WILD FISH PARASITE CONTROL IN FISHERY PRODUCTS

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INTRODUCTION

The presence of parasites in fishery products threaten their safety and quality and may represent a consumers' health hazard. Monitoring parasites in fishery products is essential to avoid commercialising contaminated fishery products.

A total of 1216 samples from different seafood products, whole fish (*n*= 278), fish steaks (*n*= 70), fish fillets (*n*= 9), fish fingers (*n*= 801), ray wings (*n*= 4) and shredded fish (*n*= 4 100.72 g) from the Portuguese markets were analysed between 2020 and 2023 (to date) at the Pathology Laboratory of Aquatic Animal.

MATERIAL AND METHODS

External surface, internal organs, visceral cavity and muscle of fresh or thawed whole fish were visually inspected for detection of parasites. The muscle tissue was directly observed under stereomicroscope, submitted to candling method (fillets and belly flaps) and artificial digestion. Thawed fish steaks and fish fillets were also visually inspected, and the muscle was weighed, sliced, observed by candling and artificially digested. Wet mount preparations of muscle tissues from fresh and thawed fish, thawed fish steaks and fish fillets were prepared and microscopically examined. Parasitological analysis of each fish finger was performed on the fish muscle, after removing the batter coating, through macroscopic observation, stereomicroscope examination, slicing and candling. Fresh smears of the muscle were examined microscopically. The collected parasites were morphologically identified[1] and their viability evaluated.

The occurrence, distribution and viability of anisakids (viable and dead larvae) which represent a risk to the consumers' health and those of other parasites that are associated with economic losses in the fish market were reported.

RESULTS

Viable *Anisakis* and *Pseudoterranova* L3 larvae were collected from different fish samples, although live larvae were only found in fresh fish. *Anisakis* L3 larvae were observed encysted in the cape horse mackerel, chub mackerel, Atlantic mackerel and blue whiting muscles. Other nematodes, *Huffmanela lusitana* and *Philometra* sp.; cestodes, pleurocercoids of *Gymnorhynchus gigas* and *Hepatoxylon trichiuri* blastocysts; crustacean copepods, *Pennella instructa* and *Lernaeocera branchialis*; mixosporean spores of *Kudoa* sp., *Kudoa thyrsites* and *Henneguya salminicola*; microsporidean *Spraguea americana*, *Spraguea lophii*, and *Dasyatispora levantinae* gen. et sp. nov. were also observed (Table 1 and 2. Figures 1 to 12).

Type of product: Whole fish					
Scientific name	**Commercial name**	**Specimens (n)**	**Analytical method**	**Total L3**	**Other parasites**
Brama brama	Atlantic pomfret	1	VI, S, C, St	-	*Gymnorhynchus gigas*
Dicenthrachus labrax	European seabass	1	VI, S, C, St	-	-
Gadus sp.	Cod	2	VI, S, C, St	3	-
Merluccius spp.	European hake	1	VI, S, C, St	8	-
Micromesistius poutassou	Blue whiting	1	VI, S, C, St	18	-
Pagellus bogaraveo	Blackspot seabream	9	VI, S, C, St	20	-
Plectorhincus mediterraneus	Rubberlip grunt	1	VI, S, C, St	-	-
Sardina pilchardus	European pilchard	81	VI, S, C, St Sm,D	-	*Kudoa* spp., *Philometra* sp.
Scomber japonicus	Chub mackerel	20	VI, S, C, St, Sm, D	352	*Kudoa* spp.
Scomber scombrus	Atlantic mackerel	22	VI, S, C, St	-	-
Scomber colias		1	VI, S, C, St	2	-
Sparus aurata	Gilthead seabream	4	VI, S, C, St, Sm, D	-	-
Sprattus sprattus	European sprat	4			
Trachurus trachurus	Cape horse mackerel	71	VI, S, C, St, Sm, D	388*	*Kudoa* spp.
Trachurus picturatus	Blue jack mackerel	1	VI, S, C, St, Sm, D	-	
Trisopterus luscus	Pouting	45	VI, S, C, St Sm	27	*L. branchialis; Kudoa* sp. *H. lusitana; Trypanorhyncha* blastocysts

(*) add one more specimen with an uncountable number of larvae

Table 1– Summary of the results obtained by parasitological analysis in whole fish. VI (visual inspection), S (slicing), C (candling), St (visual examination under a stereomicroscope), D (muscle digestion), Sm (smears). The common and scientific names of fish were cited according to www.fishbase.org, version (02/2023).

Type of product	Scientific name	Commercial name	Analytical method	Total L3	Other parasites
Fish steaks (*n*)					
2	*Aphanopus carbo*				
1	*Epinephelus aeneus*	White grouper	VI, S, C, St, Sm	-	*Philometra* sp.
51	*G. morhua*	Atlantic cod	VI, S, C, St, Sm, D	26 *Anisakis* sp.	-
				5 *Pseudoterranova* sp.	-
1	*Molva molva*	Ling	VI, S, C, St, Sm	-	
6	*Merluccius* sp.	European hake	VI, S, C, St, Sm	3 *Anisakis*	*Kudoa thyrsites, Myosporidium merluccius*
1	*Prionace glauca*	Blue shark	VI, S, C, St, Sm	-	-
6	*Salmo* sp.	Salmon	VI, S, C, St, Sm	-	*Henneguya salminicola*
1	*Seriola sp.*		VI, S, C, St, Sm	-	Trypanorhyncha blastocysts
1	*Xiphias gladius*	Swordfish	VI, S, C, St, Sm	-	*Pennella instructa*
Fish fillets (*n*)					
2	*G. morhua*	Atlantic cod	VI, S, C, St, Sm	-	-
1	*Genypterus blacodes*		VI, S, C, St, Sm	-	*Hepatoxylon trichiuri* plerocercoid
2	*Lophius litulon*	Angler	VI, S, C, St, Sm	-	*Spraguea lophii*
1	*Merluccius* sp.	European hake	VI, S, C, St, Sm	-	-
3	*Pollachius pollachius*	*Pollachius pollachius*	VI, S, C, St, Sm	-	-
Fish fingers (*n*)					
584	*Merluccius sp.*	European hake	VI, S, C, St, Sm, D	1 *Anisakis*	*Kudoa* spp.
60	*Pollachius pollachius*	Pollack	VI, S, C, St, Sm	-	-
54	*Pollachius virens*	Saithe	VI, S, C, St, Sm	-	-
103		"minced fish mix"	VI, S, C, St, Sm	1 *Anisakis*	1 Trypanorhyncha blastocyst
Shredded fish (g)					
3 898.81	*G. morhua*	Atlantic cod	VI, S, C, St, Sm, D	42 *Anisakis* sp.	-
				3 *Pseudoterranova* sp.	
201.91	*Pollachius pollachius*	*Pollack*	VI, S, C, St, Sm	-	
Others					
4		Ray wings	VI, S, C, St, Sm	-	*Dasyatispora levantinae* gen. sp. nov.

Table 2– Summary of the results obtained by parasitological analysis in fish steaks, frozen fish fillets, fish fingers, shredded salt cod, and ray wings. St (visual examination under a stereomicroscope), D (muscle digestion), Sm (smears).

FINAL REMARKS

Commercially valuable fish are often placed on the market as fresh fishery products, and they may contain parasites. If these parasites are observed macroscopically in commercially valuable fishery product - like the nematode *Anisakis*, and the cestode, *Gymnorhynchus gigas* or if changes in the texture or color of the muscle are observed - as occurred in *Kudoa* and *Huffmanela* infection, it makes the fish disgusting, leading to the withdrawal of the fish from the supply chain, with consequent economic losses.

On the other hand, *Anisakis simplex* s.s. and *A. pegreffii* larvae of the genus *Anisakis* are the most frequently nematodes reported and the ones that are most important from a health point of view because they have zoonotic potential. Human infection (anisakiasis) occurs when raw or poorly prepared fish is consumed, containing live L3 larvae, which cause vomit, nausea, abdominal pain, diarrhoea and even potential allergic reactions that can vary from an allergic reaction to anaphylactic shock[2]. Raw fishery product should undergo a previous temperature treatment to kill viable parasites before consumption, accordingly to the European Union legislation which establishes that the temperature in all parts of certain fishery products must be lowered to at least -20 °C for not less than 24 hours or -35 °C for not less than 15 hours, in order to kill viable parasites. (Commission Reg. EC No 1276/2011).

It is through the dissemination of knowledge and raising awareness on the issue of parasites in fishery products to health inspectors, economic agents and consumers that infection prevention must be carried out[3].

LEGENDS:

Figure 1 – European pilchard. Muscle degeneration and gelatinization (1a) due to the presence of *Kudoa* spores (⟶) (1b).
Figure 2 – Atlantic pomfret. Larval cestodes of *Gymnorhynchus gigas* in the muscle (2a) and after removed (2b).
Figure 3 – Pouting. Darkened flesh of the hypaxial muscle (3a) due to the presence of *Huffmanela lusitana* eggs (3b).
Figure 4 – White grouper. Presence of nematode females -yellow body full of eggs (⟶) of the genus *Philometra*.
Figure 5 – Swordfish. Parasitic cyst with sections of the copepoda *Pennella instructa*.
Figure 6 – Angler. Cysts (⟶) in the spinal nerves with microsporidia spores of *Spraguea lophii* (Microsporidia).
Figure 7 – *Seriola* sp. steak. *Hepatoxylon trichiuri* plerocercoid embedded in the muscle (⟶) (7a). Close-up view of the cestode (7b).
Figure 8 – Ray wing. Tumor-like protubrances (⟶) induced by the microsporidea, *Dasyatispora levantinae* gen. sp. nov.
Figure 9 and 10 – Atlantic cod. L3 *Anisakis* larvae (⟶) observed by candling and visual inspection, respectively.
Figure 11 – Fish finger. *Merluccius* sp. muscle with L3 Anisakis larvae (11a). Close-up view of the larvae (11b)
Figure 12 – Salmon steak. White cysts (⟶) containing spore-filled white fluid (12a). Close-up view of *Henneguya salminicola* spores (12b).

References

1. B. Berland, *Sarsia* (1961) 1-50. J. Lom & I. Dyková, *Protozoan parasites in fishes* (1992). ISBN 0444 89434 9. Henning, S.S., Hoffman, L.C., Manley, M., *South African Journal of Sciences* (2013) 109,11/12: 1-5.
2. M.T. Audicana, M.W. Kennedy, *Clin Microbiol Ver*. (2008) 360-379.
3. P. Ramos, *Experimental Parasitology* (2020) 1-8.

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