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Review

# Human health, legislative and socioeconomic issues caused by the fish-borne zoonotic parasite *Anisakis*: Challenges in risk assessment

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ABSTRACT

*Background:* Nematodes of the genus *Anisakis* parasitize many commercial fish species and are responsible for a fish-borne zoonosis (anisakiasis) and allergic reactions. *Anisakis* can also cause consumer distrust in fishery products and economic losses to the fish industry.

*Scope and approach:* We review current socioeconomic, legislative, risk management and human health problems caused by the occurrence of *Anisakis* in fishery products and discuss possible strategies to mitigate them.

*Key findings and conclusions:* Visual inspection (and candling) of fishery products as required by EU legislation is not efficient for parasite detection. Consequently, visible (and non-visible) *Anisakis* reach the market and may be detected (and eaten) by consumers. Marine fish appears to be the only industrial food product that is at high risk of containing parasites when placed on the market.

Anisakiasis and allergy to *Anisakis* are hidden, underestimated emerging zoonoses worldwide. There is a need to better understand the impact of these zoonoses on individual health and particularly exposed human populations, and to assess the risk posed by *Anisakis* allergens in fishery products. Quantitative risk assessment (QRA) is identified as an appropriate methodology as it estimates the risk from fishing ground to human disease.

Improvements in parasite control legislation and procedures (e.g. establishment of research-based and standardized parasite detection methodologies, appropriate sampling strategies, development of non-destructive methods for detection and removal of nematodes from fish products), suitable for use by seafood businesses, are recommended to improve protection of consumers and to protect the industry by minimizing *Anisakis*-associated economic losses. QRA may help to provide the scientific basis for improved food safety legislation and strategies to reduce the risk of anisakiasis/allergy in humans.

## 1. Introduction

Some nematodes of the family Anisakidae (Nematoda: Ascaridoidea), particularly species of the genera *Anisakis*, *Pseudoterranova* and *Contracaecum*, are parasites of a wide range of aquatic organisms and have indirect life cycles in the aquatic (mainly marine) environment (Mattiucci & Nascetti, 2008). These anisakids (especially *Anisakis* spp.) are of medical and economic concern worldwide since they are responsible for 1) a fish-borne zoonosis known as anisakidosis (anisakiasis when caused by *Anisakis* spp.) and allergic reactions, as well as 2) economic losses to the fishing industry due to reduced marketability of fishery products (Audicana & Kennedy, 2008; Buchmann & Mehrdana, 2016; EFSA-BIOHAZ, 2010).

This review focusses on the fish-borne zoonotic parasite *Anisakis* spp. and associated socioeconomic, legislative, risk management and human health issues, and discusses possible mitigation strategies.

### 1.1. *Anisakis* spp. biologic aspects

*Anisakis* spp. have complex heteroxenous life cycles comprising five stages and four moults (Smith & Wootten, 1978). Cetaceans are the

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definitive hosts where *Anisakis* spp. fourth stage larvae (L4) and adults can often be found forming clusters of worms embedded within a single lesion in the mucosa and submucosa of the stomach. A cetacean stomach is divided in three compartments (forestomach, main or fundic stomach and pyloric stomach) (Smith & Wootten, 1978; Ugland, Strømnes, Berland, & Aspholm, 2004; Young, 1972) and it appears that gastric lesions mainly occur in the forestomach and less frequently in the main stomach, while larval stages can also be found free in all three stomach compartments, causing no obvious damage to the gastric wall (Hrabar, Bocina, Kurilj, Duras, & Mladineo, 2017). *Anisakis* spp. eggs are released with the host's faeces into the marine environment (Young, 1972) where they hatch. Apparently, larvae in the third stage (L3) emerge from the egg, after having completed two moults within the egg prior to hatching (Højgaard, 1999; Køie, Berland, & Burt, 1995). The free-swimming L3 larva may be ingested by planktonic or semi-planktonic crustaceans (mainly euphausiaceans and copepods), which become the intermediate hosts (Gregori, Roura, Abollo, González, & Pascual, 2015; Klimpel, Palm, Rückert, & Piatkowski, 2004; Smith, 1983b). Infected crustaceans are generally believed to be directly infective to final hosts (e.g. baleen whales), when eaten by these cetaceans, in which the L3 grows and moults to reach the fourth larval stage and then adulthood, thereby closing the life cycle (Klimpel et al., 2004; Mattiucci & Nascetti, 2008; Smith, 1983a).

In the marine food chain, infected crustaceans may be eaten by small fish and cephalopods which thus become second intermediate or paratenic hosts which, in turn, may be predated upon and so transfer the larvae to larger piscivorous fish which also serve as paratenic hosts (Klimpel et al., 2004; Levsen & Berland, 2011). The ability of the larvae to reinfect fish through the food chain together with their long life expectancy in fish hosts (they can survive for at least two years in Atlantic cod (Hemmingsen, Lysne, Eidnes, & Skorping, 1993) or three years in Atlantic herring (Smith, 1984)) may enable accumulations of hundreds to thousands of *Anisakis* spp. larvae in large and old predatory fish feeding extensively on infected intermediate and paratenic crustacean/fish hosts (Berland, 2006; EFSA-BIOHAZ, 2010; Levsen & Berland, 2011). Many fish and cephalopod species have been identified as intermediate or paratenic hosts for *Anisakis* spp. (Mattiucci, Cipriani, Levsen, Paoletti, & Nascetti, 2018). Indeed, the European Food Safety Authority (EFSA) Panel on Biological Hazards (BIOHAZ) concluded that "*For wild-catch fish, no sea fishing grounds can be considered free of* A. simplex" (EFSA-BIOHAZ, 2010). The use of fish and cephalopod intermediate and/or paratenic hosts facilitates the dispersion of *Anisakis* spp. larvae in the ocean and their successful transmission to piscivorous and teuthophagous final hosts (i.e. toothed and some baleen whales), so completing the life cycle (Abollo, Gestal, & Pascual, 2001).

To date, nine species of *Anisakis* have been confirmed using genetic and/or biochemical methods, i.e. *A. simplex sensu stricto*, *A. pegreffii*, *A. berlandi*, *A. ziphidarum*, *A. nascettii*, *A. paggiae*, *A. physeteris*, *A. brevispiculata* and *A. typica* (Mattiucci, Cipriani, Paoletti, Levsen, & Nascetti, 2017; Mattiucci et al., 2018). Morphological identification of *Anisakis* spp. to species level is difficult and sometimes impossible (i.e. not all species can be identified in this way), especially for the larval stage occurring in fish (those infective for humans), which is mainly based on the shape and length of the ventriculus and the presence/absence of a caudal spine (mucron). The former morphological characters, the presence/absence of a boring tooth in the anterior body of the nematode, and the relative distance from the boring tooth to the excretory pore, are also used to distinguish between *Anisakis* spp. developmental stages and the other frequently occurring ascaridoid nematode species present in fish, i.e. *Pseudoterranova* spp., *Contracaecum* spp. and *Hysterothylacium* spp. (Berland, 1961, 1989; Levsen & Berland, 2011; Mattiucci et al., 2018). Thus, while accurate identification of larval *Anisakis* spp. is essential for understanding their epidemiological significance, since they are the causative agents of human anisakiasis, this can currently only be reliably achieved by molecular methods (Mattiucci, Cipriani, et al., 2017; Mattiucci et al., 2018).

Humans may become accidentally infected with *Anisakis* spp. through consumption of parasitized raw or lightly cooked fish and fishery products containing viable larvae. However, humans are not part of its natural life cycle, and the parasite cannot develop further and eventually dies (Audicana & Kennedy, 2008; EFSA-BIOHAZ, 2010). As obvious as it may sound, this fact is the key to solving food safety and product quality issues related to *Anisakis* spp. infections in fish.

## 2. The problem of *Anisakis* spp. in the fish industry – socioeconomic, legislative and parasite control issues

### 2.1. Socioeconomic aspects

The presence of *Anisakis* spp. larvae compromises the quality and safety of fishery products, representing a cause for concern for consumers, official control authorities and seafood businesses (SB) in the fishery value chain (D'Amico et al., 2014). Indeed, the appearance of the parasite in the viscera and muscle of fish can cause rejection of fishery products by fish sellers and consumers, notable negative effects on fish marketability and an increasing lack of confidence in fishery products by consumers (Abollo et al., 2001; D'Amico et al., 2014; Karl, 2008; Levsen, Lunestad, & Berland, 2005; Llarena-Reino, Abollo, & Pascual, 2013; Llarena-Reino, Abollo, Regueira, Rodríguez, & Pascual, 2015), which may result in economic losses for the industry and loss of fishery jobs, as observed in Germany during the "nematode crisis" in 1987 (see Karl (2008)). The coverage of the "*Anisakis* problem" by the media may also generate social alarm, exacerbating distrust in consumers and monetary losses to the industry (Abollo et al., 2001; Karl, 2008).

Fishery products derived from many commercially important wild marine fish appear to be the only industrially processed food with a high risk of containing parasites when put on the market. Inspection procedures to control and remove visible parasites carried out by the industry evidently introduce additional costs to commercial processing (Abollo et al., 2001; Llarena-Reino et al., 2015; Sivertsen, Heia, Hindberg, & Godtliebsen, 2012) but, as illustrated above, the alternative is worse for both consumers and industry.

A survey-based contingent valuation study was recently performed to investigate the attitudes and opinions of fish consumers in Spain about the presence of *Anisakis* spp. in fish, associated diseases (i.e. anisakiasis and allergy), and how much they would value the removal of *Anisakis* spp. from fishery products (Bao et al., 2018). Contingent valuation is an economic methodology for estimating the value that an individual places on a product that is not currently transacted in the market, such as (guaranteed) "*Anisakis* spp.-free" fishery products. Results from online questionnaires indicated that a majority of consumers (77%) were willing to pay more for "*Anisakis*-free" fish product, and that over a quarter of consumers (>25%) had previously avoided purchasing and/or consuming fishery products due to the presence of *Anisakis* spp. Moreover, almost one third of consumers (29%) interviewed said that they would always avoid purchase/consumption of fish products in the future, while 31% would do so if there were a high chance of *Anisakis* ssp. presence (Bao et al., 2018). These findings support the premise that *Anisakis* spp. is an important health and aesthetic issue for consumers, highlighting the potential monetary losses for the industry, and imply that currently parasite control through the fish value chain is not perceived to be efficient.

### 2.2. Current parasite control by seafood businesses: legislative and risk management issues

The problem caused by the presence of *Anisakis* spp. in fishery products has been recently examined in the European context, with particular focus on the risk management issues that are of interest for SB in the fish value chain in Italy (D'Amico et al., 2014) and Spain (Llarena-Reino, Abollo, et al., 2013; Llarena-Reino et al., 2015). Under the "European Hygiene Package" (EC, 1991, 1993, 1996; 2004a; 2004b;

2004c, 2005) and its modifications (EC, 2006b; 2006a), SB at all stages of the fishery production chain (on shore and on board vessels) are responsible for ensuring consumer protection with regard to food safety, and they must ensure that fishery products have been subjected to visual examination to detect visible parasites, and that no obviously contaminated fish products reach the consumer (EC, 2004b). Commission Regulation (EC) No. 2074/2005 specifies that "*Visible parasite means a parasite or group of parasites which has a dimension, colour or texture which is clearly distinguishable from fish tissues*", that "*Visual inspection means non-destructive examination of fish or fishery products with or without optical means of magnifying and under good light conditions for human vision, including, if necessary, candling*", and that "*Candling means, in respect of flat fish or fish fillets, holding up fish to a light in a darkened room to detect parasites*" (EC, 2005). The use of confusing terminology that SB find hard to interpret, such as "visible parasites" and "obviously infected with parasites", and the lack of standardized and efficient methods and procedures for parasite detection during fish inspections represent a source of uncertainty, as parasite control is a critical control point in the SB food safety management system (D'Amico et al., 2014; Llarena-Reino, Piñeiro, et al., 2013; Llarena-Reino, Abollo, et al., 2013; Llarena-Reino et al., 2015; Llarena-Reino, González, Vello, Outeiriño, & Pascual, 2012).

Visual inspection of liver, gonads and visceral cavity of eviscerated fish, and of fish fillets (where necessary, candling must be included in the sampling plan) are required by legislation (EC, 2005). In addition, Regulation EC No 853/2004 requires that all parts of fishery products intended to be consumed raw, almost raw, marinated, salted or cold-smoked must be frozen at −20 °C or less, for at least 24 h (EC, 2004b)). Commission Regulation (EC) No. 1276/2011 states that SB do not need to carry out the above-mentioned freezing procedures for products derived from farmed fish and intended to be consumed raw or lightly processed, if they are cultured from embryos and have been fed exclusively on a diet that cannot contain viable parasites. However, SB must verify that either fish have been exclusively reared in an environment that is free from viable parasites or that these fishery products do not represent a health hazard with regard to the presence of viable parasites (EU, 2011).

In addition to EU regulations, the Codex Alimentarius of the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO), through its International Food Standards, guidelines and codes of practice, also contributes to ensure the safety and quality of fishery products and, although not legally binding, these are internationally accepted recommendations (Codex Alimentarius, 1989a; 1989b, 1995, 2004, 2013) with which SB normally comply (Llarena-Reino, Abollo, et al., 2013). These standards set out the procedures for detection of parasites in certain fishery products and criteria to identify fish lots that should be rejected as defective. The standard for quick frozen fish fillets recommends the candling method for the detection of parasites in skinless fillets, and that a sample unit shall be considered as defective when the presence of two or more parasites with a capsular diameter greater than 3 mm or a parasite not encapsulated and greater than 10 mm in length, per kg of sample unit, is detected (Codex Alimentarius, 1995). The standards for salted herring and sprat and for smoked fish recommend that the flesh shall not contain living nematodes, the viability of which shall be examined by means of artificial peptic digestion (i.e. nematodes survive the digestion process) (Codex Alimentarius, 2004; 2013). If living nematodes are present, freezing procedures to kill the nematodes must be applied prior to placing the product on the market for consumption. Salted fish should be examined for visible parasites by normal visual inspection (Codex Alimentarius, 2004) and smoked fish by candling (Codex Alimentarius, 2013), and the sample shall be considered defective as explained above (see Codex Alimentarius (1995)) for salted fish, and if readily visible parasites are detected in the sample for smoked fish.

The visual inspection methods specified by EU legislation and FAO guidelines, in practice, are not efficient for detection or quantitative determination of parasites in fish. In fish processing plants, the fast non-destructive candling method is commonly applied to detect and remove visible nematodes from fish fillets but it has low detection efficiency. Hence, only a few superficial *Anisakis* spp. larvae may be detected in skinless fillets (EFSA-BIOHAZ, 2010; Karl & Leinemann, 1993; Levsen et al., 2005) and the procedures applied by the industry cannot completely guarantee the absence of *Anisakis* spp. larvae in fishery products reaching the market (Levsen & Lunestad, 2010; Llarena-Reino et al., 2012). In addition, Llarena-Reino et al. (2012) indicated the low efficiency of visual inspection of visceral nematodes as a predictor of the number of parasites in flesh.

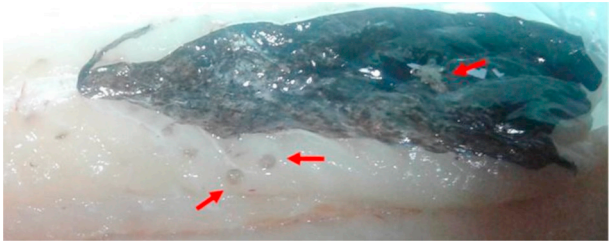

**Fig. 1.** Ventral fillet of European hake purchased in a supermarket (Aberdeen, Scotland) clearly parasitized by *Anisakis* spp.

The previously mentioned results of the contingent valuation study (Bao et al., 2018) which revealed that more than a quarter (27%) of respondents (among Spanish fish consumers) had avoided purchasing or consuming fish due to the presence of *Anisakis* spp. in fishery products, emphasises the inefficiency of the current risk management procedures carried out by SB, and makes clear that fish contaminated with visible *Anisakis* spp. are reaching the market (Fig. 1). Thus, the development of efficient, low cost and fast technologies for nematode detection in fish and fishery products should be of great interest to the fishing industry and seafood businesses, regulatory authorities and consumers.

### 2.3. How to improve the control and management of risk posed by fish parasites?

Llarena-Reino, Abollo, et al. (2013) proposed a scoring system approach to categorize parasite infection in fish lots, therefore, helping the SB to use a standardized protocol to ensure food quality and safety during parasite control. The scoring system ranked the parasite risk from 0 to 10 (from high to lower risk) depending on four criteria: site of infection (e.g. visceral body cavity, hypaxial and epaxial flesh), quality (i.e. visual parasite problems), demography of infection (density of parasites per kg of flesh (determined by a combination of visual followed by peptic digestion methods)) and epidemiology relevance (i.e. zoonotic or non-zoonotic parasite). In terms of demography of infection, samples were divided into three groups, D0: density >5 parasites/kg, D1: density 2–5 parasites/kg, D2: density <2 parasites/kg.

Rodríguez, Abollo, González, and Pascual (2018) developed further this scoring system into a new Fish Parasite Rating (FPR) score which allows the classification of fish lots into five categories of risk based on *Anisakis* spp. exposure (from "poor" (0–3 marks) to "excellent" (10 marks, "parasite free" fish)). They compared three approaches to identify fish with any visible parasites (even if there was only one parasite), which should be rejected under EU legislation (i.e. EC (2004b)): 1) the FPR score, 2) the visual inspection currently applied by SB and 3) the UV press and artificial peptic methods, which are considered 100% accurate. They concluded that the UV-press/peptic digestion method had the highest rejection rate of fish lots, as expected, followed by visual inspection, which penalized fish samples with high viscera infection but failed to reject samples with high infection in flesh. The FPR standard had the lowest rejection rate, even though it would reject some samples with high flesh infection that would not have been

rejected by the visual inspection method. The FPR standard would probably achieve better levels of food quality compared to the currently applied visual parasite screening, but further work is required to also ensure food safety of fishery products.

Further work is required to determine what level of parasite exposure (i.e. number of parasites and/or their allergens per kg of fish flesh) would represent 1) acceptable, 2) tolerable and 3) unacceptable health risks for human consumption (see section 5.3).

Thus, improvements in the parasite control legislation of fishery products are recommended in order to establish standardized and research-based (e.g. dose-response relationship modelling) parasite detection procedures, which can then be implemented by SB in the fishery chain to protect consumers by ensuring the quality and safety of fishery products, as well as to protect the fishing and food industries by minimizing the parasite-associated economic losses (see future work section).

## 3. Anisakiasis and its incidence in the human population – an underestimated zoonosis globally

Human anisakiasis is a fish-borne zoonotic disease caused by members of the genus *Anisakis* (Audicana & Kennedy, 2008). To date, the only *Anisakis* species genetically confirmed as aetiological agents of human anisakiasis are *A. simplex* s.s. and *A. pegreffii* (Mattiucci et al., 2018). The transmission of *Anisakis* spp. to humans is typically associated with the consumption of parasitized raw or undercooked fish meals, such as traditional anchovies in vinegar consumed in Spain and Japanese sushi and sashimi (Audicana & Kennedy, 2008). *Anisakis* spp. infection can cause mild to severe gastrointestinal symptoms (e.g. epigastric pain, vomiting, diarrhoea, etc.), which may be associated with hypersensitivity symptoms (e.g. urticaria, angioedema and anaphylaxis) due to the combined result of the direct penetration of the larva (e) in the gastrointestinal tract and the complex interaction between the human immune response and the parasite excretory-secretory, somatic and cuticular antigens (Audicana & Kennedy, 2008). It is therefore considered that anisakiasis can have ectopic, gastric, intestinal and gastro-allergic clinical forms (Audicana & Kennedy, 2008).

Anisakiasis is considered to be an emerging zoonosis of worldwide concern (EFSA-BIOHAZ, 2010; McCarthy & Moore, 2000). Since anisakiasis was firstly described in The Netherlands in the 1960s by Van Thiel (Van Thiel, 1960), there has been a marked increase in the reported prevalence and geographic range of anisakiasis throughout the world (EFSA-BIOHAZ, 2010). Ishikura et al. (1998) estimated that the total number of anisakidosis cases (including zoonosis caused by *Anisakis* spp. and other Anisakidae family members (i.e. *Pseudoterranova* and *Contracaecum*)) up to 1997 was around 35,000 worldwide, of which 97.11% (32,300 cases) were from Japan. EFSA-BIOHAZ (2010) gave a somewhat lower estimate, of approximately 20,000 cases of anisakiasis reported worldwide up to 2010, of which over 90% were from Japan, where it was estimated that around 2000 cases were diagnosed annually. It is considered that the anisakid most frequently involved with human infection is *Anisakis* spp. (Buchmann & Mehrdana, 2016; EFSA-BIOHAZ, 2010). Sohn and Chai (2011) estimated that over 50,000 anisakidosis cases have been reported worldwide, more than doubling the estimate by EFSA-BIOHAZ. Recently, Yorimitsu et al. (2013) estimated approximately 3000 cases of anisakiasis in Japan per year.

New cases of anisakiasis have subsequently been reported in several countries where they were not previously reported, such as Croatia, Portugal, China, Malaysia and Taiwan, as well as in many countries in where the disease had already been reported (Table 1 at Supplementary materials).

The increase in the number of reported anisakiasis cases and geographical range observed over the last three decades is probably due to changes in dietary behaviour (increasing global demand for seafood and growing interest for raw or lightly cooked fish) as well as to improved techniques and knowledge/expertise to diagnose infection (Audicana & Kennedy, 2008; EFSA-BIOHAZ, 2010; McCarthy & Moore, 2000). However, the actual burden of disease (i.e. total number of annual anisakiasis cases and incidence) in each country is still poorly estimated or unknown, and anisakiasis (and anisakidosis) is believed to be an underestimated zoonosis globally (Baird, Gasser, Jabbar, & Lopata, 2014; Buchmann & Mehrdana, 2016; Del Rey; EFSA-BIOHAZ, 2010; Hernandez-Prera & Polydorides, 2012; Hochberg & Hamer, 2010; Mladineo, Popović, Drmić-Hofman, & Poljak, 2016; Del Rey Moreno et al., 2013; Shamsi, 2016; Shimamura et al., 2016; Wiwanitkit & Wiwanitkit, 2016).

Recently, a probabilistic quantitative risk assessment (QRA) study of anisakiasis caused by consumption of raw and marinated home-made anchovies in Spain estimated that the total number of anisakiasis cases requiring medical attention was approximately 8000 annually (20 cases per 100,000 inhabitants/year) (Bao, Pierce, et al., 2017). Thus, there is strong reason to believe that anisakiasis is a highly underestimated and underdiagnosed fish-borne zoonosis, and this is basically due to misdiagnosis (i.e. disease symptoms are not specific and may be confounded with other gastrointestinal diseases), undiagnosed (e.g. lack of clinical investigation during anamnesis, mild and asymptomatic cases may occur) and not reported cases (the notification of anisakiasis is not mandatory) (Bao, Pierce, et al., 2017; Del Rey; Del Rey Moreno et al., 2013; EFSA-BIOHAZ, 2010; Kim et al., 2013; Toro et al., 2004).

Finally, if we consider the Sohn and Chai (2011) estimates as accurate, the total number of worldwide anisakidosis (almost all anisakiasis) cases up to December 2017 may be over 76,000 (Table 2 at Supplementary materials). It is likely that these figures would increase considerably when mild, symptomless and asymptomatic cases were diagnosed and reported. Furthermore, if the Bao, Pierce, et al. (2017) estimates of annual anisakiasis cases per year in Spain (around 8000 cases in 2013) were confirmed by epidemiological studies, it would imply that global figures would be much higher. It should be noted that this application of QRA required several assumptions to be made. For example, it was assumed that all anisakiasis cases were caused by untreated home-prepared raw and marinated anchovies due to lack of data, but of course other fish preparations might have been involved as vehicle of disease. More importantly, the QRA estimates are only accurate to the extent that the input data are valid, and the model variables represent the process (Bao, Pierce, et al., 2017). In relation to this, Herrador, Daschner, Perteguer, and Benito (2018) performed a retrospective descriptive study from 1997 to 2015 of anisakidosis-related hospitalizations using the centralized hospital discharge database which collects clinical data from all public hospitals in Spain. They identified an average of 130 anisakidosis hospitalizations per year. Considering that only 1–2% of anisakidosis cases require hospitalization, they further estimated the occurrence of between 6000 and 13,000 cases requiring medical attention per year (numbers that increased to 10,000 to 21,000 cases in the last years of the study). The estimates performed by Herrador et al. (2018) based on anisakidosis-related hospitalizations (6000 to 13,000 cases for the whole study period) appear to be of the same order of magnitude as those of Bao, Pierce, et al. (2017) (approximately 8000 cases estimated for 2013), thus supporting the assertion that QRA is a useful and valid methodology for estimating the burden of disease. There is therefore a need for a better data and understanding to allow reliable quantitative determination of the burden of disease of this emerging and hidden zoonosis globally, as well as to identify strategies to help to reduce its incidence (see future work section).

## 4. Allergy and subclinical sensitization to *Anisakis* spp

In their scientific opinion on risk assessment of parasites in fishery products, the European Food Safety Authority concluded that, in a sensitized individual, *A. simplex* can provoke two main clinical responses, namely gastro-allergic anisakiasis and allergy to *A. simplex* (EFSA-BIOHAZ, 2010). It is generally believed that, in most cases,

*Anisakis* spp. infection must occur to initiate allergic sensitivity to *Anisakis* spp. in humans. Nevertheless, the possibility that sensitization can occur via exposure to the antigen alone, in the absence of live infection, has not been excluded (e.g. Audicana, Ansotegui, Fernández de Corres, & Kennedy, 2002; Baird et al., 2014; EFSA-BIOHAZ, 2010; Ivanović et al., 2017; Nieuwenhuizen, 2016; Pravettoni, Primavesi, & Piantanida, 2012).

In gastro-allergic anisakiasis, an acute parasite infection with live *Anisakis* spp. larvae can provoke gastric symptoms together with an immunoglobulin E (IgE)-mediated immune response, which results in the presentation of allergic symptoms, such as urticaria, angioedema or life-threatening anaphylaxis (Daschner, Alonso-Gómez, Cabañas, Suárez de Parga, & López-Serrano, 2000; Daschner, Cuellar, & Rodero, 2012). Immunoglobulin E is involved in Type I immune hypersensitivity as well as the immune response of humans against helminth parasites (e.g. *Ascaris* spp.) (Daschner et al., 2012; EFSA-BIOHAZ, 2010). Other immunoglobulins (e.g. IgG, $IgG_4$) are also involved in the immunological response against *Anisakis* spp. (Daschner et al., 2014).

Allergy to *Anisakis* spp., in which the severity of symptoms varies from acute urticaria, angioedema to anaphylaxis, has been also described in sensitized patients after consumption of fish contaminated with dead larvae or with their allergens ("true" food allergy). This implies that some allergic individuals can have allergic symptoms even when the fish meal was properly cooked or previously frozen (AAITO-IFIACI Anisakis Consortium, 2011; Audicana & Kennedy, 2008; Carballeda-Sangiao, Rodríguez-Mahillo, Careche, Navas, Moneo, et al., 2016; Moneo, Caballero, Rodriguez-Perez, Rodriguez-Mahillo, & Gonzalez-Muñoz, 2007; Montoro, Perteguer, Chivato, Laguna, & Cuéllar, 1997; Moreno-Ancillo et al., 1997; Trujillo et al., 2002; Ventura, Tummolo, Di Leo, D'Ersasmo, & Arsieni, 2008). Allergic manifestations, such as chronic urticaria, rhinitis, conjunctivitis, contact dermatitis, asthma, rheumatologic disorders, etc. have also been reported (Audicana & Kennedy, 2008; Daschner, Vega; de la Osada, & Pascual, 2005; EFSA-BIOHAZ, 2010; Mattiucci, Colantoni, et al., 2017; Ventura, Napolitano, Menga, Cecere, & Asero, 2013).

Occupational allergy has been described in fishmongers, fishermen, fishery and aquaculture workers and cooks (Añíbarro & Seoane, 1998; Armentia et al., 1998; Fernández-Delgado, Martínez-Castillo, Lasanta-Melero, Gaitero-Reina, & Domínguez-Escobar, 2015; Mazzucco et al., 2012; Nieuwenhuizen et al., 2006; Ventura et al., 2008) and, recently, a case of non-occupational airborne-induced anaphylaxis caused by *Anisakis* spp. was described in Spain (Barbarroja-Escudero, Sanchez-Gonzalez, Antolin-Amerigo, Rodriguez-Rodriguez, & Alvarez-Mon, 2016). A recent review of the *Anisakis* spp. allergy issue, concluded that occupational and non-digestive exposure seems to be clinically relevant and that *Anisakis* spp. is an important agent for chronic urticaria (Moneo, Carballeda-Sangiao, & González-Muñoz, 2017).

To date, 17 *Anisakis* spp. allergens are considered, several of them being heat- and/or pepsin-resistant (Caballero & Moneo, 2004; Carballeda-Sangiao, Rodríguez-Mahillo, Careche, Navas, Caballero, et al., 2016; Kobayashi, Kakemoto, Shimakura, & Shiomi, 2015; Moneo et al., 2005; Vidaček et al., 2009), according to the AllFam Database of allergen families and the WHO/IUIS Allergen Nomenclature Database. Several new potential allergens have also been suggested, some of them showing thermostable characteristics (Arcos et al., 2014; Baird et al., 2016; Fæste et al., 2014; Llorens et al., 2018). Heat- and pepsin-resistant allergens of *Anisakis* spp. have been detected in frozen, cooked and canned fish (Klapper et al., 2018; Rodríguez-Mahillo, González-Muñoz, de las Heras, Tejada, & Moneo, 2010; Tejada et al., 2015). In addition, *Anisakis* spp. proteins have been detected in farmed fish (Atlantic salmon), processed fishery products (Fæste, Plassen, Løvberg, Moen, & Egaas, 2015) and *Anisakis* spp. peptides in fish feed, which is an important food component in fish farming and in the poultry industry (Fæste, Levsen, et al., 2015). The presence of *Anisakis* spp. allergens in the above-mentioned fishery products, even if the parasite is dead or not physically present in the product, may explain why some sensitized patients suffered allergic reactions after consumption of canned fish (Montoro et al., 1997), industrial tuna preparations (AAITO-IFIACI Anisakis Consortium, 2011), farmed fished (Carballeda-Sangiao, Rodríguez-Mahillo, Careche, Navas, Moneo, et al., 2016) and even chicken meat (Armentia et al., 2006).

The diagnosis of allergy to *Anisakis* spp. is based on a compatible medical history showing allergic reactions within 24 h after fish consumption. Allergy suspicion is confirmed by a positive skin-prick test and/or detection of specific IgE against *Anisakis* spp. (enzyme immunoassay) with values > 0.7 KU/l (some allergists used a threshold level >0.35 KU/l in accordance with the manufacturer's instructions). In addition, absence of allergy to fish and other possible cross-reacting allergens has to be confirmed (Carballeda-Sangiao et al., 2014; Daschner & Pascual, 2005; EFSA-BIOHAZ, 2010; Lorenzo, Iglesias et al., 2000). Cross-reactivity between *Anisakis* spp. and carbohydrates, phosphorylcholine, anisakids and other ascarid nematodes (e.g. *Ascaris suum*, *A. lumbricoides*, *Toxocara canis*, *Hysterothylacium aduncum*), shrimps, insects (e.g. wasps, cockroaches) and mites might lead to misdiagnosis of *Anisakis* spp. allergy, and the clinical importance of such cross-reactions is not yet well understood (Audicana & Kennedy, 2008; Audicana et al., 2002; Bernardini et al., 2005; Daschner et al., 2012; EFSA-BIOHAZ, 2010; Johansson, Aponno, Lundberg, & van Hage-Hamsten, 2001; Lorenzo, Romaris, et al., 2000; Lozano Maldonado et al., 2004; Pascual et al., 1997; Rodríguez-Pérez et al., 2014). Thus, it frequently occurs that patients attending allergy clinics present positive skin-prick tests and/or specific Ig-E against *Anisakis* spp., but they are not finally diagnosed as having *Anisakis* spp. allergy because they do not fulfil the criteria presented above. The presence of IgE against *Anisakis* spp. in otherwise healthy individuals also occurs (i.e. subclinical or asymptomatic sensitization). It has been suggested that previous subclinical or undiagnosed anisakiasis (i.e. previous episode of parasite infection) would be the probable cause for the existence of *Anisakis*-specific IgE in the majority of asymptomatic individuals, even though cross-reactions might also occur but seem to be less probable (Audicana & Kennedy, 2008; Moneo et al., 2017).

Allergy to *Anisakis* spp. has been reported to be relatively common in some areas of Spain (Audicana & Kennedy, 2008; Fernández de Corres, Del Pozo, & Aizpuru, 2001; López Sáez et al., 1999; Puente et al., 2008), being the most important hidden food allergen in those members of the adult population who are suffering acute urticaria and anaphylaxis (Table 3 at Supplementary materials) (Añíbarro, Seoane, & Múgica, 2007; Audicana & Kennedy, 2008; Del Pozo et al., 1997). Studies carried out in Spain also showed that subclinical sensitization in blood donors and healthy populations ranged from 0.4% to 22% (Table 3 at Supplementary materials) (Fernández de Corres et al., 2001; Puente et al., 2008; Valiñas et al., 2001). Extrapolation of the seroprevalence data to the total Spanish population (approximately 46 million inhabitants) would suggest that thousands to millions of healthy individuals may have IgE sensitization to *Anisakis* spp. and thus have experienced previous subclinical or undiagnosed anisakiasis.

*Anisakis* spp. hypersensitivity was screened in 10,570 individuals attending 34 allergy clinics in Italy during 2010, of which 474 (4.5%) were found to be sensitized to *Anisakis* spp. and 66 of these (0.6% of the total) were diagnosed as having *Anisakis* spp. allergy (AAITO-IFIACI Anisakis Consortium, 2011). In that study, *Anisakis* spp. sensitization prevalence ranged from 0.4 to 12.7% depending on the geographic area, variation which appeared to be related with different consumption habits of marinated anchovy among different Italian populations. A study carried out in Sicily (Italy) showed that 527 out of 3419 (15.4%) patients attending the allergy clinic had *Anisakis* spp. sensitization, of which 29 (0.8%) patients were diagnosed to have allergy to *Anisakis* spp. (Heffler et al., 2016). Serodiagnosis data from a recent IgE immunoblotting (WB) study showed that some Italian individuals suffering from gastro-allergic anisakiasis or presenting allergic symptoms (e.g. acute urticaria) after fish consumption, without any confirmed parasitism by *Anisakis* spp. larvae, had IgE sensitization to *A. pegreffii*

(Mattiucci, Colantoni, et al., 2017).

*Anisakis* spp. sensitization has been reported in other European countries (Croatia, France, Portugal and Norway) and in Africa (South Africa and Morocco), Asia (Japan and South Korea), North America (Greenland) and South America (Brazil) (Table 3 at Supplementary materials) (Abattouy, Valero, Martín-Sánchez, Peñalver, & Lozano, 2012; Bønløkke et al., 2012; Choi et al., 2009; Dupouy-Camet et al., 2016; Falcão, Lunet, Neves, Iglesias, & Barros, 2008; Figueiredo Jr et al., 2013; Kasuya & Koga, 1992; Kimura, Takagi, & Gomi, 1999; Kinoshita et al., 2014; Lin, Nepstad, Florvaag, Egaas, & Van Do, 2014; Mladineo, Poljak, Martínez-Sernández, & Ubeira, 2014; Nieuwenhuizen et al., 2006). Moneo et al. (2017) recently concluded that in endemic countries (i.e. Spain and Italy) the percentage of highly sensitized individuals could be as high as 7% of the general population. They proposed that, apart from the four recognised clinical manifestations of anisakiasis (i.e. gastric, intestinal, ectopic and gastro-allergic), a fifth clinical group (sensitized asymptomatic patients) should be included, and that this group of patients should be the real concern for the health authorities. Moreover, Moneo et al. (2017) concluded that hypersensitivity to *Anisakis* spp. should be considered as a disease affecting a high number of otherwise asymptomatic individuals, probably waiting only for an episode of *Anisakis* spp. infection or an encounter with an undetermined amount of *Anisakis* spp. allergens to develop acute or chronic disease.

Thus, *Anisakis*-specific IgE seroprevalence in patients and otherwise healthy individuals can be relatively high, although variable among different countries and even among regions of the same country (Table 3 at Supplementary materials). This variation in seroprevalence between human populations may be explained by a number of factors. Most obviously it may arise due to different patterns (amounts, frequencies and habits) of consumption of raw and/or undercooked fish in individuals from different countries/regions (i.e. it is consequence of human behaviour). This might explain the low *Anisakis* spp. sensitization found in Norwegians (Lin et al., 2014) and Galicians (inhabitants of NW Spain) (Valiñas et al., 2001), who do not usually eat raw fish meals even though they have a high *per capita* fish consumption, compared to other countries like Japan (Kasuya & Koga, 1992) or other regions in Spain in which the consumption of raw fish is much more frequent (Fernández de Corres et al., 2001; Del Rey Moreno et al., 2006; Fernández Puente et al., 2008).

A second source of variation may be differences in the sensitivity and specificity of the methods used to detect *Anisakis*-specific IgE in the sera of individuals, and ultimately, for the *Anisakis* spp. allergy diagnosis in different studies (i.e. the reliability of the method). Some studies proved the higher specificity of immunoblotting and ELISA compared to a skin-prick test and immunoCAP when employing *Anisakis* spp. crude extracts as target antigens, because they may be affected by cross-reactivity (Mattiucci, Colantoni, et al., 2017; Puente et al., 2008). Recently, it has been shown that by increasing the cut-off value for the *Anisakis*-specific IgE tested by immunoCAP to 0.71 KU/l and using the *Anisakis/Ascaris* IgE ratio, the specificity of the method improves remarkably (Carballeda-Sangiao et al., 2014). The cross-reactions interfering with the accuracy of the *in vitro* tests for *Anisakis* spp. allergy based on crude parasite extracts can be avoided with the use of single native or recombinant allergens or a cocktail of them (Carballeda-Sangiao, Rodríguez-Mahillo, Careche, Navas, Caballero, et al., 2016; Cuellar et al., 2012; González-Fernández et al., 2017; Moneo et al., 2017).

Thirdly, the variation in seroprevalence against *Anisakis* spp. allergens in humans may be also determined by genetic predisposition (i.e. human genetic susceptibility) (Audicana & Kennedy, 2008; Mattiucci, Colantoni, et al., 2017; Mattiucci et al., 2018). A significant association between Human Leucocyte Antigen (HLA) class II alleles (i.e. DRB1*1502-DQB1*0601 alleles) and hypersensitivity to *Anisakis* spp. allergens has been shown in a northern Spanish population (Sánchez-Velasco et al., 2000). It has been suggested that the low frequency of those alleles in some populations, such as in Norway and Morocco, might also explain the absence of clinical cases (Abattouy et al., 2012; Lin et al., 2014; Sánchez-Velasco et al., 2000). In addition, Caballero et al. (2013) found that *Anisakis* spp. hypersensitivity shows different clinical and immunological patterns between Spanish and Italian *Anisakis* spp. allergic patients. This leads us to the fourth factor: the different *Anisakis* species may have different pathogenic potential (i.e. pathogenicity/allergenicity of *Anisakis* species). In relation to this, *A. simplex* s.s. and *A. pegreffii* have been proved to have allergenic capacity (Llorens et al., 2018), but the variability and severity of the allergenic symptoms caused in humans by the different allergens needs further investigation.

In conclusion, it appears that sensitization to *Anisakis* spp. is frequent worldwide, even though it may occur in an asymptomatic form (hidden pandemic). It cannot be discarded that those sensitized but asymptomatic individuals may eventually develop allergic symptoms after passing a certain threshold for the specific *Anisakis* spp. allergen due to frequent contact with the parasite and its allergens through consumption of parasitized/contaminated fishery products. If this is the case, a rise of allergenic cases might be expected due to continuous consumption of parasitized wild marine fishery products. Changes in human behaviour may also lead to a rise (or indeed a fall) in the number of cases, e.g. new trends and habits of eating raw/marinated fish delicacies.

## 5. Future work

The problems caused by the presence of *Anisakis* spp. in fishery products have been reviewed both here and elsewhere, but what more can be done to address them? We propose a number of future research lines to solve outstanding problems. We need a better understanding of (a) the real scale of the *Anisakis* spp. associated health problems in human populations (i.e. *Anisakis* spp. allergy and anisakiasis) and (b) the risks arising from consumption of parasitized fish and fish products, and to determine how these risks could be mitigated (see 5.1 and 5.2). Such studies may help to provide the scientific basis for improved legislation and/or codes of practice to manage and control the parasite risk, thus better protecting both the consumers and the fish/food industries by assuring the quality and safety of fishery products (see 5.3 and 5.4). We also need a much better understanding of the ecology of *Anisakis* spp. transmission through the marine food web, including the roles of external drivers (i.e. anthropogenic abiotic and biotic stressors (e.g. climate change, pollution, habitat loss, etc., see Cable et al. (2017)) in order to understand whether there are plausible options for intervention (see 5.5).

### *5.1. Quantitative risk assessment for Anisakis spp. allergens from parasitized fish and fish products*

Quantitative risk assessment is a science-based methodology that can be applied to estimate the probability and severity of known or potential adverse health effects resulting from human exposure to foodborne hazards (Codex Alimentarius Commision, 1998; EC, 1997), such as exposure to the parasite *Anisakis* spp. and its allergens through contaminated fishery products. QRA provides a numerical estimation of the risk across a defined population, identifies factors that may influence it, and gives an indication of the attendant uncertainties. If any risk is characterized, QRA proposes strategies to mitigate it, and the efficacy of these interventions may be evaluated *in silico*. Recently, a QRA study was performed to determine the probability of anisakiasis from exposure to *Anisakis* spp. larvae through consumption of home-made raw and marinated anchovies in Spain (Bao, Pierce, et al., 2017).

The other main human health risk from *Anisakis* spp. concerns allergens. Although there have been no specific studies on *Anisakis* spp. allergens to date, QRA is increasingly applied to assess the risk derived from food allergens (Crevel et al., 2014; Crevel, Ronsmans, Marsaux, &

Bánáti, 2018). Several studies have estimated the probability of an allergic reaction following allergen exposure from food, such as allergic reactions to peanut due to consumption of cross-contaminated chocolate, vegetable oils and other food products (Blom et al., 2017; Rimbaud, Heraud, La, Leblanc, & Crepet, 2013; Rimbaud, Heraud, La Vieille, Leblanc, & Crepet, 2010).

What data are required to perform a QRA study for allergy to *Anisakis* spp.? The QRA process consists of the following steps: 1) hazard identification, 2) hazard characterization, 3) exposure assessment and 4) risk characterization (Codex Alimentarius Commision, 1998; Crevel et al., 2014; EC, 1997). In the hazard identification step the *Anisakis* spp. allergens of concern and the fishery product(s) acting as vehicle of transmission need to be identified. Humans ingest *Anisakis* spp. allergens through their diet and it has been recently shown that consumption of contaminated fishery products (even if they were previously frozen or derived from farmed fish) may increase the level of *Anisakis* spp.-specific IgE in sensitized patients (Carballeda-Sangiao, Rodríguez-Mahillo, Careche, Navas, Moneo, et al., 2016). It therefore appears that the main health risk might arise from the encounter with heat- and pepsin-resistant *Anisakis* spp. allergens due to eating parasitized fish. In hazard characterization, the quantitative evaluation (if possible) and the nature of the adverse effect (e.g. allergic reaction) associated with the biological agent (e.g. *Anisakis* spp. allergen) present in food is characterized. The relationship between the magnitude of exposure (dose) to the *Anisakis* spp. allergen and the severity and frequency of associated allergic reaction (response) should be mathematically described by a suitable dose-response model.

*Anisakis* spp. allergens have been identified in European hake (*Merluccius merluccius*) and European anchovy (*Engraulis encrasicolus*) muscle (Rodríguez-Mahillo, González-Muñoz, de las Heras, Tejada, & Moneo, 2010). Considering the high infection levels of *Anisakis* spp. in the muscle of many commercially important species of European waters (Levsen et al., 2018), it is likely that *Anisakis* spp. allergens occur in the muscle of many other fish species. In addition, *Anisakis* spp. proteins have been detected in different food matrices (see section 4), evidencing the leftovers of *Anisakis* spp. allergens (Fæste et al., 2016; Fæste, Plassen, et al., 2015). Exposure assessment will require additional data on prevalence and concentration of *Anisakis* spp. allergens in fish fillets (or selected fishery products) and how these changes over time and through different processes such as cooking (i.e. determination of the level of allergen contamination in the fishery product, including the cooked product). In addition, data on consumption patterns (amount, frequency and habits of fish intake by consumers) in the population will be needed. The distribution of the amount of *Anisakis* spp. allergens consumed per fish meal (single serving portion) can then be determined (i.e. the dose). In the risk characterization step, the quantitative information resulting from the exposure assessment and hazard characterization steps are integrated to provide an estimation of the probability and severity of health risk (i.e. allergic reaction) that may occur in a given population (e.g. allergic and/or asymptomatic individuals).

To sum up, probabilistic QRA studies of allergy to *Anisakis* spp. should be performed to assess the risk posed by the presence of *Anisakis* spp. allergens in fish meals to allergic and subclinically sensitized fish consumers. QRA will provide a better understanding of the burden of allergic disease in the human population (i.e. prevalence/incidence of *Anisakis* spp. allergy). The difficulties involved here should not be underestimated. While some up-to-date information is available on the occurrence of *Anisakis* spp. in captured fish, similar data are required for fish which have already passed the existing screening processes and allergen levels need to be measured in these fish. Then, robust human health data are needed. One of the issues encountered by Bao, Pierce, et al. (2017) was that data on anisakiasis incidence were available from only certain hospitals, and large differences were seen between different studies. In order to obtain a representative picture for a country, ideally more extensive data are needed, assuming that such data are collected. In countries where human health risks caused by *Anisakis* spp. are, rightly or wrongly, considered to be minimal, the concern is that these data will not be routinely collected.

Finally, QRA can be used to propose strategies to mitigate the risk of allergy (Crevel et al., 2014; Taylor et al., 2014), e.g. avoidance of consumption of highly contaminated parts of fish, establishment of an accepted reference dose (mass of *Anisakis* spp. allergen in contaminated fish that would protect a percentage of the allergic population from suffering allergic reaction after fish consumption), etc. In addition to QRA, it is also recommended to carry out seroepidemiological studies, especially in those countries with high fish consumption *per capita* or where the consumption of lightly cooked fish is common. For instance, the respective epidemiological impacts of gastroallergic anisakiasis and allergy to *Anisakis* spp. are unknown (EFSA-BIOHAZ, 2010).

### 5.2. Assessing the risk of anisakiasis in other fish preparations/species and countries

Bao, Pierce, et al. (2017) performed a QRA study in which the risk of anisakiasis caused by consumption of untreated raw and marinated anchovy meals was assessed, and the burden of disease in the Spanish population estimated. This was performed assuming (based on literature) that all anisakiasis cases occurring in Spain in 2013 were caused by home-prepared raw and marinated anchovy meals. Obviously, this is an oversimplification: consumption of raw and marinated anchovies at restaurants, or other fish species and recipes at home or at restaurants may also represent a risk for disease. After implementation of the Spanish Royal Decree 1420/2006, establishments that offer raw and marinated fish meals were obliged to freeze the fish prior to consumption by customers (Ministerio de Sanidad y Consumo, 2006). However, this may not always happen, perhaps due to lack of awareness among sellers. Considering that anchovies in vinegar are widely and frequently consumed in Spain, and that home-made meals were suggested to be the food vehicle for thousands of anisakiasis cases each year, the risk of acquiring anisakiasis at bars/restaurants should be assessed.

Undercooked hake has been implicated in a number of anisakiasis cases reported in Spain (Alonso-Gómez et al., 2004; Jurado-Palomo, López-Serrano, & Moneo, 2010). Cooked and non-cooked hake has also been implicated in *Anisakis* spp. associated anaphylaxis cases in Spain (Audicana & Kennedy, 2008). Considering the high level of *Anisakis* spp. infection in the viscera and muscle of hake, as well as the high levels of human consumption of hake in Spain (Pascual, Rodríguez, Pierce, Hastie, & González, 2018), a QRA study to assess the risk of anisakiasis caused by undercooked hake in Spain is also strongly recommended. While this is likely to reveal a significant risk associated with consuming hake, it should not be assumed that risks from eating anchovy were overestimated in the 2017 study, since it remains likely that anisakiasis is routinely underreported.

New cases of anisakiasis have been recently reported in Europe in Spain, Italy, Portugal, France and Croatia (see Table 1 at Supplementary materials). Raw and marinated anchovies are also frequently consumed in Italy and Croatia. As a part of the EU FP7 PARASITE project (GA no. 312068), an online questionnaire (see Bao, Pierce, et al. (2017) supplementary materials) was used to gather information about the frequency and nature of fish consumption amongst the Spanish population. The questionnaire was adapted and disseminated in Croatia, and a simplified version of the questionnaire was disseminated in Italy. In an unpublished preliminary study, the QRA model used by Bao, Pierce, et al. (2017) was adapted for Italian and Croatian cases using results from questionnaires as well as *Anisakis* spp. epidemiology data for anchovies from the Mediterranean Sea obtained in the PARASITE project. Monte Carlo simulations indicated that raw and marinated anchovies would cause approximately 3500 and 170 anisakiasis cases in Italy and Croatia, respectively, suggesting that anisakiasis is a highly underestimated zoonosis in both countries (Unpublished results). However, a more extensive QRA study is needed to confirm these preliminary findings.

### 5.3. Categorizing the health risk posed by parasitized fishery products

Seafood businesses are required to inspect for "visible parasites" so that any fishery products "obviously contaminated" with parasites do not reach the market for human consumption (EC, 2004b). This ignores the potential presence of non-visible parasites (even though these are zoonotic), or their remains, leaving consumers to manage this risk, by freezing or adequately cooking the fish to prevent anisakiasis. However, the consumption of parasitized fishery products (even if the meal was properly cooked or previously frozen) may still pose a risk for health (probably mainly derived from ingestion of *Anisakis* spp. thermostable allergens) and it is likely that this risk increases with the intensity of the infection (i.e. a meal containing 100 *Anisakis* spp. larvae probably poses a higher risk than a meal containing one larva). Moreover, it is likely that a fish meal containing 100 larvae contains higher levels of *Anisakis* spp. thermostable allergens, therefore posing a higher risk for consumers. Thus, putting aside flaws in the current screening process and its legal basis, a QRA study should be performed to determine the possible human health risk posed by the presence of non-viable *Anisakis* spp. (and/or its allergens (section 5.1)) in fishery products, to then categorize fish lots depending on this risk. This might be pursued by categorizing the risk, based on information obtained from dose-response assessments. The relationship between the magnitude of exposure (e.g. number of parasites or concentration of allergen per 100 g of fish flesh)) and the frequency and severity of associated health effects (i.e. allergic response (e.g. urticaria, etc.) in humans, or antibody response in human blood) should be determined. Ideally, if any dose-response relationship is found, the risk might then be categorized into high, medium, and low by ranking fish lots depending on potential parasite/allergen exposure (i.e. density of muscle infection or allergen contamination). The results of this kind of study might help to mitigate public health, legislative and socioeconomic issues caused by the presence of *Anisakis* spp. in fish, by providing some scientific basis to develop a standardized protocol of parasite inspection in fishery products based on dose-response assessments, which would benefit policy, seafood businesses and consumers. The implications for screening are twofold. Firstly, there is a need for more effective screening for the presence of viable parasites (considering that what is "visible" depends on where and how you look for it). Secondly, screening should be extended to measure allergen content of fish flesh.

### 5.4. Contingent valuation ("willingness to pay") and cost-benefit analyses for "Anisakis spp. free" fishery products

Bao et al. (2018) performed a survey-based contingent valuation study, finding that many Spanish consumers have avoided fish in the past and would avoid fish in the future due to *Anisakis* spp. presence in fishery products. This behaviour causes monetary losses to the industry that should be quantitatively estimated in a more extensive study. *Anisakis* spp.-associated diseases not only compromise human health and quality of life, but also generate a cost for the health services that should also be estimated.

Results of the Bao et al. (2018) study showed that most consumers were willing to pay more for "*Anisakis*-free" fish, suggesting a potential economic benefit to the industry for offering such products. Preliminary results from an equivalent (smaller-scale) contingent valuation study performed in Croatia within the EU FP7 PARASITE project showed a similar distribution of the willingness to pay for "*Anisakis* spp.-free" fish of Croatian consumers (Unpublished results). A more extensive study is needed for comparison with the Spanish case.

Contingent valuation studies are recommended for other European countries with high levels of fish consumption and/or history of *Anisakis* spp. -associated health problems (e.g. Italy, France, Denmark, Norway, Germany, and Portugal), to investigate possible regional similarities or differences in European consumer demand for *Anisakis* spp.-free fish products, and to better understand consumer attitudes and opinions towards parasitized fish.

Ultimately, a cost-benefit analysis should be performed to estimate (in monetary units) the potential benefits (e.g. reduction of the incidence of disease, ensuring food safety and quality, increased consumer trust in fishery products, increased value of fishery products, decreased fish rejections/claims, etc.) versus costs (e.g. purchase/development of technology for parasite detection and removal, fish parasite surveillance programs, training of employees, etc.) of implementing measures to reduce *Anisakis* spp. presence in fishery products. It is also important to consider the "externalities", for example costs to the health service of treating anisakiasis and allergic reaction cases.

### 5.5. Interventions to reduce the amount of Anisakis spp. and allergens entering the human food chain

A strategy to reduce human exposure is likely to include some or all of the following components: (1) measures to reduce the incidence of *Anisakis* spp. in free-living fish, (2) improved screening procedures to avoid infected fish proceeding along the value chain, (3) improved advice on treatment of fish after it reaches the consumer, (4) improved monitoring of *Anisakis* spp. and their allergens in fish at all steps of the value chain, (5) monitoring of the incidence of anisakiasis and allergic reactions in the human population, (6) improved legislation to underpin stages 1–5 and (7) publicity to increase awareness amongst consumers and in the industry.

The abundance of *Anisakis* spp. in fish is expected to reflect the life-cycle biology and ecology of these nematodes and their various hosts. For many regions, ecosystem models exist to describe the flow of energy through the marine ecosystems (Colleter et al., 2015) and it would be useful to adapt such models or develop new models to include the transfer of nematodes along the food chain. Des Clers and Wootten (1990) developed a simple model to describe the transfer of sealworm *Pseudoterranova decipiens* across different trophic levels during its life cycle but there appear to be no equivalent models for *Anisakis* spp. It may also be useful to develop statistical models of habitat suitability for *Anisakis* spp. (Kuhn, Cunze, Kochmann, & Klimpel, 2016), an approach already widely applied to the host species (Redfern et al., 2006; Valavanis et al., 2008).

González et al. (2018) proposed direct intervention to reduce the amount of *Anisakis* spp. in the marine food web, specifically by destroying viable parasites present in fish viscera prior to discarding of this waste by fishing vessels. In the absence of better knowledge of the dynamics of the parasites the likelihood of success remains unknown, but it is the only current proposal. Other options might be considered. It is plausible that high abundances of cetaceans (final hosts) would help to support high nematode burdens in fish, as has been proposed for seals (Zuo, Kania, Mehrdana, Marana, & Buchmann, 2017). However, as discussed above, our understanding of the dynamics of the transfer of *Anisakis* spp. between hosts over their life cycle, including the roles of external drivers, is very limited.

In terms of understanding the relationship between *Anisakis* spp. infection intensity and host population abundances, the links between individual health and *Anisakis* spp. burdens also require investigation, in both fish and cetaceans. For example, does the presence of many worms compromise health or does poor health due to some additional factor such as disease or contaminant bioaccumulation facilitate parasite infection by compromising the immune system? Alternatively, are more parasites found in fish in better condition purely because high food intake promotes both? While it can readily be established whether parasite load is correlated, positively or negatively with body condition (Ferrer-Maza et al., 2016; Pierce et al., 2018), elucidating the mechanism is less straightforward.

In the context of resource competition between fisheries and cetaceans, certain nations, notably Japan, have proposed culling of cetaceans to "protect" fisheries, while Buchmann and Mehrdana (2016) and Zuo et al. (2017) mention the possibility of regulating seal populations

in order to reduce the impact of nematodes on fish. However, the broad consensus of scientific opinion is that the benefits of such action would at best be speculative (see ICES (2018)), especially considering, as explained above, our very limited knowledge upon the ecology and dynamics of *Anisakis* spp. life cycle and its transmission in the marine realm. Large-scale ecosystem manipulations such as culling marine mammals, even if designated to protect fisheries, can have unpredictable and undesirable consequences (see, e.g. Houle et al., 2016; Morissette, Christensen, & Pauly, 2012; Olsen, Galatius, & Härkönen, 2018). In any case the International Whaling Commission's moratorium on whaling remains in place and all cetacean species in EU waters are strictly protected.

In relation to parasite screening, various candidate procedures that can achieve high levels of detection efficiency are available, notably invasive artificial enzymatic digestion, and combined pressing and UV illumination methods (Karl & Leinemann, 1993; Levsen et al., 2005; Llarena-Reino, Piñeiro, et al., 2013). Gómez-Morales et al. (2018) suggest that further development of the UV-press method may be the most effective option to increase detection of *Anisakis* spp. in fillets. However, the UV-press method is time-consuming, expensive and destructive (it destroys the fish flesh so tested fish are no longer available for commercial purposes), so it cannot we used to remove the parasites from fillets during industrial processing of fish, and this is possibly its biggest handicap. Evidently not every fish can be tested in this way and an appropriate sampling strategy is needed. Since, in many commercial fish species (but not all) the number of *Anisakis* spp. increases with body size and varies within fish fillets (i.e. much higher relative proportion of nematodes in the belly flaps) (Levsen et al., 2018), screening should always include some of the largest fish. By including fish across the (legal) size spectrum it should be possible to infer the typical rate of accumulation in relation to body size and potentially estimate the potential total number of *Anisakis* spp. in a batch of fish and whether there is a cut-off size below which fish should be free of *Anisakis* spp. At worst, the absence of *Anisakis* spp. in the largest fish is a good indication that the batch (unless obviously of mixed origin) should be relatively "clean".

Non-destructive hyperspectral imaging system was evaluated under industrial conditions at a fish processing plant in Norway for automatic detection of nematodes (most likely *Anisakis simplex* and *Pseudoterranova decipiens*) in cod fillets. The method appears to detect 52.4% and 61.5% of the nematodes before and after the trimming process (i.e. removal of nematodes using candling) respectively, even though the false alarm rate was high (60%). The method appears promising and may reduce the workload for the trimmers (Sivertsen et al., 2012), and most importantly, facilitate removal of any nematodes present in cod fillets. Further research is required to determine if the method can improve the nematode detection rates and if it could be applied to fillets from other fish species, and to whole fish individuals.

Recently, Bao, Strachan, et al. (2017) showed the potential of Magnetic Resonance Imaging (MRI) as a non-invasive and non-destructive nematode detection tool in whole fish specimens and fish muscle samples, although the approach requires further development before it could be used for routine parasite inspection by the fish industry. This development of non-destructive methods for parasite detection (and removal) from fish fillets and/or whole fish that can be used by the fish industry would be a most valuable achievement.

Screening for and removing allergens in fish is a key area for further development. Olivares et al. (2015) described the conditions for an optimal removal of *Anisakis* spp. allergens from fish flesh as a function of the washing steps during surimi production. They concluded that this procedure might provide a solution for the utilization of those heavily infected fish or fish parts (e.g. belly flaps) which currently must not be placed on the market for human consumption. While the efficacy of screening for removing fish containing visible worms can be easily verified (e.g. by artificial digestion of sampled fillets), the effect on allergen concentrations in fish flesh requires additional research and monitoring. The efficacy of freezing and cooking at different temperatures and for different length of time, in relation to killing the worms and reducing the allergenic effects, requires further investigation.

As explained in section 2.2, the visual inspection method for the detection of parasites in fishery products required by EU legislation, and with which SB must comply, is not efficient. In addition, the use of confusing terminology and lack of standardized protocol for parasite inspection in legislation is causing lack of consensus and use of different diagnostic procedures for parasite control by SB (Llarena-Reino et al., 2015). These issues undoubtedly contribute to the current situation in which some fish with visible *Anisakis* do reach the market. Furthermore, it is not known to what extent SB carry out the required parasite screening, what they do with parasitized fish and if they record these in their HACCP data sheets. If such data exist and were collected, they would be useful for QRA as well as tracing back to where and when the fish were caught. From our experience and conversations with SB, they are very concerned about rejection of their products and the risk of increasing consumer distrust in fishery products (at least in Spain and Norway), and they have told us that many highly infected fish go directly to the trash bin (pers. comm.). Thus, further efforts are needed to explore the efficacy of current control practices by SB, identifying where problems arise as well as further exploring the geographical, seasonal, species and size-related variation in parasite abundance in fish, which may help to minimise the number of parasites present in landed fish and to identify those fish most in need of screening, before we can make further progress with the parasite control problem. Whatever measures are developed, food safety legislation may need to be modified to ensure that it is fit for purpose and that it is followed.

Finally, good risk communication strategies (e.g. well-designed educational campaigns) are crucial and needed, striking a balance between scaring the consumer (the perception of risk differs between individuals and is not easily understood) and potentially harming the market for fish, and ensuring that fishery products are safe at the point of consumption.

## 6. Conclusion

The presence of *Anisakis* spp. in fishery products is an important health and aesthetic issue for consumers, and this is of relevance for both the fishing and food industries as well as for food safety authorities. An unknown quantity of visible (and non-visible) *Anisakis* spp. currently reaches the market and may be observed (and eaten) by consumers. This shows the inefficiency of the current parasite control procedures performed by seafood businesses which comply with European regulations. It is the opinion of the authors of this review that the health standards and screening procedures required for the control of fish parasites in fishery products as stated in EU regulations need to be improved. This might be achieved by establishing research-based and standardized parasite detection methodologies and procedures (e.g. development of non-destructive methods for detection and removal of nematodes from fish products; appropriate sampling strategies), which can then be implemented by seafood businesses during parasite controls. Anisakiasis and allergy to *Anisakis* spp. are identified as underestimated, hidden and emerging fish-borne zoonoses globally. The risk for human health posed by the presence of *Anisakis* spp. and their allergens in fishery products needs to be assessed. Efforts should be pursued to better understand the burden of these zoonoses in the human population, and quantitative risk assessment has been identified as an appropriate methodology. Results from these QRA studies may help to provide the scientific basis to modify food safety legislation and to identify strategies to mitigate the risk of anisakiasis/allergy in the population.

## Conflicts of interest

None.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tifs.2019.02.013.

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