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Anisakis spp. larvae in black scabbardfish (*Aphanopus carbo*) muscle: Consumer health risk and recommendations along the value chain

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ABSTRACT

The black scabbardfish, *Aphanopus carbo*, is a deep-sea Atlantic fish with socioeconomic value, but it is often heavily infected with *Anisakis* spp. larvae. The aim of the present work was to ascertain the distribution and quantification of *Anisakis* spp. larvae in the viscera and muscle of *A. carbo*, and to evaluate whether larval intensity in the viscera can be used to predict parasite load in the muscle. UV-press and pepsin-HCl digestion methods were complemented by molecular techniques to detect and identify the larvae. Four batches of four individuals each, one batch per season ($n = 64$), were obtained. Each batch, with a different parasite intensity based on visual inspection, was selected for comparison. Larval subsamples randomly selected from the viscera, belly flaps, and dorsal muscle were identified by PCR-RFLP, marking the first study of this kind in this species. Data showed that the higher the larval load in the visceral cavity and internal organs, the greater the number of larvae in the belly flaps, allowing us to predict *Anisakis* burden in this site. The low number and unpredictability of the zoonotic *A. simplex s.s* in the dorsal muscle pose a risk to consumers. Epidemiological data support discarding the belly flaps from fish with relatively high parasite intensity. Additional recommendations to mitigate anisakids presence in fishery products are presented for improvement and implementation along the black scabbardfish value chain.

1. Introduction

The black scabbardfish, *Aphanopus carbo*, is a deep-sea Atlantic fish (Froese and Pauly, 2025) with high socioeconomic value,

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mainly due to its firm texture and distinctive flavour. In mainland Portugal, *A. carbo* is mainly caught off Sesimbra. Once landed, the catch is sent to fish auction, where it is distributed and sold as fresh whole fish. Despite its culinary and economic importance, *A. carbo* from the NE Atlantic is frequently infected with nematodes of the genus *Anisakis*. This parasitic burden has serious implications for public health. One of the most severe cases of human anisakiasis reported in Portugal was linked to the consumption of grilled scabbardfish, with 140 larvae extracted from a single patient (Baptista-Fernandes et al., 2017). Other cases have also been associated with raw fish consumption (Bernardo and Castro-Poças, 2018; Carmo et al., 2017). Globally, infections are increasingly recognized as an emerging zoonotic disease (Robertson, 2018; Mladineo, 2019; Adroher-Auroux and Benítez-Rodríguez, 2020; Rahmati et al., 2020).

Historically, anisakids were primarily linked to gastric ulcers in marine mammals, and human cases were confined to regions with raw fish consumption habits. Parasitological analyses for *Anisakis* were requested only for fish destined for export. However, over the recent decades, laboratory surveillance has revealed a growing prevalence of anisakids in fishery products from both the Portuguese coast and imported sources (Ramos, 2020). It was previously suggested that the "turning point" in public and scientific attention came with increased awareness of the parasite's impact on consumer health (Ramos, 2020).

Understanding the biological cycle of *Anisakis* (Køie et al., 1995; Gómez Sáenz et al., 1999) is essential for controlling and mitigating its presence throughout the fishery product value chain. In this context, the EFSA (European Food Safety Authority) Panel on Biological Hazards (BIOHAZ) et al. (2024) compiled a comprehensive overview of diagnostic methods for detecting anisakids in fishery products, alongside physical and chemical treatments for parasite inactivation. Building on this foundation, two complementary strategies: first, the acquisition of epidemiological data on infection along the fish value chain, and second, the implementation of educational initiatives to raise awareness among stakeholders and consumers – have proven to be *sine qua non* conditions for the effective control and mitigation of anisakids in fishery products (Ramos, 2020; Caldeira and Santos, 2022). Eight species of *Anisakis* are currently recognized including *A. simplex*, *A. pegreffii*, *A. berlandi*, *A. ziphidarum*, *A. nascettii*, *A. oceanica*, *A. similis*, and *A. typical* (WoRMS Editorial Board, 2025). Of these, only *A. simplex* s.s., *A. pegreffii*, and *A. typica* have been implicated in human anisakiasis (EFSA, BIOHAZ panel, et al., 2024).

The present study aimed to (1) detect and identify *Anisakis* spp. larvae in the viscera, belly flaps and dorsal muscle of black scabbardfish using the UV-press and pepsin-HCL digestion methods, complemented by molecular techniques; (2) quantify the occurrence and describe the distribution of *Anisakis* spp. larvae in the viscera and muscle (belly flaps and dorsal muscle); (3) evaluate whether the number of *Anisakis* spp. larvae in the viscera can be used to predict parasite load in belly flaps and dorsal muscle; (4) assess consumer health risk and provide recommendations and outputs for stakeholders along the black scabbardfish value chain.

2. Material and methods

2.1. Fish sampling and parasitological analysis

A total of 120 adult black scabbardfish specimens were obtained seasonally (30 fish per season) between July 2023 and May 2024 from Sesimbra, mainland Portugal. All fish were transported under refrigerated conditions to the Pathology Laboratory of Aquatic Animals at IPMA. An average of 48 to 72 h was allowed to pass between capture and sampling. Upon arrival, each specimen underwent parasitological examination to enumerate anisakids. Biological data were recorded for each fish, including total length (to the nearest 0.1 cm; lt ± SD, cm) and weight (to the nearest 0.1 g; wt ± SD, g). The visceral cavity was opened, visually inspected, and photographed.

Parasite intensity was classified into four grades based on a visual, non-destructive inspection of the visceral cavity and internal organs. While Grades 1 and 2 represented light parasitism, characterized by scattered larvae on the surface of internal organs (Grade 1: $n < 30$; Grade 2: $n < 50$ and/or small clusters, Grade 3 and 4 indicated heavy parasitism. This was defined by the presence of larval clusters that perforate the peritoneal serosa in Grade 3, progressing to extensive clusters that lined the entire visceral cavity in Grade 4.

Each fish was dissected, and the viscera were individually stored in plastic bags and frozen at −20 °C for later analysis. The left-side muscle was divided into dorsal (epaxial) and belly flap (ventral/hypaxial) portions, which were also frozen. For comparative analysis, fish of comparable lengths were selected, to minimize the confounding effects of host size or age (Poulin, 2000). Four batches of four individuals ($n = 4$ per batch), one per season, were selected based on parasite intensity Grades 1 to 4, totalling 64 specimens. Parasite distribution was analysed across internal organs and tissues using visual inspection, stereomicroscopy, UV-press, and pepsin-HCl digestion. After thawing, the stomach and intestine were examined macroscopically and under a stereomicroscope (3× magnification). Thirty third-stage larvae (L3) from the viscera of each fish were removed and identified to genus level using a light microscope and the key of Berland (1961). The liver, gonads, and dorsal muscle were inspected using the UV-press method, following ISO 23036-1, 2021. After thawing and removing the skin and peritoneum, each left dorsal muscle was compressed to 2 mm thickness, frozen for 24 h, and examined under 366 nm UV light in a dark room. Larvae were identified by their fluorescence.

Pepsin-HCl digestion was performed on the belly flap muscle following the ISO 23036-2, 2021 standard. This artificial digestion method uses pepsin in hydrochloric acid (HCl) to digest the muscle tissue, facilitating the isolation and enumeration of *Anisakis* spp. larvae. All larvae observed, whether by visual inspection, UV-press, or digestion were counted and preserved in vials containing 98 % ethanol at 4 °C for subsequent molecular identification.

2.2. DNA isolation and species identification by PCR-RFLP

Subsamples for molecular analysis consisted of five *Anisakis* spp. larvae randomly selected from the viscera of different fish within each seasonal batch (totalling $n = 20$), and five larvae from the UV-press analysis of dorsal muscle. Additionally, larvae ($n = 50$) were

obtained from pepsin-HCl digestion of belly flaps.

Individual L3 larvae were washed in sterile 8 % (*w/v*) NaCl solution, mechanically fragmented with a sterile scalpel, and transferred into 1.5 mL microcentrifuge tubes. Genomic DNA (gDNA) was extracted using a 10 % Chelex® resin-based protocol (Walsh et al., 1991). Briefly, samples were mixed with 150 µL of 10 % Chelex® resin, incubated at 100 °C for 20 min with intermittent vortexing at 5-min intervals, and centrifuged at 13,000 ×*g* for 10 min. The resulting gDNA-containing supernatant was recovered for subsequent use.

Species identification was performed using PCR–restriction fragment length polymorphism (PCR–RFLP) targeting the nuclear ribosomal internal transcribed spacer (ITS) region (ITS1, 5.8S rRNA, ITS2) (D'Amelio et al., 2000).

The ITS region was amplified in 25 µL reactions containing 2 µL of gDNA, 400 nM of each primer (NC5: 5′-GTAGGTGAACCTGCGGAAGGATCATT-3′; NC2: 5′-TTAGTTTCTTTTCCTCCGCT-3′) (Gasser et al., 1996), and NZYTaq II 2× Green Master Mix (NZYTech, Lisbon, Portugal). The PCR program consisted of an initial denaturation at 95 °C for 3 min; 35 cycles of 95 °C for 50 s, 55 °C for 50 s, 72 °C for 100 s and a final extension at 72 °C for 7 min.

ITS amplicons were digested with *Hin*fI at 37 °C for 3 h to discriminate among *A. simplex/A. berlandi*, *A. pegreffii*, and hybrid forms (Abollo et al., 2003). Subsequent digestion with *Hha*I at 37 °C for 3 h enabled distinction between *A. simplex* and *A. berlandi*. Restriction fragments were separated on 2 % agarose gels, stained with GreenSafe Premium (NZYTech), and visualized under UV light using a Gel Doc™ XR+ system (Bio-Rad, Hercules, CA, USA). Fragment sizes were determined by comparison with NZYDNA Ladder VI (50–1500 bp; NZYTech).

2.3. Statistical analysis

Analyses were performed using IBM SPSS Statistics v29.0. Data distribution and variance homogeneity were assessed using Shapiro-Wilk and Levene's tests, respectively. Fish biometric data (length and weight) met parametric assumptions and were analysed by one-way ANOVA with Tukey's post-hoc test across seasons and Grades 1–4. Larval count data not meet normality assumptions and were analysed using Kruskal-Wallis tests to assess differences across Grades 1–4 within each tissue type, with Dunn's post-hoc tests for significant results. A planned comparison between combined low-intensity (Grades 1–2) and high-intensity (Grades 3–4) groups was conducted using the Mann-Whitney *U* test. Significance was at $p < 0.05$ for all tests.

3. Results

3.1. Fish characterization

Subsamples of black scabbardfish collected across four seasons in 2023–2024 ($n = 16$ per season) were analysed for weight and total length. In summer 2023, fish weighed 1821.56 ± 399.34 g (range: 1220–2845 g) and measured 110.2 ± 7.25 cm in length (range: 97–125.6 cm). In fall 2023, specimens weighed 2212.81 ± 512.92 g (range: 1290–2915 g) and measured 109.66 ± 5.68 cm (range: 100.5–118.7 cm). In winter 2024, fish weighed 1752.81 ± 305.76 g (range: 1365–2345 g) and measured 108.71 ± 5.77 cm (range:

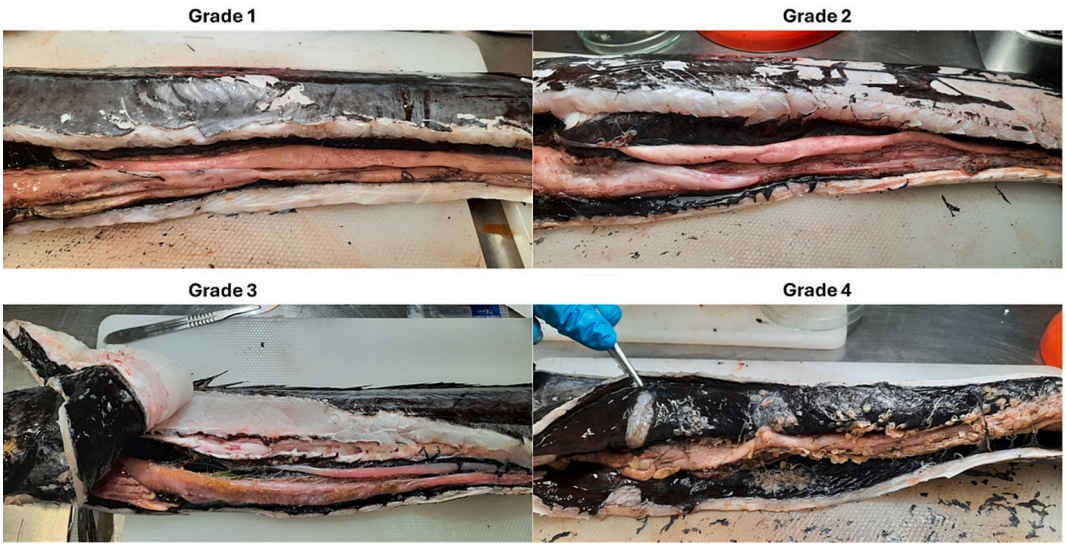

Fig. 1. Visual and non-destructive inspection of the visceral cavity and internal organs of black scabbardfish, used to classify parasite intensity into four grades based on the number and distribution of *Anisakis* larvae. Grade 1 corresponds to scattered larvae ($n < 30$) on the surface of internal organs; Grade 2 includes scattered larvae ($n < 50$) or small clusters; Grade 3 is characterized by clusters of larvae, with some perforating the peritoneal serosa; and Grade 4 shows dense clusters of larvae both on the surface and perforating the serosa, sometimes lining the entire visceral cavity.

98–118 cm). In spring 2024, weights averaged 1441.88 ± 264.34 g (range: 1055–2000 g), with lengths of 105.17 ± 5.31 cm (range: 98–117 cm).

Seasonal differences in total length and mean weight, as well as differences across parasite intensity Grades 1–4, were investigated. No significant variation in fish length was detected across seasons ($F = 2.302$, $p = 0.086$) or among grades ($F = 0.907$, $p = 0.443$). In contrast, mean weight varied significantly across seasons ($F = 10.965$, $p < 0.001$), but not across Grades 1–4 ($F = 0.055$, $p = 0.983$).

3.2. Infection levels with Anisakis spp. larvae

The prevalence of anisakids in the 120 examined black scabbardfish was 100 %. Larvae were located predominantly in the visceral cavity and internal organs. Exceptionally high infection intensities were observed in some Grade 4 fish, ranging from 2545 to 3018 worms per fish. In these heavily infected individuals, numerous L3 larvae were observed to perforate the peritoneum simultaneously and *Anisakis* L3 larvae were visibly coiled beneath the skin, forming prominent subcutaneous bulges.

Infection Grades 1–4 were defined based on visual inspection of the visceral cavity and internal organs (Fig. 1). Among the 64 black scabbardfish analysed in detail, the total number of *Anisakis* spp. larvae recorded was 38,466 in the viscera and visceral cavity, 14015 in the belly flaps, and 223 in the dorsal muscle. Larval counts in the viscera and visceral cavity increased progressively with infection grade (Grade 1: 1913; Grade 2: 5416; Grade 3: 9477; Grade 4: 21660). Statistical analysis confirmed significant differences in larval counts across Grades 1–4 (Kruskal-Wallis test, $\chi^2 = 47.347$, df = 3, $p \leq 0.001$) (Fig. 2). When fish were grouped into low-intensity (Grades 1–2) and high-intensity (Grades 3–4) categories, the number of larvae differed significantly between groups (Mann-Whitney test, $U = 928$, $p \leq 0.001$).

Larvae recovered from pepsin-HCl digestion of belly flaps also increased with infection grade (Grade 1: 1068; Grade 2: 1969; Grade 3: 3594; Grade 4: 7384). These differences were statistically significant (Kruskal-Wallis test, $\chi^2 = 38.893$, df = 3, $p \leq 0.001$) (Fig. 3). A total of 223 L3 larvae were recovered from the dorsal muscle. However, statistical analysis revealed no significant differences in dorsal muscle infection across Grades 1–4 (Kruskal-Wallis test, $\chi^2 = 7.230$, df = 3, $p = 0.065$).

3.3. Morphological and molecular identification of Anisakis larvae

All collected larvae were visually confirmed as *Anisakis* L3. Molecular identification revealed *A. simplex sensu stricto* (s.s.) as the predominant species across all tissue types, although distribution patterns varied by location. Among larvae from the viscera ($n = 20$), 90 % were *A. simplex* s.s. and 10 % were *A. pegreffii*. Belly flap samples ($n = 50$) showed greater diversity, with 82 % *A. simplex* s.s., 14 % hybrids (*A. simplex* × *A. pegreffii*), and 4 % *A. pegreffii*. In contrast, the limited dorsal muscle samples ($n = 3$) contained 66 % *A. simplex s.s.* and 33 % hybrids, with no *A. pegreffii* detected.

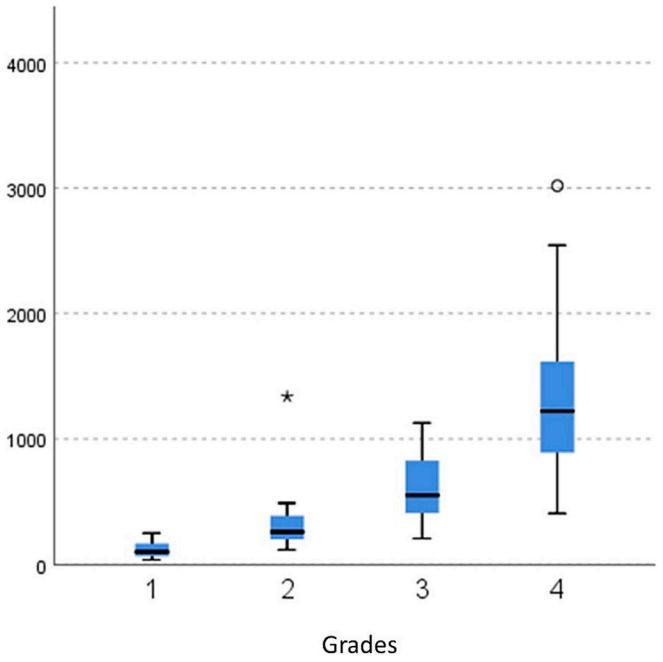

Fig. 2. Distribution of *Anisakis* larvae in the visceral cavity and internal organs, showing a progressive increase in parasite burden from Grade 1 to Grade 4.

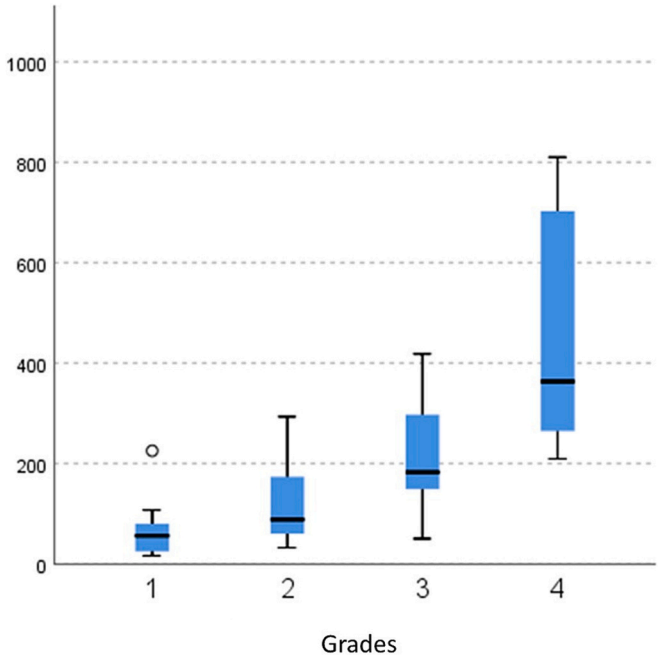

Fig. 3. Distribution of *Anisakis* larvae in the belly flaps across grades, showing significant differences in the number of parasites recovered.

4. Discussion

4.1. Anisakis infection dynamics and tissue distribution

This study confirms the black scabbardfish as a highly susceptible host for anisakids, showing 100 % prevalence across all seasonal batches, consistent with previous findings in Portuguese waters (Costa et al., 2003; Santos et al., 2009; Cruz et al., 2009; Hermida et al., 2020). In this study, fish of comparable length were selected for seasonal comparison to minimize host-size confounding, as this parameter significantly influences *Anisakis* spp. abundance (Hermida et al., 2020).

The visceral cavity and internal organs were the primary infection sites, yet a substantial proportion of larvae (approximately 37 % of the total visceral count) was recovered from the muscle tissues (belly flaps and dorsal muscle). Evidence of *intra-vitam* migration was observed, with larvae encapsulated in spiral-shaped, tightly coiled forms in both belly flaps and dorsal muscle. In heavily infected (Grade 4) fish, larvae were found immediately beneath the skin, forming visible subcutaneous prominences (Fig. 4) similar to those as previously described in European anchovy by Cipriani et al. (2016). The strong positive correlation between worm counts in the viscera and belly flaps, in contrast to the lack of such a relationship for the dorsal muscle, suggests that the visceral burden is a reliable predictor of infection intensity in adjacent muscle tissues, a pattern also observed in European hake and grey gurnard (Cipriani et al., 2015; Karl and Levsen, 2011). The predominance of larvae in belly flaps over dorsal muscle, especially in heavily infected fish (Grades 3 and 4), suggests the high collagen content of belly flaps in this species may act as a migration barrier. Although refrigeration below 2 °C inhibits *post-mortem* migration (Cipriani et al., 2016, 2024), the observation of a high number of larvae simultaneously piercing the peritoneum suggests other factors, like extended gear soaking times (24–72 h) may initiate *post-mortem* migration before chilling.

Several biological and abiotic factors have been proposed to influence larval migration, including host feeding behaviour, the

Fig. 4. Macrophotograph showing coiled and prominent L3 *Anisakis* larvae encapsulated beneath the skin of the belly flaps.

richness of fatty acids in fish muscle, stomach wall thickness, fish size, immune responses (Smith, 1983; Cruz et al., 2009; Hermida et al., 2020), and post-capture storage conditions (Cipriani et al., 2016, 2024). Although no studies have specifically addressed storage temperature effects in *A. carbo*, fish in this study were placed on ice immediately after capture, with temperatures maintained near 2 °C throughout the handling chain. Under these conditions, no significant differences in dorsal muscle infection were observed across grades, suggesting limited larval penetrability into fillets.

Molecular identification revealed that *A. simplex* s.s. was the predominant species across all tissues, exhibiting greater invasive capacity (Suzuki et al., 2010). This finding aligns with the known geographical distribution of *Anisakis* species, where *A. simplex* s.s. is typically the dominant species in colder Atlantic waters, such as those where the black scabbardfish in this study were captured, while *A. pegreffii* is more common in warmer Mediterranean and southern hemisphere waters. Nevertheless, the species distribution varied, with belly flaps showing a more diverse composition that included *A. pegreffii* and hybrid genotypes. Intriguingly, the limited dorsal muscle samples contained *A. simplex* s.s. and hybrids, but no *A. pegreffii*, suggesting potential differences in invasive capability or tissue tropism between the species. The higher prevalence of hybrids in muscle tissue compared to viscera may indicate an adaptive advantage for muscular penetration. In our study, we used the PCR-RFLP method for distinguishing *A. simplex s.s.* from *A. pegreffii*, which remains widely applied, especially in large-scale studies. However, we acknowledge its limitations in reliably identifying hybrid genotypes. Intermediate profiles may suggest hybridization, but definitive confirmation requires mitochondrial *COX2* gene sequencing.

The precise triggers for larval migration remain multifactorial (EFSA, 2010), involving host, parasite, and environmental factors. While our standardized refrigeration protocol aimed to inhibit *post-mortem* migration, the potential influence of factors such as

Table 1
Preventive recommendations and expected outcomes to improve or implement across the black scabbardfish value chain, aimed at mitigating the presence of zoonotic *Anisakis* spp. in fishery products.

Black scabbardfish Value chain	Recommendations to improve/implement	Outputs
Fishing	Pre-freezing the baitfish	Prevent further infection of fish
	Decrease the soaking period Promote the use of ice to keep fish refrigerated	Control of anisakids *post-mortem* migration
	Good handling and hygiene practices on board	Improve fishing art management Prevent predation of fish in gear Physical removal of larvae
Transport	Refrigerated road transport	Control of anisakids *post-mortem* migration
	Cooling temperature control	Prevent fish spoilage
Fish auction	Remove visible parasites and anomalous /damaged fish	Improve fish quality
	Add ice/keep the fish refrigerated	Control of anisakids *post-mortem* migration
Wholesale market[1]	Distribution of bulk fish in boxes	Improve storage conditions
	Good practices	Prevent fish spoilage
	Cooling temperature control	Control of anisakids *post-mortem* migration
Multiple national retailing[2]	Fish line operators with specific training - Profissional training	Improve technical decision making
	Compliance with the European Union legislation	Avoid the presence of visible parasites in fish
Retail markets[3]	Access to cold chain	Control muscle deterioration and larvae migration
	Evisceration and cleaning if necessary	Physical removal of larvae
	Remove belly flaps if necessary	Control of anisakids *post-mortem* migration
	Filleting if necessary	Avoid food waste
	Good practices	Avoid physical damage to fish
	Compliance with the European Union legislation	Avoid the presence of visible parasites in fish
Restaurants & catering	Evisceration and cleaning if necessary	Control of anisakids *post-mortem* migration
	Good practices	Avoid physical damage to fish
	Compliance with the European Union regulations	Avoid infection - anisakiase
Consumption at home	Evisceration and cleaning if necessary	
	Freeze fish intended to be eaten raw	Avoid infection - anisakiase
	Make the fish before consuming	
Research institutes/ unversity	Awareness campaigns	Increase awareness of zoonotic fish parasites Increase consumer confidence
	Strengthen the link research/industry	Improve technical decision making
	Improve technology in the industry	Avoid infection - anisakiase
	Training programs for operators - Professional training	
	Improve parasitological analysis to support FBOs* decisions	Control and mitigation of fish parasites
	Developing tools for effective communication with stakeholders	
Food loss / waste	Belly flaps discard / filleting dorsal muscle Raise awareness among fishermen	Reduce food waste
Services	Strengthen the application of laws in this issue	Control and mitigation of fish parasites
	Implement official controls	Compliance with the European Union regulations

[1]local whole sale trade
[2]hypermarket chain
[3]markets, mini-markets, fish mongers shops, itinerant fish sellers
FBOs* - food business operators

extended gear soaking times cannot be discounted. Recent research highlighting how temperature modulates gene expression for invasion-related peptidases and the release of extracellular vesicles in *Anisakis* (Palomba et al., 2020, 2023) underscores the need for further study into the mechanisms governing this critical behaviour.

4.2. Consumer risk and practical mitigation

In our study, *Anisakis* larvae were frequently observed lodged in the visceral cavity and encysted within the internal organs of black scabbardfish. In such cases, evisceration combined with proper handling and hygiene practices can effectively reduce the risk of infection by removing visible parasites prior to consumption. However, our findings indicate that belly flaps, particularly in fish classified as Grade 3 and 4, harbour a high number of anisakids and should therefore be removed and discarded to minimize consumer exposure. Although the number of larvae detected in the dorsal muscle was low, the unpredictable presence of the zoonotic species *A. simplex s.s* that cause gastric, intestinal, and allergic anisakiasis, (Audicana and Kennedy, 2008), means this risk cannot be ignored.

Storage temperature is indeed a factor known to affect *post-mortem* migration of *A. pegreffii.* in several fish hosts, including anchovies (Cipriani et al., 2016), Atlantic herring, Atlantic mackerel, and blue whiting (Cipriani et al., 2024). In anchovies, larval migration increases with rising storage temperatures (2 °C, 5 °C, and 7 °C), further underscoring the importance of temperature control (Cipriani et al., 2016). However, no related investigation exists in black scabbardfish. Therefore, strict adherence to existing food safety regulations is paramount. To ensure consumer safety, particularly for raw or undercooked products, fish must be frozen at −20 °C for a minimum of 24 h or − 35 °C for at least 15 h, or cooked to a core temperature of 60 °C for a minimum of 1 min, as stipulated by Commission Regulation EU (No. 1276/2011).

4.3. Stakeholder recommendations for managing Anisakis risk in the black scabbardfish value chain

This study confirms that black scabbardfish harbour a high number of anisakids in the viscera and belly flaps, and a lower, though not negligible number in the dorsal muscle. This poses a public health risk and a commercial challenge. Beyond the potential for anisakiasis and allergic reactions, visible parasites can provoke consumer disgust, leading to food waste and reputational damage for Food Business Operators (FBOs). This fish–parasite–human interface underscores the need for integrated "One Health" strategies.

Beyond existing Codes of Good Practice for both fishing vessels (Sousa et al., 2021) and commercial areas; and EU legislation regarding parasites in fishery products (Commission Regulation EU, No. 1276/2011), we propose targeted improvements for the black scabbardfish value chain to mitigate parasite risk (Table 1). At capture, where the species is caught using deep-sea longlines with soaking periods of 36–48 h (Campos et al., 2025) that may promote *post-mortem* migration, pre-freezing baitfish like sardines and mackerel could help prevent the transmission of zoonotic parasites, as most wild fish are potential carriers (EFSA-BIOHAZ, 2010, EFSA, BIOHAZ panel, et al., 2024). Onboard, rapid evisceration and immediate chilling are critical to remove larvae and inhibit migration. Viscera should be retained on the vessel, particularly in protected areas like Sesimbra, to limit environmental parasite cycling (Bozzano and Sardá, 2002), and technologies like TEDEPAD® could inactivate parasites in discards (González et al., 2018), though cost-effective solutions are needed for small-scale operators. On land, refrigerated transport is essential, and all operators must be trained to comply with EU legislation (Commission Regulation EU, No. 1276/2011). Furthermore, discarding belly flaps in highly parasitized fish (Grades 3–4) is a key practical measure to enhance safety. For outreach and policy, Universities and Research Institutes are key to bridging science and industry, promoting awareness to mitigate risk (Ramos, 2020), while government institutions must enforce legislation. Visible parasites cause economic losses and consumer complaints (Llarena-Reino et al., 2015; Bao et al., 2019), underscoring the need for awareness campaigns to reduce food waste. A holistic "One Health" approach involving all stakeholders is essential for the sustainable management of black scabbardfish and the broader challenge of zoonotic fish parasites.

5. Conclusions

This study establishes a clear association between visual infection of the visceral cavity and internal organs (Grade 1–4) and *Anisakis* L3 larvae abundance in belly flaps of black scabbardfish. Although larval presence in the dorsal muscle was low and unpredictable, the detection of the zoonotic species of *A. simplex s.s.,* underscores a persistent consumer risk. Based on our epidemiological data, we recommend the mandatory discard of belly flaps in Grade 3–4 fish to substantially reduce consumer exposure. Furthermore, we provide specific recommendations to enhance control measures across the value chain, aiming to mitigate zoonotic anisakids presence while improving product safety, quality, and consumer confidence.

CRediT authorship contribution statement

P. Ramos: Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **C. Ayra-Pardo:** Writing – review & editing, Investigation. **M.M. Oliveira:** Investigation, Data curation. **L. F. Rangel:** Writing – review & editing, Investigation. **F. Atroch:** Investigation. **C. Sirin:** Writing – review & editing, Investigation. **M.J. Santos:** Writing – review & editing, Project administration, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

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