100% of the specimens
None	Whole fish	Sand smelt	Visual inspection of viscera and muscle of 29 specimens for each batch (Circular Letter VS8/C790/94 of the Lombardy Region; Circular (1997) n. 1 of Liguria Region); restaurants' information and feedback
		Perch >400 g	Visual inspection of viscera and muscle of 5% of the captured fish; restaurants' information and feedback on 100% of the sold specimens
		Eel (10% of the production)	
		Pumpkinseed sunfish	

*only eel

Table 3. Total fish filleted, number of examined specimens in the 5-year period of the retrospective analysis. Prevalence, mean abundance, and mean intensity.

Year	Species	Total processed fish (Kg)	Total fish examined (n)	Number positive	P% (95% CI)	MA	MI
2016	**Perch <400g**	11587	111235	4696	4.22 (4.10–4.34)	0.04	1
	Largemouth black bass	3792	30334	143	0.47 (0.39–0.55)	0.005	1
	Black bullhead	159	680	n.d.	-	-	-
	Carp	7841	1867	n.d	-	-	-
	Tench	4470	5551	n.d	-	-	-
	Goldfish	2927	6539	n.d	-	-	-
2017	**Perch <400g**	2429	23318	961	4.12 (3.86–4.38)	0.04	1
	Largemouth black bass	10246	81964	148	0.18 (0.15–0.21)	0.002	1
	Black bullhead	238	1079	n.d	-	-	-
	Carp	11441	2720	n.d	-	-	-
	Tench	4681	5812	n.d	-	-	-
	Goldfish	1496	3291	n.d	-	-	-
2018	**Perch <400g**	9281	89094	12188	13.68 (13.54–13.91)	0.17	1.26
	Largemouth black bass	5037	40296	162	0.40 (0.34–0.46)	0.004	1
	Black bullhead	370	1386	1	0.07 (0.00–0.21)	0.0007	1
	Carp	17944	4062	1	0.02 (0.00–0.07)	0.0002	1
	Tench	2258	2801	1	0.036 (0.00–0.11)	0.0004	1
	Goldfish	3139	7045	n.d	-	-	-
2019	**Perch <400g**	5934	56962	19016	33.38 (32.99–33.77)	1.14	3.42
	Largemouth black bass	9687	77494	485	0.63 (0.57–0.68)	0.01	1
	Black bullhead	789	2623	1	0.04 (0.00–0.11)	0.0004	1
	Carp	21266	4931	1	0.02 (0.00–0.06)	0.0002	1
	Tench	1452	1801	1	0.06 (0.00–0.16)	0.0006	1
	Goldfish	3960	8723	n.d	-	-	-

2020	**Perch <400g**	13435	128979	71522	55.45 (55.18–55.72)	3.91	7.05
	Largemouth black bass	2937	23498	72	0.30 (0.24–0.38)	0.003	1
	Black bullhead	1486	2820	1	0.04 (0.00–0.10)	0.0004	1
	Carp	24479	5719	1	0.02 (0.00–0.05)	0.0002	1
	Tench	2442	3052	3	0.10 (0.00–0.21)	0.001	1
	Goldfish	2541	5706	n.d	-	-	-
2021*	**Perch <400g**	4273	41021	27890	67.99 (67.54–68.44)	6	8.28
	Largemouth black bass	241	1929	5	0.26 (0.03–0.49)	0.003	1
	Black bullhead	22	110	3	2.72 (0.00–5.77)	0.0273	1
	Carp	24874	5777	1	0.02 (0.00–0.05)	0.0002	1
	Tench	626	792	1	0.13 (0.00–0.37)	0.0013	1
	Goldfish	1312	3123	n.d	-	-	-

*January, February, March, April

Table 4. Total fish commercialized as whole ungutted, number of examined specimens in the 5-year period of the retrospective analysis. Prevalence (P), mean abundance (MA) and mean intensity (MI).

Year		**Total processed fish (Kg)**	**Total examined (n)**	**N of positive**	**P% (95% CI)**	**MA**	**MI**
2016	**Sand smelt**	57633	1660	1	0.06 (0.00–0.18)	0.0002	1
	Pumpkinseed	540	900	53	5.89 (4.35–7.43)	0.059	1
	Perch >400 g	144	300**	2	0.67 (0.00–1.59)	0.007	1
	Eel	210	839**	n.d.	-	-	-
2017	**Sand smelt**	27729	1332	1	0.08 (0.00–0.22)	0.0002	1
	Pumpkinseed	180	300	14	4.67 (2.28–7.05)	0.06	1.29
	Perch >400 g	12	25**	n.d.	-	-	-
	Eel	176	704**	n.d.	-	-	-
2018	**Sand smelt**	30929	1332	2	0.15 (0.00–0.36)	0.0004	1
	Pumpkinseed	400	667	48	7.19 (5.24–9.16)	0.092	1.27
	Perch >400 g	44	96**	1	1 (0.00–3.07)	0.01	1
	Eel	139	557**	1	0.18 (0.00-0.53)	0.00	1
2019	**Sand smelt**	10750	1332	76	5.71 (4.46–6.95)	0.057	1
	Pumpkinseed	220	367	36	9.81 (6.77–12.85)	0.19	1.89
	Perch >400 g	25	53**	n.d.	-	.	-
	Eel	271	1084**	2	0.18 (0.44-0.00)	0.00	1
2020	**Sand smelt**	29966	2000	421	21.05 (19.26–22.84)	0.21	1
	Pumpkinseed	1400	2333	865***	37.08 (35.12–39.04)	0.87	2.34
	Perch >400 g	159	353**	2	0.57 (0.00–1.35)	0.006	1
	Eel	213	851**	1	0.12 (0.00-0.35)	0.00	1
2021*	**Sand smelt**	794	1005	396	39.40 (36.38–42.42)	0.39	1
	Pumpkinseed	110	580	576	99.31 (98.64–99.98)	2.55	2.57
	Perch >400 g	20	44**	n.d.	-	-	-
	Eel	57	228**	2	0.87 (0.00-2.08)	0.01	1

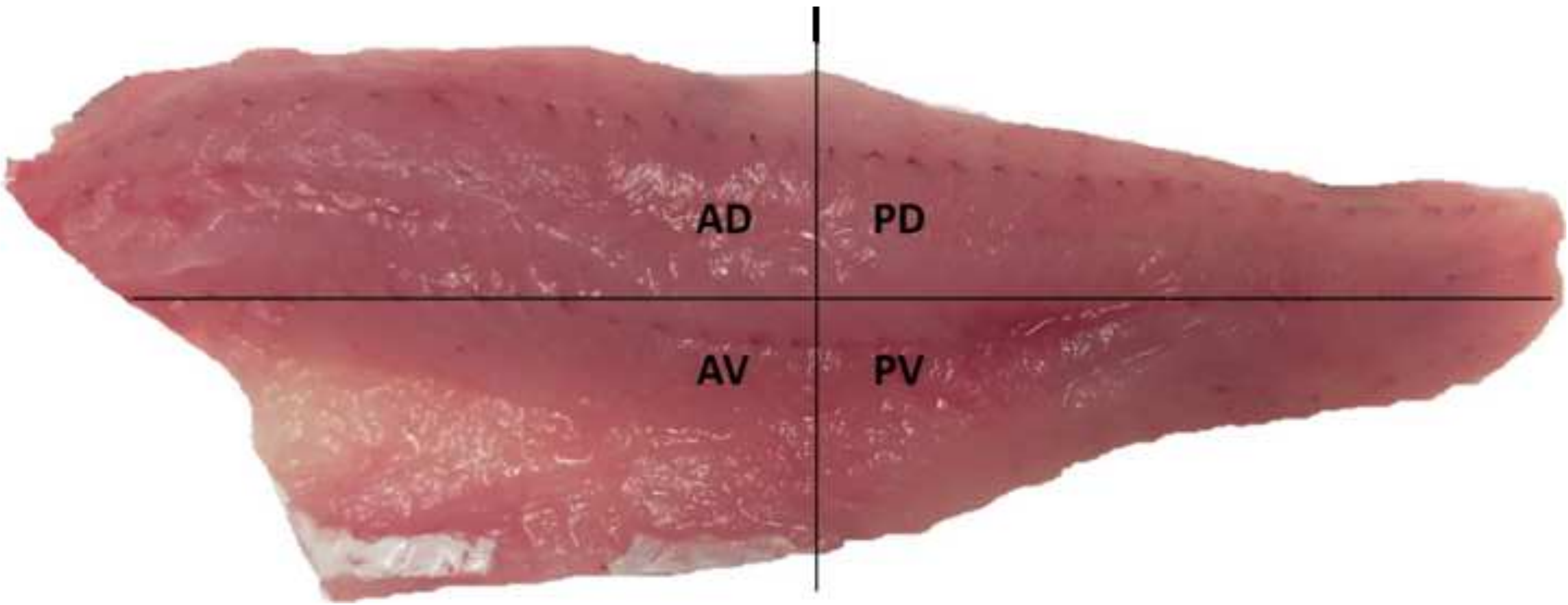
*January, February, March, April

** The results consider the parasitological examination (visual inspection), as well as customer complaints due to the presence of nematodes.

*** The results consider the parasitological examination (visual inspection) of the FBO


FISHERMEN'S COOPERATIVE
1
2
3
4
1
2
1 – LANDING AREA
2 – RECEPTION AREA
3 – TEMPERATURE STABILIZATION ROOM
4 – PROCESSING PLANT

Figure 2




a
b

Figure 4

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CRediT authorship contribution statement

Raffaella Franceschini: Data curation, Formal analysis, Writing - original draft.

Lisa Guardone: Conceptualization, Writing - review & editing

Andrea Armani: Conceptualization, Writing - review & editing, Supervision.

David Ranucci: methodology, investigation

Rossana Roila: methodology, investigation

Andrea Valiani: visualization

Francesca Susini: Formal analysis**,** Funding acquisition,

Raffaella Branciari: Conceptualization, Writing - review & editing, Supervision.