



## IMPLICATIONS OF ZONOTIC PARASITE, *Anisakis* spp. TO SEAFOOD SAFETY: A REVIEW

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### ABSTRACT

*Anisakis* spp. are marine parasitic nematodes commonly found in seafood, particularly in coastal fish that can cause anisakiasis and allergic reactions in humans. Despite increasing clinical reports, the allergenic profiles of different *Anisakis* species and their hybrids remain incompletely characterised. This review aims to consolidate and analyse current knowledge on the identification, classification, and molecular characteristics of allergens present in *Anisakis simplex* s.s., *Anisakis pegreffii*, and their hybrid haplotypes to better understand their roles in allergic pathogenesis. A systematic bibliographic search was conducted using scientific databases including PubMed Central, ScienceDirect, Springer Nature Link, Wiley Online Library, and Google Scholar. A total of 89 relevant peer-reviewed articles were selected based on inclusion criteria focusing on allergen identification, immunological reactivity, and diagnostic development. Data were extracted and analysed based on allergen type, molecular properties, and relevance to clinical allergy. 14 allergens were identified across the studied *Anisakis* species, with Ani s 1, Ani s 7, and Ani s 12 consistently reported as major allergens due to their strong IgE-binding properties and resistance to heat and digestion. Minor allergens, including Ani s 4, Ani s 5, Ani s 6, Ani s 8, and Ani s 11 were also noted, showing variable immunogenicity and potential cross-reactivity with other nematodes and invertebrate allergens. Current diagnostic limitations were observed in serological testing and allergen standardisation. The review highlights the emerging role of advanced techniques such as Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), immunoblotting, and transcriptomic profiling in improving allergen detection and characterisation. This review emphasises the need for integrated molecular and immunological approaches to improve allergen identification in *Anisakis* spp. The findings can inform clinical diagnosis, therapeutic development, and seafood safety regulations. Better understanding of the allergen profiles supports targeted public health interventions and risk assessments for anisakiasis and *Anisakis*-related allergies.

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### Introduction

*Anisakis simplex* is one of the anisakid nematodes third stage larvae (L3) in marine fish product which also consist of *Pseudoterranova decipiens* and may infect consumers that ingest raw or and *Contracaecum osculatum* that occur as undercooked fish products, resulting in severe

illnesses (Buchmann & Mehrdana, 2016). Many commercial fish species are parasitised by anisakid nematodes, which are responsible for a fish-borne zoonotic disease (anisakiasis) as well as allergic reactions (Bao et al., 2019). In today’s world, anisakiasis is becoming more likely to occur in nations like Spain, Italy, and Japan, in which raw or barely cooked fish consumption is common (Garcia-Perez et al., 2015). On the other hand, the number of cases of anisakiasis is estimated to be around 8,000 per year in Spain using a quantitative risk assessment model, since raw anchovies are consumed far too frequently.

The worldwide infection rate of anisakiasis is estimated to be 0.32 cases per 100,000 people, with cases of anisakiasis diagnosed in over 20 countries (Adroher-Auroux & Benítez-Rodríguez, 2020). *A. simplex* is a nematode found in a wide variety of marine creatures, primarily in the Arctic-Boreal region (Kochanowski et al., 2020). The L3 larvae of this parasites have been discovered in many economically important fish species but the prevalence is varying across geographic location fishing grounds and periods surprisingly in the same fish host species (Aibinu et al., 2019). This case happens as the invertebrate hosts of *Anisakis* spp. at certain places are not in the diet

of the predator because they much more prefer other species (Serracca et al., 2014).

*Anisakis* spp. is a genus of highly dispersed nematodes that parasitise a diverse variety of marine vertebrate and invertebrate hosts such as crustaceans, fish, cephalopods, and cetaceans (Gomes et al., 2020). Their life cycle begins with marine mammal such as whales and seals (cetaceans) (Buchmann & Mehrdana, 2016; Aibinu et al., 2019), which stated that adult stage of the parasite are presents in the cetaceans, where copulation and oviposition occur with a complex life cycle (Figure 1). These cetaceans, which are also the nematode’s final definitive hosts, then secrete the unembryonated eggs with faeces into the free ocean, where they can develop into L2 parasite larvae (Aibinu et al., 2019). The larvae then hatch and swim freely in the pacific. Buchmann and Mehrdana (2016) suggested that these larvae will be consumed by a variety of vertebrates, including crustaceans, causing them to act as a transport host for these larvae. Fish become infected when they consume these invertebrates, and the cycle is continued when larger fish consume smaller infected fish. When infected fish or crustaceans are consumed by cetaceans and the larvae are transferred to them, the cycle is complete.

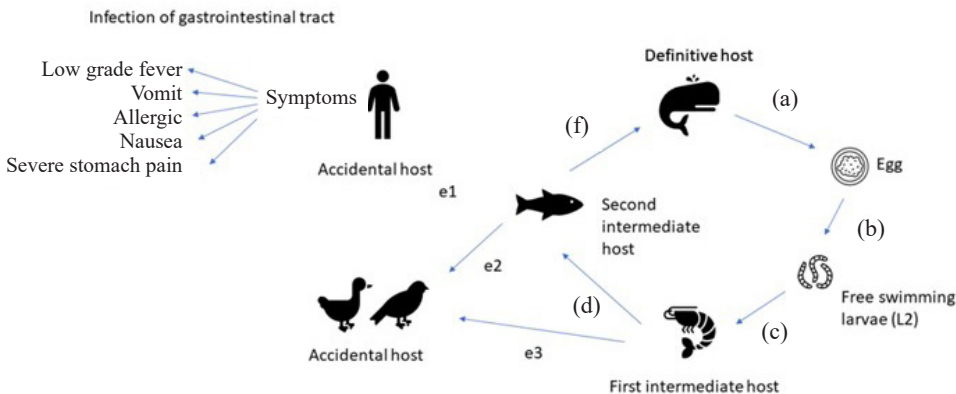


Figure 1: The *Anisakis* spp. life cycle

Note: Location of the L3 larvae may be gastric, intestinal, or ectopic. (a) Adults release eggs into the water along with the faeces of marine mammals; (b) the eggs hatch and release free-swimming L2; (c) the L2 is then ingested by first intermediate hosts and grows into L3; (d) e1, e2, and e3 infected intermediate and paratenic hosts are then ingested by accidental hosts; and (f) infected second intermediate and paratenic hosts are then ingested by marine mammals.

Source: Totoiu et al. (2018)

Infection to humans begins when people eat raw or lightly cooked seafood carrying third-stage larvae (Gomes *et al.*, 2020). Despite the fact that *Anisakis* spp. cannot finalise its life cycle in humans who serve as unintentional hosts, it can still cause other side effects such as diseases and allergies (Łopieńska-Biernat *et al.*, 2019). Smaldone *et al.* (2020) stated that despite the fact that many precautions are taken to keep food from spoiling for every food need, there is still possibility for *Anisakis* spp. parasite to survive and infect humans as there is thermostable allergens in the edible part of the fish. The presence of these allergens should be highlighted as a potential danger for certain overly sensitive persons, and that visible parasites, even if nonviable, could represent a defect, altering the quality of the product and rendering it impractical for human ingestion. *Anisakis* spp. can be found in the edible parts of fish due to its ability to penetrate the musculature and accumulate in these areas, even if the parasite initially targets part of the organs (Godínez-González *et al.*, 2021) and affects human if ingested. Morozinska-Gogol (2019) stated that some *Anisakis* spp. are thermostable, resistant to pepsin digestion, and have a slightly acidic pH. Therefore, allergic reactions are possible even after consuming dead larvae in fish flesh, for instance, after conventional or microwave heat treatment. This makes eradicating these latent parasites challenging.

## Materials and Methods

This review was conducted using electronic databases including the National Centre for Biotechnology Information (NCBI), ScienceDirect, PubMed Central, MDPI, and ResearchGate to identify peer-reviewed

articles related to *Anisakis* spp. in canned fish, with an emphasis on seafood safety. The search strategy incorporated key terms such as “*Anisakis*”, “anisakiasis”, “fish”, “marine”, “seafood”, “*Anisakis* spp. infection in humans”, “distribution of anisakiasis”, “disease therapy”, “allergens”, “allergic reaction”, “HACCP”, “consumer prevention”, and “food processing”. Only full-text articles written in English and published between 2010 and 2021 were considered. Articles were excluded if they were not in English, outdated, irrelevant to the review topic, or not full-length articles. Zotero reference management software was used to screen and remove duplicates, and the remaining articles were assessed for eligibility based on their relevance to the subtopics. Thirty-six relevant articles were selected based on specific subtopics and keywords, excluding articles published before 1987 or after 2000 that did not align with the review’s scope (Figure 2). The data were organised in accordance with the subtopics and keywords of this review study.

The review covered key areas such as the biological and epidemiological characteristics of *Anisakis* spp., its distribution, host associations, clinical manifestations in humans, and therapeutic approaches. In addition, special attention was given to the allergenic properties of *Anisakis* spp., including the identification and classification of allergens, mechanisms of allergic responses, and detection methods. The review also examined consumer-related issues such as the stigma associated with raw fish consumption, prevention methods through food processing (e.g., freezing and cooking), consumer education, and the role of Hazard Analysis and Critical Control Points (HACCP) in minimising risks linked to anisakiasis and seafood allergies.

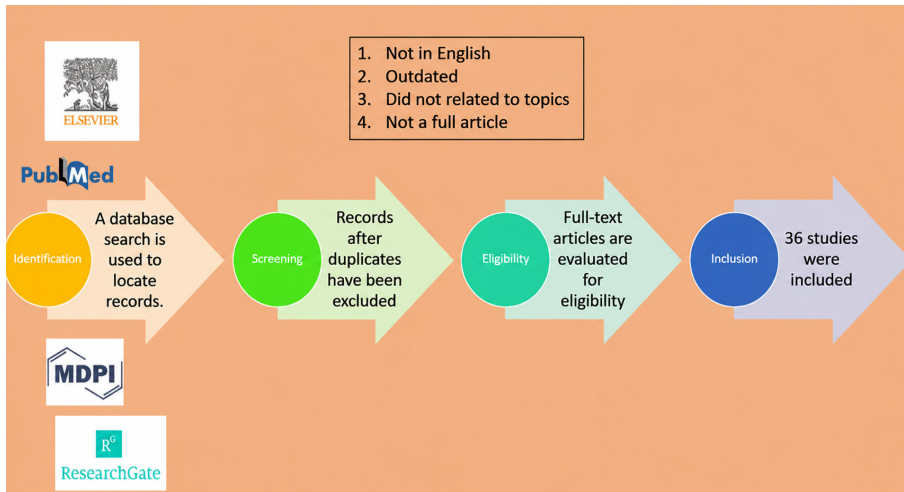


Figure 2: Search strategy for paper selection

## Results and Discussions

### *Impacts on Human Health*

*Anisakis* spp. life cycle has proven to involve human as their accidental host when human consumed parasitised seafood. They have a damaging impact on humans and can cause food-borne zoonotic infection or known as anisakiasis or anisakidosis. Data from the World Health Organization (WHO) in 2012 showed that anisakiasis is included among 56 million cases of zoonotic parasite infection, and the current situation is rapidly spreading worldwide (Pampiglione *et al.*, 2002; World Health Organization, 2012; Aibinu *et al.*, 2019). All of this can occur if they swallow *Anisakis* spp. or its larvae that are infectious to human unknowingly. Because these parasites feed on marine species such as shrimp, fish, and squid, items containing these parasites are often seafood.

Daschner (2016) stated that while other parasite-related health risks are frequently regionally confined, and if of international significance, it pertains only to imports and entering tourists, *Anisakis* spp. may be found in a diverse range of fish and cephalopods and has a global distribution. As a result, the sickness is frequently connected with fish species that are marketed to be eaten raw or partially cooked. Despite outbreaks have been recorded

in other regions around the world, Japan has the greatest frequency of anisakiasis disease in humans (Ünüvar, 2018). Raw or undercooked seafood like fish, squid, and crab, especially contaminated sushi and sashimi is a common cause of the disease. According to Mazzucco *et al.* (2018), the motile *Anisakis* spp. larvae reach the mucosal layers of the gastrointestinal system and burrow through the intestinal walls within hours of ingestion, producing immediate tissue injury that may lead to this illness. Local inflammation would react, causing the consumer to suffer symptoms such as acute ulceration, nausea, vomiting, and epigastric discomfort, sometimes with hematemesis, which will subsequently be diagnosed as anisakiasis symptoms (Daschner, 2016; Ünüvar, 2018).

Severe anisakiasis is regarded by the production of granulomas as an immune system response to an exogenous body and can extend from days, weeks, and even to several months. In extreme situations, the larva enters extra intestinal organs such as the peritoneum or pleura. Because *Anisakis* spp. larvae cannot live in humans for more than a few days beyond, most severe instances of anisakiasis are detected after surgical excision of the infected area. By year

2000, it was known that the third-stage larvae of *Anisakis* spp. causes acute allergic symptoms which also included anaphylaxis, angioedema, as well as urticaria. This new condition was also known as gastroallergic anisakiasis (GAA). Urticarial and abdominal symptoms had been identified as allergy response to the *Anisakis* spp. due to the detection of certain type of immunoglobulin E (IgE), which is linked with Type I immune hypersensitivity in some of the patients (Daschner, 2016).

With the advent of these symptoms' occurrence in consumers, doctors and physicians have had an easier time to determine the source

of their discomfort after ingesting some food. However, it later became ineffective because the same symptoms that occurred are also frequent in any other allergies including non-food related. Aibinu *et al.* (2019) stated that the occurrence of cross-reactivity of *Anisakis* spp. allergens with antigens out of different nematodes as well as allied invertebrates such as crustaceans, insects, and dust mite pose a significant issue in diagnosing and interpreting allergic responses. Some *Anisakis* spp. allergens such as tropomyosin and paramyosin, have shown strong cross-reactivity towards homologous proteins from various invertebrates such as midge and insects.

Table 1: The allergen in *Anisakis* spp. and the cross-reactivity with other invertebrates

<i>Anisakis</i> Allergen	Cross-Reactivity with Other Invertebrates	Invertebrates Common Name	
Ani s 2 (Paramyosin)	<i>Blomia tropicalis</i> (Blo t 11)	Dust mite	
	<i>Dermatophagoides pteronyssinus</i> (Der p 11)		
	<i>Dermatophagoides farina</i> (Der f 11)		
	<i>Periplaneta americana</i> (Per a 7)		
Ani s 3 (Tropomyosin)	<i>Dermatophagoides farina</i> (Der f 10)	Dust mite	
	<i>Dermatophagoides pteronyssinus</i> (Der p 10)		
	<i>Lepidoglyphus destructor</i> (Lep d 10)		
	<i>Charybdis feriatus</i> (Cha f 1)	Crustaceans	
	<i>Homarus americanus</i> (Hom a 1)		
	<i>Panulirus stimpsoni</i> (Pan s 1)		
	<i>Metapenaeus ensis</i> (Met e 1)		
	Ani s 9 (SXP-RAL-2 family)	<i>Mimachlamys nobilis</i>	Mollusc
		<i>Heliotis diversicolor</i>	
		<i>Helix aspersa</i> (Hel as 1)	
<i>Perna viridis</i>			
<i>Crassostrea gigas</i>			
<i>Turbo cornutus</i> (Tur c 1)			
Ani s 9 (SXP-RAL-2 family)	<i>Chironomus kiiensis</i> (Ch k 10)	Midge	
	<i>Lepisma saccharina</i>	Insects	
Ani s 9 (SXP-RAL-2 family)	<i>Hymenoptera</i> spp.	Insects (wasps)	

Source: Aibinu *et al.* (2019)

As a result, it is very easy to mistakenly identify an *Anisakis* spp. allergy with another type of allergy. This is compounded by the fact that some treatment facilities are not very well

taught about *Anisakis* spp. and their related diseases. Frankly, specialists in very diverse sectors do not prioritise basic understanding of parasite immune biology and clinical allergy,

causing an incorrect interpretation of allergic features, that could have significant negative impacts on the worldwide implementation of a healthy fish-inclusive diet (Daschner & Cuéllar, 2020). Even if their allergy test results are positive, patients are still at risk of being misdiagnosed by doctors. After all, this is why this parasite-related disease remain hidden in society and less attention is paid to efforts to eradicate them.

If the symptoms are left unchecked for an extended period, this could cause severe impact on human's health. Patients parasitised with

this parasite will experience extreme continual epigastric pain accompanied by nausea and also vomiting. The presence of these parasites beneath the mucosal layer of the antrum can be discovered during an examination and endoscopy. The parasite can cause injury to the surrounding area, causing oedematous and erythematous mucosa. Another effect of this parasite's infiltration is gastric erosion (Madi *et al.*, 2013). Presence of *Anisakis* spp. can be seen in the stomach of a patient from endoscopic image along with the erosion that eventually leads to other worse symptoms (Figure 2).

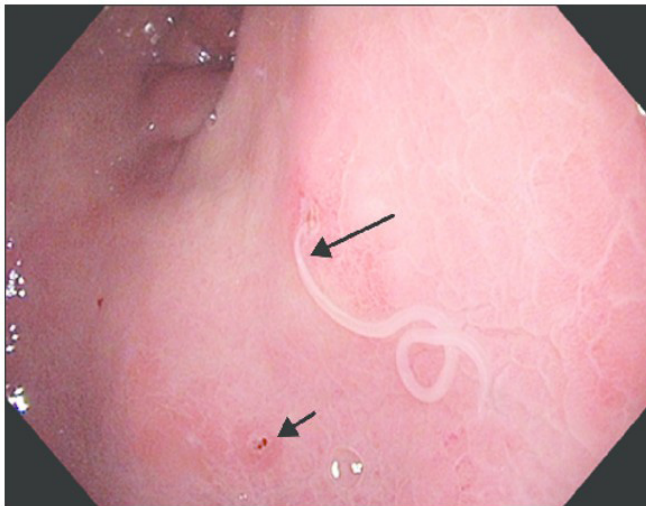


Figure 2: Endoscopic image of stomach demonstrating *Anisakis simplex* (long arrow) erosion from *Anisakis simplex* (short arrow)  
Source: Madi *et al.* (2013)

As for the processed food industry, additional costs is required to prevent the presence of this parasite. The manufacturing facilities must establish inspection procedures to ensure that the products manufactured are free of parasites. This is to ensure that customers can still have confidence in their products. The expenses incurred are substantial, potentially amounting to thousands and, in some cases, even reaching millions. It is due to the cost of controlling, removing, and eliminating the parasite, not to mention the additional cost of re-commercialising the parasite-free products produced by the manufacturer. As stated by

Bao *et al.* (2018). Atlantic coast Canadian fish processors spent 26.6 to 50 million dollars a year to downgrade and dispose contaminated products. This demonstrates the significant global impact of *Anisakis* spp. and the diseases associated with it.

### ***The Stigma to Raw Fish Meal***

Simsek *et al.* (2020) stated that globalisation opened new market areas and altered food consumption habits, which results in rapid and extraordinary societal change. The consumption of raw fish has been popularised worldwide, which then increased the threats of illness

associated with parasitic infection. Numerous traditional dishes from various countries feature raw fish, including sushi and sashimi from Japan, gravlax from Nordic countries, fish carpaccio from Italy, and umai and hinava from Malaysia. In recent years, these dishes have gained international recognition and have been introduced in other regions as healthy and distinctive culinary offerings. Unfortunately, when these foods are introduced in other regions or countries, they are not prepared as safely and hygienically as they are in their place of origin. Furthermore, traditional preparation techniques are often complex and require appropriately trained experts, from the selection of raw materials to the storage of the prepared food. Consequently, the proportion of raw fish products that are free from parasites remains relatively low. (Šimat & Trumbić, 2019).

Several articles and health websites have published their thoughts and findings on the dangers of eating raw seafood as well as the impacts of the disease. This can eventually lead to a negative perception of traditional foods and discrimination against certain cultures. Japan's traditional foods have been among the most severely affected by this issue with annually reported cases exceeding 10,000 to 20,000 cases (Furuya *et al.*, 2018). Cases of anisakiasis in Japan are also caused by raw foods that are not properly stored and prepared such as when the fish stomach contents are not removed, and the food is not deep-frozen. It is claimed that this method is performed to preserve appearance and to sustain the rich taste of the seafood (Kondo, 2018).

However, most cases in Japan are caused by the consumption of cheap fish which are served at home. Food industry such as sushi restaurants normally serves expensive and high-quality fish like big-eye tuna, Atlantic bluefin tuna, puffer fish, broadbill swordfish, and horse mackerel. Most of these selected fish is parasite-free and safe to be consumed raw. The authentic sushi industry has vast experience in preparing raw foods, where the trained sushi chefs are well-versed in food safety and proper handling of

raw foods. Hence, there is no necessity to avoid or sushi restaurants out of fear of this parasite (Oshima, 1987).

### **Food Processing and Prevention Strategies**

Various food safety measures have been implemented to reduce the risk of parasitic contamination, especially in restaurant practices and processed seafood products like canned fish. These strategies include careful selection of fish species, monitoring of the effectiveness of thermal and non-thermal processing and ensuring proper storage to extend shelf life and inactivate parasites. However, studies have shown that conventional processing methods such as light cooking, brining, marinating with high salt (NaCl) or acetic acid, and even canning or freezing may not completely inactivate all parasitic threats. For example, *Anisakis* spp. larvae and their allergenic proteins have demonstrated resistance to some of these processes, with allergen activity still detectable post-processing (Lunestad *et al.*, 2011; Mazzucco *et al.*, 2018; Bao *et al.*, 2019). This resistance underscores the importance of strict processing standards, including adequate freezing ( $-20^{\circ}\text{C}$  for  $\geq 24$  hours) and thorough cooking ( $\geq 63^{\circ}\text{C}$ ) to ensure consumer safety. Preventive measures should also include routine inspection and parasite monitoring during food preparation, especially for high-risk products like raw, lightly cooked, or marinated seafood.

### **Global Awareness of *Anisakis* spp. and Associated Health Risks**

*Anisakis* spp., the parasitic worms found in seafood, pose a significant health risk to consumers, yet public awareness of the disease varies widely across regions. A survey conducted in Spain (Table 2) highlighted the awareness level of respondents regarding *Anisakis* spp. and the associated health risks, showing varying levels of knowledge. However, this survey alone does not capture the broader global picture. Studies from other countries, including Japan, Norway, and the United States revealed that awareness of anisakiasis and related allergic reactions is inconsistent, with some regions

having higher awareness due to the prevalence of raw fish consumption, while others remain underinformed.

For instance, in Japan, where sushi consumption is common, public health campaigns have been highly effective in educating consumers about the risks and necessary precautions such as freezing seafood at -20°C for 24 hours to kill larvae. On the other hand, in countries where seafood consumption

is lower or less focused on raw preparations, awareness is often limited, increasing the risk of anisakiasis in unsuspecting consumers.

This variation in awareness underlines the need for global public health initiatives to improve consumer education, enhance food safety measures, and implement consistent standards for seafood processing worldwide. Such efforts are crucial in reducing the incidence of anisakiasis and minimising the public health burden associated with this parasitic infection.

Table 2: The protein features in different allergen of *Anisakis* spp.

Allergen	Origin	Protein Features
Ani s 1	ES	Kunitz-type serine-protease inhibitor; domain lustrine-type cystein-rich. Major allergen. Thermostable.
Ani s 2	Somatic	Paramyosin. Cross-reactivity with another helminth and arthropod paramyosins.
Ani s 3	Somatic	Tropomyosin. Cross-reactivity with arthropod tropomyosins.
Ani s 4	ES	Cysteine protease inhibitor. Thermostable. Resistant to pepsin digestion.
Ani s 5	ES	SXP/RAL-2 family protein. Thermostable.
Ani s 6	ES	Serine protease inhibitor. Resistant to pepsin digestion.
Ani s 7	ES	Glycoprotein with tandem repeat sequences. Major allergen. Specific for <i>Anisakis</i> -infected patients.
Ani s 8	ES	SXP/RAL-2 family protein. Thermostable. Cross-reactivity with Ani s 5.
Ani s 9	ES	SXP/RAL-2 family protein. Thermostable. Share identity partial with Ani s 5 and Ani s 8.
Ani s 10	Somatic	Unknown function. With repeat sequences. Thermostable.
Ani s 11	Unknown	Unknown function. With repeat sequences. Ani s 11-like 78% similarity (17 kDa)
Ani s 12	Unknown	Unknown function. With repeat sequences.
Ani s 13	Unknown	Haemoglobin. No cross-reactivity to <i>Ascaris suum</i> haemoglobin.
Ani s 14	Unknown	Unknown function. Share identity partial with Ani s 7 and Ani s 12.

Source: Adroher-Auroux and Benítez-Rodríguez (2020)

Through research and experiments, it is found that the best and most effective way to get rid of these parasites is to deep-freeze or cook the seafood in high heat before eating it. This method is required for seafood that is to be eaten raw or partially cooked (lower than 60°C for 10 minutes). This is done first to ensure that *Anisakis* spp. larvae have been completely killed before consumed. To ensure the safety of fish product line in terms of *Anisakis* spp. larvae mortality, a fast-freezing rate is preferred.

Quicker frozen parasites are predicted to undergo less cell damage and soak up malachite green to a lower extent than slowly frozen larvae (Podolska et al., 2019; Łopieńska-Biernat et al., 2020). The ventriculus, which is the posterior part of the *Anisakis* spp. larva and contains the rectal and mucron glands, is usually the region affected when this method is applied effectively. When frozen, the part is thought to be the most vulnerable. This is beneficial because these parasites use the ventriculus to swarm into

the intestine lining (Podolska *et al.*, 2019). According to Lunestad *et al.* (2011), although it appears that a fast-freezing rate is recommended and effective in contexts of larvae mortality, it is believed that a longer deep freezing of the raw material is the ultimate solution in terms of lowering the IgE-mediated allergic reaction in sensitive consumers.

However, in the end, the freezing process is a fruitful and acceptable method of combating anisakiasis. The process must be carried out by the authorities' specifications, which are frozen in all parts of the seafood by  $-35^{\circ}\text{C}$  (or below) for not less than 15 hours or, if frequently frozen,  $-20^{\circ}\text{C}$  (or below) for not less than 24 hours for a maximum of 7 days. If consumers do not follow the guidelines set, there is still a high possibility for this parasite and its allergens to still exist in seafood and infect the eater later. Towards the problem of temperature inhomogeneity in freezers or freezers with low cooling capacity, it is advisable for the consumer to often measure the internal temperature of the seafood with a suitable thermometer rather than just rely solely on the freezing point set on the freezer (Łopieńska-Biernat *et al.*, 2020).

### ***Prevention by Consumer***

While this review aims to provide a global overview of public awareness and preventive

behaviours related to *Anisakis* spp., available data on the subject remain limited. To address this gap, a focused case study from Spain is presented to illustrate how public knowledge and preventive practices are manifested in a country with high seafood consumption and has relatively high incidence of anisakiasis. Spain serves as a useful example for exploring levels of public understanding, common misconceptions, and behavioural shortcomings regarding *Anisakis* prevention. This case highlights the broader need for international comparisons and coordinated public health efforts to raise global awareness and mitigate infection risks.

Public awareness plays a pivotal role in minimising the risks associated with *Anisakis* spp., as it directly influences consumer choices and safety practices. Although comprehensive global data are lacking, findings from Spain offer valuable insight into current awareness levels (Bao *et al.*, 2018). As shown in Table 3, 95% of respondents were aware of *Anisakis* and 82% claimed knowledge of preventive methods. However, further analysis reveals gaps: Only 52% correctly identified cooking as a preventive method, 78% recognised freezing, and just 15% cited gutting as effective. Alarmingly, some respondents mistakenly believed that marinating or smoking fish would eliminate the parasite.

Table 3: Survey conducted in Spain on respondents' awareness about *Anisakis* spp. and disease related

Variable Definition	Respondents' Choices from Questionnaire	Frequency of Responses (%)
Awareness of <i>Anisakis</i>	Yes	612 (95%)
	No	27 (4%)
	Not available	6 (1%)
Knowledge of <i>Anisakis</i> prevention methods	Yes	528 (82%)
	No	104 (16%)
	Not available	13 (2%)
Prevention methods (contingency multi-response question)		
Raw or lightly cooked fish	Yes	2 (0%)
	No	629 (98%)
	Not available	14 (2%)
Gutting the fish	Yes	94 (15%)
	No	537 (83%)
	Not available	14 (2%)
Cooking the fish	Yes	333 (52%)
	No	298 (46%)
	Not available	14 (2%)
Marinating or smoking fish	Yes	9 (1%)
	No	622 (96%)
	Not available	14 (2%)
Freezing the fish	Yes	505 (78%)
	No	126 (20%)
	Not available	14 (2%)

Source: Bao *et al.* (2018)

This case study underscores the urgent need for more targeted public education campaigns even in regions with relatively high awareness. It also highlights the importance of broader, cross-cultural studies to inform the development of globally relevant public health strategies.

Efforts to prevent anisakiasis should not rest solely on the shoulders of consumers; they require a coordinated, multi-sectoral approach. Authorities tasked with monitoring and controlling food-borne diseases must play an active role in identifying risk sources, enforcing regulations, and promoting effective interventions across the entire food supply chain from harvesting to consumption (Simsek *et al.*, 2020). For example, routine inspections

in Spain found no evidence of *Anisakis* spp. larvae in sampled anchovies, a result likely attributed to regular staff training and effective food safety protocols. The implementation of Hazard Analysis and Critical Control Points (HACCP) systems in food processing and handling has demonstrated success in improving food safety and reducing the incidence of food-borne illnesses. Given the critical role of such frameworks, it is essential that all stakeholders collaborate to continually review, update, and enforce regulations and best practices.

#### **HACCP Role and Importance**

Efforts to prevent anisakiasis require coordinated involvement from consumers, food industries,

and regulatory authorities. While consumer education is essential, effective prevention depends largely on systematic monitoring and safety protocols throughout the seafood supply chain (Simsek *et al.*, 2020). A cornerstone of this approach is the Hazard Analysis and Critical Control Points (HACCP) system, which helps identify and control potential hazards such as *Anisakis* spp. in seafood. In high-risk products like raw or lightly processed fish, HACCP strategies include proper gutting, freezing at -20°C for at least 24 hours, and temperature monitoring during storage and transport. These steps are enforced under European Union (EU) regulations, and similar measures are adopted in Japan, where anisakiasis cases are more common due to traditional dietary practices. In Malaysia, although data on *Anisakis* is still emerging, HACCP principles are incorporated into national seafood export standards, especially for frozen and processed fish. An illustrative case from Spain showed no *Anisakis* larvae in anchovy samples, a result attributed to consistent freezing protocols, routine inspections, and trained personnel which are the core elements of HACCP compliance. Third-party audits and certifications also enhance accountability and food safety performance.

To effectively reduce anisakiasis risks, all stakeholders must ensure that *Anisakis*-specific controls are embedded into HACCP plans. Regular updates, staff training, and cross-sector collaboration are essential for maintaining high safety standards. Strengthening and harmonising HACCP implementation globally, especially in regions with high seafood consumption, will be key to reducing the public health burden of anisakiasis.

## Conclusions

This review underscores the complex challenges posed by *Anisakis* spp., encompassing its allergenic potential, zoonotic threat, and economic burden on the seafood industry. Allergens such as Ani s 1, Ani s 7, and Ani s 12 emerged as key contributors to *Anisakis*-induced hypersensitivity, while other proteins including Ani s 4 to 6 and Ani s 8 to 11 play supporting roles in eliciting immune responses. These findings are crucial in addressing the growing public health concern surrounding

seafood-borne allergic reactions and highlight the urgent need for improved diagnostic tools and therapeutic approaches.

Furthermore, the review reveals notable disparities in consumer awareness and preventive practices, particularly regarding safe seafood consumption. Even in high-consumption countries like Spain, misconceptions persist about the effectiveness of marination or smoking, while proven methods such as cooking, freezing, and gutting are not consistently adopted. Such behavioural gaps increase the risk of infection and sensitisation, pointing to an unmet need for more effective education and communication strategies.

To address these issues, this review offers the following evidence-based recommendations:

- For consumers: Adopt preventive behaviours such as avoiding raw or undercooked seafood, freezing fish to -20°C for a minimum of 24 hours, and cooking seafood thoroughly (to at least 60°C). Increased public access to accurate information through online platforms, food labels, and point-of-sale materials is also essential.
- For the seafood industry: Strengthen the implementation of Hazard Analysis Critical Control Point (HACCP) systems specifically tailored to monitor and manage *Anisakis* contamination. Regular training for food handlers and quality assurance personnel can enhance detection and minimise risks along the supply chain.
- For policymakers and regulators: Support the development of harmonised regulations and surveillance systems to track *Anisakis* prevalence, improve inspection protocols, and fund public health campaigns. International collaboration is vital for establishing globally consistent standards for seafood safety.
- For clinicians and researchers: Focus on allergen characterisation, development of rapid and reliable diagnostic assays, and epidemiological studies to better understand exposure risks in different regions. Further research on cross-reactivity with other parasites and allergens is also needed.

By consolidating current knowledge on *Anisakis* spp. allergens, awareness trends, and mitigation strategies, this review offers a comprehensive foundation for enhancing seafood safety and public health globally. The findings underscore the need for coordinated, multi-stakeholder action including consumers, industries, scientists, and regulators to reduce the burden of anisakiasis and prevent future outbreaks. In the long run, raising awareness and promoting evidence-based practices are key to mitigating this under-recognised but significant health risk.

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### Conflict of Interest Statement

The authors declare no conflict of interest.

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