Literature research regarding parasites in marine white fish species

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ABSTRACT

This report includes the analysis of regulations, international guidelines and recommendations, and available scientific literature on parasites in fishery products, marine species affected, life cycle, effects, etc., as well as the possible mechanisms of prevention, reduction and/or inactivation of viable forms in fish.

Specifically, this document is focused on zoonotic fish-borne parasites of the highest public health concern found in commercially important marine white fish species. In Europe, the most important fish parasites causing illness in humans are nematodes from the Anisakidae family, and the species most commonly associated with human infection is *Anisakis simplex (A. simplex)*, followed by *Pseudoterranova decipiens (P. decipiens).* Although rankings for risk management of food-borne parasites show the relative low importance of anisakids regarding public health concerns among all food-borne parasites, they have a more prominent importance for several countries that trade or consume fish in large quantities.

All wild caught and seawater white fish must be considered at risk of containing any viable parasites of human health concern if these products are to be eaten raw or almost raw. In fact, no sea fishing grounds can be considered free of *A. simplex* larvae.

Anisakiasis (human infection by anisakids) is easy to avoid if the treatments stated in international regulations are followed. Specifically, freezing (at least -20°C for 24 hours) or heat treatments above 60°C for 1 minute or equivalent remain the most effective processes guaranteeing the inactivation of parasitic larvae. Other physical and chemical treatments and their effect on parasites viability are also reviewed in this report. Traditional processing methods such as salting, curing, marinating, pickling, smoking, and addition of food additives may be effective for the control of certain other food-borne pathogens, but they are generally not sufficient for the control of food-borne parasites and to kill *A. simplex* larvae. Salting has demonstrated to be a valid treatment for parasites inactivation as long as the concentration of salt and the time of the treatment are applied according to the recommendations. However, there is an infection risk for humans if they eat raw, uncooked, lightly salted, cold smoked or pickled fish, which has not been frozen before processing.

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TABLE OF CONTENTS

INTRODUCTION

All wild caught seawater and fresh water fish must be considered at risk of containing any viable parasites of human health concern if these fishes are to be eaten raw or almost raw, as in fact, no sea fishing grounds can be considered free of *A. simplex* larvae (EFSA, 2010). Several types of parasites have been found in white fish species like cod (*Gadus morhua*), hake (*Merluccius* spp.), saithe (*Pollachius virens*), ling (*Molva molva*), tusk (*Brosme brosme*) or haddock (*Melanogrammus aeglefinus*). The presence of *A. simplex* has been reported in all these species, while *Pseudoterranova decipiens* and another anisakid nematode, *Hysterothylacium aduncum*, have been detected in some of them.

Although it is out of the scope of this document, it is interesting to point out that the situation is different in general for farmed fish, as they do not present a health hazard if they have been fed exclusively on a diet that cannot contain viable parasites. Specifically, for farmed Atlantic salmon reared in floating cages or onshore tanks and fed on compound feedstuffs. These animal feed are unlikely to contain live parasites, so the risk of infection with larval anisakids is negligible unless changes in farming practices occur. There is no sufficient monitoring data are not available for other farmed fish, it is not possible to identify if such fish species do not present a health hazard with respect to the presence of parasites.

Human fishery product-borne parasitic diseases primarily include those caused by cestodes, trematodes and nematodes. These diseases are either caused by an infection following ingestion of viable parasites, or as an allergic (hypersensitivity) reaction against parasite antigens. In Europe, the most important fish parasites causing illness in humans are nematodes from the Anisakidae family, which comprises 24 genera. However, the most common species associated with human infection is *Anisakis simplex*, followed by *Pseudoterranova decipiens* (FAO/WHO, 2014) causing symptoms as abdominal pain, nausea, vomiting, diarrhoea and can simulate gastric ulcer or even abdominal tumours. The only parasite in fishery products that is implicated in allergic reaction is *A. simplex*, but although the different forms of allergy to this species are relatively common in some regions in Spain, they are rarely reported in other parts of Europe (EFSA, 2010).

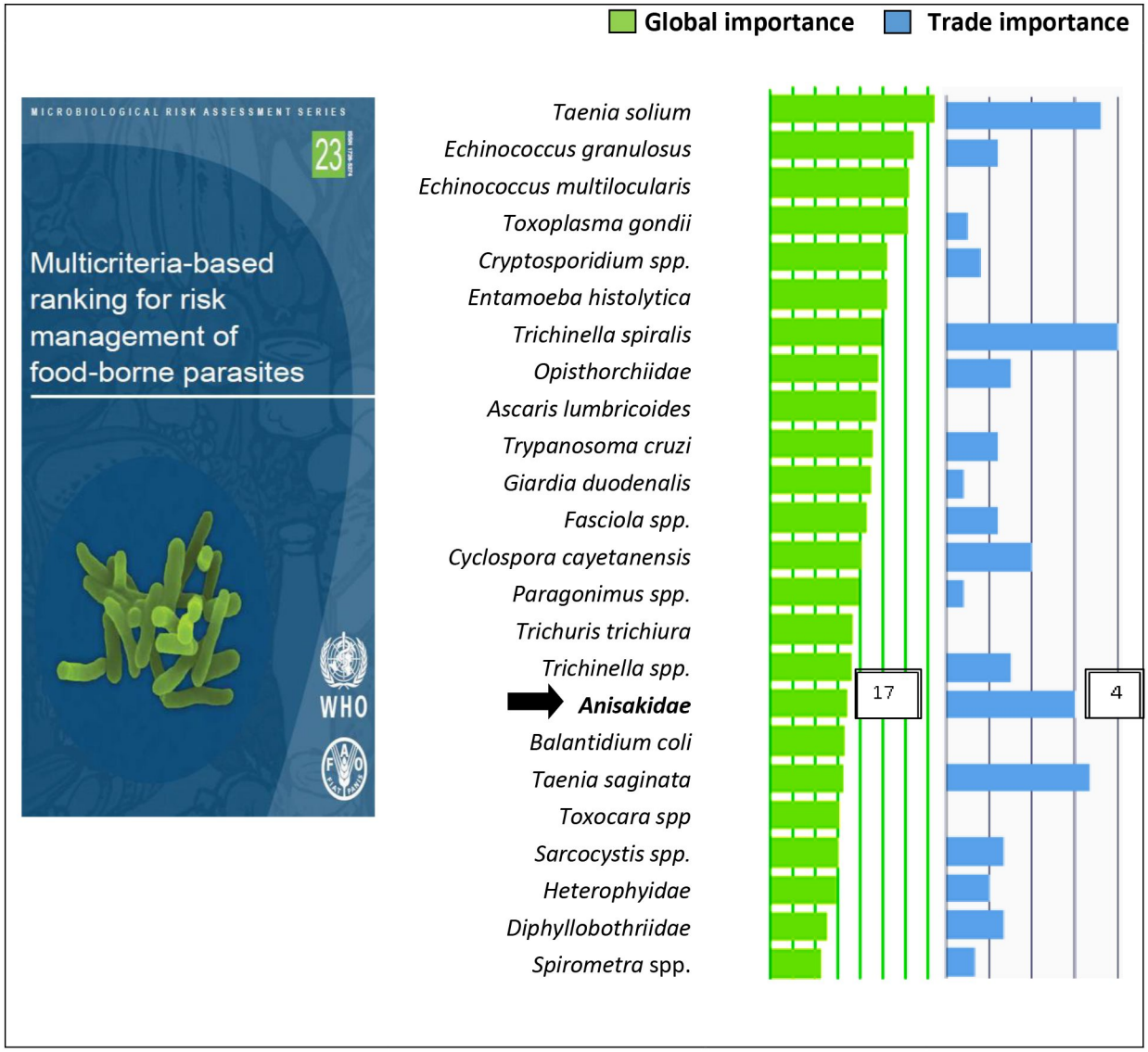

Figure 1. The most common animal parasites, ranked by global importance and trade importance (FAO/WHO, 2014).

According to the report "Multicriteria-based ranking for risk management of food-borne parasites", based on a Joint FAO/WHO Expert Meeting held in Rome in September 2012 (FAO/WHO, 2014), Anisakidae ranked 17th in the global ranking of food-borne parasites using a multicriteria ranking tool for scoring parasites, according to the inputs provided by expert meeting participants. But when only the average trade criteria scores for each parasite were considered, the Anisakidae were mentioned in the 4th place in the relative ranking of international trade importance of parasites in primary food vehicles (marine fish, crustaceans and cephalopods in the case of anisakids). This

shows the relative low importance of anisakids regarding public health concerns among all food-borne parasites, but the higher importance anisakids have for several countries that trade or consume fish extensively (Figure 1).

Human anisakiasis may be gastric or pharyngeal which are most often associated with *A. simplex* or *Pseudoterranova decipiens* respectively (EFSA, 2010). Morphologically speaking, there are two categorized types of Anisakis larvae in their third-stage form (L3): Anisakis Type I and Anisakis Type II larvae. The former has a longer ventriculous and a mucron at the posterior tip and the latter has a shorter ventriculous and a non-existent mucron (Quiazon *et al.,* 2009). Table 1 shows the different species of every type of larvae.

TYPE OF LARVAE (L3)	SPECIES
Type I	• *Anisakis simplex* s.s. • *Anisakis simplex* C • *Anisakis pegreffii* • *Anisakis typical* • *Anisakis ziphidarum* • *Anisakis nascettii* • *Anisakis berlandi*
Type II	• *Anisakis physeteri* • *Anisakis brevispiculata* • *Anisakis paggiae*

Table 1. Types of larvae species

Different publications (Murata *et al.*, 2011, Mattiuci *et al.*, 2008) show that *Anisakis* larvae type II may be divided into 3 more categories by genetic and morphological identification. The ventriculous of this species is short, but the tails are morphologically different. The *Anisakis* Type II would be *A. physeteris,* the *Anisakis* Type III would be *A. brevispiculata* and *Anisakis* Type IV would be *A. paggiae*, so these 3 species could also been identified by morphological differentiation and not only by genetic identification.

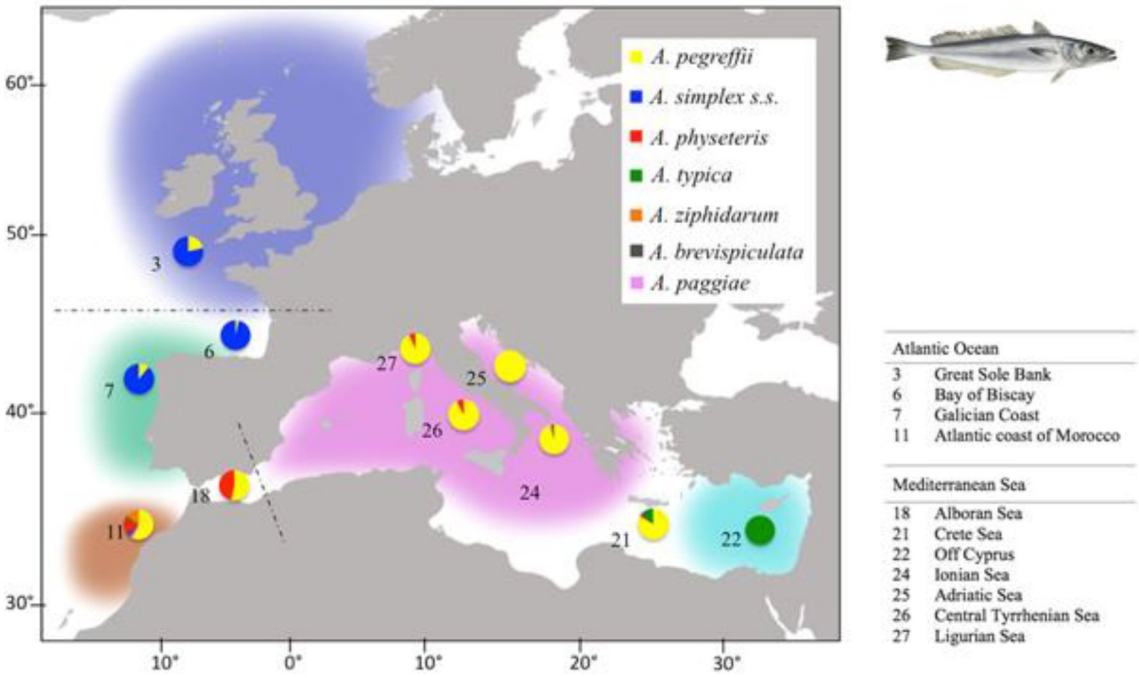

Figure 2. Distribution of different *Anisakis* species in Europe

Anisakis species are widely distributed all over the oceans. Figure 2 shows the distribution of different species in Europe, identified in hake (Mattiucci *et al.,* 2015). Main species identified are *A. simplex* in the north and central European waters, and *A. pegreffii* in the south, mainly in the Mediterranean Sea. The other species where identified in small amounts and at different Mediterranean regions.

1. ANISAKIS TYPE AND OTHER PARASITE OF PUBLIC HEALTH IMPORTANCE IN COD AND OTHER WHITE FISH SPECIES. HAZARD IDENTIFICATION AND CHARACTERISATION

A parasite is an organism that survives at the expense of another living organism, usually more complex, feeding from its nutrients and offering no benefit in return. This organism acts as a host while the parasite is the infectious agent. When both come into contact, the host defends itself against the parasite and three situations can occur: destroy it and eliminate it; living in balance, becoming an asymptomatic carrier of the pathology; or be negatively altered by the appearance of clinical symptoms.

Thus, parasitism is understood as the interrelation between two organisms, parasite and host, relationship in which man can interfere by becoming an accidental guest.

European Commission Guidance on viable parasites in fishery products indicates that parasites may represent a risk to the health of the consumer (EC, 2011), and establishes the larval stages of such parasites representing a health hazard to the consumer: (1) nematodes, mainly larvae of *Anisakis* species and *Pseudoterranova decipiens*, (2) larvae (plerocercoids) of *Diphyllobothrium* cestodes and (3) larvae (metacercariae) of trematodes.

In relation to marine fish species, and specifically in white fish species, the most important fish parasite causing illness in humans is *Anisakis simplex*, followed by *Pseudoterranova decipiens*, both from the Anisakidae family. Among trematodes, only Echinostomatidae have been found in pouting (*Trisopterus luscus*), but they are of less clinical importance than diseases caused by other fish-borne zoonotic trematodes. Finally, some myxosporean fish parasites of the genus *Kudoa* may occur in white fish species like cod, hake and blue whiting. *Kudoa* infections from consumption of unfrozen raw fish have been reported only recently in Japan (FAO/WHO, 2014).

About 650 species of nematodes are parasites of fish in their adult phase and many other species use these hosts as intermediaries, in which the development of larval phases takes place.

In fish, these parasites are almost always located in the digestive tract and only some are found in the peritoneal cavity, the gonads or the swim bladder of the host fish. Larvae can be present in any organ, although they are more frequently seen in the viscera, muscles and peritoneal cavity. As it was said before, in marine fish, infections by nematode larvae are mainly produced by species belonging to the family Anisakidae (Ascaridida), commonly called anisakids, whose definitive hosts are vertebrates.

Anisakiasis refers to infection of people with larval stages of these nematodes belonging to this family. Although mammalian hosts (including human) have been experimentally infected with worms from a number of species within the family Anisakidae, human infections almost always involve *A. simplex* and *P. decipiens.* Therefore these two are the parasites most often associated with anisakiasis, and both species are considered the most zoonotic for human within this group.

This is currently a serious zoonotic disease, as there has been a dramatic increase in its reported prevalence throughout the world in the last decades (Lymbery *et al.,* 2007). Anisakiasis occurs throughout the world, but is reported most frequently from Asia (especially Japan) and Western Europe, and also along the Pacific coast of South America, where risky food behaviour customs (i.e., eating raw, lightly cooked, or marinated fish in dishes such as sushi, salted or smoked herring, gravlax, and ceviche) are common (Lymbery *et al.,* 2007; FAO/WHO, 2014; Ryder *et al.,* 2014).

The genus *Anisakis*

The genus *Anisakis* belongs to the phylum Nematoda, class Secernentea, order Ascaridida and family Anisakidae. These are aquatic parasites whose life cycle develops in crustaceans, cephalopods, fish and mammals, being human and seabirds regularly accidental hosts (Shokoofeh *et al.*, 2017). The geographical distribution of *Anisakis* is practically universal due to the vector that transmits it to man, it is found in a greater percentage in the digestive tract of fish such as cod, sardines, anchovy, herring, salmon, haddock, hake, whiting, etc. There are at least ten *Anisakis* species described although the main cause of anisakiasis is considered the *A. simplex.*

Adult stages of *Anisakis simplex* reside in the stomach of marine mammals, where they are embedded in the mucosa, in clusters. Unembryonated eggs produced by adult females are passed in the feces of marine mammals. The eggs become embryonated in water, and first-stage larvae are formed in the eggs. The larvae molt, becoming second-stage larvae, and after the larvae hatch from the eggs, they become free-swimming. Larvae released from the eggs are ingested by crustaceans. The ingested larvae develop into third-stage larvae that are infective to fish and squid. The larvae migrate from the intestine to the tissues in the peritoneal cavity and grow up to 3 cm in length. Upon the host's death, larvae migrate to the muscle tissues, and through predation, the larvae are transferred from fish to fish. Fish and squid maintain third-stage larvae that are infective to humans and marine mammals. When fish or squid containing third-stage larvae are ingested by marine mammals, the larvae molt twice and develop into adult worms. The adult females produce eggs that are shed by marine mammals. Humans and seabirds become infected by eating raw or undercooked infected marine fish. After ingestion, the anisakid larvae penetrate the gastric and intestinal mucosa, causing the symptoms of anisakiasis (CDC, Centers for Disease Control and Prevention).

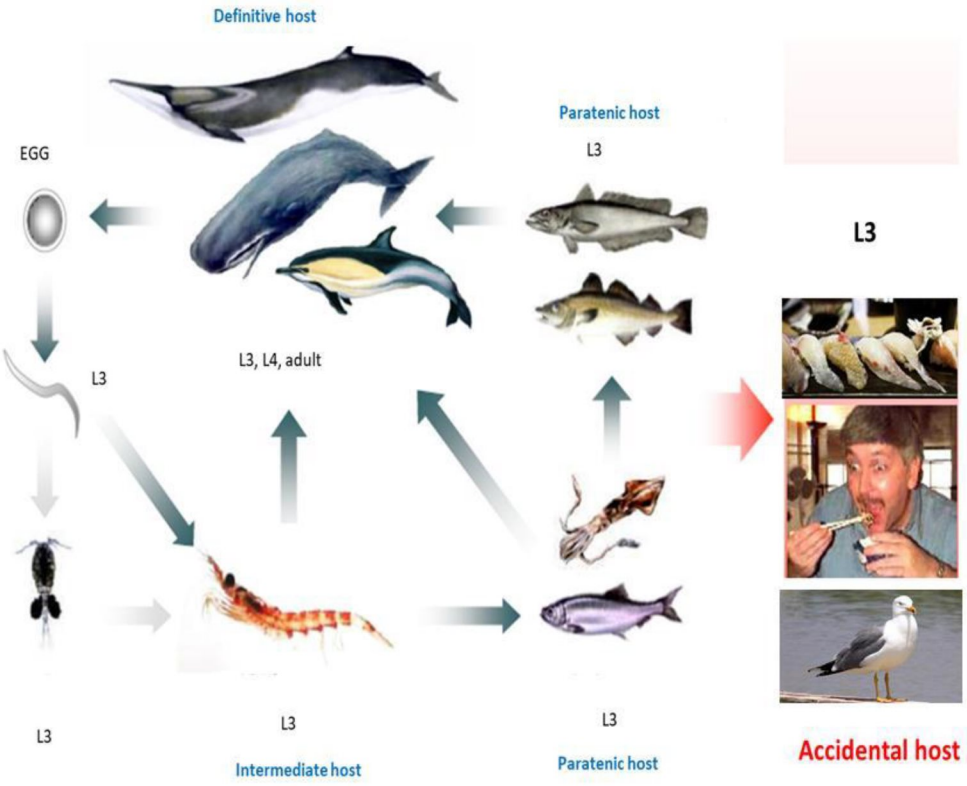

Figure 3. Live cycle of genus *Anisakis*

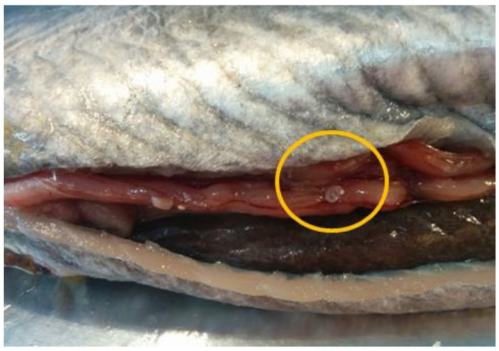

Figure 4. *Anisakis* in hake

The genus *Pseudoterranova*

The genus *Pseudoterranova* also belonging to the family Anisakidae and is considered the second most important fish parasite causing human illness after *Anisakis* genus (FAO/WHO, 2014). When the L3 larvae are consumed, they can penetrate the walls of the stomach and intestine, causing stomach pain, fever, diarrhoea, vomiting between other symptoms.

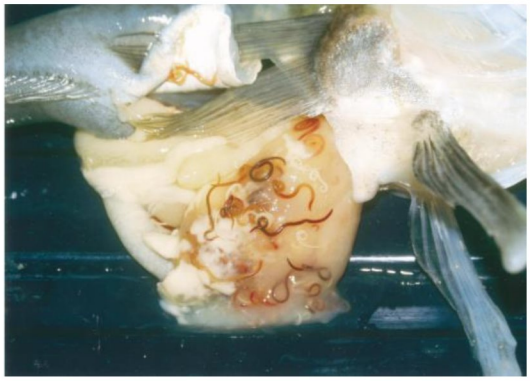

Figure 5. Liver of a *Chaenocephalus aceratus infested with Pseudoterranova decipiens* (large, red-colored) and *Contracaecum* spp. (small, white specimens). (Palm, 1999)

Also, the life cycle of this species is almost the same as that of *A. simplex*, both having always a marine mammal as final host; the only difference is that for *A. simplex* it is a cetacean (whales), whereas *P. decipiens* have their adult stage in pinnipeds (seals or sea lions).

Adults live in the stomach of seals and eat food in the stomach. Adults are often found in groups with their forward edge deep in the depressions in the wall of the stomach. The eggs pass in the faeces and develop in the water. L2 is the infective larva, as happens with other ascarids. When eaten by a fish, the L2 migrates to the fish's musculature and changes to become an L3. The big L3 rests rolled in the flesh. L3 can infect seals or humans that ingest infected fish, although in humans the infection is usually transitory. Molting to L4 and then to adults occur in the stomach of the seal.

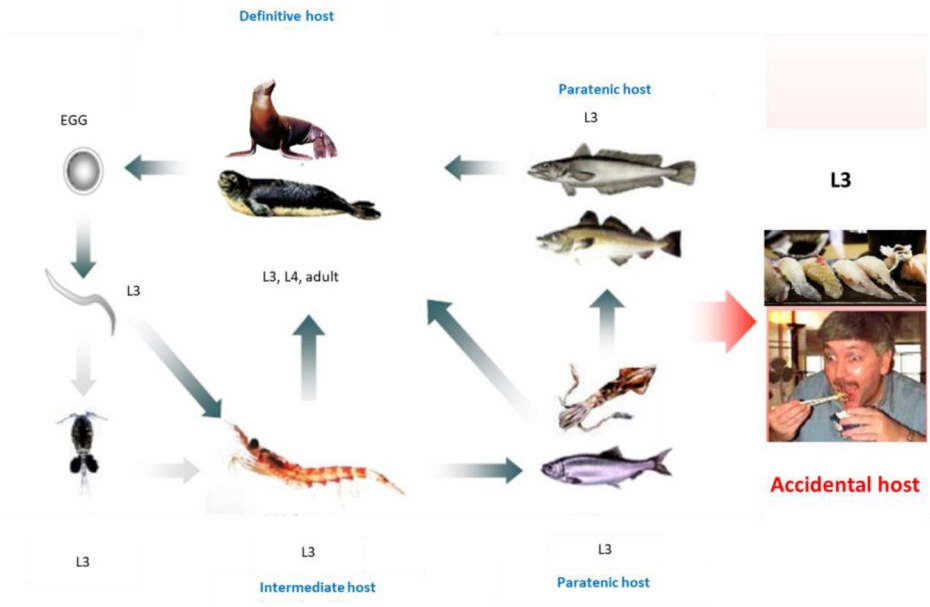

Figure 6. Live cycle of *Pseudoterranova*

Other species have been identified and included in the *Pseudoterranova* genus: *P. decipiens* (Krabbe, 1878); *P. krabbei* (Paggi *et al.,* 2000); *P. cattani* (George-Nascimento & Urrutia, 2000); *P. kogiae* (Johnston & Mawson, 1939); and *P. ceticola* (Deardorff & Overstreet, 1981). These larvae are difficult to differentiate and identify to the level of species based solely on morphology because there are few or no diagnostic characters at this level; hence, the need for species assignation based on genetic analyses (Mafra *et al.,* 2015).

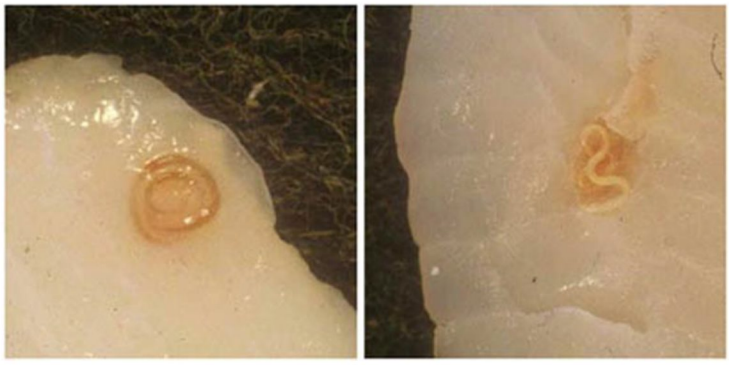

Figure 7. *Anisakis simplex* (left) and *Pseudoterranova decipiens* (right) – both in cod. Photos courtesy of S. Mellergaard (Huss *et al.*, 2004).

The main differences with *Anisakis*, at first sight and without using the microscope, are size and colour (Figure 7). Differences between both species are described in the table below (Huss *et al.*, 2003; EFSA, 2010)

Species	Size	Colour	Common		
			name	feature	Definitive host
Anisakis simplex	18-36 mm long 0.3-0.7 mm wide	White	Herring worm / Whale worm	Curled up in a spiral	Mostly cetaceans
Pseudoterranova decipiens	25-60 mm long 0.3-1.2 mm wide	Yellowish, brownish or reddish	Cod worm / Seal worm	Straight or in S-shape	Mostly pinnipeds

Table 3. Differences between *Anisakis simplex* and *Pseudoterranova decipiens.*

Anisakiasis

The anisakiasis is the parasitization of the man by the live larva in development stage (L3) of his biological cycle (Figure 3), acquired by the ingestion of raw or semi-crude fish. The larva is normally found in the viscera of the fish but some studies have shown that it can migrate to the fish muscle after the death of the fish.

Clinically, it is manifested by episodes of abdominal pain, nausea, vomiting, diarrhoea and can simulate gastric ulcer or even abdominal tumours. Due to this relationship with problems for public health, nematodes themselves, and especially anisakids, have been the subjects of numerous studies during the last years.

The main specie of this genus is *P. decipiens* that cause the same symptoms as *A. simplex.*

Although the first case of anisakids infection was described by Leuckart in Groenland in 1876, the disease was more widely described in the 1950s and 1960s when epidemics of anisakidae occurred in the Netherlands following ingestion of "green" (i.e. lightly salted) herring (154 proven cases between 1955 and 1968). Of the approximately 20,000 cases of anisakiasis reported until 2010 worldwide, over 90% were from Japan (where approximately 2,000 cases are diagnosed annually), with most of the rest from Spain, the Netherlands and Germany (EFSA, 2010).

Over the last 30 years, there has been a marked increase in the reported prevalence of anisakiasis throughout the world. This increase is probably due to: more widespread application of diagnostic techniques, particularly endoscopy (previously many cases of gastric anisakiasis were probably misdiagnosed increasing global demand for seafood; a growing preference for raw or lightly cooked food, especially in many Western countries, with increased risk of parasite exposure.

Human anisakiasis may be gastric or pharyngeal which are most often associated with *A. simplex* or *P. decipiens* respectively. Invasion by the parasite into the gastrointestinal tract may lead to

eosinophilic granuloma formation in the mucosa with severe symptoms of disease. Because the symptoms of anisakiasis are non-specific, the disease is often misdiagnosed.

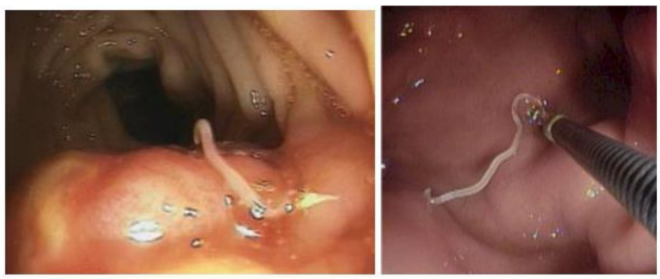

Figure 8. Right, example of colonic anisakiasis, a live worm (*Anisakis simplex*) found on biopsy of a large submucosal poly [in the descending colon of patient with abdominal pain and nausea; Left, *Anisakis* sp. being removed from the digestive tract. Medical corporation MCC (http://www.med-chem.com/para-site.php?url=org/anisakis)

A presumptive diagnosis can be made on the basis of the patient's food history. Definitive identification is based on larval recovery or histologic examination of infected tissue. Although serologic reagents have been developed, they are not commercially available. Molecular biology-based methods may also provide some additional diagnostic tools. A rise in the levels of total and specific IgE in the first month after an allergic reaction, consistent with the patient's history of gastroallergic anisakiasis, can provide valuable information, particularly if the parasite cannot be seen by fibre-optic gastroscopy.

There is no recommended therapy other than removal of the larvae, often through surgery. Gastric endoscopy is usually effective in larval location and removal. If the diagnosis is confirmed and there is no ileus, then surgical intervention can be avoided; the larvae will die and become absorbed within several weeks. (Medical corporation MCC http://www.med-chem.com/para-site.php?url=org/anisakis).

Observations suggest that parasites and the human immune system (as well as other hosts immune systems), have co-evolved over millennia in order to minimize collateral damage of the host tissue, and to remain alive to ensure the reproduction of parasite. Thus, some common helminthic parasites of man such as *Schistosoma* and *Ascaris* show evidence of adaption to the human immune system despite their inherent antigenicity.

Allergic reactions caused by fish-borne parasites

The only parasite in fishery products that is implicated in allergic reaction is *A. simplex*, and the primary initiator of the different forms of allergy is via infection by live larvae. Once sensitisation has occurred, response to nematode allergens can be highly aggressive and generate severe allergic

disease, with clinical symptoms including anaphylactic shock. Some authors have shown that an infection can provoke a concurrent allergic episode in a sensitised individual, and claim this is the principal mechanism for disease. However, others consider that allergic episodes can not only be elicited by infection, but also by exposure to allergen remaining in food with no viable larvae. The relative epidemiological impact for each route of provoking an allergic episode is unknown. However there is general agreement that consumption of fishery products containing viable *A. simplex* larvae presents a greater risk for allergy than consumption of fishery products containing non-viable parasites (EFSA, 2010).

But it is important to point out that currently the different forms of allergy to *A. simplex* are only relatively common in some regions in Spain, while they are rarely reported in other parts of Europe (EFSA, 2010). Thus, allergy to *A. simplex* cannot be considered an important food safety and public health problem in other countries nowadays, apart from Spain (Lin, 2015).

Allergic reactions to *A. simplex* antigens are associated with the production of specific IgE, although specific IgE is detected in all patients following anisakiasis including patients without allergic symptoms.

Although freezing and other physical and chemical treatments can be appropriate for killing viable parasites in fishery products, *A. simplex* allergens are highly resistant to heat and freezing, therefore treatments which kill Anisakidae in fishery products may not protect the consumers against allergic hazards due to the ingestion (EFSA, 2010). But according to the **Report from the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) on allergy to *Anisakis*** (September 2016), the scientific bibliography published and consulted up to that date does not show current clinical evidence to confirm that the dead parasite is a health concern for consumers who are allergic to *Anisakis.* Nevertheless, the immunological recognition of different thermostable antigens, demonstrated even after applying the recommended methods of treatment, does not permit the total rejection of the possibility of risk of allergic reaction in those individuals who are sensitive to them.

Distribution of anisakids parasites in fish body, pre and post mortem

There have been conflicting reports on whether anisakid nematodes migrate from the viscera into the muscle of fish after death of the host. Van Thiel (Van Thiel *et al.,* 1967) suggested that this occurred in herring and subsequently other authors reported a significant increase in the proportion of *A. simplex* larvae in the muscle of herring after fish were kept in ice for up to 48 hours after capture. These observations suggest that encapsulation (formation of a cyst) of larvae from the viscera is followed by migration into the muscle. Other authors also found a significant increase in numbers of *A. simplex* larvae in the muscle of cold smoked Pacific herring with time after capture. It was hypothesized that this apparent migration was due to post-mortem changes in the

decomposing viscera and/or the exposure of larvae to the cold smoking temperatures and brining salinities.

However, Roepstorff (Roepstorff *et al.,* 1993) found no evidence of migration of *A. simplex* larvae post-mortem in herring when fish were maintained on ice, in chilled sea water, or in 10°C sea water. The reasons for the discrepancy between different studies are unknown. Karl (Karl *et al.,* 2002) found no evidence of post-mortem migration of *A. simplex* larvae into the muscle of haddock, saithe and ocean perch after capture.

In pelagic fish, for example North Sea and NSS herring, the largest proportion of flesh residing *A. simplex* larvae were found in the belly flaps and no significant difference between the left and right flesh side was found. However, Cipriani et al., 2016 suggest that temperature plays an important role in the *post-mortem* motility of *A. pegreffii* larvae in anchovies.

It is therefore not clear when, under what conditions and in which fish species post-mortem migration of *A. simplex* larvae occurs. Therefore the effects of processing practices on the exposure to consumers cannot be predicted (EFSA, 2010).

Presence of parasites of public health concern in the main edible white fish species

After a scientific literature review, Table 4 shows a detailed list of parasite species of the highest public health concern found in commercially important specific marine white fish species, all of them belonging to Order Gadiformes.

FAMILY	NAME / SPECIES	PARASITES
Merluciidae	Merluccid hakes / *Merluccius* spp.	• *Anisakis simplex* • *Kudoa rosenbuschi* • *Kudoa thyrsites* • *Pseudoterranova decipiens*
Gadidae	Atlantic cod / *Gadus morhua*	• *Anisakis simplex* • *Kudoa thyrsites* • *Pseudoterranova decipiens*
	Whiting / *Merlangus merlangius*	• *Anisakis simplex* • *Kudoa spp.*
	Haddock / *Melanogrammus aeglefinus*	• *Anisakis simplex* • *Pseudoterranova decipiens*
	Blue whiting / *Micromesistius potassou*	• *Anisakis simplex* • *Kudoa spp.*
	Pouting / *Trisopterus luscus*	• *Anisakis simplex* • Echinostomatidae
	Poor cod / *Trisopterus minutus*	• *Anisakis simplex*
	Pollack / *Pollachius pollachius*	• *Anisakis simplex*
	Saithe / *Pollachius virens*	• *Anisakis simplex*

Phycidae	Phycid hakes / *Phycis spp.*	• *Anisakis simplex*
Lotidae	Tusk/Cusk / *Brosme brosme*	• *Anisakis simplex* • *Pseudoterranova decipiens*
	Common ling / *Molva molva*	• *Anisakis simplex*
	Molva blennoides	• *Anisakis simplex*
	Blue ling / *Molva dipterygia*	• *Anisakis simplex*

Table 4. Species of parasites mainly found in white fish

2. QUALITY ISSUES IN RELATION TO THE PRESENCE OF PARASITES IN MARINE WHITE FISH SPECIES

As it has been stated before, the zoonotic parasites of fish represent only a minority of the many parasite species that infect fish, even though the former are a widespread and diverse group (Ryder *et al.,* 2014). Regardless of the parasite species present in fish, Chapter V of Section VIII of Annex III to Regulation (EC) No 853/2004, regarding "Health standards for fishery products", states in Part D that "Food business operators must ensure that fishery products have been subjected to a visual examination for the purpose of detecting visible parasites before being placed on the market. They must not place fishery products that are obviously contaminated with parasites on the market for human consumption".

So, although the presence of parasites different from those ones which may cause fish-borne human disease (the ones detailed in the previous chapter) is not a question of public health importance, it must be considered a quality concern that has to be taken into account. In fact, it should be pointed out that the presence of readily visible parasites in the edible portion of fish products, detected by normal visual inspection of the fish flesh, even if the public health risk is low. According to Regulation (CE) 853/2004 Food business operators must ensure that fishery products have been subjected to a visual examination for the purpose of detecting visible parasites before being placed on the market. They must not place fishery products that are obviously contaminated with parasites on the market for human consumption.

The previous Table 4 shows a list of parasite species of the highest public health concern found in commercially important specific marine white fish. Below there is a list of different species that have been identified in two important fish species: Atlantic cod (*Gadus morhua*) and Tusk/Cusk (*Brosme brosme*).

The following species of parasites can be found in Atlantic cod: *Abothrium spp., Aega psora, Ascarophis filiformis, Ascarophis morhuae, Caligus curtus, Clavella adunca, Clavella brevicollis, Contracaecum spp., Cryptocotyle lingua, Cucullanus cirratus, Derogenes varicus, Echinorhynchus gadi, Hemiurus levinseni, Hysterothylancium aduncum, Lepidapedon gadi, Lernaeocera branchiallis, Phoscascaris spp., Podocotyle atomon, Podocotyle reflexa, Pyramicocephalus phocarum, Scolex polymorphus* and *Udonella caligorum.*

In Tusk/Cusk, the following species of parasites can be found: *Ascaris sp., Ascarophis filiformis, Lernaeocera lusci, Prosorhynchus squamatus* and *Sphaeromyxa hellandi.*

Of all these species, the anisakidae nematodes of **genus Hysterothylacium** have special interest because of its increasing appearance in fish products. This genus comprises a type of nematode which is commonly found in the digestive tract of teleosts of the marine environment (Navone *et al.,* 1998). The main species of this genus is *H. aduncum*, and depending on the author, the taxonomy of the different species can be considered as unresolved (Køie, 1993). Most of authors just recognise *H. aduncum* as the single species parasiting marine teleosts, although other authors

(Hartwich, 1975) consider at least three species of *Hysterothylacium*: *H. aduncum* parasiting cupleid fishes, *H. gadi* identified from gadoid fishes and *H. auctum* from eelpouts and some flat fishes.

The life cycle of these nematodes is also partially unknown. The investigations developed by Køie (1993) provided a detailed account of the life cycle *H. aduncum*, showing that it matures in fishes, and the first intermediate hosts are crustaceans, such as copepods, amphipods, shrimps, and isopods. The eggs with developed larvae of this parasite use to be ingested by the crustaceans, in whose intestines this larvae finally develop. The secondary intermediates are larger invertebrates and crustaceans and some fishes are also thought to be final and/or paratenic hosts. The parasitization in fishes is done by larvae in L3 shape, and seems to depend on the size of the L3 larvae, occurring just when these are at least 2.5-3 mm long.

The implication of *Hysterothylacium* spp. as a potential causal agent of anisakiasis is still under discussion (Iglesias *et al.,* 2002). It seems that this type of parasites can cause a disease similar to anisakiasis in humans by eating raw fish, but so far it has only been described as the causal agent of at least one case of non-invasive anisakiasis (González-Amores *et al.,* 2015). Although it is still an unusual parasite, reports on the parasitization from *H. aduncum* are becoming more abundant (Klimpel *et al.,* 2005).

3. KILLING TREATMENTS AND PREVENTION METHODS FOR ANISAKIDS. REGULATIONS, GUIDELINES AND RECOMMENDATIONS DELIVERED BY COMPETENT AUTHORITIES AND SCIENTIFIC PUBLICATIONS

This chapter cites mandatory regulations as well as recommendations and guidelines issued by different national and international organizations. Among these last ones are:

- Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs. Chapter V.D: Food business operators must ensure that fishery products have been subjected to a visual examination for the purpose of detecting visible parasites before being placed on the market. They must not place fishery products that are obviously contaminated with parasites on the market for human consumption.
- The Codex Alimentarius: it is a collection of internationally recognized standards, codes of practice, guidelines, and other recommendations relating to foods, food production, and food safety. Its texts are developed and maintained by the Codex Alimentarius Commission (CAC), a body established by the FAO and WHO. Although they are voluntary reference standards and there is no obligation on countries to adopt them, an increasing number of countries are aligning their food standards with those of Codex.
- The European Food Safety Authority (EFSA) is an agency of the European Union (EU), which became operational in 2002. Its main objective is the responsibility of providing scientific methods to alert and detect all those problems that affect food safety; this authority assesses the risks that may affect the EU member states. As the risk assessor, EFSA produces scientific opinions and advice that form the basis for European policies and legislation in terms of Food and feed safety, nutrition, animal health and welfare, plant protection and plant health.
- The Spanish Agency for Consumption, Food Safety and Nutrition (AECOSAN) integrates and performs within the framework of the general administration of the state, functions related to the promotion of the rights of consumers and users in goods and services, as well as food security and healthy nutrition. It is an autonomous body, attached to the Spanish Ministry of Health. Between its main functions are promoting food safety, offering guarantees and objective information, as well planning, coordination and development of strategies and actions to provide information, education and health claims in the field of nutrition, and in particular, in the prevention of obesity.
- Codex Standard for Quick Frozen Fish Fillets (CODEX STAN 190 – 1995, last modified in 2017). The sampling of lots for examination of the product and the procedure for the detection of parasites in skinless fillets is described, as well as the lot acceptance. Among the properties that make a sample unit to be considered as defective are: the presence of two or more parasites per kg of the sample unit detected by the method described in the standard with a capsular diameter greater than 3 mm or a parasite not encapsulated and greater than 10 mm

in length; (this is the same that in Codex Standard for Smoked Fish, Smoke-flavoured Fish and Smoke-dried Fish, CODEX STAN 311-2013 last modified in 2016). A sample unit with pasty texture resulting from parasitic infestation affecting more than 5% of the sample unit by weight.

3.1. Killing treatments

In this chapter, different killing treatments for Anisakis are described. There is more information on the resistance to physical and chemical treatments by A. simplex than for other fishery parasites. The properties of A. simplex are likely to be similar to that of other multicellular parasites (although trematode metacercariae are considerably more heat resistant). A. simplex is sensitive to salt and high concentrations for a prolonged period could be necessary to inactivate the larvae.

Many traditional marinating and cold smoking methods are not sufficient to kill larvae of *A. simplex*, so there is a risk of infection for humans when raw, uncooked, lightly salted, cold smoked or pickled fish are consumed.

According to some studies there is also a hypersensitivity risk through consumption of cooked or frozen fish.

3.1.1. Freezing treatments with international regulations

One of the first countries in Europe that applied freezing as preventive treatment for anisakiasis was the Netherlands in 1968, with the so called "Green Herring Laws" which stated that fresh herring should be frozen in such a manner as to reach a temperature of at least -20°C within 12 hours and stored for a period of 24 hours prior to being released to the public. This resulted in a decrease of 40-50 human cases per year to less than 10 cases per year after the legislative action was implemented. In 1987 the EEC subsequently implemented legislation/recommendations for the similar freezing requirements (-20°C for 24h) as were implemented in the regulation of Netherlands.

<u>European Union regulations</u>

Annex III to Regulation (EC) No 853/2004 was amended in 2011 by **Commission Regulation (EU) No 1276/2011**, specifically as regards the treatment to kill viable parasites in fishery products for human consumption. Regulation No 853/2004 provided that food business operators must ensure that all fishery products to be consumed raw or almost raw, undergo a freezing treatment to kill viable parasites that may represent a risk to the health of the consumer. The competent authorities could only authorise an exemption from this required freezing treatment on fishery products derived from wild catches, when epidemiological data are available indicating that the fishing grounds of origin do not present a health hazard with regard to the presence of parasites. But in April 2010, the EFSA adopted a scientific opinion on risk assessment of parasites in fishery

products, including information regarding the cases where fishery products may present a health hazard with regard to the presence of viable parasites, and analysing the effects of various treatments for killing such parasites in fishery products. This brought a new exemption from the freezing treatment, now for fishery products from aquaculture (Atlantic salmon and any other farmed fish) that are fed compound feedstuffs which are unlikely to contain live parasites.

The new Part D of Chapter III of Section VIII of Annex III to Regulation (EC) No 853/2004, regarding "Requirements concerning parasites for establishments, including vessels, handling fishery products", includes the specific data of temperatures and times that must be applied to fishery products during the freezing treatment. Also alternative heating treatments to kill parasites are described:

"1. Food business operators placing on the market the following fishery products derived from finfish or cephalopod molluscs:

(a) fishery products intended to be consumed raw; or

(b) marinated, salted and any other treated fishery products, if the treatment is insufficient to kill the viable parasite;

must ensure that the raw material or finished product undergo a freezing treatment in order to kill viable parasites that may be a risk to the health of the consumer.

2. For parasites other than trematodes the freezing treatment must consist of lowering the temperature in all parts of the product to at least:

(a) – 20°C for not less than 24 hours; or

(b) – 35°C for not less than 15 hours.

3. Food business operators need not carry out the freezing treatment set out in point 1 for fishery products:

(b) that have been preserved as frozen fishery products for a sufficiently long period to kill the viable parasites;

4. (a) When placing on the market, except when supplied to the final consumer, fishery products referred to in point 1 must be accompanied by a document issued by the food business operator performing the freezing treatment, stating the type of freezing treatment that the products have undergone.

Brazilian regulations

Brazilian regulations also follow EU standards. Thus, article 216 of **Decreto 9.013, de 2017**, states that fishery and aquaculture products infected with endoparasites transmissible to humans may not

be intended for raw consumption unless they are subjected to freezing at a temperature of -20°C for 24 hours or at -35°C for 15 hours.

USA regulations

In the USA, the FDA (Food and Drug Administration) requires that all fish and shellfish intended for raw or semi-raw (e.g. marinated or partly cooked) consumption should be blast frozen to -35°C (-31°F) or below for 15 hours, or be completely frozen to -20°C (-4°F) or below for 7 days (FDA, 2001). The same freezing treatment is required in Canada. The temperature and time difference between the EU and US regulations reflects either the total storage time or the time the product core achieves the critical temperature. These preventive measures have been adopted by the fish industry in Europe and North America as part of their Hazard Analysis and Critical Control Points (HACCP) systems.

3.1.2. Heating treatments with international regulations

The new Part D of Chapter III of Section VIII of Annex III to Regulation (EC) No 853/2004, regarding "Requirements concerning parasites for establishments, including vessels, handling fishery products", includes the specific data of temperatures and times that must be applied to fishery products during the freezing treatment. Also alternative heating treatments to kill parasites are described:

3. Food business operators need not carry out the freezing treatment set out in point 1 for fishery products:

   (a) that have undergone, or are intended to undergo before consumption a heat treatment that kills the viable parasite. In the case of parasites other than trematodes the product is heated to a core temperature of 60 °C or more for at least one minute;

So instead of the freezing treatment, EU regulations also state alternative heating treatments to inactivate parasites other than trematodes. Although temperatures of -20°C or lower are not usually achieved in household freezers, consumers can avoid this hazard buying frozen instead of fresh fish products from retailers. Additionally, normal household cooking assures that fish products are heated to a core temperature of 60°C or more for at least one minute, so this process will also kill the parasites. Therefore, their possible presence is not a significant hazard if any of these two options is fulfilled.

3.2. Prevention methods to reduce and control

Among the prevention methods to avoid hazards we can identify different good handling practices, on board and/or in land, and other control measures:

- **Selective fishing:** avoid fishing in areas where it is known that there is a massive presence of this parasite or marine mammals. According to Commission Regulation (EU) No 1276/2011, Food business operators need not carry out the freezing treatment in order to kill viable parasites that may be a risk to the health of the consumer, for fishery products from wild catches provided that i) there are epidemiological data available indicating that the fishing grounds of origin do not present a health hazard with regard to the presence of parasites; ii) and the competent authority so authorises.
  But this is the least effective measure since the parasite is widely distributed. In fact, EFSA has been requested to specify criteria for determining when epidemiological data indicate that a fishing ground does not present a health hazard with regard to the presence of viable parasites, if the fishery products are to be eaten raw or almost raw (EFSA, 2010). After evaluating these criteria for wild catch fishery products, they concluded that, because of the complexity and variation in distribution of parasites, all wild caught seawater fishery products must be considered at risk of containing parasites and should not be eaten raw or almost raw without further treatment.

- **Evisceration:** Evisceration is a good measure of prevention due parasites are mainly found in stomach and intestine of commercial species. Keep in mind that it is NOT a technique that reduces to 100% the presence of parasites since those in the musculature are not, although it is true that the parasite in the musculature only occurs if there is a migration, therefore its presence in these areas is less. Ideally, the evisceration should be at the time of capture, on board, so that the parasite does not migrate to the musculature. The evisceration must be done correctly and the viscera must be totally removed.

- **Visual inspection:** on board or in land, the operators can make a visual inspection of fish products since the parasites are generally visible to the naked eye, and therefore the operators can remove them. Again, this is not a 100% effective measure since some parasites may not be visualized, or maybe they are in the musculature. Candling, trimming belly flaps and physically removing the parasite cysts will reduce the parasite hazards but may not eliminate them (*Codex alimentarius*, CAC/RCP 52-2003).

  This measure is regulated according to **Regulation (EC) No 2074/2005:**

  "Regulations (EC) No 853/2004 and 854/2004 set out the requirements governing parasite checks during handling of fishery products on shore and on board vessels. It is up to food

business operators to carry out their own checks at all stages in the production of fishery products in accordance with the rules in Chapter V(D) of Section VIII of Annex III to Regulation (EC) No 853/ 2004 so that fish which are obviously infested with parasites are not released for human consumption[1]. The adoption of detailed rules relating to visual inspections calls for the concepts of visible parasites and visual inspection to be defined and the type and frequency of the observations to be determined."

Section 1 of Annex II describes the obligations of food business operators, laying down detailed rules relating to visual inspections to detect parasites in fishery products:

*"**VISUAL INSPECTION***

1. Visual inspection shall be performed on a representative number of samples. The persons in charge of establishments on land and qualified persons on board factory vessels shall determine the scale and frequency of the inspections by reference to the type of fishery products, their geographical origin and their use. During production, visual inspection of eviscerated fish must be carried out by qualified persons on the abdominal cavity and livers and roes intended for human consumption. Depending on the system of gutting used, the visual inspection must be carried out:

(a) in the case of manual evisceration, in a continuous manner by the handler at the time of evisceration and washing;

(b) in the case of mechanical evisceration, by sampling carried out on a representative number of samples being not less than 10 fish per batch.

2. The visual inspection of fish fillets or fish slices must be carried out by qualified persons during trimming and after filleting or slicing. Where an individual examination is not possible because of the size of the fillets or the filleting operations, a sampling plan must be drawn up and kept available for the competent authority in accordance with Chapter II(4) of Section VIII of Annex III to Regulation (EC) No 853/2004. Where candling of fillets is necessary from a technical viewpoint, it must be included in the sampling plan[2]."

As it was described, before, the **CODEX standard for salted Atlantic herring and salted sprat** (CODEX STAN 244-2004) also states that a sample unit shall be considered as defective when it

[1] Chapter V(D) of Section VIII of Annex III to Regulation (EC) No 853/2004 states that "Food business operators must ensure that fishery products have been subjected to a visual examination for the purpose of detecting visible parasites before being placed on the market".

[2] 'Candling' means, in respect of flat fish or fish fillets, holding up fish to a light in a darkened room to detect parasites.

exhibits the presence of readily visible parasites in a sample of the edible portion of the sample unit detected by normal visual inspection of the fish flesh. For the determination of the presence of visible parasites:

- The presence of readily visible parasites in a sample unit that is broken into normal bite-size pieces 20-30 mm of flesh by the thickness of the fillet. Only the normal edible portion is considered even if other material is included with the fillet. Examination should be done in an adequately lighted room (where a newspaper may be read easily), without magnification, for evidence of parasites.
- Notwithstanding paragraph 1, the verification of the presence of parasites in intermediate entire fishery products in bulk intended for further processing could be carried out at a later stage.

Due to its importance in the international trade of white fish, references to the regulations of Brazil regarding the presence of parasites in fish are included in Annex I of this document.

Following a request from the European Commission, the Panel on Biological Hazards of the EFSA was asked to deliver a **scientific opinion on food safety related to parasites in fishery products** (EFSA, 2010). EFSA concluded that the critical control points for prevention of consumers' exposure to fishery-parasites are:

- the quality of the raw material, i.e. the catching or rearing of parasites-free stocks;
- the application of physicochemical treatments to fishery products to ensure killing of any parasites which may be present;
- or the physical separation of parasite contaminated fishery products during processing.

According to EFSA, all these three options are potential control measures for control of allergic diseases, and the second option (physical-chemical treatments to kill parasites) will also be effective at preventing infections.

3.3. International guidelines and recommendations

Recommendations of different international entities on treatments to inactivate parasites are in line with the regulations previously described. In general, it is considered that an appropriate freezing treatment is a totally effective measure for the inactivation of the larval stage of the nematode parasites. Heat treatment is also an alternative measure: the fish undergoes a thermal treatment in which the larvae in L3 state would be inactivated, becoming harmless to the human being.

In **FAO Fisheries Technical Paper 444** (Huss *et al.*, 2003), freezing at -20°C at the thermal centre of the product for 24 hours is considered a process that may be sufficient to kill parasites of the species *Anisakis* spp. and *Pseudoterranova decipiens*.

The **CODEX standard for salted Atlantic herring and salted sprat** (CODEX STAN 244-2004) states that fish flesh shall not contain living larvae of nematodes. Viability of nematodes shall be examined according to the test described in the document: Nematodes are isolated from fish fillets by digestion, transferred into 0.5% Pepsin digestion solution and inspected visually for viability. Digestion conditions correspond to conditions found in the digestive tracts of mammals and guarantee the survival of nematodes.

If living nematodes are confirmed, products must not be placed on the market for human consumption before they are treated in conformity with the methods laid down in Annex II, considered sufficient to kill living nematodes:

- e.g. freezing to -20°C for not less than 24 h in all parts of the product
- the adequate combination of salt content and storage time (To be elaborated)
- or by other processes with the equivalent effect (To be elaborated)

Sampling of lots for pathogenic microorganisms and parasites will be in accordance with the Principles and Guidelines for the Establishment and Application of Microbiological Criteria Related Foods (CAC/GL 21-1997).

Also the **Codex CAC/GL 88-2016** refers to Annex 1 of the Code of Practice for Fish and Fishery Products (CAC/RCP 52-2003) for control of parasites in fish and fishery products intended for raw consumption by freezing: conditions of –20°C or below for seven days or –35°C for about 20 hours will kill parasites.

In the Report of the Scientific Committee of the **Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN)** on measures to reduce the risk associated with the presence of *Anisakis*, reference is also made to the need for freezing and temperature and time data:

“European and national legislation establishes that fishery products that are to be consumed raw or practically raw should be frozen at a temperature equal to or lower than -20°C in the entire product, for a period of at least 24 hours, in the raw product or the finished product”.

Specifically for heat treatments, references to the data of the binomial temperature / time necessary to inactivate the larva can be found in **AECOSAN** and **EFSA** documents. In both cases, temperatures equal to or greater than 60°C (in the centre of the product) are considered, for 5 to 10 minutes in the first case, and one minute in the second one.

The **FDA** also considers that a treatment of 60°C for 1 minute is enough to kill the larvae.

After the review of different regulations and recommendations, we can summarize that treatments which provide an equivalent level of protection as freezing (-20°C for not less than 24 hours) for the killing of *A. simplex* larvae include freezing at -35°C for at least 15 hours or at -15°C for at least 96 hours at the core of the fishery products, and heat treatment at >60°C for at least 1 minute.

3.4. Effect of alternative treatments on survival of parasites

National and international organisms like Codex Alimentarius Commission, EFSA and AECOSAN have reviewed the scientific literature regarding other physical and chemical treatments, besides freezing and heating, and their effect on parasites viability. In general, although processing methods such as salting, curing, marinating, pickling, smoking, and addition of food additives may be effective for the control of certain other food-borne pathogens, they are generally not sufficient for the control of food-borne parasites. Combinations of several treatments (hurdle concept) can be effective to control parasites. When a combination of treatments is used, it should be subject to rigorous validation to ensure consumer protection (Codex alimentarius, CAC/GL 88-2016).

3.5. Chemical methods

Salting and marinating are the chemical treatments most commonly used to inactivate viable Anisakidae larvae. In any case, processes such as brining or pickling may reduce the parasite hazard if the products are kept in the brine for a sufficient time but may not eliminate it (*Codex alimentarius*, CAC/RCP 52-2003).

3.5.1. Salting

Regarding aspects of quality, some *Codex Alimentarius* standards include specific references to parasites among their definition of defectives. The only reference for salted fish is in the Codex Standard for salted Atlantic herring and salted sprat (CODEX STAN 244-2004, last modified in 2018), stating that one of the properties that makes a sample unit to be considered as defective is the presence of readily visible parasites in a sample of the edible portion of the sample unit detected by normal visual inspection of the fish flesh (Annex III describes how the determination of the presence of visible parasites must be developed). This Standard also references the Codex guidelines that must be followed for the sampling of lots for parasites, and its Annex I describes the method that must be developed for the determination of the viability of nematodes. Finally, lot acceptance is established in relation to the number of defectives and the acceptance number of the sampling plan.

Although *A. simplex* is sensitive to salt, high concentrations for a prolonged period are needed to inactivate the larvae. Some data on the effect of NaCl on the survival of nematodes in fish are listed below, according to Huss *et al.* (2003), AECOSAN (2005) and Smaldone *et al.* (2017).

NaCl, % WPS (1)	Time to inactivation	Reference
4-5	>17 weeks	Huss *et al.* (2003)
6-7	10-12 weeks	
8-9	5-6 weeks	
15	4 weeks	AECOSAN (2005)
20	3 weeks	
24.15	15 days	Smaldone *et al.* (2017)

(1) WPS: Water Phase Salt, in muscular tissue

Thus, the need for freezing will depend on the concentration of salt reached in the fish, and the time that salting is maintained. Freezing would be necessary in case that the concentration of NaCl in fish does not reach a level around 8-9% maintained for 6 weeks (AECOSAN, 2005). For this, special care should be taken in the case of the so-called "Very lightly salted fish" and "Lightly salted fish" whose NaCl levels in the fish muscle are below 10% in water phase (CODEX STAN 244-2004). The freezing would not be necessary when the salt concentration in the fish reach levels higher than 9% of NaCl and it is maintained for at least six weeks (AECOSAN, 2007).

Four to five weeks would be the maximum survival time when the concentration of salt in the fish reaches levels between 10% and 20% of NaCl. This is the case of the "Medium salted fish"(CODEX STAN 244-2004).

When the concentration of salt in the fish reaches levels of at least 20% of NaCl, the maximum survival time would be three weeks. This is the case of "Heavily salted fish" (CODEX STAN 244-2004).

The traditional semipreserved anchovies guarantee the inactivation of the larva, since the procedure is carried out by the preservation in salt during 5 to 12 months, reaching concentrations higher than 12% of salt; these time and concentration conditions are higher than those required to inactivate the larvae, i.e. 8-9% salt concentration, for five to six weeks (AECOSAN, 2007).

Dry salting does tend to kill those parasites residing on fish surfaces, but generally does not do so for those imbedded within the tissue (IFT/FDA, 2001).

A study was conducted by Bécel *et al.* (2005) to determine, under the real manufacturing conditions practiced in France for dry salted herring (between 120 and 160 grams of weight), the salting parameters (salt content and duration) to be taken into account to guarantee the destruction of the *Anisakis* larvae. Final concentrations of salt in fish are reached between 13% and 16%, and the results showed that all *A. simplex* larvae were killed after 16 days of conservation herring in salt. This meant that processes used by the salt smokers in France, with a minimum salting time of 21 days, make it possible to guarantee the destruction of *A. simplex* larvae in traditional herring fillets that have not undergone freezing.

Regarding the killing of parasites other than anisakids in fishery products, it was established that *Opisthorchis* metacercariae in fish are killed at 13.6% NaCl after 24 hours.

3.5.2. Marinating

The active ingredients usually utilized in marinating processes can include vinegar, lemon juice, wine, soy sauce, or brine, what normally brings acidic conditions. Experimental tests show that 35 days are needed to inactivate the larvae when the processing conditions are: 2.4% acetic acid (pH of the muscle water phase, 4.2) and 6% NaCl (AECOSAN, 2005). Also at least 13 days would be necessary if the conditions are: 6% acetic acid and 12% salt (AECOSAN, 2007). This shows that the traditional marinating method for anchovies in vinegar, based on their permanence in commercial vinegar, with an approximate content of 6% acetic acid, and salt for 4 to 24 hours, is insufficient for the inactivation of *Anisakis* larvae.

Other studies confirm that *A. simplex* larvae are resistant to traditionally conditions of marinating and can survive 25 days in a mixture of salt and vinegar. Depending on the salt concentrations, the survival of larvae reaches 35 to 119 days. Other marinating procedures different from traditional have been tested. A marinating procedure for anchovies with the use of 10% acetic acid (vol/vol) plus 12% salt, guaranteed destruction of *A. simplex* larvae within 5 days; 13 days would be necessary if traditional conditions (6% acetic acid and 12% salt) were used (University of Alcalá de Henares, Spain). A marinade of vinegar (6% acetic acid) and 10% sodium chloride applied for 24 h to sardines, followed by the addition of sunflower seed oil and refrigeration for 13 days, inactivates all *A. simplex* larvae.

Therefore, freezing becomes necessary for these traditional marinating processes. Salt and vinegar can reduce the danger associated with *Anisakis*, but they do not eliminate it or reduce it to an acceptable level.

3.6. Physical methods

3.6.1. High hydrostatic pressure

High hydrostatic pressure has been demonstrated to be an effective technique for treating food to reduce the number of pathogenic microorganisms and to extend shelf life. A pressure of 200 MPa for 10 minutes at 0-15°C kills *A. simplex* larvae, as well as pressures down to 140 MPa when the treatment time is increased up to 60 minutes. In addition, cycles of compression and decompression applied for a specific time were found to be more effective at killing larvae than a single pressure treatment for a similar time. It should be noted that such long treatment times would be impractical for the food industry. A pilot study was performed to determine the effect of high hydrostatic pressure on the viability of *A. simplex* larvae in raw fillets of king salmon and arrowtooth flounder, and to evaluate the effects of the treatment on the colour and texture of the fillets. Different pressure and time combinations were required to kill 100% of the larvae, and were

as follows: 414 MPa for 30-60 seconds, 276 for 90-180 seconds, and 207 MPa for 180 seconds. For 100% killing, however, a significant increase in the whiteness of the flesh was observed: this effect on the colour and appearance of the fillet may limit its application to the processing of fish for raw consumption. However, pressure treatment could be applicable to processed fish, e.g. marinated and cold-smoked fish, where the tissues are already substantially modified. In these processes, the pressure needed to kill parasites could be lower when combined with other treatments. In a recent study, the application of a pressure of 300 MPa for 5 minutes has resulted in the inactivation of *A. simplex* larvae in the tissues of mackerel, and a similar procedure has been suggested for the treatment of other fatty fish such as sardines and anchovies. These experimental studies should be extended in order to evaluate their usefulness in food processing.

3.6.2 Irradiation

In 1986, the Scientific Committee for Foods concluded that fish and shellfish could be irradiated at doses up to 3 kGy (overall average irradiation dose), as those values were considered to be acceptable from a public health standpoint. Irradiation has been applied to fresh, frozen as well as dried fish, fishery products, and shellfish.

Irradiation doses that kill *A. simplex* larvae in salted herring were reported to be higher than 6–10 kGy. Similarly, another study found *A. simplex* larvae to be highly resistant to irradiation doses of 2 kGy or 10 kGy. Another recent study based on an in vivo experiment in rats demonstrates that *A. simplex* third-stage larvae in the sea eel are not inactivated up to 1 kGy Irradiation is therefore not effective in inactivating *A. simplex* larvae, since they appear to be highly resistant to the irradiation doses which are normally recommended.

For liver flukes, investigations in Thailand demonstrated that low dose irradiation of freshwater fish can prevent infectivity of metacercariae of *O. viverrini* when such fish are prepared in local dishes made from raw or semi processed fish. At 0.5 kGy, the metacercariae could not develop in hamsters and caused no infection in their livers. The effective inactivation of *Opisthorchis* metacercariae through irradiation has also been recently reported, although high doses were used (12.5-25 kGy), much above the recommended levels.

Irradiation with 0.15 KGy inactivates metacercariae of *O. viverrini* and *C. sinensis* in fish. These experimental studies should be extended in order to evaluate their usefulness in food processing.

3.6.3 Low voltage current

A treatment to inactivate *A. simplex* larvae based on the application of electrical discharge through the fish has been patented in Spain in 2005 (ES 2 213 486 B1). The fish, either a single large fish (e.g. tuna) or pools of small fish (sardines, anchovies), are placed in an electrolyte bath. This is claimed to inactivate the larvae and leave the organoleptic properties as unaltered. Nevertheless adequate studies to prove the effectiveness of this method are not currently available. These experimental studies should be extended in order to evaluate their usefulness in food processing.

3.6.4 Smoking treatment

Smoking techniques can be categorised into hot smoking and cold smoking.

Hot smoking exposes foods to smoke and heat in a controlled environment; products are submitted to temperatures >60°C (average reference parameters: 70°C-80°C for 3-8 hours approximately). *A. simplex* larvae are unable to withstand such conditions (IFT/FDA, 2001). Cold smoking can be used as a flavour enhancer for example to salmon or scallops, and smokehouse temperatures for this process are maintained below 38°C: the process lasts from a few hours to a several days. During cold smoking, temperatures are insufficiently high for killing parasite larvae, thus the products must undergo an initial inactivation treatment.

Neither cold smoking for 12 h at 25.6°C nor refrigeration for 27 days killed *A. simplex* larvae in salmon. This analysis indicated that fresh salmon and cold-smoked salmon had 1-3 and 1-5 *A. simplex* viable larvae per 200 g of fish, respectively. A similar result was found in whole Pacific herring, where *A. simplex* larval remained viability after brining and smoking at an average temperature of 19°C for 24 h was 100% and 87.5%, respectively. Thus during hot smoking, products are treated at >60°C for some hours, and *A. simplex* larvae are unable to withstand such conditions. During cold smoking, instead, the temperature are too low (<38°C) in order to kill the parasitic larvae (EFSA, 2010).

This is in accordance to **CODEX STAN 311-2013**, where article 6.3 states that particular attention needs to be paid to cold smoked or smoke-flavoured products, which should be frozen before or after smoking if a parasite hazard is present. Annex 1 of this standard describes the procedures sufficient to kill parasites in such products.

4. REFERENCES

4.1 Regulations

4.1.1 European Union regulations

- Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin.
- Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption.
- Commission Regulation (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the organisation of official controls under Regulation (EC) No 854/2004 of the European Parliament and of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) No 854/2004.
- Commission Regulation (EU) No 1276/2011 of 8 December 2011 amending Annex III to Regulation (EC) No 853/2004 of the European Parliament and of the Council as regards the treatment to kill viable parasites in fishery products for human consumption.

4.1.2 Brazilian regulations

- Decreto Nº 9.013 de 29 de Março de 2017. Regulamenta a Lei nº 1.283, de 18 de dezembro de 1950, e a Lei nº 7.889, de 23 de novembro de 1989, que dispõem sobre a inspeção industrial e sanitária de produtos de origem animal.
- Decreto Nº 9.069, de 31 de Maio de 2017. Altera o Decreto nº 9.013, de 29 de março de 2017, que regulamenta a Lei nº 1.283, de 18 de dezembro de 1950, e a Lei nº 7.889, de 23 de novembro de 1989, que dispõem sobre a inspeção industrial e sanitária de produtos de origem animal.
- Memorando-Circular nº 2/2018/CGI/DIPOA/MAPA/SDA/MAPA (Ministério da Agricultura, Pecuária e Abastecimento. Coordenação Geral de Inspeção – CGI)
- PORTARIA Nº 1.914, DE 9 DE AGOSTO DE 2011. Aprova a Classificação de Risco dos Agentes Biológicos elaborada em 2010, pela Comissão de Biossegurança em Saúde (CBS), do Ministério da Saúde.

4.1.3 Other regulations

- *Codex Alimentarius.* Standard for Salted Fish and Dried Salted Fish of the Gadidae Family of Fishes. CODEX STAN 167 – 1989 *(last modified in 2018).*
- *Codex Alimentarius*. Standard for Quick Frozen Fish Fillets. CODEX STAN 190 – 1995 *(last modified in 2017).*
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- *Codex Alimentarius*. Guidelines on the application of general principles of food hygiene to the control of foodborne parasites, CAC/GL 88-2016.
- *Codex Alimentarius*. Code of Practice for Fish and Fishery Products. CAC/RCP 52-2003 *(last modified in 2016).*
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