# Inspective Investigation on Swordfish (*Xiphias gladius*) frozen Slices of Commerce: Anatomical-Histopatological Findings

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**Abstract**

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The aim of this work was to carry out an inspective survey on frozen slices of swordfish (*Xiphias gladius*, Linneo 1758), regularly commercialised in Messina, Sicily (Italy). 402 products were checked at retail levels; the products came from four different fishing area: 59 from the Mediterranean Sea, 155 from North-East Atlantic Ocean, 139 from the Indian Ocean, and 49 from the Pacific Ocean. Fifty-one products were sampled and carefully examined macroscopically and histologically. The histological examination was also carried out on 31 muscle portions without macroscopic alterations. 25 samples (Group A), were parasitised by larvae of *Gymnorhynchus gigas* or *Molicola (Gymnorhynchus) horridus.* The lesions described for Group B (24 samples) as well as for 31 muscle portions without macroscopic alterations were attributed, to the freezing effect. Histological and macroscopic findings on four samples suggested the presence of parasitic lesions.

**Keywords**: parasite; freezer burn; Trypanorinchae; fish

The swordfish (*Xiphias gladius* Linnaeus), the only member of the Xiphiidae family, is a pelagic and migratory fish, with a worldwide distribution. It is subject of significant attention by the seafood industry for its high commercial value. It is generally sold as fresh, frozen, or smoked pieces and slices. The frozen products are packed in single units wrapped in plastic film, in several units placed in polystyrene trays, or in the bulk form. The potential food safety issues related to the consumption of swordfish are chemical and biological. In particular, chemical hazards are linked to the biomagnification along the food chain, of pollutants such as Hg, Pb, and Cd (Severino & Russo 2007) as well as of PCB, PCDD, and PCDF (Bocio *et al.* 2007). Hygienic quality of this product, despite the potential bacterial contamination related to the harvest and post-harvest processing, is closely related to muscle parasites such as: Copepods of the genus *Pennella*, especially *Pennella instructa* (Hogans *et al.* 1985; Castro-Pampillón *et al.* 2002) and *Pennella crassicornis* (Pellegrino *et al.* 1987), cestodes such as *Molicola* (*Gymnorhyncus*) *horridus* and *Gymnorhyncus gigas* (Trypanorhyncha plerocercoid larvae) (Panebianco 1994), and flukes such as *Maccalumtrema xiphiados* (Panebianco & Giannetto 1994). Furthermore, also the presence of *Anisakis* larvae which represent, as well known, a zoonotic parasitosis, was reported in swordfish by Hogans *et al.* (1983); Gomez-Cabrera (1987) and Castro-Pampillón *et al.* (2002).

The aim of the present study was to characterise the macroscopic damage to frozen slices of *Xiphias gladius*.

## MATERIAL AND METHODS

Frozen slices (402 slices) of *Xiphias gladius* were inspected at several retail outlets or seafood industries in the province of Messina (Sicily, Italy), in order to sample the products with macroscopic modification of the appearance, including the presence of presumptive parasites. At the same time, traceability data were recorded for all frozen slices. Fifty-one products were sampled and transported to the laboratory within two hours. All samples, before and after thawing at 4°C × 24 h, were carefully examined macroscopically in order to characterise each modification of colour, shape, and consistence. When the macroscopic examination did not allow to reach a definitive diagnosis, the samples were fixed in 10% buffered formalin, embedded in paraffin, and the sections obtained were stained with hematoxylin-eosin. Histological examination was also carried out on 31 muscle portions without macroscopic alterations in order to assess the degree of the freezing damage.

## RESULTS AND DISCUSSION

The samples came from four different fishing areas; in particular: 59 came from the Mediterranean Sea (FAO Area 37), 155 from North-East Atlantic Ocean (FAO Area 27), 139 from the Indian Ocean (FAO Areas 51 and 57), and 49 from the Pacific Ocean (FAO Areas 61, 67, 71, 77, 81, and 87).

***Macroscopic findings.*** Macroscopic alterations, recorded in 51 slices (12.68%), are summarised in Table 1. In particular, 25 samples (Group A) showed a variable number of small round or oval nodules with a diameter of 0.6–1.0 cm. After thawing, these lesions were more evident and appeared as white streaks that, in some cases, according to Gelmetti *et al.* (1995), resembled myosepta or small blood vessels. In some cases, a moderate pressure allowed to enucleate a white ribbon structure, approximately 4–6 cm long (Figure 1). One sample belonging to this Group (sample No. 27) was also affected by a cystic oval lesion, bipartite and replete with a white and creamy material.

Another kind of alteration (Group B), found in 24 samples, was characterised by gray-whitish oval areas, from 0.5 cm to 3 cm diameter, which appeared isolated or confluent and not delimited from the surrounding tissue (Figure 2). These lesions, before thawing, appeared slightly hollow and, after the thawing, proved to be softer than the surrounding tissue and with a spongy-like aspect.

Finally, three samples (Nos 5, 11 and 31, this latter belonging to Group B) were characterised by the presence of areas (3 × 1 cm) well delimited and containing a blackish-brown or grey-yellow friable material.

***Histological results.*** The microscopic examination of histological preparations of Group A allowed to observe parasitic sections with a diameter ranging from 1900 μm to 2900 μm. The parasites were surrounded by a thin or thick connective capsule, with a pericapsular infiltration of inflammatory cells (Figure 3). Around the lesions, myocytes usually showed a coagulative necrosis. In some cases, the observation of cross sections of the bothridial portion of the scolex (Figure 4), allowed presumably to classify these larvae as belonging to the order of Trypanorinchae. Particularly, the cross section of the scolex showed a thick cuticle and an inner fibrillar structure that extended to

Table 1. Distribution and incidence of each kind of lesion for fishing areas

| Sea fishing (area) | Total slices observed | Slices with lesions | Lesions of Group A | Lesions of Group B | Other lesions |
|---|---|---|---|---|---|
| | | (incidence %) | | | |
| Mediterranean (FAO 37) | 59 | 4 (6.77%) | 3 (5.08%) | 0 (0%) | 1 (1.69%) |
| Indian Ocean (FAO 51, 57) | 139 | 16 (11.51%) | 12 (8.63%) | 4 (2.87%) | 0 (0%) |
| North-east Atlantic Ocean (FAO 27) | 155 | 26 (16.77%) | 8 (5.16%) | 17 (10.96%) | 1 (0.64%) |
| Pacific Ocean (FAO 61, 67, 71, 77, 81, 87) | 49 | 5 (10.20%) | 2 (4.08%) | 3 (6.12%) | 0 (0%) |
| Total | 402 | 51 (12.68%) | 25 (6.21%) | 24 (5.97%) | 2 (0.49%) |

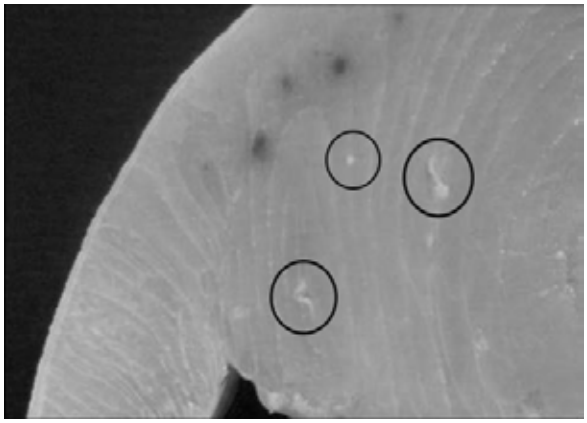

Figure 1. Group A: Presence of larvae cestoda on samples surface after thawing

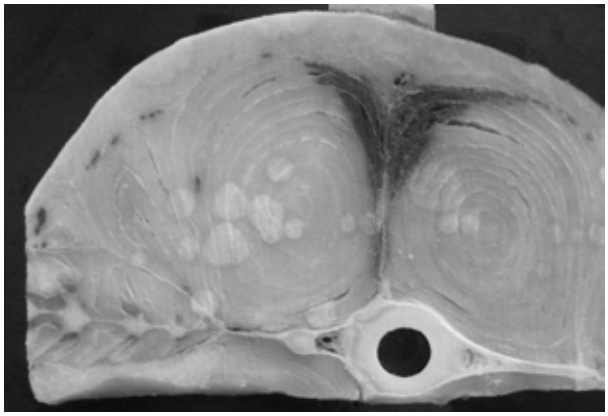

Figure 2. Group B: Macroscopic aspect of "freezer burns" after thawing

all the parasite; none significant organoids structures were found.

The examination of histological sections of Group B samples allowed to observe a complete structure tissue modification with a loss of the typical mosaic aspect. Muscle fibers appeared largely fragmented, divided, chopped, and well-spaced (Figure 5). In some cases, sarcoplasma did not show the typical fibrillar structure but appeared as a detrital mass with a granular composition. A muscle fiber vacuolation, as a further sign of the initial breakdown processes, was observed especially in the central part of cells while the peripheral zone was affected by few large vacuoles that modified the cell contour. In same sections, intracellular vacuoles appeared uniformly distributed so as to give an alveolar aspect to the muscle fibres.

Histological examination of the muscle portions without macroscopic alterations showed a set of lesions similar to those described for Group B, but in a slighter degree. In particular, the typical mosaic aspect, proper of the fresh muscle tissue, was almost maintained. The muscle fibers appeared to be shrunken and were slightly divided and fragmented, showing an irregular shape. In some cases cytoplasm appeared granular.

Finally, macroscopic lesions of samples Nos 5, 11, 27, and 31 appeared histologically characterised by a thick connective capsule infiltrated by phlogistic cells, surrounding the parasitic delaminated cuticle containing an amorphous irregularly pigmented material.

According to the histological findings and to the observed size of worms (Dollfus 1942; Vazquez-Lopez *et al*. 2001b), the larvae found in Group A samples appear referable to the Cestoda *Gymnorhynchus gigas* and *Molicola (Gymnorhynchus) horridus*, which are frequently described in *Xiphias*

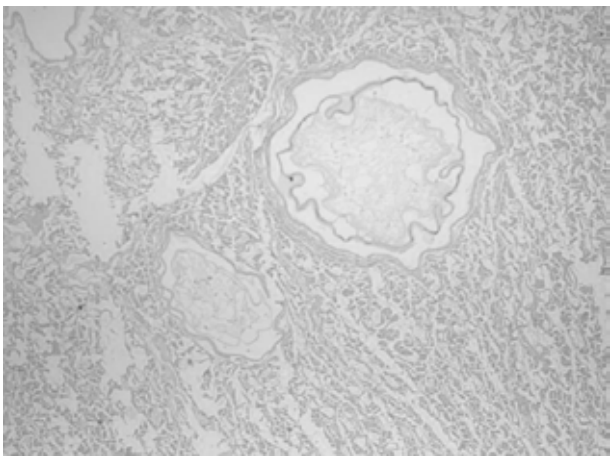

Figure 3. Group A: Cross parasites sections (Haematoxylin/eosin staining; 40×)

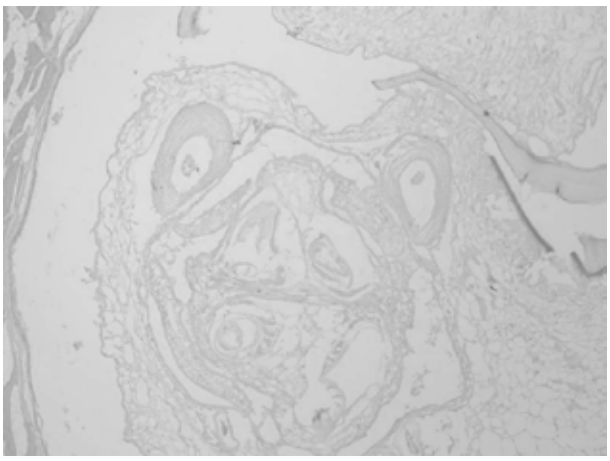

Figure 4. Group A: Cross section of bothridial portion of the scolex (Haematoxylin/eosin staining, 100×)

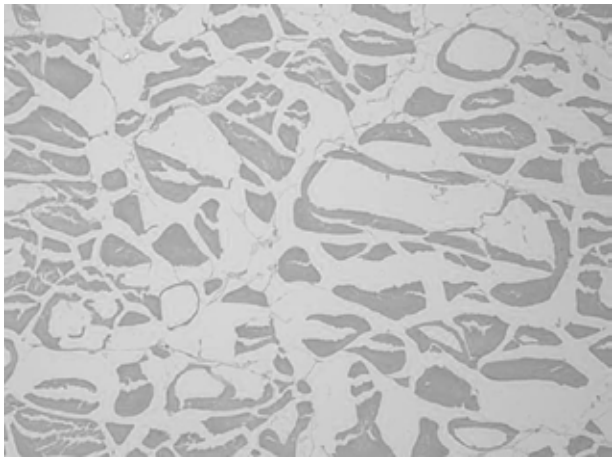

Figure 5. Group B: Histological aspect of "freezer burns" (Haematoxylin/eosin staining, 400×)

Figure 6. Histological aspect of frozen swordfish muscle (Haematoxylin/eosin staining, 100×)

*gladius* (Manfredi *et al.* 1993). Histological and macroscopic findings of samples Nos 5, 11, 27 and 31, suggest the presence of parasitic localisations.

The lesions described for group B as well as for 31 muscle portions without macroscopic alterations are to attribute, as well known (Penso 1950), to the freezing effect ("freezer burns") as well as to the formation of ice crystals, according, more recently, to Ayala *et al.* (2005) and Pavlov *et al.* (2008).

In conclusion, concerning the food safety issues, all the alterations observed should not represent any important public-health risk. Despite that generically, the consumption of parasitised seafood can represent a serious public-health risk (Chai *et al.* 2005), in this study the parasites found do not constitute a zoonotic risk for the consumer and, in any case, freezing treatment is able, as well known, to inactivate most cestodes and nematodes. On the other hand, a potential immune-mediated toxicity of tissues and body fluids should be taken into account. In this regard, only *Anisakis simplex* allergens have been studied and characterised (Moneo *et al.* 2000), and their stability to thermal or freezing treatments has been demonstrated (Audicana & Kennedy 2008; Puente *et al.* 2008). Also the ability of *Molicola horridus* and *Gymnorincus gigas* to produce an anaphylactic reaction has been reported but only in laboratory animals and with crude larvae extracts (Vazquez-Lopez *et al.* 2001a; Gomez-Morales *et al.* 2008). Therefore, the potential risk for consumers related to the conssumption of parasitic toxins and/or allergens could be considered remote in the case of parasitised swordfish frozen slices. Furthermore, according to Ziino *et al.* (2002), this kind of parasitosis does not significantly affect the lipid profile of the muscle. However, according to EC Regulation 2074/2005 Annex II, Section I, Chapter I, the parasites found in this study can be considered as "visible parasites" (because of their dimensions and morphologic characteristics such as colour or texture which are clearly distinguishable from the fish tissues). Therefore, besides to the potential risks for consumers and considering the repugnant appearance of parasitised tissues, according to the Regulation 853/2004 EC (Annex III, Section VIII, Chapter V, Part D), swordfish products "that are obviously contaminated with parasites are not to place on the market for human consumption".

Finally, "freezer burns" neither represent a sanitary risk for consumers nor affect the sensorial and nutritional characteristics of the product, in case they are not very extensive. However, a better modality of storage avoiding the contacts between the freezer surfaces and products as well as the reduction of temperature fluctuations could reduce these kinds of product damage.

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