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Abstract: Due to the social and legislative implications, the presence of *Anisakis* spp. larvae in fishery products has become a concern for both the consumers and the official Control Authorities. The issuance of a large number of provisions, aimed at better managing fish products intended to be consumed raw or almost raw and the associated risks, resulted in a very complicate legal framework. In this work, we analyzed the evolution of the normative through an overview on the local and international legislation, focusing on issues that are of practical interest for Food Business Operators (FBOs) in the fishery chain. In addition, we performed a survey across the Departments of Prevention of the Italian Local Health Authorities (LHA) and the main fish markets in Italy to collect the operating procedures and the monitoring plans. Overall, we found many differences, due to the absence of a national reference standard for the management of the *Anisakis* risk. From this examination, it turns clear that only a participation of all the involved Institutions, a strategy of synergistic interventions, as well as a correct training of FBOs, can result in an effective risk management and a proper risk communication, which should overcome states of confusion and unnecessary negative impacts on the economy.

Dear Editor,

We would like to submit the following manuscript for possible publication:

“Evolution of the Anisakis risk management in the European and Italian context”

The Anisakis risk management has recently been a source of great concern worldwide for both consumers and the Official Authorities in charge for controls. At the international levels, authorities, such as the *Codex Alimentarius* Commission, the EFSA and the U.S. FDA have recognized the need to face this issue through the adoption of preventive measures, capable to reduce, or even eliminate, the risk for public health.

The increased perception of this risk has determined, over the years, the issuance of a large number of international provisions, which have created a legal framework aimed at better managing fish products intended to be consumed raw or almost raw and its associated risks.

In this manuscript we revisited the evolution of the legislation related to the Anisakis risk, with an excursus of all European and Italian amendments issued from the late 60s to date. In particular, we addressed those aspects that, in our opinion, need to be reconsidered. Moreover, thanks to the collaboration of an Official Veterinary who has been working for more than 30 years at the wholesale market of Milan, we took into consideration many practical issues that are of interest for people working in the fishery chain.

Our aim was not only to provide an update on this topic, but to also analyze the data obtained from a survey of operating procedures and the self-monitoring plans, gathered at the Department of Prevention of the Italian Regional Health Authorities (AZ. USL) and at the main fish markets.

Other than to the academic world, this work is addressed to all those, who daily deal with the management of the fishery products as Food Business Operators (manufacturers, vendors, caterers), responsible for self-monitoring plans, and Official Authorities, to better clarify the tasks that they are called to fulfill.

Best regards,

Andrea Armani

Dear Editor,

we revised the manuscript according to the suggestions of the Reviewers. Our answers, comments and rebuttals are listed below.

The modified parts have been written in green to facilitate the revision process.

Reviewer #1: Manuscript Number: FOODRES-D-14-01201

Title: Evolution of the Anisakis risk management in the European and Italian context

This work carries out an overview on the legislation about Anisakids which are the most important fish parasites from a sanitary point of view.

The content is of interest; however, some important deficiencies are included:

(AZ. USL) The authors have to explain its meaning.

Az. USL is the acronym of Azienda Unità Sanitaria Locale. Its name, in English, is Local Health Authority (the name in the text has been modified from Regional Health Authorities to Local Health Authorities). Az. USL has been removed from the text to avoid confusion.

The authors affirm: "In fact, hypersensitivity reactions may also occur either via or with cooked fish, since allergens are thermostable". There are not evidences about this possibility. Nowadays, we can accept that allergic reactions only occur when live larva are ingested in raw or undercooked parasitized fish.

The sentence has been modified and, according to the suggestions of reviewer N.3, a short paragraph on the ongoing debate on the necessity of larvae being alive to induce allergy the first time has been added (line 134-154)

Anisakiasis should be changed by anisakiosis. According to the following paper: T. KASSAI, M. CORDERO DEL CAMPILLO, J. EUZEBY, S. GAAFAR, Th. HIEPE and C.A. HIMONAS Standardized Nomenclature of Animal Parasitic Diseases (SNOAPAD) Veterinary Parasitology, 29 (1988) 299-326 299, "the disease name is constructed solely by the suffix -osis, which is added to the stem of the name of the parasite taxon, formed from the nominative of the taxa". Besides, the reference of Audicana and Kennedy 2008 should be changed by Kassai et al 1988.

Anisakiasis has been changed in anisakiosis and Kassai et al 1988 (line 128) has been added in the text.

Hysterothylacium belongs to the family Anisakidae, but to the subfamily Raphidascaridinae Hartwich, 1954. Please include this in the text.

In this work we used the classification of Fagerholm, 1991, due to the fact that molecular analyses performed to investigate phylogenetic relationships in the superfamily Ascaridoidea support this classification (Murrell, K. Darwin, and Bernard Fried. Food-borne parasitic zoonoses: fish and plant-borne parasites. Vol. 11. Springer, 2007 report two different accepted classification for ascaridoid). For further explanations see answers to the Reviewer 2. Moreover, a new paragraph has been added in the manuscript (line 82-89).

Infestation should be changed by infection.

Infestation has been changed in infection in all the manuscript.

The following reference: De Nicola, P., Napolitano, L., Di Bartolomeo, N., Waku, M., & Innocenti, P. (2005). Su di un caso di Anisachiasi con perforazione del cieco. *Il Giornale di Chirurgia*, 26, 375-377, there is inadequate in order to justify that "infestations constitute a public health problem, especially in Spain".

The sentence has been modified and the reference has been removed (line 173-176)

Reviewer #2: The topic of this review is interesting. However, in my opinion there is too much generic information. Although this review does not present any new knowledge, after some revisions, it could give contribution in this field.

Detailed reviewer comments:

ABSTRACT

The abstract is not sufficiently informative and the objective is not clear (line 35...we analyze the data obtained etc...)

The abstract has been changed according to the suggestion

Line 34: operator working in....it could be replaced with food business operators in...

Done (line 34)

Line 37: On the basis of or Based on....

The authors should combine some sentences. (lines...37 - 42)

The sentence has been changed (line 37-42)

Line 44: European legislation or European Rules

We prefer European legislation

INTRODUCTION

There is a disconnect in several parts in the section.

Introduction has been revised

Line 55: the Authors may delete "significant" in the text.

Significant has been deleted (line 54-55)

Lines 106-110: The Authors should explain better these sentences.

The classification of Hartwich, 1974 was based on the structure of the secretory-excretory system while that of Fagerholm, 1991 was based on the male caudal morphology (see table below from Murrell, K. Darwin, and Bernard Fried. *Food-borne parasitic zoonoses: fish and plant-borne parasites. Vol. 11. Springer, 2007 report two different accepted classification for ascaridoid*). These differences in the morphological characteristics used for phylogenetic

reconstruction led to an array of contrasting interpretations and hypotheses of relationships, in turn leading to instability of ascaridoid classification, although the classification schemes of Hartwich (1974) and Fagerholm (1991) have been the most commonly used. Molecular data have been used to investigate phylogenetic relationships in the superfamily Ascaridoidea (e.g. Nadler, 1992; Nadler and Hudspeth, 1998, 2000; Zhu et al., 1998). Although these studies have not fully resolved taxonomic uncertainty within the superfamily, they tend to support the classification proposed by Fagerholm (1991). For this reason we decide to report the classification of Fagerholm, 1991, which consider the genus *Hysterothylacium* not belonging to the family Anisakidae, but to the family Raphidascarididae. A new section has been added in the text (Line 82-89).

TABLE 5.1. Contrasting classification schemes of anisakid nematodes by Hartwich (1974) and Fagerholm (1991).

Authority	Family	Subfamily	Genera
Hartwich (1974)	Anisakidae	Anisakinae	<i>Anisakis</i> , <i>Phocanema</i> (= <i>Pseudoterranova</i>), <i>Terranova</i> , <i>Sulcascaris</i> , <i>Duplicaecum</i> , <i>Galeiceps</i> , <i>Contraecum</i> , <i>Phosascaris</i>
		Geozinae	<i>Goezia</i>
		Raphidascaridinae	<i>Raphidascaris</i> , <i>Raphidascaroides</i> , <i>Thynnascaris</i> (= <i>Hysterothylacium</i>), <i>Lappetascaris</i> , <i>Aliascaris</i> , <i>Heterotyphlum</i> , <i>Paranisakis</i> , <i>Paranisakiopsis</i>
Fagerholm (1991)	Anisakidae	Anisakinae	<i>Anisakis</i> , <i>Pseudoterranova</i> , <i>Terranova</i> , <i>Sulcascaris</i> , <i>Peritrachelius</i> , <i>Pulchrascaris</i> , <i>Paranisakiopsis</i>
		Contraecinae	<i>Contraecum</i> , <i>Galeiceps</i> , <i>Phosascaris</i>
	Raphidascarididae		<i>Raphidascaris</i> , <i>Raphidascaroides</i> , <i>Hysterothylacium</i> , <i>Lappetascaris</i> , <i>Heterotyphlum</i> , <i>Paranisakis</i> , <i>Goezia</i> , <i>Sprentascaris</i> , <i>Paraheterotyphlum</i>

Line 117: "the molecular studies carried out have shown" ?

The sentence has been modified (line 82-85)

Line 152: (Genchi and Pozio, 2003 before Abe,2008)

Done Line (169)

Lines: 164-170 and: these sentences are not clear, Please could you explain these sentences better?
I think that this sentence is not pertinent for the topic of the review.

The sentence has been modified and shortened (line 178-184)

Lines 192 - 193: the authors should eliminate this part.

The sentence has been modified (line 195-197)

Line: 202....the reference?

The reference has been refined (line 211)

Lines: 215-220 The objectives need to be re-written

The objectives have been re-written and moved at the beginning of the introduction (line 62-67)

LEGISLATIVE HYSTORY

Lines: 249-254 The authors should combine this part.

The sentence has been modified (line 242-249)

This is not in contrast with the EFSA opinion.

**The reviewer did not report the line. Probably he/she refers to line 414 (original manuscript).
The sentence has been modified according to the suggestion (line 393-395)**

DISCUSSION

The Authors need to rethink the Discussion.

In my opinion this section is too long and the Authors may cut some parts or combine some sentences.

Discussion has been revised and shortened

Lines: 450-452...this part is not clear, the Authors need to rethink the sentence.

The sentence has been modified (line 427-428)

There is a disconnect in several parts in the section...please could you organize it better?

In some parts, I have found the same information reported in other sections (Introduction or Legislative Hystory)

The section has been revised and the repeated information have been deleted

Reviewer #3: The paper gives an comprehensive overview on the European legislation concerning Anisakis in fish as food and describes in detail how the visual inspection was adopted in Italy. It needs some revisions before it is acceptable for publication.

In the introduction you should add a short paragraph on the ongoing debate on the necessity of larvae being alive to induce allergy the first time.

The section has been modified and some information has been reported on this issue (line 134-154)

line 130 - 141: the paragraph should be changed because *Anisakis* larvae can be found in most marine species caught in European waters and not only in the species you mentioned, e.g. herring can also be heavily infected.

According to the suggestions we decided to remove the paragraph from line 137 to line 141 (original manuscript) which is not fundamental for the aim of our review. This to avoid to add an excessive number of references, due to the fact that a multitude of fish species can be infected by *Anisakis*.

It is correct that according to the RASFF data, the species you mentioned are the most frequently parasitized species, but these species were mainly imported to Spain and Italy, other European countries simply do not check for nematodes in species landed in their harbours. So a general ranking could be different.

The sentence has been modified highlighting that the data mainly come from Italian and Spanish notification (Line 116-123)

line 142-148: This paragraph should be moved and included in the explanation given in line 176 - 185.

In this section we discussed the reason why the *Anisakidosis* is an emerging parasitosis and the factors that have led to an increase of the human cases of parasitosis. Among these factors, there is the decrease of INTERMEDIATE hosts of the larvae. The paragraph previously included in lines 142-148 is about the prevalence of infected species, which is higher where cetaceans are more numerous. This is an issue correlated more to the epidemiology of the infected species than to the increase of parasitosis cases in these last years. Therefore, we think that it should be included in the paragraph 1.1.

line 208-9: it was also my opinion that farmed *Salmo salar* presents an irrelevant risk of infestation, but Mo et al: *Journal of Fish Diseases* 37, 135-140 (2014) showed the farmed *S.salar* can be infected with *Anisakis*. So your conclusions throughout the paper concerning farmed salmon should be slightly revised according to this new findings.

The section about farmed salmon has been modified in the light of the results of Mo *et al.*, 2014. Moreover, to avoid repetition, the section has been deleted from introduction and moved to line 450-457).

line 490 - 495. *Anisakis* free does not mean parasite free (trematodes, cestodes). It would be nice if you could add one sentence on possible consequences the consumption of fresh raw fish may have concerning these parasites.

A new sentence has been added at line 468-469

- The presence of Anisakids larvae in fishery products is an emerging issues
- We carried out a survey on European and local laws on Anisakis risk management
- We analyzed the approach of different control systems to face the Anisakis risk
- The management of the Anisakis risk show differences in local laws
- A reassessment of the Anisakis issue is needed to yield common strategies

1 **Evolution of the Anisakis risk management in the European and Italian context.**

2

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27 **ABSTRACT**

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4 29 fishery products has become a concern for both the consumers and the official Control
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7 30 Authorities. The issuance of a large number of provisions, aimed at better managing fish
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10 31 products intended to be consumed raw or almost raw and the associated risks, resulted in a
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12 32 very complicate legal framework. In this work, we analyzed the evolution of the normative
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14 33 through an overview on the local and international legislation, focusing on issues that are of
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17 34 practical interest for Food Business Operators (FBOs) in the fishery chain. In addition, we
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19 35 performed a survey across the Departments of Prevention of the Italian Local Health
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21 36 Authorities (LHA) and the main fish markets in Italy to collect the operating procedures and
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24 37 the monitoring plans. Overall, we found many differences, due to the absence of a national
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27 38 reference standard for the management of the *Anisakis* risk. From this examination, it turns
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29 39 clear that only a participation of all the involved Institutions, a strategy of synergistic
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31 40 interventions, as well as a correct training of FBOs, can result in an effective risk management
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34 41 and a proper risk communication, which should overcome states of confusion and
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36 42 unnecessary negative impacts on the economy.

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41 44 Keywords: Anisakids, risk management, European legislation, raw fish products.
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1. INTRODUCTION

Parasitic infections associated to the consumption of fish products have always been a concern for the public health and for economy. Recently, the World Health Organization (WHO) has estimated a prevalence of approximately 56 million cases worldwide and a population exposed to the risk of about 400 million individuals (World Health Organization, 2013). The main food-borne zoonoses associated with the consumption of fishery products are mainly attributable to trematodes, cestodes and nematodes. Among the latter, the Anisakids are the most important parasites from a sanitary point of view, since capable to induce pathologies in humans (Chai, Darwin Murrell, & Lymbery, 2005).

In this work, we made an excursus on the European, Italian and regional legislation and analyzed the operating procedures and the monitoring plans for prevention and control of Anisakidosis, the information on which were provided by the Italian Local Health Authorities (LHA) or collected at the main fish markets. We then highlighted the peculiarities in the current normative and the differences in the risk management strategies, implemented by the Official Authorities and by the FBOs.

1.1. Anisakids: classification, biological cycle and epidemiology

The identification of Anisakid species is very difficult due to the limited species-specific differences in morphological characters. Moreover, these differences are only visible in the adult worm and not in the larvae (Fagerholm, 1991; Mattiucci, Abaunza, Damiano, Garcia, Santos, & Nascetti, 2007). For this reason, in the past, only two species were considered responsible for zoonotic forms: *Anisakis simplex*, known as “herring worm”, and *Pseudoterranova decipiens*, known as “cod worm” (Smith and Wootten, 1978a; Murrell and Fried, 2007). However, the molecular studies based on genetic markers have shown that many morphospecies of *Anisakis* and *Pseudoterranova* include a certain number of sibling species with identical morphology, but different genetic make-up and geographical location (Mattiucci and Nascetti, 2008). Currently, 9 species of the *Anisakis* genus, (Mattiucci and

79 Nascetti, 2008; Mattiucci *et al.*, 2011) and 6 of the *Pseudoterranova* genus (Paggi *et al.*,
80 1991; Mattiucci *et al.*, 1998; George-Nascimento and Urrutia, 2000; McClelland, 2002;
81 Murrell and Fried, 2007; Mattiucci and Nascetti, 2008) have been described.

82 Even though the taxonomic uncertainty within the superfamily has not been still
83 completely solved, the molecular analyses used to investigate phylogenetic relationships tend
84 to support the classification proposed by Fagerholm (1991) based on the male caudal
85 morphology (Murrell and Fried, 2007). This classification considers the genus
86 *Hysterothylacium* not to belong to the family Anisakidae, but to the family Raphidascarididae.
87 In spite of this, there is a tendency to use the term Anisakidosis also for infections provoked
88 by species belonging to this genus, because likewise hazardous to public health (Audicana, *et*
89 *al.*, 2002; Genchi and Pozio, 2003; Griglio *et al.*, 2012).

90 The Anisakids are characterized by different stages of larval development, located in
91 different hosts. In general, the adult forms are located in the stomach of marine mammals,
92 while the eggs are shed in the feces and require an incubation period before hatching into
93 second stage larvae (L2). The L2 are ingested by invertebrates, especially crustaceans, such as
94 Euphausiacea and Copepoda, where they grow inside the hemocoel (McClelland, 2002;
95 Murrell and Fried, 2007; Smith and Snyder, 2005). These larval forms do not seem to be
96 hazardous to the human health as it is the third stage larvae (L3), which, after being ingested
97 by a paratenic host, can directly encyst in the edible tissue (Nagasawa, 1990; Anderson and
98 Anderson, 2000), representing a risk when fishery products are consumed raw or
99 undercooked. In addition, some studies have shown that L3 larvae are able to migrate from
100 the viscera to the muscle, even after the death of the fish (Van Thiel, 1962; Smith and
101 Wootten, 1978a; Adams, Murrell, & Cross, 1997; Abollo, Gestal, & Pascual, 2001; Murrell
102 and Fried, 2007; Rello, Adroher, Benitez, & Valero, 2009; Pravettoni, Primavesi, &
103 Piantanida, 2012).

104 At the global level, the *Anisakis* larvae were found in at least 200 different species of fish
105 and 25 species of cephalopods (Abollo, Gestal, & Pascual, 2001; Klimpel, Palm, Rückert, &
106 Piantkowsky, 2004; Murrell and Fried, 2007). In the Mediterranean Sea, the L3 larvae were
107 observed in 31 different species out of 99 species examined belonging to 47 families (about
108 15.000 specimens), with a prevalence ranging from 1.3 to 100% and with an infection
109 intensity from 1 to over 300 larvae per specimen (Ponzio, 2004). Moreover, some studies
110 have found that fishes caught in areas with abundant populations of cetaceans show a greater
111 degree of infection. For instance, in the Ligurian Sea, which is part of the Marine Mammal
112 Sanctuary and where the presence of whales and dolphins is stable, the prevalence of infection
113 is 5 times greater than those found in the South Atlantic or in other Mediterranean areas
114 (Notarbartolo-di-Sciara, Agardy, Hyrenbach, Scovazzi, & Van Klaveren, 2008; Rello *et al.*,
115 2009).

116 In the European Union, in the period 2009-2013, the Rapid Alert System for Food and
117 Feed (RASFF) (Rapid Alert System for Food and Feed, 2014) has reported a total of 333
118 notifications for the presence of parasites: of these, 262 (78.5%) were due to *Anisakis* spp.
119 These notifications were mainly forwarded by Italy (147) and Spain (49) and refer to 108
120 border rejections, 86 information and 68 alerts. The RASFF data, mainly based on Italy and
121 Spain notification, show that the most frequently parasitized fish, among the species
122 commercialized in this countries, were: *Merluccius merluccius*, *Scomber scombrus*, *Engraulis*
123 *engrasicolus*, *Lophius* spp., *Xiphias gladius* and *Lepidopus caudatus*.

124 **1.2 Human health issues**

125 In 1988, Kassai *et al.* recommended the use of three different terms to indicate zoonoses
126 caused by nematodes of the Anisakidae family:

- 127 - Anisakidosis: for zoonoses from species belonging to the Anisakidae family (Anisakids);
- 128 - Anisakiosis: for zoonoses from species belonging to *Anisakis* genus;
- 129 - Pseudoterranovosis: for zoonoses from species belonging to the *Pseudoterranova* genus.

130 Humans, who are accidental hosts of the L3 larvae, may develop diseases, which presents
131 either non-invasive forms, generally asymptomatic, or invasive forms, characterized by
132 gastrointestinal symptoms (acute or chronic), nowadays well identified and extensively
133 described and discussed in the literature.

134 What is less known is the “gastroallergic anisakidosis”, an IgE-mediated allergic reaction
135 that can be developed after ingestion of Anisakids. It is characterized by clinical
136 manifestations ranging from urticaria to anaphylactic shock (Daschner, Alonso-Gomez,
137 Cabanas, Suarez-de-Parga, & Lopez-Serrano, 2000; Dominguez-Ortega *et al.*, 2001). Despite
138 two episodes of allergy have been described without the involvement of living parasites
139 (Audicana *et al.*, 1995; Aníbarro *et al.*, 1997), the ingestion of alive larvae is usually required
140 for sensitization and allergic reactions (Daschner, Alonso-Gomez, Cabanas, Suarez-de-Parga,
141 & Lopez-Serrano, 2000; Audicana, *et al.*, 2002; Alonso-Gomez *et al.*, 2004), while the
142 exposure to Anisakis proteins alone may suffice to elicit allergic reactions in sensitized
143 individuals (Nieuwenhuizen *et al.*, 2006). This possibility makes the “gastroallergic
144 anisakidosis” a professional pathology in people who have frequent contacts with infected
145 fish (Aníbarro *et al.*, 1997; Aníbarro and Seoane, 1998; Armentia *et al.*, 1998; Scala *et al.*,
146 2001). Still, considering that some allergens have been shown to be heat-resistant, cooking
147 might not provide an effective protection against allergic manifestations (Moneo, Caballero,
148 GómezOrtega, & Alonso, 2000; Caballero and Moneo, 2004; Moneo *et al.*, 2005). For
149 instance, it has been reported that in northern Spain the consumption of cooked hake is one of
150 the main cause of allergies, closely followed by cooked or raw anchovies (Fernández de
151 Corres *et al.*, 1996; Audicana, Del Pozo, Iglesias, & Ubeira 2003). On the contrary, it has also
152 been reported that freezing could reduce the risk of allergic reactions (Garcia *et al.*, 2001).
153 However, the exposure to Anisakid antigens is still a debated issue, due to the many clinical
154 manifestations that are involved (Audicana and Kennedy, 2008).

155 The 90% of diseases related to the ingestion of Anisakids worldwide is determined by
156 *Anisakis simplex*, *A. pegreffii* and *Pseudoterranova* spp. (Bouree, Paugam, & Petithory, 1995;
157 Rosales et al., 1999; Smith, 1999; Audicana, et al., 2002; Murrell and Fried, 2007). In Italy,
158 the species most frequently associated to health issues is *A. pegreffii* (D'Amelio,
159 Mathiopoulosv, Brandonisio, Lucarelli, Doronz, & Paggi, 1999; Moschella et al., 2004;
160 Fumarola et al., 2009; Mattiucci et al., 2011; Bernardi, Gustinelli, Fioravanti, Caffara,
161 Mattiucci, & Cattaneo, 2011; Mladineo and Poljakb, 2013). *A. physeteris*. *Contracaecum* spp.
162 and *Hysterothylacium* spp. are other species of *Anisakis* spp. that can be involved in human
163 infections, even though they are only seldomly involved in pathological forms (Ishikura et al.,
164 1993; Yagi et al., 1996; Rosales et al., 1999; Smith, 1999; Lima dos Santos and Howgate,
165 2011).

166 ***1.3 The oxymoron of Anisakidosis: a classic but emerging parasitosis.***

167 In the last decade there have been about 20,000 cases of human Anisakidosis, of which more
168 than 90% located in Japan, where, every year, about 2.000 infections occurs (Genchi and
169 Pozio, 2003; Abe, 2008). In the United States, a recent survey of patients with generic
170 gastrointestinal disorders showed that these symptoms were ascribable to parasitic diseases of
171 fish origin, with such a frequency to require preventive controls throughout the national
172 territory (Hochberg, Hamer, Hughes, & Wilson, 2010).

173 In Europe, the estimated incidence is about 0.038% (Orphanet, 2009 and Lima dos Santos
174 and Howgate, 2011). Most of the cases have been registered in Spain, Netherlands and
175 Germany, (European Food Safety Authority, 2010 and Pravettoni, Primavesi, & Piantanida,
176 2012). The exact incidence is difficult to establish, but it seems to average 20 cases per
177 country per year. In France, a report of 2003 estimated an incidence of eight cases per year
178 (French Agency for Food, Environmental and Occupational Health and Safety, 2011). In
179 Italy, 54 cases had been reported from 1996 to 2011 (Ponzio, 2004; De Rosa, 2011; Griglio et
180 al., 2012), most of which occurred in Puglia, Abruzzo, Molise and Sicily, especially in coastal

181 areas. Only few sporadic cases have been reported in other regions as Emilia Romagna,
182 Liguria, Lombardy, Tuscany, Marche, Lazio and Campania. All cases were associated with the
183 consumption of traditional dishes made with anchovies, herring and mackerel served raw or
184 "almost raw" (Ponzio, 2004; De Rosa, 2011; Griglio *et al.*, 2012).

185 The factors that have led to an increase of the Anisakidosis cases over the past 30 years are
186 many and interdependent. First of all, the food scare crises, such as the "mad cow disease"
187 and the "avian influenza", which have shifted the orientation of consumers' attention towards
188 proteins of fish origin, have increased the consumption of fishery products.

189 Another factor to be taken into consideration is the spread of ethnic food on the Western
190 tables has led to the availability of a variety of Oriental dishes, especially Japanese,
191 characterized by preparations of raw seafood. In fact, many Japanese restaurants are not really
192 authentic, but managed by operators of different ethnicity, especially Chinese. These latter
193 tend to increasingly convert their restaurant activities into Sushi Bars or Sushi Restaurants,
194 offering cheaper products, often at the expense of quality (Masotti, Amadei, & Lanni, 2010;
195 Armani, Castigliego, Gianfaldoni, & Guidi, 2011). **However, the lack of a proper knowledge
196 on the microbiological and parasitic risks associated to dishes based on raw fish could lead to
197 an inappropriate manipulation and treatment of the raw materials.**

198 Not to be undervalued is also the change, in recent years, of the control responsibility in
199 the fishery chain. In fact, while once the veterinary inspector was the person in charge for
200 controls, nowadays, this task is delegated to the FBOs, who are not always adequately trained
201 in detecting infections.

202 Finally, the unconditioned increase in the supply of wild fishery products has often
203 determined adverse effects on the availability of natural fisheries, resulting in a depletion of
204 marine resources. Therefore, the reduction of the species that have "historically" represented
205 the intermediate hosts, together with the low specificity that these parasites have towards their
206 intermediate hosts (LyMBERY and Cheah, 2007; Murrell and Fried, 2007), have likely

207 determined the progressive increase of both the number of affected species and the prevalence
208 of infection.

209 Due to the increasing difficulties in the management of the "Anisakis risk", related to the
210 involvement of wider geographic areas and to the significant number of individuals affected
211 by anisakidosis, the European Food Safety Authority (EFSA), in its report (EFSA, Panel on
212 Biological Hazards (BIOHAZ), 2010), highlighted that no maritime area can be considered
213 free from Anisakids and that the presence of larvae in fishery products is a natural condition
214 that claims the "presumption of infection" throughout the supply chain. In conclusion, the
215 group of EFSA experts stated that to reduce Anisakis infection it is essential to provide to all
216 the operators involved in the fishery chain and to the final consumers the necessary
217 information for the proper management of the risks associated to the presence of parasites in
218 fishery products.

2. LEGISLATIVE HISTORY

2.1. Before the "Hygiene Package"

221 In Europe, the first regulation related to the management of the *Anisakis* spp. associated
222 risk was issued by the Dutch Health Authorities in the years 1968/69. This law allowed the
223 marketing of not-eviscerated herrings only if previously subjected to treatments for
224 devitalization of the larvae (Panebianco and Lo Schiavo, 1985).

225 In the early 70's, in Germany traders and processors decided to adopt the regulations issued
226 by the Dutch Authorities (Horst, 2008) but, in the course of the '80s, some companies decided
227 to no longer adhere to this voluntary agreement. This led to an increase of cases of infection
228 and to the collapse of sales. Since then, the German government started a series of efforts in
229 order to minimize the Anisakids risk in the domestic fish products (Horst, 2008). On August
230 the 8th 1988, the Federal Republic of Germany issued an Ordinance (Federal Republic of
231 Germany, 1988), which ordered the removal of parts containing nematodes (dead or alive)
232 and their marketing ban.

233 In the same period, both the French and the Dutch Ministry of Agriculture and Fisheries
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2 234 instituted specific rules for herrings processing (Renon and Malandra, 1991). In Italy, the first
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5 235 specific Circular Letters (CL) on Anisakis were enacted around the 80's, but already in 1962,
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7 236 with the national Law n.283, it was forbidden to *"use in the preparation of food or drink, sell,*
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10 237 *hold to sell, administer or otherwise distribute for consumption, soiled, invaded by parasites,*
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12 238 *altered or harmful foodstuff"*. The parasites considered by the Law n. 283 were not Anisakids,
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15 239 but all those that could be found in the products, as a consequence of a poor state of
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17 240 preservation and thus destined to seizure and destruction.

19 241 The first specific legislation on the management of *Anisakis* spp. in fishery products was
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22 242 enacted in 1992, when the presence of the parasite strikingly emerged in bluefish. In order to
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24 243 draw the attention of the industry, consumers and Health Authorities on the need to adopt
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27 244 systems to prevent this zoonosis, the CL No. 10 of 11/03/1992 was issued by the Ministry of
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29 245 Health: *the Italian government recommended the FBOs to proceed with a rapid evisceration*
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31 246 *of the fish at risk of infection (with a size exceeding 18 cm) and not to throw the entrails into*
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34 247 *the sea. On the other hand, the veterinary inspectors were asked to carry out a check of the*
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36 248 *fish through random sampling and visual inspection, after opening the coelomic cavity*
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39 249 *(Circular Letter No. 10/92)*. Finally, if the invasion by the parasites of the edible parts was
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41 250 such as to give a repugnant appearance to the fish, according to the judgment of the veterinary
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44 251 inspector, the Letter No. 10 settled the obligation to destroy the products. In case of slight
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46 252 infection, the veterinary inspector could instead dispose the treatment of products to kill
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49 253 parasites. Such treatment consisted in the freezing at -20 °C for at least 24 hours or,
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51 254 alternatively, in the heat treatment at 60 °C for 10 minutes at least. As for the visual
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54 255 inspection, the aforesaid Letter did not include particular obligations for the retailers, but a
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56 256 verification that the product had been monitored by the Veterinary Service. These provisions
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58 257 were resumed in the Ministerial Decree of 12/05/1992, which stated that preventive
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2 258 treatments would have been only carried out in authorized establishments and that they had to
3 be certified (Ministerial Decree of 12/05/1992).

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5 260 Subsequently, the Legislative Decree No. 531 of 30/12/1992 introduced the obligation of
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7 261 the visual inspection and the research of visible parasites, before the introduction on the
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9 262 market. The Decree No. 531 also ordered that the fish or their parts, obviously **infected** with
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11 263 parasites, were not intended for human consumption. Fishes, to be consumed raw or almost
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13 264 raw, cold-smoked (with a core fish temperature of less than 60° C), as well as marinated
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15 265 and/or salted, had to be treated with low temperatures if the process did not guarantee the
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17 266 death of the larvae (Decreto Legislativo No. 531/92).

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21 267 In 1993, Commission Decision No. 93/140/EEC defined the concept of "visible parasite"
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23 268 as *"a parasite or a group of parasites which has a dimension, color or texture which is clearly*
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25 269 *distinguishable from fish tissues"* and "visual inspection" as *"a non-destructive examination of*
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27 270 *fish or fishery products without optical means of magnifying and under good light conditions*
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29 271 *for human vision, including, if necessary, candling"*. The recipients of the aforesaid Decision
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31 272 were required to implement controls for the detection of visible parasites in whole products
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33 273 and in fish fillets or slices. They must determine the scale and frequency of the inspections,
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35 274 depending on the nature of the fishery products, their geographical origin and their use. For
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37 275 eviscerated fish, the search for visible parasites must be conducted by professionals on the
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39 276 abdominal cavity, livers and roes, intended for human consumption. According to the type of
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41 277 evisceration, the visual inspection must be performed in a continuous manner at the time of
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43 278 evisceration and washing (manual evisceration) or by sampling a representative number of
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45 279 specimens, being not less than 10 fish per batch (mechanical evisceration). Fish fillets and
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47 280 fish slices must be inspected by qualified personnel during trimming after filleting or slicing.
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49 281 Where an individual examination is not possible, due to the size of the fillets or to the filleting
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51 282 operations, operators must draw up a sampling plan.
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283 In later years, some Italian regions introduced appropriate measures into their local
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2 284 legislation, in order to protect the health of the consumers. In 1994, the Region of Lombardy
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4 285 defined the procedures for carrying out a statistically significant sampling for the detection of
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6 286 the parasite in fish products (Table 1). The protocol established that, through an initial visual
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8 287 inspection, the Control Authority must ensure that there were no migrating larvae on the outer
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10 288 surface of the fish. Otherwise, the lot of fish should have been withdrawn and subsequently
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12 289 destroyed. As for the anchovies, if no larvae were found on the surface, the Authority would
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14 290 have proceeded with the sampling, on a representative number of specimens, according to the
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16 291 "Table of statistical significance" (Table 1). In 1997, the Liguria Region stated that in all
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18 292 business activities where raw anchovies were commercialized, an informative billboard must
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20 293 have been displayed reporting "*.....the specimens of anchovies (Engraulis encrasicolus) used*
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22 294 *in food preparation as raw, raw marinated "the flagship acciughette" or with lemon etc.,*
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24 295 *must be previously frozen at -20 °C for at least 24 hours"* (Decree of the President of the
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26 296 Regional Council n. 282/49671). Moreover, the Liguria Region defined how the controls must
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28 297 be carried out on lots of anchovies, as well as the appropriate measures to be taken following
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30 298 the outcome of the inspection. In particular, specific procedures were defined for the sampling
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32 299 of specimens (Circular Letter No. 1 of 1997).
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34 300 In 1996, an European Regulation took into consideration, for the first time, the relationship
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36 301 between the presence of parasites and the negative effect that they have on the quality of the
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38 302 product, establishing the downgrade of the products despite the excellent organoleptic
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40 303 conditions and the favorable judgment of edibility (Council Regulation (EC) No. 2406/96).
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42 304 In 1999, the Norwegian Directorate of Fisheries, on the basis of the European rules,
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44 305 established that a visual control of random samples must be carried out to detect and remove
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46 306 visible parasites. The controls must be performed after gutting and rinsing of the belly cavity
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48 307 and any liver/roe and during filleting and slicing, possibly using the candling. The person in
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50 308 charge of the establishment would set up a plan, as part of the self-monitoring, specifying the
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309 scope and frequency of the controls in relation to the type of product, geographical origin and
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2 310 use. Herrings, mackerels, sprats, wild salmons caught in the Atlantic or Pacific Ocean,
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4 311 marinated and/or salted herrings (when the treatment has not been sufficient to destroy
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6 312 nematode larvae) intended to be eaten raw or almost raw, must undergo a freezing treatment
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9 313 at a core temperature of -20° C or lower for at least 24 hour. Moreover, the fish species listed
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11 314 above, if not sold as fresh or frozen products, must be accompanied by a certificate from the
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13 315 producer indicating the type of treatment that they had undergone. Finally, special provisions
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15 316 were required for packed capelin roe, which must, on examination of a minimum of 6
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17 317 samples, contain a maximum average of 1 nematode larva per 100 g in the samples, and a
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19 318 maximum of 3 nematode larvae per 100 g in any single sample (Norwegian Directorate of
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21 319 Fisheries, 1999).

26 320 *2.2. After the “Hygiene Package”*

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29 321 The new provisions introduced by the “Hygiene Package” led to a radical change in the
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31 322 system of parasites control in fishery products. At the fish market, the figure of the official
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33 323 veterinarian, who was previously responsible for carrying out the sampling and the visual
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35 324 inspection of the fish, has been replaced by the FBO, who has become the first responsible for
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37 325 the risk management. The FBO is required to set, implement and maintain the procedures
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39 326 based on HACCP principles (Hazard Analysis Critical Control Points), through an analysis of
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41 327 risks associated with food production. He should also identify operational techniques to
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43 328 eliminate or reduce the presence of Anisakids in the finished product. Unfortunately, the new
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45 329 regulatory approach does not define the specific methods and procedures that the FBOs have
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47 330 to apply, but it only defines the objectives that they have to attain, possibly assisted by the
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49 331 Competent Authorities. This transposition of tasks, if on one side has streamlined the **official**
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51 332 inspection practices, on the other implies an adequate training of FBOs. In fact, at present, the
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53 333 FBOs are called to perform a statistically significant and appropriately documented sampling,
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55 334 in order to demonstrate that they have taken all possible precautions to detect parasites. Such
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335 a sampling should be performed by qualified persons, who are required to submit their
336 products to a control for the detection of visible parasites, without placing on the market those
337 *'obviously contaminated'*.

338 The Regulation (EC) No. 853/2004 states that fishery products to be consumed raw, almost
339 raw, marinated, salted and cold smoked with an internal temperature not more than 60° should
340 be frozen at a temperature not higher than -20 °C in all parts of the product for at least 24
341 hours. Subsequently, Regulation (EC) No. 2074/2005 gave further **explanations** about the
342 visual inspections, clarifying the concept of candling: *“Candling means, in respect of flat fish
343 or fish fillets, holding up fish to a light in a darkened room to detect parasites”*.

344 In 2006, in Spain, the Royal Decree 1420/2006 was issued, following both the EU policies
345 and the data provided by the Scientific Committee of the Spanish Agency for Food Safety
346 (AESAN) and the National Center for Epidemiology, which showed a higher incidence of the
347 parasite in fish and an increase in Anisakidosis prevalence in the population. With this Decree
348 all the operators dealing with preparation and catering activities were called to accomplish a
349 preventive treatment of fishery products to be consumed as raw or almost raw, cold-smoked,
350 pickled or salted. Afterwards, in 2007, the AESAN provided some recommendations
351 concerning the best criteria to determine if freezing is necessary in marinated or salted fish
352 products (Comité Científico de la Agencia Española de Seguridad Alimentaria y Nutrición,
353 2007).

354 In May 2008, the Italian Ministry of Health, following the remarks received by the
355 European Commission about the activation procedure of the national alert system for the
356 detection of *Anisakis* spp. in fishery products coming from Third Countries, issued the CL
357 DGSAN No. 10776-P. This document provided that the alert system must be activated only
358 after detection of viable larvae in the coelomic cavity and in the flesh of fresh fish products or
359 in those products subjected to heat treatment insufficient to kill larvae. Moreover, if during

1 controls, products unsuitable for human consumption were found (regardless of the larvae
2 were dead or alive), the authorities should proceed with the rejection of the goods.
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5 362 Moreover, in 2008, with the amendments introduced by Reg. (EC) No. 1020/2008 to the
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7 363 Reg. (EC) No. 853/2004, the visual inspection become mandatory also for FBOs who operate
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9 364 at the retail level.
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12 365 In Italy, following the EFSA opinion of 2010, the Ministerial CL 4379-P and 4380-P of
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14 366 17/02/2011 were enacted. The Letter 4379-P-17/02/2011 stresses the obligation for FBOs to
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16 367 freeze at a temperature not higher than -20 °C and for almost 24 hours, the fish (even fresh
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18 368 water fishes) intended to be administered raw. The type of the treatment must be declared in
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20 369 the self-monitoring plan of the food business activities and, the fishery products always be
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22 370 accompanied, during their placing on the market, by a certificate of the FBO, stating the
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24 371 treatment performed. Moreover, the Letter 4379-P, in order to maintain the correct
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26 372 information on freezing treatment and due to the fact that reporting the term “defrosted” on
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28 373 products previously frozen for health safety purposes might negatively influence the
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30 374 consumer, allowed to report: *"Conforms to Regulation EC No. 853/20054, Annex III, Section
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32 375 VII, Chapter 3, point D, point 3"* on products label.
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39 376 The Letter 4380-P1 stated that, if in presence of **infected** products, the operators prove they
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41 377 acted in good faith and in accordance with the law the theory of liability is not "perfectible"
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43 378 against them and therefore the Article 5 of Law 283/62 is not applicable. Before this CL, due
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45 379 to the numerous detections of *Anisakis* spp. in fishery products, the Regional Directorate of
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47 380 Public Health of Piedmont Region issued, in 2010, the Note No. 16294. This note established
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49 381 that retailers *"... have to communicate to the local competent authority the detection of viable
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51 382 larvae found during self-monitoring and selling"*.
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55 383 In 2011, in Europe the Reg. (EC) No. 1276/2011 was issued, which amended and
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57 384 expanded the Annex III of Regulation (EC) No. 853/2004. The FBOs were called to respect
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59 385 the requirements also when place on the market *"Fishery products derived from finfish and
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386 *cephalopod molluscs*" (in the [Reg. \(EC\) No. 853/2004](#) generically defined as "fish") at the
387 retail or catering level. This clarification was necessary, considering that cephalopods, as
388 already stressed above, may be infected by Anisakids. Moreover, according to this [Reg.](#), the
389 freezing treatment should be aimed at killing the "viable parasite" and not the "nematode
390 larvae", as defined by [Reg. \(EC\) No. 853/2004](#). Furthermore, for parasites other than
391 trematodes, the new provisions introduce the possibility to apply other time/temperature
392 combinations for the freezing treatment, such as -35° C for at least 15 hours.

393 According to the new provisions, the FBOs can place on the market fresh and not treated
394 fishery products only if they are intended for cooking or if they come from fishing area and/or
395 "Anisakis free" farms. [However](#), the FBOs must ensure that the fishing or aquaculture area of
396 origin are in conformity with the requirements of [Reg. \(EC\) No. 1276/2011](#), through a check
397 of the information provided by the accompanying documents. In fact, as previously stated by
398 the CL 4379-P, if the freezing treatment is carried out in the intermediate steps of the product
399 chain, the FBO supplier is obliged to provide to the buyer a certificate that states the freezing
400 method applied. In 2011, both the Piedmont and Puglia Regions, with the Note Circular [No.](#)
401 2998 and 8499, respectively, tried to clarify some aspects of application of [Reg. \(EC\) No.](#)
402 853/2004 regarding the sale and supply of fishery products. In particular, both notes pointed
403 out the role of the FBOs and their responsibility for visual inspection, to be carried out for
404 self-monitoring and during the sales activities, stressing the importance for the operators to
405 record all the non-conformances detected and the corrective actions taken.

406 In 2012, at the Italian national level, the Ministerial Note DGISAN 0024111-P drew
407 attention to the official control of parasitic infections, reaffirming that it represents an
408 effective measure for the risk management during the marketing and administration of fishery
409 products. Subsequently, the Italian Decree [No. 158/2012](#), known as "Balduzzi" Decree,
410 ordered that: *"The food business operator, who offers for sale to the final consumer fresh fish*
411 *and cephalopods as well as products of fresh water, is required to expose a clearly*

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412 *visible billboard bearing the information specified by the Minister of Health, after*
413 *consultation with the Minister of Agriculture and Forestry, concerning the correct conditions*
414 *of employment".* Recently, this provision has been further specified by the Decree of July 17,
415 2013 of the Italian Ministry of Health, which stated that, at the retail level, a billboard should
416 be displayed in a prominent position reporting: *"In case of consumption as raw, marinated or*
417 *not fully cooked, the product must first be frozen for at least 96 hours at - 18 ° C in a domestic*
418 *freezer marked with three or more stars".*

419 In order to facilitate understanding, the laws related to the standards considered in this
420 work are listed in chronological order in the Table 2.

421 **3. DISCUSSION**

422 The different geographical origin, species and size, method of capture, and post-capture
423 operations are among the main factors that could influence the chances of fishery products to
424 be contaminated by Anisakids larvae. On the other hand, good manufacturing practices and
425 HACCP programs, applied from the primary production to the administration and/or use by
426 the final consumer, are the two key factors to maximize the safety of fishery products,
427 reducing the *Anisakis* spp. risk to acceptable levels. **However, in our opinion, some points**
428 **should be pointed out to better understand the risk management of this zoonoses.**

429 **3.1 Preventive pre-capture measures.**

430 **The preventive measures that FBOs can apply before the capture are very limited and are**
431 **mainly related to farming. In fact, only sometimes there is the possibility to raise "Anisakis**
432 **free" fish, through the implementation of management procedures, validated by the**
433 **Competent Authority and aimed at controlling the environmental and ecological factors that**
434 **regulate the relationship between hosts and parasites (French Food Safety Agency, 2007).**

435 **One of the factor to be taken into account is the feeding plan.** As reported by **Reg. (EC)**
436 **No. 1276/2011, after the approval by the Competent Authority, fishery products raised**
437 **exclusively in free area and fed with a diet without parasites can be considered exempt from**

438 *Anisakis* spp. In particular, it is essential that the farmed fish are not fed with wet fee, but only
439 with pellet (U.S. Food and Drug Administration, 2013). The geographical location is another
440 important factor to be considered. In this context, epidemiological studies in marine areas of
441 interest, aimed at assessing the level of infection of the species are a prerequisite to the
442 installation of the farm.

443 Despite Peñalver *et al.* (2010) reported the absence of Anisakids larvae in sea bass, EFSA,
444 based on scientific data examined (Deardorff and Kent, 1989; Angot and Brasseur, 1993; and
445 Inoue, Oshima, Hirata, & Kimura, 2000; Lunestad, 2003; Wootten, Yoon, & Bron, 2009;
446 Lima dos Santos and Howgate, 2011), asserted that the risk of parasites can be considered
447 negligible only for Atlantic salmon (*Salmo salar*) and that the possibility that parasites may
448 also affect farmed fish grown in open sea must not be underestimated. In fact, it is possible
449 that invertebrates and fishes could enter into the cages and transfer the parasite to the species
450 farmed (Marty, 2008; Skov, Kania, Olsen, Lauridsen, & Buchmann, 2009). However, a recent
451 work of Mo *et al.* 2014, reported the presence of *A. simplex* and *H. aduncum* both in viscera
452 and in muscle of farmed Atlantic salmon (*Salmo salar*). However, the larvae have been only
453 found in runts not processed for human consumption. Therefore, even though these
454 observations should be considered in future risk analyses, currently, the risk of infection for
455 the Atlantic salmon farmed in floating cages or onshore tanks and fed with feed that does not
456 contain live parasites can be considered irrelevant (Marty, 2008; European Food Safety
457 Authority, 2010; Norwegian Food Safety Authority, 2011).

458 In Spain, a research project conducted during 2011-2012 showed that national aquaculture
459 products, such as sea bass, bream, turbot and Croaker are free of *Anisakis* spp. larvae
460 (Asociación Empresarial de Productores de Cultivos Marinos, (APROMAR), 2012). It is not
461 to be rejected the hypothesis that, in this Country, these species could be marketed without the
462 need to apply the preventive freezing treatment. In Italy, only the Region of Puglia has
463 indicated cases of exclusion from the obligation of freezing treatment, in relation to the local

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464 culinary habits and to the frequent consumption of raw or marinated fish products in its own
465 territory. The possibility to obtain "Anisakis free" products, would not only avoid the freezing
466 treatment, but also allow the sale of a fresh product, characterized by a better commercial
467 appeal.

468 However, considering that "Anisakis free" does not mean "parasite free", the presence of
469 some other kind of parasites, such as trematodes or cestodes, cannot be excluded a priori.

470 **3.2 Preventive post-capture measures**

471 **3.2.1 Fish handling on board.** Since no fishing area can be considered free from zoonotic
472 parasites (European Food Safety Authority, 2010), the only post-capture measure that can
473 reduce, or rather, not increase the spread of the parasitic infection is related to good
474 manufacturing practices on vessels. In particular, in order to break the cycle of the parasite, it
475 is crucial not to dispose the entrails into the sea. With this regard, the Reg. (EC) No.
476 1069/2009, "laying down health rules as regards animal by-products and derived products
477 not intended for human consumption...", clarifies specific measures for the disposal of
478 materials derived from evisceration of fish with signs of disease on board fishing vessels. It
479 follows that the fish parasitized by *Anisakis* spp. must necessarily be regarded as Category 1
480 material. In fact, the Article 8, paragraph a), makes clear reference to "wild animals, when
481 suspected of being infected with diseases communicable to humans or animals".

482 **3.2.2 Inspection control.** During inspection controls, the possible infection in fishery
483 products can be detected by non-destructive or destructive methods. The first, such as visual
484 inspection and candling, can be used for gutted and filleted products, while, in case of whole
485 fish, inspection of the viscera can be performed by opening the coelomic cavity. The
486 enzymatic digestion (destructive method), despite more sensitive in identifying the parasites,
487 is not considered an official method, as a consequence of the small number of samples that
488 can be processed and to the long time requested (Karl and Leinemann, 1997). The visual
489 inspection method does not suffice to detect all the larvae, but only 50% of the parasites

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490 detectable by other techniques, such as candling or enzymatic digestion (Llarena-Reino,
491 González, Vello, Outeiriño, & Pascual, 2012). The U.S. FDA considers candling as a
492 subjective technique (U.S. Food and Drug Administration, 2001), whose outcome can be
493 affected by many factors, such as: thickness of the fillet, oil content, presence of skin,
494 pigmentation, and training level and competence of the FBOs (Smith, 1999; Stormo,
495 Sivertsen, Heia, Nilsen, & Elvevoll, 2007; Milligan, 2008; Angelucci *et al.*, 2011). However,
496 candling is considered an official method by the *Codex Alimentarius* (Codex Alimentarius
497 Commission, 1995).

3.2.3 *Sampling plans*. Currently, no European or Italian legislation defines a statistically
significant sampling system to be used for the collection of samples during the inspection. In
this context, the sampling procedures for the visual search of the parasite often refer to the
internationally accepted protocols, such as those of the *Codex Alimentarius*. Specifically, the
Codex Alimentarius (Codex Alimentarius Commission, 1989) establishes that the presence of
two or more parasites per kg of sample unit with a capsular diameter greater than 3 mm, or the
detection of a parasite not encapsulated and greater than 10 mm in length, determine the non-
conformity of the product. In addition, based on the requirements of the *Codex* sampling plans
for prepackaged products (Codex Alimentarius Commission, 1969), Canada has set limits
stating that, for batches of 1 kg or more, the presence of 2 or more parasites per kg of sample
unit entails the rejection of the sample. In case of prepackaged products of less than 1 kg, the
rejection is due to the detection of 1 parasite per kg calculated on the total sample (Canada
Food Inspection Agency, 2013).

In Italy, some Regions have set up specific procedure to look for the *Anisakis* spp.
parasites in fishery products, especially focusing on anchovies. In 1994, the Lombardy Region
provided a statistical method to be used for the detection of *Anisakis* larvae, which is still
used. This protocol, developed for the veterinary inspector, but today also applicable by the
FBOs, provides criteria for sampling of a statistically significant number of specimens to be

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516 inspected, given a degree of sensitivity of 10% and confidence of 95%. From the total weight
517 of the lot it is possible to estimate the number of specimens that compose it, using a
518 conversion ratio, which differs depending on the species. Then, once the size of the lot has
519 been determined, and given a sensitivity of 5 or 10%, it is possible to determine the number of
520 subjects to be examined (Table 1). For the visual inspection, the head and viscera are removed
521 from the body (edible portion) and left at room temperature for at least 10 minutes. In this
522 way, the larvae start moving and become visible. The inspection procedure adopted by the
523 Liguria Region is very similar, but the sampling of the specimens must be performed on the
524 basis of the number of boxes (Circular Letter No. 1 of 1997). When the lot is composed of
525 less than 10 boxes, 3 specimens per box (up to 28) were to be sampled, while from 10 to 150
526 boxes 1 specimens per box (up to 28), and for more than 150 boxes, 1 specimen per box (up
527 to 40) (Tab3). A basic prerequisite, for a statistically representative sampling control, is that
528 the lot is uniform with regard to the specimen size and origin. Furthermore, the larger the size,
529 the greater the risk of infection.

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530 Therefore, among the criteria for identifying the lots to be sampled it could be considered:
531 fish species with higher *Anisakis*-related risk, geographical origin, commercial size and
532 destination. In addition, it would be appropriate to conform the criteria of sampling by:

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- ensuring the uniformity of the lot;
 - considering the number of crates, carrying out sampling evenly distributed throughout the lot, taking samples at several points;
 - collecting a statistically significant number of specimens;
 - examining the viscera removed from the cavity and the cavity itself after 30/40 minutes after their evisceration and exposure.

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539 The experience gained in decades of sampling shows that the degree of infection within a
540 lot is constant and this allows to state that the visual examination of the viscera has a value
541 statistically reliable for the purposes of a judgment. Therefore, it is possible to demonstrate a

1
2 542 directly proportional correlation between the number of specimens parasitized and the number
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4 543 of larvae detectable: in general, to a high number of parasitized subjects corresponds a high
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6 544 number of larvae per subject (and vice versa). Therefore, it is essential to identify the
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8 545 percentage of parasitized subjects that can be tolerated, or rather the percentage that is not
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10 546 perceptible to the observation of the consumer. For instance, on the basis of a confidence level
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12 547 of 95% associated with the sampling method, there is the possibility that in 5% of cases the
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14 548 10% of **infected** subjects (occult infection) may not be detected, a percentage of additional
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16 549 tolerance of 10% could be proposed. This means that, during the inspection, it could be
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18 550 tolerated the presence of up to a maximum of 3 parasitized subjects, up to a maximum of 3
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20 551 larvae each, when the sampling presupposes the collection of the 29 specimens (lot > 600
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22 552 specimens). For batches in which the percentage of parasitized individuals exceeds the level
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24 553 of tolerability, the FBOs should choose the destination depending on the species, size and
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26 554 final use.

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31 555 In the other Italian regions, the provisions laid down are more or less those **adopted by the**
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33 556 **Regions of Lombardy and Liguria**. Regarding Sardinia, the sampling is carried out in a
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35 557 different manner depending on the size of the species. For instance, for specimens weighing
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37 558 less than 20 grams, the procedure applied is the same as that applied in the Liguria Region for
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39 559 anchovies. For species weighing more than 20 grams, new criteria have been established
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41 560 (Table 3). Even in the Region of Marche, the Operating Unit (OU) for Fishing Products of the
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43 561 **LHA** has developed a procedure similar to that provided by the **Liguria Region** (Tab3). The
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45 562 procedure laid down by the Veneto Region provides that research for parasites must be
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47 563 carried out only on fish at risk if they are intended for cold processing and if not previously
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49 564 frozen (Tab 3). An interesting aspect to highlight is that, in case of non-compliance, the
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51 565 procedure requires that at least the three successive batches from the same supplier must be
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53 566 checked.

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567 The procedures that must be put in place after the visual inspection are different in the two
568 Regional laws. In this regard, it may be interesting to consider the weight of a hypothetical
569 sample of anchovies. Based on the data provided by the CL of the Liguria Region, we can
570 infer that a sample of 28-30 anchovies may have a weight ranging between 200 and 300
571 grams depending on the season. Therefore, the approach of the Lombardy Region seems to be
572 more in line with the provisions of the Codex than with those of the Liguria Region, even
573 though no distinction is made between encysted and free parasites. In fact, for a sample with a
574 weight less than 1 kg, the entire batch should be rejected following the discovery of a single
575 parasite. On the other hand, this procedure could be considered overly restrictive, whereas, at
576 present, only the batches obviously infected should be subjected to restrictions on marketing.
577 For this reason, the Veterinary Services of the Lombardy Region, and especially those present
578 at the wholesale fish market of Milan, have adopted an approach that, while bestowing greater
579 tolerance, allows to identify, at the same time, the lots manifestly contaminated.

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580 As for the other Regions, Sardinia provides that batches invaded by clearly visible
581 parasites should be seized and destroyed. In this regard, the Region of Marche states that the
582 destruction of the consignment is to be carried out only in case of massive infection and
583 repulsive appearance of the products, applying the same criteria adopted in Liguria (Table 3).
584 Unfortunately, the expression “massive infection” is not easily correlatable to “obviously
585 contaminated”, adopted by the European Commission to indicate a product not suitable for
586 human consumption.

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587 *3.2.4 Obviously contaminated.* The wording "obviously contaminated" introduced by
588 Council Reg. (EC) No. 853/2004 has been the topic of several discussions in Europe. In fact,
589 the European Commission has noted that the term "obviously" can be interpreted in different
590 ways within each Member State. This led the Commission to produce the draft document
591 SANCO/10137/2013-rev1. In this document, it is reported that a distinction should be made
592 based on the location of the visible parasite. If during the visual inspection larvae are found in

593 the edible part, such as the muscles, the product is considered to be obviously contaminated.

594 If the visible parasites are found only in the non-edible parts, such as viscera, the raw material
595 is not to be considered "obviously contaminated" and can be placed on the market.

596 Another concept closely related to the definition of "obviously contaminated" is those of
597 "visible parasite". On the basis of the definition given by the Decision 93/140/CE and by the
598 *Codex Alimentarius* (Codex Alimentarius Commission, 1971), the larvae must have a size that
599 make them detectable and must be clearly differentiable from the tissues of the fish, even in
600 the absence of optical instruments. In this regard, it must be underlined that the larvae of
601 *Hysterothylacium* spp. (0.7 mm -2 cm) (Køie, 1993; Borges, Cunha, Santos, Monteiro-Neto,
602 & Santos 2012) can often co-infect fish together with the larvae of *Anisakis* spp. (10-30 mm
603 in length and 0.44-0.54 mm in diameter) (Sakanari and McKerrow, 1989; Genchi and Pozio,
604 2003), from which are not easily distinguishable macroscopically. Thus, even though
605 *Hysterothylacium* spp. are only rarely responsible for diseases, they fall within the concept of
606 "visible parasite" and for this reason contribute to determine the total infection degree. On the
607 basis of these findings, when talking about nematode larvae, it would be more appropriate to
608 refer to visible larvae, including *Anisakis* spp. and *Hysterothylacium* spp., rather than about
609 Anisakids larvae.

610 *3.2.5 Processing that guarantee the inactivation of the parasites. At present, the freezing*
611 *treatment can be also done at the retail level, such as catering establishments, restaurants and*
612 *similar administration activities that have been registered under the current legislation.*

613 In Italy, if the FBOs want to directly perform the preventive treatment, they have to
614 provide the necessary guarantees of compliance and effectiveness (*Ministerial Circular Letter*
615 *4379-P-17/02/2011*). First, the FBOs, after setting up a written procedure aimed at controlling
616 the presence of parasites based on the principles of the HACCP system, must give a prior
617 communication to the Competent Authority concerning the preventive treatment to be
618 applied. Then, they are required to be equipped with an appropriate blast chiller, certified for

1 619 the reduction of the temperature at -20° C, which must be exclusively used to perform the
2 620 freezing treatment and not to preserve fish or other product. About the freezing method, the
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4 621 U.S. FDA stated that the effectiveness of the treatment is related to several factors, the most
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7 622 important of which are the type of parasite involved, the temperature and the duration of the
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10 623 treatment (U.S. Food and Drug Administration, 2013). Also, the time required to reach the
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12 624 intended core temperature and the percentage of fat of the fish can affect the preventive
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14 625 treatment. With regard to the type of parasite involved, it should be underlined that some
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17 626 cestodes, for instance, are more sensitive to freezing than nematodes, which, however, are less
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19 627 resistant than trematodes. According to the U.S. FDA ([U.S. Food and Drug Administration,](#)
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21 [2013](#)), in order to kill the nematode parasite, the product may be subjected to various types of
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24 629 preventive treatments, which provide different time/temperatures combination, such as:

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26 630 - Freezing at -20° C followed by a storage period of 7 days at - 20° C (or lower);
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29 631 - Freezing at -35° C (or lower) associated with a period of storage at -35° C (or lower) for
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31 632 15 hours;
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34 633 - Freezing at -35° C (or lower) and at -20° C (or lower) for 24 hours.

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36 634 The freezing, as preventive treatment, is a procedure expressly required by law and,
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39 635 according the provisions about the correct information to consumer, the data related to the
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41 636 process have to accompany the product up to the retail sale. However, this information is
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43 637 usually omitted, especially in catering and food service. This is because the defrosted fish
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46 638 creates mistrust among consumers, who consider it of second-rate compared to fresh fish,
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49 639 showing a reluctance to purchase and consume it. In this regard, the term "defrosted" should
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51 640 not appear on fishery and aquaculture products subjected to a preventive treatment for food
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53 641 safety and health purposes ([Regulation \(EC\) No. 404/2011](#)). Beyond this, it would be
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56 642 desirable that, at the retail level, the [FBOs](#) exposed an indication to clearly differentiate the
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58 643 product defrosted from the remaining fish retained. At the same time, it would be also
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2 644 desirable that not “Anisakis free” fish were distinguished by an indication, informing the
3 645 consumer that the product must be “consumed after cooking or after freezing”.

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5 646 While the European legislation does not deal with the problem of consumer’s information,
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7 647 in Italy, this issue has been considered many times (Decree of the President of the Regional
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9 648 Council No. 282/49671, Ministerial Circular Letter 4379-P-17/02/2011, "Balduzzi" Decree
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11 649 and Decree of July 17, 2013).

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14 650 Some EU countries, such as Spain and France, have specified the technical parameters of
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16 651 salting and pickling to kill the larvae of the parasite, thus excluding the preventive freezing of
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18 652 the products. In Spain, the Scientific Committee of AESAN, following the entry into force of
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20 653 the Royal Decree No. 1420/2006, asserted that freezing is not necessary for those fishery
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22 654 products that reach a concentration of NaCl above 9% for at least six weeks (Comité
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24 655 Científico de la Agencia Española de Seguridad Alimentaria y Nutrición, 2007), between 10
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26 656 and 20% for four/five weeks or more than 20% for at least three weeks. With regard to the
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28 657 salting, the French Food Safety Agency (Afssa), with the opinion No. 2007-SA-0379,
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30 658 reported that in traditional preparations and for small quantities salinity levels of 20% result in
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32 659 the inactivation of the parasite within 21 days, while concentrations of 15% require 28 days
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34 660 (French Food Safety Agency, 2007). Afssa also reported that, according to some authors, fish
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36 661 marinated with 10% acetic acid and 12% salt, maintained for 5 days at 4° C, are not
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38 662 hazardous to health as well as marinated products with 12% salt and 6% of acetic acid for 13
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40 663 days at 4° C. Finally, Afssa affirmed that the freezing treatment can also be replaced by either
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42 664 irradiation or treatments with high pressures. Unfortunately, the irradiation procedure
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44 665 requested to kill the parasite seems to induce changes in the organoleptic characteristic
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46 666 (Farkas, 1998). In addition, in the EU the use of ionizing radiation for fishery products is not
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48 667 approved by most of the Member States, including Italy (Directive 1999/3/EC and Notice No
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50 668 2009/C 283/02). The organoleptic quality of the products is also compromised by too high
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52 669 pressures (French Food Safety Agency, 2007 and U.S. Food and Drug Administration, 2013).
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670 Twenty-five years ago, this kind of alternative approaches were already implemented by
671 Germany (Ordinance of Federal Republic of Germany, 1988). In Italy an alternative technique
672 was never defined and from the very beginning it was established the unconditional
673 application of preventive treatment to all products to be consumed raw or almost raw.

674 *3.2.7 Training of personnel.* Considering the complexity related to the management of
675 zoonotic parasites, the training of FBOs is fundamental for a thorough understanding of the
676 issues associated to *Anisakis* spp.. However, this does not imply that the figure in charge for
677 carrying out checking of goods ought to be the FBO, since the *Anisakis* risk needs scientific
678 knowledge to be best managed. Therefore, it is essential that the FBOs are supported by
679 specific professionals, such as veterinarians, who can assist them during controls. The "know
680 how" of the veterinarian is also crucial to implement all the necessary preventive and
681 corrective measures and to define the inspective outcomes. In this regard, the Local
682 Veterinary Departments could consider the possibility that the responsible of large-scale retail
683 or wholesale fish markets, where large quantities of fishery products are handled, could be
684 supported in the management and control of parasites by a veterinarian, as the most suitable
685 competent figure.

686 **4. CONCLUSIONS**

687 The *Codex Alimentarius* defines food hygiene as "*all conditions and measures necessary*
688 *to ensure the safety and suitability of food at all stages of the food chain*". In this context,
689 food safety, defined as "*the assurance that food will not cause harm to the consumer when it*
690 *is prepared and/or eaten according to its intended use*", represents the priority aspect.
691 However, food has to be suitable for human consumption, in accordance to the purpose for
692 which it is intended, beyond its safety. The presence of Anisakids larvae in fishery products is
693 not only a public health issue, but it also determines a deterioration of the quality product
694 making it repugnant to the consumer. Therefore, on the one hand it is necessary to avoid that
695 products containing viable larvae can arrive to the consumer, on the other, it is also

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696 appropriate to implement corrective measures aimed at reducing the perception of the
697 problem by the consumers as recently occurred (Anonymus, 2013 and La Pira, 2013). In fact,
698 even though the larvae had lost their pathogenic power, the products were found not to be
699 suitable for sale, since repugnant. For this reason, and also in the view of current economic
700 conditions and limited fish stocks, it would be desirable that alternatives were considered by
701 the legislation in order to improve the management of the products affected by visible
702 parasites. For example, the medium/large fish could be eviscerated in an equipped
703 establishment, while those of small size could be exclusively marketed without viscera and
704 the retailers could carry out a deferred evisceration at the time of retail.

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Among the emerging critical issues there is also the need to implement harmonized policies during the inspections and controls: with regard to the sampling, the international laws do neither explain in detail how the visual inspection should be carried out nor specify a reference value to identify the "obviously contaminated" products. This uncertainty is largely due to the different perception/acceptance of the risk associated to Anisakidosis that characterizes each Countries, which is also reflected at the level of political bodies. Therefore, in Europe, there is an autonomy on measures to be taken, which are left to the Competent Authorities of each Member States. In Italy, beyond the inevitable local differences, the Competent Authorities have so far adopted a balanced approach between what is expected in terms of prevention by the European and Italian legislation and the need to protect, besides the public health, the production activities related to fishing and their economy. However, Anisakids do no longer involve a risk only for the lesions induced by the viable larvae, but also for the allergic reactions in sensitive subjects, which should be taken into consideration by establishing preventive measures, such as the reporting of Anisakids as allergenic in fishery products, as well as their inclusion in the list of all common allergens by the specific Community regulation (Directive 2003/89/EC).

1 721 Finally, more than 20 years after the CL No. 10 of the Italian Ministry of Health, in which
2 722 it was stated the importance of the veterinary inspector in the evaluation of an infected
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4 723 product, in a complex framework, such as that related to the *Anisakis* risk management, this
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7 724 professional figure is still essential, especially when a discriminating judgment is required,
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10 725 based on scientific knowledge and professional skill.

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NUMBER OF SPECIMENS OF THE LOT	NUMBER OF SPECIMENS TO USE FOR VISUAL INSPECTION	
	SENSIBILITY 10 %	SENSIBILITY 5 %
10	All	All
20	16	19
30	19	26
40 – 45	21	31
46 – 50	22	35
51 – 60	23	38
61 – 70	24	40
71 – 80	24	42
81 – 90	25	43
91 – 100	25	45
101 – 120	26	47
121 – 140	26	48
141 – 161	27	49
161 – 180	27	50
181 – 200	27	51
201 – 250	27	53
251 – 350	28	54
351 – 450	28	55
451 – 600	28	56
601 – 1200	29	57
1201 – 4000	29	58
> 4000	29	59

Table 1. Sampling protocol for the visual inspection to detect *Anisakis* larvae in fish batches, according to the Circular Letter VS8/C790/94 of the Lombardy region.

STANDARD OF REFERENCE	DISPOSITIONS
<i>Law n. 293 of 30 April 1962</i>	<ul style="list-style-type: none"> • Prohibition of use in the preparation of food or drink, sell, hold to sell, administer or otherwise distribute for consumption foodstuffs soiled, invaded by parasites, altered or harmful.
<i>Ordinance of 8 August 1988 of German Federal Republic</i>	<ul style="list-style-type: none"> • Removing parts containing nematodes (dead or alive) and their marketing ban. • Official procedures for frozen, cooked, salted or pickled products.
<i>Italian Ministry of Health Circular Letter No. 10 of 11 March 1992</i>	<ul style="list-style-type: none"> • Prompt evisceration of fishery products at risk of infestation, longer than 18 cm. • The official veterinarian must perform a check of the fish, through random sampling and visual examination, with the opening of the coelomic cavity. • Freezing treatment at -20 ° C for at least 24h
<i>Italian Ministry of Health Ministerial Decree of 12 May 1992</i>	<ul style="list-style-type: none"> • The treatment for killing parasites must be certified.
<i>Legislative Decree No. 531 of 30 December 1992</i>	<ul style="list-style-type: none"> • Obligation to carry out the visual inspection with the purpose of visible parasites detection, prior to the placement on the market of fishery products.
<i>Decision 93/140/EEC of 19 January 1993</i>	<ul style="list-style-type: none"> • Definition of "<i>visible parasite</i>" and "<i>visual inspection</i>".
<i>Region of Lombardy Regional Council prot. No. 790/94</i>	<ul style="list-style-type: none"> • Establishment of sampling procedures for <i>Anisakis</i> spp
<i>Council Regulation (EC) No. 2406/96 of 26 November 1996</i>	<ul style="list-style-type: none"> • Negative impact of parasites on the product quality.
<i>Region of Liguria Decree of the President of the Regional Council n. 282/49671 of 2</i>	<ul style="list-style-type: none"> • Display of an informative billboard at retail outlets (including points of direct sale from the fisherman to the consumer or retailer) where are marketed and/or sold raw

<i>May 1997</i>	anchovies.
<i>Region of Liguria</i> <i>Circular Letter No. 1/97 of 24 March 1997</i>	<ul style="list-style-type: none"> • Detailing on sampling procedures for <i>Anisakis</i> spp
<i>Regulation (EC) No. 852/2004 of the European Parliament and of the Council of 29 April 2004</i>	
<i>Regulation (EC) No. 853/2004 of the European Parliament and of the Council of 29 April 2004</i>	<ul style="list-style-type: none"> • The FBOs must ensure that fishery products are subjected to a visual inspection for the detection of visible endoparasites, before placing on the market. • FBOs must not place on the market, for human consumption, fishery products that are obviously contaminated with parasites. • Prohibition to trade products obviously contaminated with parasites • Obligation of the freezing treatment at -20 ° C (core temperature) for at least 24 hours, to kill the "nematode larvae."
<i>Commission Regulation (EC) No. 2074/2005 of 5 December 2005</i>	<ul style="list-style-type: none"> • Clarification about the “visible parasite” definition and on how to conduct the visual inspection.
<i>Spanish Ministry of Health and Consumer Affair</i> <i>Real Decreto No. 1420/2006</i>	<ul style="list-style-type: none"> • Obligation for bars, restaurants, cafes, hotel, hospitals, schools, residences, canteens and schools, catering services and similar, to perform a freezing treatment of the fish, to be consumed raw or almost raw, cold-smoked, marinated or salted.
<i>Italian Ministry of Health</i> <i>DGSAN 10776-P-26/05/2008</i>	<ul style="list-style-type: none"> • The alert system for the detection of <i>Anisakis</i> in fishery products from Third Countries, should only be activated upon detection of viable larvae of <i>Anisakis</i> in the coelomic cavity or in the flesh of fresh fish products

<p><i>Commission Regulation (EC) No. 1020/2008 of 17 October 2008</i></p>	<ul style="list-style-type: none"> • The visual inspection becomes mandatory for all the FBOs, including those that operate at the retail level.
<p><i>Region of Piemont</i> <i>Directorate of Public Health Note No. 16294 of May 25, 2010</i></p>	<ul style="list-style-type: none"> • Obligation for retailers to communicate to the local competent authority the detection of larvae found alive and vital during self-monitoring procedures and during sale.
<p><i>Italian Ministry of Health</i> <i>DGSAN 4379-P-17/02/2011</i></p>	<ul style="list-style-type: none"> • The preventive treatment carried out must be declared in the operators' self-monitoring plan. • The fishery products, that have been subjected to the treatment, should be always accompanied, up to the placing on the market, by a declaration of the operator responsible of the treatment. • Lettering for products treated "<i>Conforms to Regulation EC no. 853/20054, Annex III, Section VII, Chapter 3, part D, paragraph 3</i>".
<p><i>Italian Ministry of Health</i> <i>DGSAN 4380-P-17/02/2011</i></p>	<ul style="list-style-type: none"> • Where in the presence of infested products, the operator proves that he acted in good faith and in accordance with the law, implementing all the possible measures to detect parasites, it is not "perfectible" the theory of liability and therefore art. 5 of 283 in '62 is not applicable
<p><i>Region of Puglia</i> <i>Note Prot. AOO152/1 MAR 2011/2998</i></p>	<ul style="list-style-type: none"> • Recommendations about the timely fulfillment of the Ministerial Circular no. 4379-P and 4380-P in the regional territory. • Clarifying the role of the FBOs and its responsibility for the detection of larvae alive and vital during self-monitoring and during sale.
<p><i>Region of Piemonte</i></p>	

<i>Note Prot. No. 8499/DB2002 014.140.20</i>	
<i>Commission Regulation (EU) No. 1276/2011</i>	<ul style="list-style-type: none"> • Anisakis risk extended to cephalopods. • The preventive treatments must be targeted to the killing of the "viable parasite". • Possibility to perform the freezing treatment even at -35 ° C for at least 15 hours.
<i>Italian Ministry of Health</i> <i>DGISAN 0024111-P-05/07/2012</i>	<ul style="list-style-type: none"> • Importance of official control as an effective measure for managing the health risk of parasites, especially during administration and retail.
<i>“Balduzzi Decree”</i> <i>Decree Law No. 158 September 13, 2012</i>	<ul style="list-style-type: none"> • The FBOs are required to display a visible billboard with the relevant information concerning the correct operating conditions.
<i>Region of Puglia</i> <i>Regional Council Resolution prot. 1675/2013</i>	<ul style="list-style-type: none"> • Establishment of: <ul style="list-style-type: none"> - Technical Working Group "<i>Prevention and surveillance of Anisakidosis associated to fishery products</i>"; - Program "<i>Monitoring and control System for human diseases caused by Anisakis spp. in Puglia</i>"; - Allergology Regional Network for the surveillance of the Anisakidosis
<i>Italian Ministry of Health</i> <i>Decree 17 July 2013</i>	<ul style="list-style-type: none"> • At the point of sale of fishery products a sign must be displayed in a prominent position with the following information: "In the case of consumption as raw, marinated or not fully cooked, the product must first be frozen for at least 96 hours at - 18 ° C in domestic freezer marked with three or more stars. "

Table 2. Chronological evolution of the European and Italian legislation, with regard to risk management of Anisakis (in white: Italian and Regional disposition; gray: European dispositions; light gray: others Member States' dispositions)

ITALIAN REGION	NORMATIVE REFERENCE	FISH SPECIES AT RISK	DISPOSITIONS	OUTCOMES OF THE VISUAL INSPECTION
Lombardy	Regional Council Circular Sector Health and Hygiene Veterinary Service Protocol No. 201145 / G and N. 47193 of July 20, 1994	Anchovy Sardine Mackerel Melva Bonito/Skipjack Skipjack /Tunny Lanzardo Hake Whiting/Melu Whiting/ Pier Pout /Chaplain Mullet Suro	The sampling must be carried out only for species at risk. <ul style="list-style-type: none"> • Determine the total number of subjects that compose the lot *; • From the total number of subjects that compose the lot, is obtained the statistically significant number of samples and the percentage of desired sensitivity (5% or 10%) ^. Then the specimens are subjected to visual inspection through the evisceration: <ul style="list-style-type: none"> • Check for the presence of the parasite moving the viscera. 	If only one specimen is found parasitized, the whole lot must be withdrawn.
Liguria	Regional Circular Letter 1/97	Anchovy	1) Visual inspection of the lot to detect migrant parasites on the external surface of the fish; 2) Collection of specimens in a representative number, according to the number of box, composing the lot: <ul style="list-style-type: none"> • < 10 boxes: 3 specimens (MAX 28); • 10 to 150 boxes: 1 specimens per box (Max 28); • > 150 boxes: 1 specimens per box (Max 40). 3) Opening of the coelomic cavity.	Positive health inspection The lot is withdrawn from the market or destroyed or intended for other uses than human consumption. Visual Inspection <ul style="list-style-type: none"> • Detection of numerous larvae and repulsive appearance of the product: the lot must be withdrawn from the market . • Lot intended for consumption if the n. larvae per anchovy is ≤ 3, up to a maximum of 10% of the specimens examined; • Lot intended to preventive treatment if are detected: <ul style="list-style-type: none"> -3 larvae per anchovy in the 10% of specimens examined; - the infested specimens are <10% of the specimens sampled.
Lazio	Internal procedure of the Local Health Authority		See Liguria Region	
Emilia Romagna	Self-cmonitoring Procedure	Anchovies Sardines Mackerel Herring Mulletts	Sampling Method From each lot of the species at risk, according to the number of boxes that composes it, are sampled: <ul style="list-style-type: none"> • From 1 to 6 boxes: select at random a total of 10 specimens; 	

		Hake Blue Whiting Scabbardfish Suro	<ul style="list-style-type: none"> • From 7 to 12 boxes: take 1 or 2 specimens per box (minimum of 10 specimens); • Boxes > 12: select at random at least 12 specimens from 12 different boxes. 	
Sardinia	Internal procedure of the Local Health Authority	Fish Sabre Horse Mackerel Blue Whiting Mackerel Hake Mulletts Anchovies	<p>The sampling and the visual inspection are performed for all species, not just those at risk.</p> <p>Fish size of less than 20 grams:</p> <ul style="list-style-type: none"> • 3 specimens per box up to a maximum of 10 boxes; • 1 specimen from 30 boxes, for lot of 150 boxes; • 1 specimen from 40 boxes, for lot of above 150 boxes. <p>Fish size greater than 20 grams:</p> <ul style="list-style-type: none"> • 1 copy per cassette for a maximum of 10 cassettes • 1 specimen per 15 boxes for batches up to 150 cassettes • 1 specimen per 20 boxes for batches of more than 150 boxes. 	<p>In case of detection of fishery products obviously contaminated, seizure and destruction of the consignment.</p> <p>Condition for which the parasites is obviously contaminated is that the parasite is visible and that for size, color or texture is clearly distinguishable from fish tissues (Reg. 854/2004).</p>
Marche	Internal procedure of the Local Health Authority	Suro Blue Whiting/Whiting Scabbardfish Hake Chaplain/Pout Mackerel Lanzardo Anchovy Sardine Mullet Bonito Squid	<p>Determination of a representative sample:</p> <ul style="list-style-type: none"> • For lot up to 100Kg 3 specimens per box (minimum 28) are sampled • For lot from 110 to 1500Kg 1 specimen per box (minimum 28) is sampled • For lot above 1500kg is sampled 1 specimen per box (minimum 40). 	<p><u>Threshold of acceptability for the products</u></p> <p>For Anchovies:</p> <ul style="list-style-type: none"> • In case of massive infestation that results in a repulsive appearance of the product, this must be destroyed; • If > 3 parasites/specimen are found in > 10% of the specimens, the lot is sent to preventive treatment; • If <3 parasites/specimen are found in <10% of specimens, the lot is suitable for human consumption. <p>For all other species (including those at risk):</p> <ul style="list-style-type: none"> • In case of massive infestation that results in a repulsive appearance of the product, this must be destroyed; • In case of non-massive infestation, the products are subjected to preventive treatment.
Veneto	Self-monitoring Procedure	Herring Sardines Anchovies Sprat Mackerel Lanzardo Suro Hake	<p>The search for Anisakis spp larvae is performed by sampling the seafood at risk during the exposure, the storage and/or prior to sale, on all specimens intended for cold processing (pickling, smoking, salting) and that have not previously subjected to freezing treatment.</p> <p>The search shall be carried out on a sample of at least:</p> <ul style="list-style-type: none"> • 3 specimens, in case of large species (blue whiting- 	<p>Presence of superficial parasites/modest infestation: presence of parasites in small quantities (max 3 non-viable larvae)</p> <ul style="list-style-type: none"> • Freezing treatment (-20 ° C for 24 hours); • Identification and seizure of the lot and subsequent waste disposal. <p>Presence of deep parasites/invasive infestation:</p>

		Blue Whiting/Whiting Scabbardfish Monkfish St. Peter's Fish Other species, object of specific controls	whiting, hake, monkfish, St. Peter's fish, Spanish mackerel, scabbard fish) and for lot <50kg <ul style="list-style-type: none"> • 5 specimens in the case of lots between 50 - 500 kg; • 10 specimens in the case of lots > 500 kg; • 150 specimens for small species (anchovies, sardines). 	presence of parasites in large quantities (greater than 3 larvae) found on the surface that even deep in the viscera and penetration into the muscle <ul style="list-style-type: none"> • Preventive treatment before putting it on sale; • Identification and seizure of the lot and subsequent waste disposal with activation of the procedure for recall-retirement. <p>The detection of Anisakis larvae just in a single specimen inspected (3, 5, 10 depending on the size) determines the non-compliance and therefore the preventive treatment must be performed as described above.</p>
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Table 3 - Sampling protocols (and related outcomes) adopted in some of the Italian regions to control the Anisakis' risk.

* Based on the total weight of the lot, in relation to the average weight of the species or to a conversion index per species, provided in the Circular Letter

^ Based on the table attached to the Circular Letter (for lot with specimens > 600, up to a maximum of 29 specimens are sampled)