SCIENTIFIC OPINION

Scientific Opinion on risk assessment of parasites in fishery products[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Human fishery product-borne parasitic diseases are caused by cestodes, trematodes and nematodes and are caused by infection following ingestion of viable parasites, or as allergic (hypersensitivity) reactions against parasite antigens. For allergy, the only parasite in fishery products implicated is the nematode *Anisakis simplex*, and sensitisation occurs via infection by live larvae. Once sensitised, response to nematode allergens can be highly aggressive and generate severe disease. In a sensitised individual, infection can provoke a concurrent *A. simplex* allergic episode or can be elicited by exposure to allergen alone from killed parasite: the relative epidemiological impact for each is unknown. Allergy to *A. simplex* is relatively common in some regions in Spain but rarely reported in other parts of Europe. Prevention of sensitisation is most likely to be effective by control of *A. simplex* infection. There is more information on the resistance to physical and chemical treatments by *A. simplex* than for other fishery parasites, and the properties of other parasites are likely to be similar. Many traditional marinating and cold smoking methods are not sufficient to kill *A. simplex* and freezing or heat treatments remain the most effective processes guaranteeing killing. All wild caught seawater and freshwater fish are must be considered at risk of containing any viable parasites of human health concern if these products are to be eaten raw or almost raw. For wild-catch fish, no sea fishing grounds can be considered free of *A. simplex*. For farmed Atlantic salmon reared in floating cages or onshore tanks and fed on compound feedstuffs however, the current risk of infection with anisakids is negligible. Apart from farmed Atlantic salmon, sufficient monitoring data are not available for any other farmed fish therefore it is not possible to identify which fish species do not present a health hazard with respect to the presence of parasites.

KEY WORDS

Fishery products, *Anisakis*, allergy, farmed Atlantic salmon

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SUMMARY

Following a request from the European Commission, the Panel on Biological Hazards was asked to deliver a scientific opinion on food safety related to parasites in fishery products.

In particular EFSA was asked to: assess the food safety concerns due to possible allergic reactions in consumers to parasites that may be present in fishery products; evaluate alternative treatments for killing viable parasites in fishery products and evaluate their effectiveness compared to the freezing method described in the hygiene regulations; set criteria, if any, for when products are eaten raw almost raw or cold smoked from fishing grounds for wild catch and from aquaculture do not present a health hazard with regard to the presence of parasites, and an assessment of available documentation for farmed Atlantic salmon

EFSA concluded that human fishery product-borne parasitic diseases primarily include those caused by cestodes, trematodes and nematodes. These diseases are either caused by an infection following ingestion of viable parasites, or as an allergic (hypersensitivity) reaction against parasite antigens. The only parasite in fishery products that is implicated in allergic reaction is the nematode *Anisakis simplex* and that the primary initiator of the different forms of allergy is via infection by live larvae. Once sensitisation has occurred, response to nematode allergens can be highly aggressive and generate severe allergic disease. Some authors have shown that an infection can provoke a concurrent allergic episode in a sensitised individual, and claim this is the principal mechanism for disease. However, others consider that allergic episodes can not only be elicited by infection, but also by exposure to allergen remaining in food with no viable larvae. The relative epidemiological impact for each route of provoking an allergic episode is unknown. However there is general agreement that consumption of fishery products containing viable *A. simplex* larvae presents a greater risk for allergy than consumption of fishery products containing non-viable parasites. The different forms of allergy to *A. simplex* are relatively common in some regions in Spain but rarely reported in other parts of Europe.

EFSA concluded that there is more information on the resistance to physical and chemical treatments by *A. simplex* than for other fishery parasites. The properties of *A. simplex* are likely to be similar to that of other multicellular parasites (although trematode metacercariae are considerably more heat resistant). Freezing or heat treatments remain the most effective processes guaranteeing the killing of parasitic larvae, under well defined conditions. Treatments which provide an equivalent level of protection as freezing (-20ºC for not less than 24 hours) for the killing of *A. simplex* larvae include freezing at -35ºC for at least 15 hours or at -15°C for at least 96 hours, at the core of the fishery products and heat treatment at >60ºC for at least 1 minute. Many traditional marinating and cold smoking methods are not sufficient to kill *A. simplex* larvae.

All wild caught seawater and freshwater fish must be considered at risk of containing any viable parasites of human health concern if these products are to be eaten raw or almost raw. For wild catch fish, no sea fishing grounds can be considered free of *A. simplex* larvae. For farmed Atlantic salmon, if reared in floating cages or onshore tanks and fed on compound feedstuffs, which are unlikely to contain live parasites, the risk of infection with larval anisakids is negligible unless changes in farming practices occur. Apart from farmed Atlantic salmon, sufficient monitoring data are not available for any other farmed fish therefore it is not possible to identify which farmed fish species do not present a health hazard with respect to the presence of parasites.

EFSA recommends that co-ordinated studies to improve surveillance and diagnostic awareness of allergic reactions to parasites in fishery products should be implemented, and encourage epidemiological studies on a European scale to assessing the impact of *A. simplex* parasitized fish on human associated disease, including all allergic forms.

EFSA recommends that research should be improved on:

- The infectivity as well as inactivation of parasites in fishery products in relation to treatments, host fish species, and effects of passage through different hosts
- The effects of different farming practices on the prevalence of anisakids in aquaculture if products are to be consumed raw or almost raw.
- The collection of systematic data on the complete life cycle, geographical and seasonal distribution, prevalence, intensity, and anatomical location of parasites of public health importance in wild caught fishery products.

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BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

To assess the food safety concerns due to possible allergic reactions in consumers to parasites that may be present in fishery products

In 1998, the former Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) provided an opinion on "Allergic reactions to ingested *Anisakis simplex* antigens and evaluation of the possible risk to human health[4].

One of the conclusions was that "the real incidence of the allergic accidents after eating contaminated fish [……] should be carefully assessed. […….] Well conducted and coordinated studies should be encouraged […….].[5]

The EFSA Opinion of 2004 of the Scientific Panel on Dietetic Products, Nutrition and Allergies, following a request from the Commission relating to the evaluation of allergenic foods for labelling purposes, also highlights possible allergic reactions due to parasites that may be present in fishery products[6][7].

To date, one Member State has drawn the attention of the Commission to a large number of illnesses related to allergic reactions caused by Anisakidae. No other Member State has reported similar problems.

It is therefore appropriate to ask EFSA to provide an opinion on this issue.

To identify alternative treatments for killing viable endoparasites in fishery products and evaluate their effectiveness compared to the freezing method described in the hygiene regulations

According to Point D. 1 of Chapter III of Section VIII of Annex III to Regulation (EC) No 853/2004 certain fishery products must be frozen to a temperature of not more than -20°C in all parts of the product for not less than 24 hours.

The objective of this treatment is to kill viable parasites that may be present in the fishery products and that might result in a risk to health of the consumer when the fishery product is to be eaten raw, almost raw or cold-smoked. Furthermore, the legislation lays down a freezing requirement for marinated and salted products, if the processing is insufficient to destroy nematode larvae.

Chapters VII and VIII of the same Section of the Regulation specify temperatures for storage and transport of frozen fishery products, which differ from the one above.

Before amending the section on the treatment for killing viable parasites in fishery products, the Commission requests a scientific opinion on relevant alternative treatments that ensure the same level of protection as the current standard treatment, i.e. freezing at a temperature of not more than -20°C in all parts of the product for not less than 24 hours.

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4 http://ec.europa.eu/food/fs/sc/scv/out05_en.html

5 Studies conducted after 1998 have proven that allergic symptoms appear in relationship with acute parasitism by the live third stage larva of *Anisakis*, when it enters the gastric mucosa after eating improperly handled fish (see chapter 2.4, 2.8.2 and 3.2 of the present opinion).

6 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620761196.htm

7 Even in this EFSA opinion, those articles published from the year 2000 addressing the necessity of live larva in order to produce allergic reactions, were not mentioned.

It should be noted that the French Food Safety Agency (AFSSA) has provided an opinion (2007-3A-0379) which assesses the effectiveness of certain alternative treatments to freezing to kill viable Anisakidae, in particular salting and marinating.

Criteria determining when a fishing ground and farmed fishery products do not present a health hazard with regard to the presence of parasites, if eaten raw or almost raw, and an assessment of available documentation for farmed Atlantic salmon

According to Article 11.8 of Regulation (EC) No 853/2004 criteria may be specified for determining when epidemiological data indicate that a fishing ground does not present a health hazard with regard to the presence of parasites and food business operators may be authorised not to freeze fishery products.

Furthermore, it is desirable to specify criteria for determining under which conditions fishery products from aquaculture production can be recognised as being free of viable parasites that might result in a risk to health of the consumers, if eaten raw or almost raw.

It is also relevant to assess the available documentation regarding farmed Atlantic salmon, whose feed has been under strict control, in order to determine whether it is feasible to recognise the fishery products as being free of parasites that might result in a risk to health of the consumers.

It is therefore appropriate to ask EFSA to provide an opinion on these issues.

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

In view of the above, EFSA is requested to provide scientific opinions in response to the questions below.

1. **Assessment of the food safety concerns due to possible allergic reactions in consumers to parasites that may be present in fishery products**

EFSA is requested to assess the food safety risk relating to allergic reactions due to antigens from parasites in fishery products. In particular, the opinion should include prevalence rates for allergic reactions due to intake of:

- viable parasites in fishery products;
- non-viable parasites in fishery products;
- fishery products where parasites have only been present in parts of the fish that are not consumed (e.g. in intestines).

2. **Identification of alternative treatments for killing viable parasites in fishery products and evaluate their effectiveness compared to the freezing method described in the hygiene Regulations**

EFSA is requested to identify relevant alternative methods/treatments for killing viable parasites and evaluate their effectiveness compared to the freezing method described in the hygiene Regulations. These methods should provide the same level of protection as freezing at a temperature of not more than -20°C in all parts of the product for not less than 24 hours.

3. Criteria for when fishing grounds for wild catch and fishery products from aquaculture do not present a health hazard with regard to the presence of parasites, and an assessment of available documentation for farmed Atlantic salmon

EFSA is requested to:

- Specify criteria for determining when epidemiological data indicate that a fishing ground does not present a health hazard with regard to the presence of viable parasites, if the fishery products are to be eaten raw or almost raw;
- Specify criteria for determining under which conditions fishery products from aquaculture production can be recognised as being free of viable parasites that might result in a risk to health of the consumers, if to be eaten raw or almost raw;
- In view of the above assess the available documentation for farmed Atlantic salmon in order to determine whether it is feasible to recognise such fishery products as being free of parasites that might result in a risk to health of the consumers, if to be eaten raw or almost raw;
- If EFSA should be unable to draw a conclusion regarding farmed Atlantic salmon to indicate which additional data are needed and which requirements need to be met in order to recognise such fishery products from Atlantic salmon as being free of parasites.

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INTRODUCTION

For millennia a wide variety of products derived from marine and freshwater animals have been used for human consumption and to feed animals raised with the intention to be consumed by humans. Regulation (EC) No 853/2004 defines 'Fishery products' as all seawater or freshwater animals (except for live bivalve molluscs, live echinoderms, live tunicates and live marine gastropods, and all mammals, reptiles and frogs) whether wild or farmed and including all edible forms, parts and products of such animals. Consequently, the term 'fishery products' includes, among others, live finfish and crustaceans, their products as flesh and edible organs (particularly liver and gonads of fish) and products such as fish oil. The term also includes bivalve molluscs, echinoderms, tunicates and marine gastropods not marketed alive.

Parasitic diseases are those caused by eukaryotic organisms (both unicellular of multicellular). Human fishery product-borne parasitic diseases primarily include those caused by cestodes, trematodes and nematodes. These diseases are either caused by an infection following ingestion of viable parasites, or as an allergic (hypersensitivity) reaction against parasite antigens which occurs for nematodes of the family Anisakidae. While many in industrialised countries will recognize the importance of meat-borne parasitic zoonoses such as trichinellosis and cysticercosis, fishery product-borne parasitic disease like opisthorchiasis, intestinal trematodiasis, anisakiasis or diphyllobothriasis have received less attention despite the large numbers of human infections (Chai et al., 2005a). Human fishery product-borne parasitic infections have generally been limited to populations living in low- and middle-income countries, but the geographical limits and populations at risk are expanding because of growing international markets, improved transportation systems, globalisation of the food supply and demographic changes (Chai et al., 2005a). The World Health Organization has estimated that the number of people currently infected with fish-borne trematodes exceeds 18 million (World_Health_Organization, 1995), but worldwide the number of people at risk, including those in developed countries, is more than half a billion. The recognition of the public health significance of these diseases is increasing, because of intensification of aquaculture; environmental damage; a lack of appropriate tools for control; links with poor sewage treatment and poverty; and cultural traditions of eating raw or minimally processed fishery products (World_Health_Organization, 1995, 2004).

In order to prevent and control transmission of fishery product-borne parasites to humans, in particular *Anisakis* spp., Section VIII of Annex III to Regulation (EC) No 853/2004 lays down provisions for fishery products to be consumed raw or almost raw, fishery products that are to undergo a cold smoking process, and marinated and/or salted fishery products, if the processing is insufficient to destroy nematode larvae. Such products must be frozen at a temperature of not more than -20°C in all parts of the product for not less than 24 hours. The treatment must be applied to the raw product or the finished product. A document from the manufacturer, stating the type of process they have undergone, must accompany fishery products when placed on the market, except when supplied to the final consumer. A competent authority may, however, authorise that the food business operators need not carry out the freezing treatment required if epidemiological data are available indicating that the fishing grounds of origin do not present a health hazard with regard to the presence of parasites.

The onset of allergic urticaria and anaphylactoid syndromes associated with gastrointestinal infection by *A. simplex* was first described in Japan (Kasuya et al., 1990). Subsequently, this parasite was identified as an etiological agent of other allergic reactions mediated by IgE (Audicana et al., 1995). Following these initial descriptions, Spanish allergists became aware of this newly described syndrome and a series of reports after 1995 described cases involving clinical symptoms ranging from isolated angioedema and urticaria to life-threatening anaphylactic shock (Audicana et al., 1995). Furthermore, reports from Spain and from other countries (Alonso et al., 1997; Daschner et al., 1998; Foti et al., 2002; Pecquet et al., 2002) described syndromes combining simultaneous allergy and infection (gastroallergic anisakiasis, see section 3). Other immunologically mediated pathologies were

described including eosinophilic gastroenteritis, occupational respiratory diseases (Nieuwenhuizen et al., 2006; Scala et al., 2001), rheumatic disease and contact dermatitis. In addition, sensitization defined by specific IgE against the *A. simplex* in asymptomatic individuals was also described in Europe and Japan (Kasuya et al., 1990).

Parasite life cycles - definitive, intermediate and paratenic[*] (or transport) and accidental hosts

In this document the above terms will be used to describe the natural or accidental hosts that are infected by parasites. This document focuses to a considerable extent on larval anisakid nematodes as these parasites represent the major parasitological risk to humans from the consumption of fish. *Anisakis* and *Pseudoterranova*, the genera involved with human disease, have various life-cycle stages which are found in intermediate, paratenic or definitive hosts. Definitive hosts are animals in which the sexually reproducing adult form of the parasite occurs and from which offspring are shed. Intermediate hosts are those that harbour juvenile stages of the parasite (their larvae), and allow the parasite to moult one or more times. Parasites may have various larval stages which, in parasitic nematodes like anisakids are denominated first-stage larva (L1), second-stage larva (L2), etc. In paratenic host, no larval development occurs but it may be crucial for successful transfer of the parasite to the next host level, e.g. to another transport host in the food chain, or to the definitive host. Accidental hosts are those that are not part of the natural chain of infection and do not normally lead to infection of the definitive hosts, but are accidentally infected and are dead ends in the life cycle of the parasite. In the case of anisakid infections, humans are therefore accidental hosts.

[*] See glossary

1. HAZARD IDENTIFICATION AND CHARACTERISATION

1.1. Parasites of public health importance in fishery products

1.1.1. Nematodes

Anisakis spp. is a genus of nematode that causes human parasitic infection most commonly associated with consumption of fishery products. It belongs to the family of Anisakidae together with other genera including *Pseudoterranova, Phocascaris, and Contracaecum*. These worms, commonly called anisakids, utilize aquatic mammals, aquatic birds, reptiles and fish as definitive hosts, and aquatic crustaceans (krill) as intermediate hosts. Fish and molluscs (particularly squid) act also as transport hosts, which carry the infective larvae. The species most commonly associated with human infection is *A. simplex*, followed by *Pseudoterranova decipiens*. *A. simplex* is about 2 cm long, easy to see in the viscera, but difficult to see in the fish musculature and in the belly flaps of white fish.

1.1.1.1. *Anisakiasis* in humans

Although the first case of anisakid infection was described by Leuckart in Groenland in 1876, the disease was more widely described in the 1950s and 1960s when epidemics of anisakiasis occurred in the Netherlands following ingestion of "green" (i.e. lightly salted) herring (154 proven cases between 1955 and 1968; (Van Thiel, 1960 1962). Of the approximately 20,000 cases of anisakiasis reported to date worldwide, over 90% are from Japan (where approximately 2,000 cases are diagnosed annually), with most of the rest from Spain, the Netherlands and Germany (Audicana et al., 2002; Bouree et al., 1995).

Over the last 30 years, there has been a marked increase in the reported prevalence of anisakiasis throughout the world. This increase is probably due to: more widespread application of diagnostic techniques, particularly endoscopy (previously many cases of gastric anisakiasis were probably misdiagnosed; (Oshima, 1987); increasing global demand for seafood; a growing preference for raw or lightly cooked food, especially in many Western countries, with increased risk of parasite exposure (McCarthy and Moore, 2000).

Human anisakiasis may be gastric or pharyngeal which are most often associated with *A. simplex* or *Pseudoterranova decipiens* respectively. Invasion by the parasite into the gastrointestinal tract may lead to eosinophilic granuloma formation in the mucosa with severe symptoms of disease (Oshima, 1987).

Because the symptoms of anisakiasis are non-specific, the disease is often misdiagnosed. For example, in a single study, over 60% of the cases were diagnosed preoperatively as appendicitis, acute abdomen, gastric cancer or Crohn's disease (Sakanari and McKerrow, 1989). The clinical diagnosis is usually performed through endoscopy or radiological examination, whereas various immunologic assays have been used for indirect diagnosis, including the skin-prick test, complement fixation test (CFT), immunofluorescent-antibody test(IFAT), immunodiffusion test (IDT), immunoelectrophoretic assays, enzyme-linked immunosorbent assay (ELISA), and radio-allergosorbent test (RAST). Interpretation of the serological tests may be difficult because anisakiasis patients' sera cross-react with antigens from closely related nematode species (e.g. *Ascaris* and *Toxocara* species) and because sera from unaffected individuals may contain specific antibodies which can give false-positive results against *Anisakis* antigens. Of all serodiagnostic assays, the RAST is the most sensitive and specific (Sakanari and McKerrow, 1989).

For acute gastric anisakiasis, endoscopic removal of the parasite is usually necessary, while treatment for all other forms depend on the complications produced, e.g. surgical removal of granuloma or

corticosteroids to reduce inflammation. No specific pharmacological treatment has been identified for effective killing of live parasites in vivo and the most effective treatment remains prevention.

Observations suggest that parasites and the human immune system (as well as other hosts immune systems), have co-evolved over millennia in order to minimize collateral damage of the host tissue, and to remain alive to ensure the reproduction of parasite. Thus, some common helminthic parasites of man such as *Schistosoma* and *Ascaris* show evidence of adaption to the human immune system despite their inherent antigenicity (Falcone and Pritchard, 2005).

Human infections with nematodes and other helminth parasites are typically associated with tissue eosinophilia and elevated levels of IgE, signs that may also occur in patients showing type I hypersensitivity against certain antigens. Tropomyosin, a highly conserved protein in the muscle and skin of many invertebrates, is a well recognised potent allergen since the molecular structure of certain regions of invertebrate tropomyosin differs considerably from the corresponding stretches of the vertebrate (human) type (Sereda et al., 2008). Paradoxically, both epidemiological studies and experimental animal models imply that chronic infections with some helminth parasites may protect against, or at least alleviate allergic reactions (Falcone and Pritchard, 2005; Trujillo-Vargas et al., 2007; van den Biggelaar et al., 2004; Wohlleben et al., 2004). This again suggests that the basic elements for allergic reactions are present but clinical reactivity is down regulated (Jackson et al., 2006; Mangan et al., 2006; Turner et al., 2005; van den Biggelaar et al., 2000; Wilson et al., 2005). However, since neither adult nor larval anisakids are natural human parasites, no mutual adaptation has evolved and, consequently, possible modulating or immune down regulation are absent. Moreover, due to the similarity of invertebrate tropomyosins, the specifically generated IgE antibodies directed against *Anisakis* larvae, cross-react widely, particularly among seafood allergens and other allergens such as shrimp and house dust mite, respectively (Reese et al., 1999).

The *Anisakis*-associated allergic response has only been identified as associated with *A. simplex* and may lead to severe clinical symptoms including anaphylactic shock (Alonso et al., 1997; Audicana et al., 2002). Allergic reactions to *A. simplex* antigens are associated with the production of specific IgE, although specific IgE is detected in all patients following anisakiasis including patients without allergic symptoms. These aspects will be more fully described in the chapter 3. The allergic forms are usually treated symptomatically with antihistamines and corticosteroids and sometimes with epinephrine.

1.1.1.2. Life cycle and life history

Anisakids typically utilise marine mammals or piscivorous birds as definitive hosts, with planktonic or benthic crustaceans acting as intermediate hosts and fish as main transport hosts. A wide range of fish species can carry larval anisakids including in the fish flesh thus representing the pathway for human infections. Adult *A. simplex* are found mainly in the gastrointestinal tract of cetaceans (dolphins, porpoises and baleen whales), while the adults of *Pseudoterranova* spp. and *Phocascaris* spp. live in pinnipeds (seals, sea lions and walrus), with the latter occurring only in the northern hemisphere including arctic waters. Some species of *Contracaecum* reach maturity in pinnipeds while others mature in fish-eating birds such as cormorans, pelicans and herons. However, the definitive host range of many anisakid species is still incompletely understood (Anderson, 1992). Additionally, there is some controversy whether or not any alternative transmission routes exist such as direct infection of fish by ingesting free-swimming larvae, or the transfer of larvae from crustaceans such as krill, to plankton-eating or omnivorous cetaceans, i.e. by skipping the fish transport host.

After final moulting, maturation and copulation, the female worms shed eggs within the definitive host's faeces, which embryonate and hatch in the water releasing free-swimming 3rd stage larvae (Koie et al., 1995). The larvae are ingested by crustaceans such as decapods, copepods or amphipods in which they grow within the haemocoel. Fish and cephalopod molluscs (squids) become infected by eating planktonic or benthic crustaceans containing third stage larvae which bore through the wall of

the digestive tract into the viscera and body cavity followed by host induced encapsulation (Anderson, 1992). When an infected fish is eaten by another fish, the encapsulated larvae become digested thus repeating the larval fish host cycle. This is important from an epidemiological and food safety perspective since the repeated transfer of larvae between fish within the natural food-chain may result in extensive accumulation, especially in large and older fish, sometimes harbouring hundreds or even thousands of encapsulated larvae (Smith and Wootten, 1978). However, the number of fish host cycles which individual larvae may carry through without loosing infectivity, has not yet been investigated. The definitive hosts become infected by eating fish or cephalopods containing the larvae. The generalised anisakid life cycle is shown in the Figure 1.

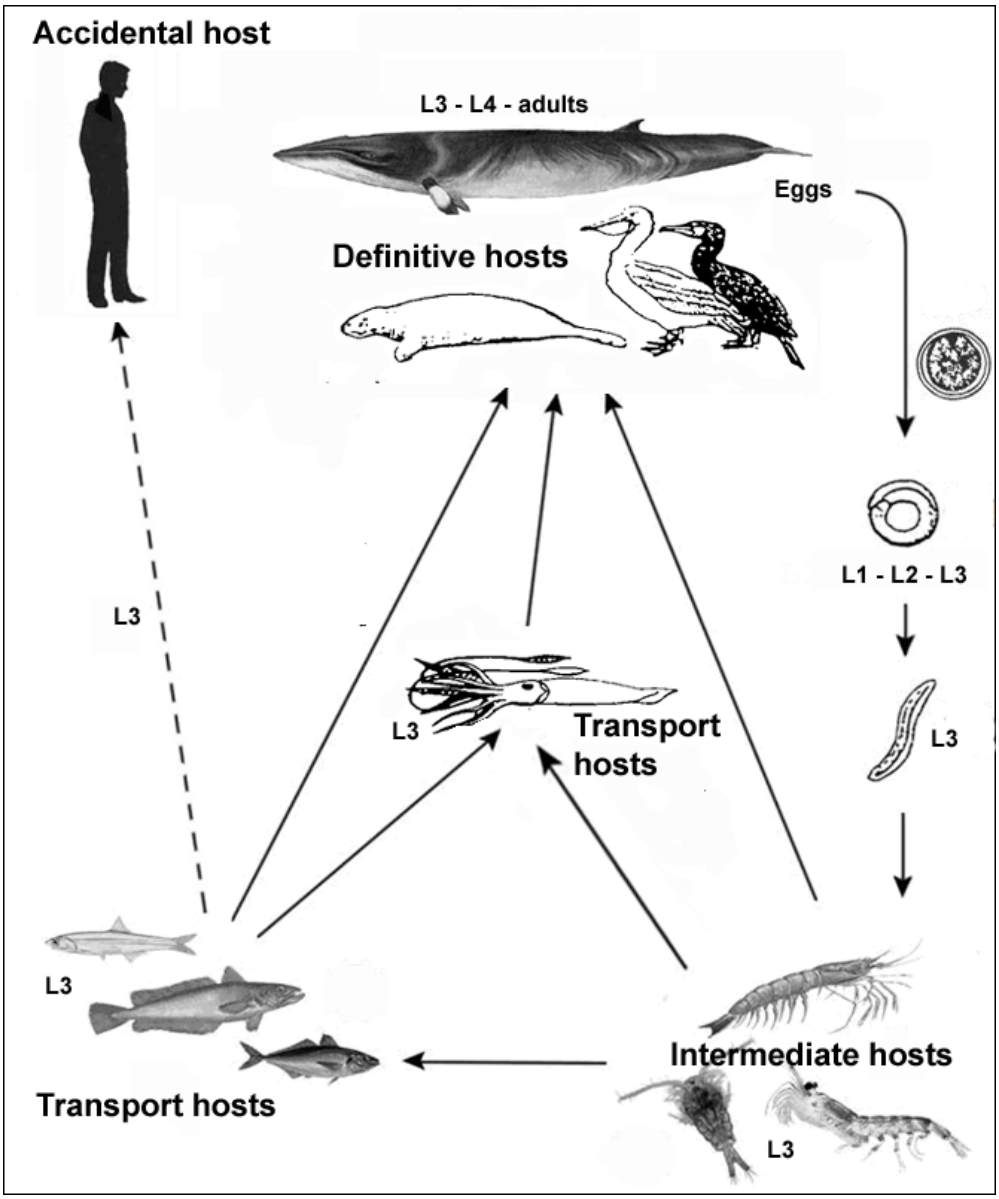

Figure 1: Life cycle of anisakid parasites (Levsen, 2010, modified from S. Mattiucci).

Species identification in the Anisakidae has traditionally been complicated by a lack of distinguishing morphological characteristics, particularly in larval worms. Distinct species of *Anisakis* and *Pseudoterranova* are known although they cannot be reliably distinguished morphologically at the larval stage and molecular differentiation is required. Historically, only two species were recognised

as pathogenic to humans: the 'herring worm' *A. simplex* and the 'codworm' *Pseudoterranova* (syns *Phocanema*, *Terranova*) *decipiens*, both with potentially cosmopolitan distributions (Oshima, 1987; Smith and Wootten, 1978). Molecular genetic studies, however, have shown that both of these morphospecies comprise several different species, often with distinct geographical and/or host ranges. At least three species have been described within the *A. simplex* complex: *A. simplex* (sensu stricto), found in the northern Atlantic, *A. simplex* C, found in the northern Pacific and southern waters below 30°N; and *A. pegreffi*, found in the Mediterranean Sea (Mattiucci et al., 1997). In addition to these three geno-species, four other species of *Anisakis* have been confirmed using genetic markers: *A. typica* from Indian Ocean and Mediterranean Sea; *A. physeteris*, from the Atlantic Ocean and Mediterranean Sea; *A. brevispiculata*, from south east Atlantic; *A. zyphidarum* from south east Atlantic and Mediterranean (Mattiucci et al., 2005).

Paggi (1991) described three 'species' within the *P. decipiens* sensu lato complex: *P. decipiens* A in the north east Atlantic and Norwegian Sea, *P. decipiens* C in the north west Atlantic and Barents Sea, and *P. decipiens* B throughout northern waters; where the ranges of these species overlapped, they appeared to preferentially utilise different definitive host species. Further genetic studies are required to confirm the geographical and host ranges of these species, and to establish their relationships with other species of *Anisakis* and *Pseudoterranova*, which have been described on morphological criteria.

Many fish species act as paratenic hosts for *Anisakis* and *Pseudoterranova* species and differences in host range between species have been found. For example, *A. simplex* and *Pseudoterranova* spp. occur most often in benthic or demersal fish, while *A. pegreffi* is found more frequently in pelagic fish (Abollo et al., 2001; Anderson, 1992; Mattiucci et al., 1997; Paggi et al., 1991). These differences appear to be more related to geographic distribution and the feeding habits of hosts rather than to behavioural or physiological host preferences of the parasites. Because of the difficulties in species identification, these two groups of parasites will be subsequently referred to as *A. simplex* and *P. decipiens* sensu lato in this document unless stated otherwise.

In an individual fish, the majority of anisakid larvae are typically encapsulated as flat tight spirals, measuring 4 to 5 mm in cross section, as well as on or within visceral organs, mesenteries and peritoneum. However, a smaller number of larvae can migrate from the abdominal cavity resulting in the presence of worms in the fish musculature, which may be noticed by the final consumer and/or food safety authorities. Most of the flesh-invading larvae seem to reside in the belly flaps, some may, however, penetrate deeply into the dorsal musculature of their fish host.

An on-going investigation of the occurrence and spatial distribution of *A. simplex* third stage larvae in three commercially important pelagic fish species from the NE Atlantic has so far revealed significant differences as to various larval infection parameters between the fish hosts (Levsen and Midthun, 2007). Preliminary data suggest that the overall *A. simplex* prevalence in blue whiting is 100%, reaching 90% in the flesh. A significant difference in larval abundance* in the flesh (per individual) was found between the smallest and the larger blue whiting, i.e. 7 ± 5 and 4 ±4 larvae per fish, respectively. The liver seems to be the most commonly infected organ carrying 69%, 50% and 43% of the total *A. simplex* burden in the smallest, medium sized and larger fish, respectively. There was also a significant decrease in larval abundance in the liver with increasing fish size. In Atlantic mackerel, the overall prevalence was 97%, while 70%, 57% and 24% of the smallest (< 300 g), medium sized (300-500 g) and larger fish (> 500g) carried *A. simplex* in the flesh, respectively. The mean abundance in the flesh was 2±3 larvae per fish in the smallest size group. In herring, both prevalence* and abundance increased with body size. The abundance in the flesh was low, reaching a maximum of 0.5±2 larvae per fish in the largest size group (150-300g). The most prominent infection site in both mackerel and herring was the pylorus area including the posterior stomach blind-sack, carrying between 57% and 81% of all larvae. The findings suggest that the *A. simplex* infection pattern in

* See glossary

pelagic fish is related to specific life history, e.g. the feeding habits and age of the host species, and, probably, host specific immunological characteristics. Additionally, the visceral organ topography seems to be important. For example, the relatively larger liver in small blue whiting probably "entraps" most of the larvae immediately after their emergence in the visceral cavity. These findings further suggest that the larvae encapsulation site is not dependent on the availability of nutrients, e.g. in the liver, but rather on the immunological capacity of each individual host to control parasite development and migration.

Although there are large differences in the prevalence and abundance of larval anisakids between species of demersal fish, there is no evidence of physiological host specificity. Rather, as described earlier for pelagic fish, the pattern of infection between and within fish species is driven by features such as feeding habits and habitat utilisation. Thus, cod and monkfish probably acquire much of their parasite burden via larvae from other fish hosts (Petrie, 2009). A number of authors have demonstrated how the larvae of *A. simplex* and *P. decipiens* are able to transfer between fish hosts, and in the case of *A. simplex* between multiple hosts (Scott, 1954; Smith, 1974). Other demersal species, such as whiting, which do not feed directly on the seabed, do not acquire *Pseudoterranova*, but do acquire *A. simplex* probably through feeding of invertebrate hosts such as euphausiids. As with some pelagic species such as herring, many demersal fish show an increase in prevalence and abundance of larval anisakids with age and size (Wootten and Waddell, 1977) and this may reflect an increase in the ingestion of larvae with prey and the longevity of larvae which are known to be able to survive for a minimum of 60 weeks in fish (Smith, 1984). However, the ability of individual larvae to establish and survive in some fish species such as Atlantic mackerel seems to be governed, at least in part, by the host's immune system (Levsen and Midthun, 2007). Thus, the apparent trade off between the need to cope with the infection and the ability of individual larvae to escape the host's immune response may result in a negative non-linear relationship between fish host age and parasite abundance (Fig. 2).

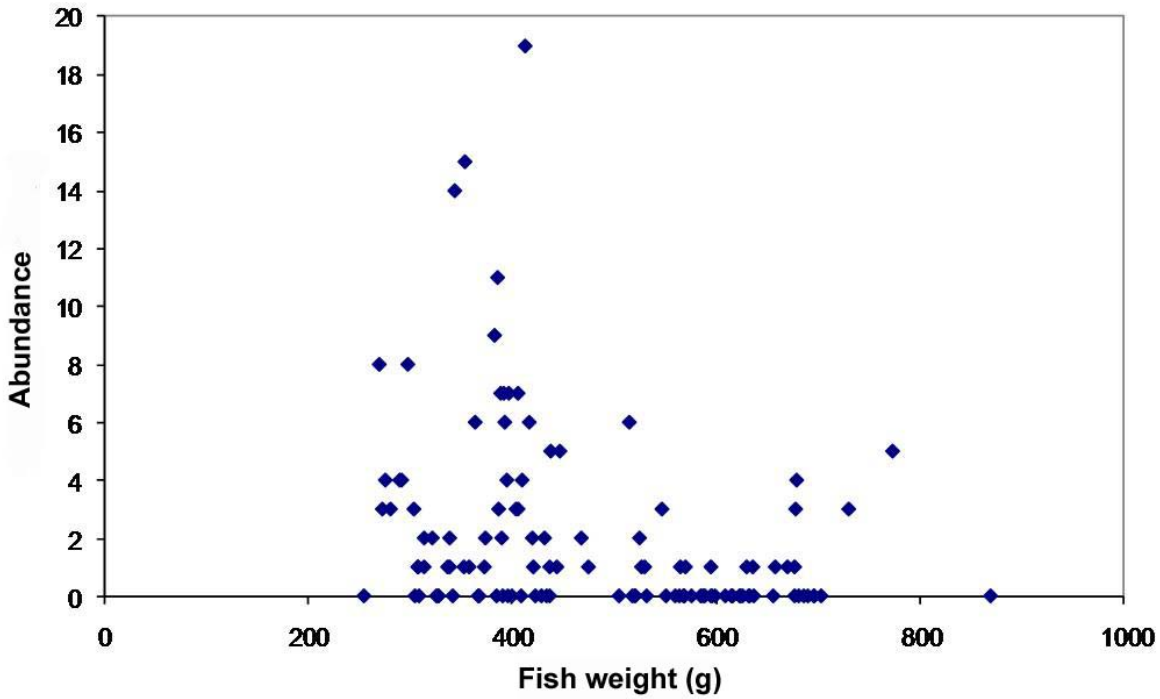

Figure 2: Relationship between abundance of A. simplex third stage larvae in the fillets and body weight of Atlantic mackerel (Levsen, 2007).

A positive relationship between body size or age and larval nematode prevalence and/or abundance has been demonstrated in several commercially important fish species from different areas of the North Atlantic, including cod and herring (Banning and Becker, 1978; Bussmann and Ehrlich, 1979; Davey, 1972; Levsen and Midthun, 2007; McGladdery, 1986; Platt, 1975; Smith, 1984; Smith and Wootten, 1978; Valero and Martín-Sánchez, 2000). However, additional information on the possible relationships between fish body size and larval abundance in the flesh (or other edible parts of fish such as the liver and roe of cod) appears to be scarce. Except for the above mentioned study on Atlantic mackerel which revealed higher *A. simplex* abundances in the flesh of smaller mackerel (Levsen and Lunestad, 2010; Levsen and Midthun, 2007) recently found that in Norwegian spring spawning (NSS) herring, fish size and larval abundance in the flesh were positively correlated only in the largest size group (> 400g). Thus, even if data on the overall larval prevalence or abundance in fish species from given areas was available, it is not possible to reliably predict the presence or absence, or level of infection of *A. simplex* larvae in the flesh of the specific wild fish host species. Indeed, the findings show that samples of NSS herring with high larval abundance in or on the organs of the visceral cavity, may be free of larvae in the fish flesh, or vice versa (Levsen and Lunestad, 2010). In demersal fish there appears to be differences in *A. simplex* distribution between host species. In whiting for example, Wootten and Waddell (1977) found that significantly more larvae were present in the muscle in some samples, whereas in cod and other demersal species relatively few parasites are present in the flesh.

P. decipiens appears to show trophism for the musculature in that the largest proportion of the total worm burden is found in this site in many species, especially of demersal fish (McClelland, 2002a). Within the musculature of cod and monkfish, a higher proportion is found in the hypaxial* muscles (belly flaps) than in the epaxial* muscles (fillets) (Petrie, 2009; Wootten and Waddell, 1977; Young, 1972). The reasons for the difference in distribution are not known.

1.1.1.3. Distribution of parasites in fish body, pre and post mortem

There have been conflicting reports on whether anisakid nematodes migrate from the viscera into the muscle of fish after death of the host. Van Thiel (van Thiel, 1962) suggested that this occurred in herring and subsequently other authors (Smith and Wootten, 1975) reported a significant increase in the proportion of *A. simplex* larvae in the muscle of herring after fish were kept in ice for up to 48 hours after capture. These observations suggest that encapsulation of larvae from the viscera is followed by migration into the muscle. Hauck (Hauck, 1977) also found a significant increase in numbers of *A. simplex* larvae in the muscle of cold smoked Pacific herring with time after capture. It was hypothesized that this apparent migration was due to post-mortem changes in the decomposing viscera and/or the exposure of larvae to the cold smoking temperatures and brining salinities.

However, Roepstorff (Roepstorff et al., 1993) found no evidence of migration of *A. simplex* larvae post-mortem in herring when fish were maintained on ice, in chilled sea water, or in 10ºC sea water. The reasons for the discrepancy between different studies are unknown. Karl (Karl et al., 2002) found no evidence of post-mortem migration of *A. simplex* larvae into the muscle of haddock, saithe and ocean perch after capture. A similar lack of any evidence for post-mortem migration of *A. simplex* larvae was reported in Chilean hake (Cattan and Carvajal, 1984).

In pelagic fish, for example North Sea and NSS herring, the largest proportion of flesh residing *A. simplex* larvae were found in the belly flaps and no significant difference between the left and right flesh side was found (Karl et al., 2002; Levsen and Lunestad, 2010).

It is therefore not clear when, under what conditions and in which fish specie post-mortem migration of *A. simplex* larvae occurs.

* See glossary

Therefore the effects of processing practices on the exposure to consumers can not be predicted.

1.1.1.4. Detection methodologies in fishery products

Fish can be examined for the presence of parasites by a variety of methods including visual inspection, slicing, candling, pressing, digestion and recently by Polymerase Chain Reaction (PCR) (Lopez and Pardo, 2010; Mossali et al., 2009). Visual inspection of fillets will reveal worms embedded near the surface which can be removed easily with a knife during processing. Worms embedded deep in the flesh however are not immediately obvious, but some can be detected by candling, that is shining a bright light through the fillet. The simplest kind of candling table is a box about 50 cm square with a ground glass or perspex top about 6 mm thick. The inside of the box is white, and is lit by two fluorescent tubes giving a white uncoloured, light. To use the box, the fillet is laid down on the illuminated top; worms show up as dark shadows in the flesh, and can be removed with forceps or a knife. Light from above the box should be restricted. The box is unsuitable in bright sunlight and although an experienced operator can handle up to 300 fillets an hour, user fatigue can result in reduced detection rates (Wootten and Cann, 2001). In commercial practice, visual inspection and candling is effective at detecting *Pseudoterranova* in thin skinless fillets of white fish, particularly cod; however the method does not work well on thick fillets with the skin on. Candling is less effective in detecting *A. simplex*. Time can be saved by candling a sample of fillets from a batch of suspect fish to determine the level of infestation which can be used to establish whether the whole batch needs to be candled, and whether the batch is more suitable for other purposes.

A comparison of the efficacy of detection methods showed that visual inspection and candling of fillets detected only approximately 50% of the numbers of parasites detected by combining both candling and destructive slicing. Candling was demonstrated to be effective in fillets of up to 2.5 cm in thickness, after which effective detection could not be achieved. In contrast, when applying successively more accurate detection methods i.e. candling, artificial digestion and UV illumination, Levsen (Levsen et al., 2005) showed that only 7 to 10% of the *A. simplex* larvae in the fillets of herring, mackerel and blue whiting were detected by candling. However, visual inspection and candling appear to be sufficiently effective in detecting worms in the belly flaps recovering at least 75% of the larvae present. In herring and mackerel it was found that there was no significant difference in the numbers of *A. simplex* recovered by digestion and pressing (Petrie, 2009).

The pressing method is widely used for systematic detection of nematode larvae in the flesh of fish in specific surveys.

This method utilises the fluorescence of frozen *A. simplex* larvae (Pippy, 1970) and is based on visual inspection of flattened/pressed and deep-frozen fish fillets or viscera under UV-light (Karl and Leinemann, 1993). Prior to the pressing process, each fish is gutted, manually filleted and deskinned before placing the visceral organs and both left and right flesh side (fillets incl. belly flaps) into clear plastic bags. The fillets are then pressed to 1–2 mm thickness in a hydraulic press. The bags containing the flattened fillets or viscera are then deep-frozen (≤ -18 °C) for at least 12 h prior to visual inspection under a 366 nm UV-light source. Any *A. simplex* larvae present appear as more or less brightly fluorescent spots in the samples (Fig. 3). Additionally, the method allows the approximate determination of the larval infection site in the fillets, i.e. whether they are situated in the dorsal (upper) or ventral (lower) portion of the fish flesh (Levsen and Lunestad, 2010).

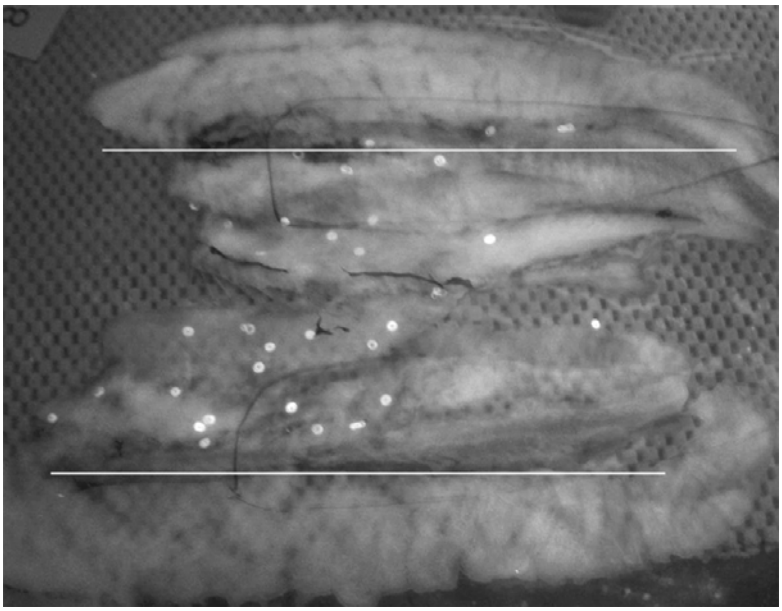

Figure 3: Pressed and subsequently frozen untrimmed fillets of blue whiting (*Micromesistius poutassou*) seen under UV-light. The *A. simplex* larvae present emerge as brightly fluorescent spots. The straight lines mark the approximate boundary between the belly flaps and the dorsal musculature of each fish side (Photo by A. Levsen).

The digestion method involves the use of a pepsin/hydrochloric acid solution to free anisakid larvae from muscle or other tissues (Jackson et al., 1981; Smith and Wootten, 1975). Pepsin is added to a 0.85 NaCl solution to a concentration of 10 mg/l. Pieces of fish for examination are then placed in a suitable glass container and pepsin solution added. The pH of the solution is then adjusted to pH 2 with concentrated hydrochloric acid and the solution incubated overnight at 38ºC. The solution is then sieved (1.5 x 1 mm mesh) and the contents of the sieve examined for larval nematodes. Live parasites survive the process unharmed and are easily detected. Dead worms, e.g. from frozen material, are also recovered using this method. The method recovers virtually all anisakid nematodes although it is time consuming and thus used for specific surveys rather than mass screening.

A real-time polymerase chain reaction (PCR) method has recently been developed for the identification of *A. simplex* in seafood products (Lopez and Pardo, 2010) combined with an optimised DNA extraction procedure. The method is highly specific and sensitive with a detection limit of 40 ppm parasite in 25g of sample, and may be used for fresh and processed material. This method is likely to be most suitable for testing of batches of fish products in specific surveys rather than screening of industrial fish.

1.1.2. Trematodes

It is estimated that more than 50 million people are infected with food-borne trematodes worldwide. Most of these infections result from the consumption of raw or undercooked freshwater fishery products. The highest prevalence of these infections is in southeast and east Asia, but increasing numbers of infections are being recognised in areas previously considered non-endemic, due largely to increased importation of contaminated seafood and travel to endemic regions.

There is a range of trematodes which may cause fishborne human disease. Among them liver flukes belonging to the family Opisthorchiidae are of the highest public health concern and will receive the most attention in this chapter. Intestinal flukes of the family Heterophyidae and Echinostomatidae are parasites of birds and mammals and about 35 and 15 species, respectively are also pathogenic to

humans. Infections in humans with intestinal flukes are of less clinical importance than diseases caused by liver flukes. Recent studies highlight the importance of these flukes (Chai et al., 2005a).

Trematodes belonging to the family Opisthorchiidae play an important role as fish-borne parasitic zoonoses in humans and cause serious disease such as cholangitis, choledocholithiasis, pancreatitis, and cholangiocarcinoma in certain areas of the world. Prevalence in the population may vary from <1% to >90% between regions. The causative agents of human infections include *Clonorchis sinensis* and trematodes belonging to the genus *Opisthorchis* and *Metorchis* (Chai et al., 2005a). The future impact of these fish-borne zoonotic parasites may be linked to the likely growth of aquaculture in Asia, where nearly 90% of freshwater production is localized (World_Health_Organization, 2004).

C. sinensis, the Chinese liver fluke, is the most important species of fish-borne zoonotic parasite and is widely distributed in East Asia. Current endemic areas of clonorchiasis include South Korea (Korea-Association-of-Health-Promotion, 2004), all of China except the Northwest (Xu et al., 1995), Taiwan (Chen, 1991), northern Vietnam (De et al., 2003), and the far eastern part of Russia (Chen, 1994). Estimated number of infected people worldwide is about 7–10 million (Crompton, 1999).

Opisthorchis viverrini is highly prevalent in Southeast Asia including Thailand (Sripa et al., 2003), Laos (Chai et al., 2005b), Cambodia (World_Health_Organization, 1995) and South Vietnam (De et al., 2003) and the estimated number of infected people is currently about 6 million. *Opisthorchis felineus* is a parasite which occurs in central, eastern and south-eastern Europe (Armignacco et al., 2008; Bernhard, 1985), the Asiatic parts of Russia (Rim, 1982; Skripova et al., 1991) and Turkey. The number of infected people reported is estimated to be about 1.6 million (Yossepowitch et al., 2004).

Metorchis conjunctus, the North American liver fluke is autochthonous in Canada and USA (MacLean et al., 1996) and particularly occurs in aboriginal populations from Quebec to Saskatchewan, and the eastern coast of Greenland (Behr et al., 1998). *Metorchis bilis* is also recognised to be infectious to human (Kuznetsova, 2000). Although metorchiasis is a rarely diagnosed parasitic infections in Europe, recent findings in animal reservoirs (cats, dogs and foxes) suggest that exposure to this parasite is more frequent than generally recognised (Schuster et al., 1999; Schuster et al., 2007).

The adult stages of liver flukes live in the biliary tract of definitive hosts (cats, dogs, foxes, wolves and others) and produce eggs, which pass out through the common bile duct and intestines into faeces. The eggs are ingested by freshwater snails where the miracidia and sporocysts with rediae develop. About 14 days after egg ingestion, cercariae develop within the rediae and migrate to the intrahepatic lymph space of the snail. After maturation, cercariae are released into the water and actively penetrate beneath fish scales where they migrate and encyst, chiefly in muscles, less frequently under the scales, fins or gills, and transform into encysted metacercariae. More than 100 species of freshwater fishes belonging to 13 families, especially the Cyprinidae, and three species of freshwater shrimp can serve as a second intermediate host (Chai et al., 2005a; Park et al., 2004). In the definitive host, the metacercariae excyst in the duodenum and migrates to the common bile duct where they develop to the adult worms.

The presence of appropriate snail, fish and mammalian hosts (including human) is essential for the fluke transmission and this combination must be sustainable to remain endemic in a region. The prevalence of human liver fluke infections is related to eating raw fish or shrimps and the prevalence of infection may vary by age and sex in endemic areas. Examples of major dietary risk factors for liver fluke infection in a certain region include consumption of: rice gruel with slices of raw freshwater fish in southern China and Hong Kong; slices of raw freshwater fish with red pepper sauce in Korea; half roasted or undercooked fish in Guangdong Province; raw shrimps in Fujian Province China (Chen, 1994); 'Koi pla' dish consisting of raw fish flesh chopped with garlic, lemon juice, fish sauce, chili, roasted ground rice, and local vegetables in north-eastern Thailand and Laos, (*O. viverrini*, (Rim, 1982).

Immigration and demographic changes are associated with emerging patterns of infection as occurs in immigrants, for example from the former Soviet Union to Germany and infected with *O. felineus*. Such cases do present challenges to clinicians unfamiliar with diagnosis and treatment of these parasites (Günther, 2006).

Clinical manifestations of human infection are similar for all the liver fluke infections. After ingestion of infected fish, metacercariae excyst in the duodenum and migrate to the bile duct where they grow to the adult stage in about 4 weeks. Pathogenesis in the biliary tract is caused by mechanical, chemical, and immunological irritation by adult flukes (Li et al., 2004). In the early stage of infection, bronchial asthma and allergy-like symptoms are encountered whereas painful enlargement of the liver, congestion of the spleen, and local eosinophilia in the wall of bile ducts may occur in the chronic stage (Rim, 1982).

Cholangiocarcinoma due to metaplastic changes of the biliary epithelial cells is the most serious complication (Watanapa and Watanapa, 2002). Moderate infections with less than 100 adult worms (e.g. *O. viverrini*) are usually asymptomatic however infection with hundreds or thousands of worms can cause severe illness (Mairiang and Mairiang, 2003). The incubation period is usually one to four weeks after primary infection but may vary with the number of adult worms developed.

Diagnosis in human is usually achieved by detection of the fluke's eggs in faeces. However, diagnosis may be problematic due to incorrect recognition of eggs and a failure to differentiate them from eggs of other parasites (e.g. intestinal flukes) or false negative results in case of low or intermittent shedding (Upatham and Viyanant, 2003). Molecular methods have recently been developed for the detection of *O. viverrini* and other fluke species in human stool specimens (Lovis et al., 2009; Wongratanacheewin et al., 2002). For routine diagnosis, serological tests (ELISA based on excretory-secretory antigens) have been developed for the detection of specific antibodies. Coproantigen detection based on antibody reagents provides high specificity (Upatham and Viyanant, 2003).

It is difficult to estimate infection intensities in fish especially when individuals harbour only a few metacercariae. Furthermore, species identification of the larval stages is not feasible since these trematodes are morphologically indistinguishable (Haas et al., 1990). Conventional methods for detection of metacercariae in fish include microscopic examination of compressed flesh samples. However, this method is time consuming and lacks sensitivity. The artificial digestion technique facilitates the extraction of metacercariae from large quantities of fish (Sithithaworn et al., 1997). PCR-based techniques to detect development stages of the liver flukes in snail and fish infections have been described. For example, a PCR targeting mitochondrial CO1 and ribosomal ITS2 genes was developed for detection of *O. viverrini* metacercariae in fish meat (Parvathi et al., 2008). In another study, a PCR has been developed from the ribosomal ITS2 gene for the detection of *C. sinensis* rediae in snails and metacercariae in fishes (Mueller et al., 2007).

For prevention and control of liver fluke infections, education campaigns are important for risk communication to consumers and should advise consumption only of cooked fish. Environmental sanitation through the building and use of latrines in endemic areas is also important. More recently, mass chemotherapy of people at risk in endemic areas was recommended as the most effective control strategy (World_Health_Organization, 2004).

Paragonimus westermani is a lung fluke (Trematoda) that infects humans as the definitive hosts and can cause serious lung damage. It is widespread across South East Asia and Japan, and related species occur in Africa and South America. Infections occur via contamination of food or drink, or during food preparation of freshwater crustaceans (mainly crabs) in which live larvae (metacercariae) are present. The complete life cycle is not known to occur in Europe, but it has been estimated that in parts of South East Asia, up to 80% of freshwater crabs are infected with the parasite. Although cooking will kill the metacercariae, other food preparation methods such as pickling, salting, or soaking in wine may not. Human infections are now being reported in the USA in immigrants arriving

from endemic areas. Infection risk in Europe may arise from the import of infected freshwater crabs from endemic areas following consumption without proper handling or preparation. With increasing numbers of people from South East Asia and Japan settling in Europe, and the increasingly widespread consumption by the general public of imported crustaceans that are raw, undercooked, or otherwise insufficiently treated to kill the metacercariae, an increased risk of infection in the EU by *P. westermani* must be considered a possibility.

1.1.3. Cestodes

Diphyllobothrium is a genus of cestode, and at least 13 species have been reported infecting humans. These cestodes are widely distributed in fish, mammal and bird hosts (Dick et al., 2001) and are generally associated with intermediate and definitive hosts living in cold water habitats. *D. latum* is most often reported from humans from the holoarctic region. Globally, the incidence of diphyllobothriasis has declined in recent years, particularly in North America (Dick et al., 2001). Diphyllobothriasis is a common infection in Far-Eastern Russia (Muratov, 1990) and is frequently reported in Japan (Dick et al., 2001). In Europe the incidence has declined except in Switzerland, Sweden, Finland and Estonia (Dupouy-Camet and Peduzzi, 2004).

The life cycles of these cestodes is complex and requires three hosts for completion. After the egg hatches in water, the motile embryo (coracidium) is ingested by a copepod, and develops to the first larval stage (procercoid*). When an infected copepod is ingested by the freshwater fish, the larva is released, enters the tissue of the host (mainly flesh or viscera) and develops to the second stage (plerocercoid*) which is 1-3 cm long. Plerocercoids can remain inactive for several years but can re-encyst several times in other predatory fish. After ingestion of the infected fish, the parasite develops in the intestine of fish-eating mammals or birds into the adult tapeworm within about one month after ingestion.

Consumption of raw or insufficiently cooked or marinated fish is the main source of infection with *Diphyllobothrium* for humans. Wild-caught fish prepared in a variety of ways such as for sushi or sashimi may pose a high risk of exposure (Deardorff, 1991). Another important factor in introducing or sustaining this parasite is the contamination of the local aquatic environments, e.g. through improperly treatment sewage (Dupouy-Camet and Peduzzi, 2004).

Diphyllobothrium tapeworms can live for several years in the human intestine and reach a length of more than 10 meters. Symptoms of infection include abdominal discomfort (abdominal pain, diarrhoea), weight loss, asthenia, and vertigo. Anaemia due to vitamin B-12 deficiency has been described in a case of prolonged infestation (VonBonsdorff, 1977). However, human diphyllobothriasis is considered a mild illness and is not normally reported.

The diagnosis of *Diphyllobothrium* infection is based upon detection of the characteristic eggs in the stool. Due to the enormous production of eggs, diagnosis is usually rapid and egg concentrations techniques are usually not needed. As the morphology of the *Diphyllobothrium* egg is very characteristic, there should be no problem with differential diagnosis from other human parasites' eggs. It is impossible, however, to distinguish between different *Diphyllobothrium* species on the basis of egg size and morphology (Kamo, 1986).

As performed for other parasites, intermediate stage plerocercoids can be detected by microscopic examination of compressed fish flesh or organ samples. In Southern Argentina, *D. latum* and *D. dendriticum* were detected in 28% and 58% of trout respectively with 1.4 and 7.2 plerocercoids per fish (Revenga, 1993).

* See glossary

1.1.4. Microsporidia

No microsporidian or protozoan fish parasites have been demonstrated to pose any direct consumer health risk. However, some microsporidians of the genera *Nosema*, *Enterocytozoon* and *Pleistophora* which may occur in fish, have been isolated from AIDS patients and others who suffer from severe immunodeficiency. These cases were, however, considered opportunistic infections of unknown origin (Cali, 1991).

2. ALLERGY CAUSED BY PARASITES IN FISHERY PRODUCTS

2.1. Introduction

The human host defence system is comprised of numerous cellular and protein components that interact in a highly complex manner in order to preserve self and to neutralize or destroy non-self. However, this system sometimes induces an overreaction by a specific defence mechanism responding inappropriately to environmental encounters. Allergy is a clear example of this over-reaction that occurs in certain individuals responding inappropriately to non-self molecules termed allergens. Allergens are antigens that provoke a specific allergic reaction, and antigens are generally defined as moities that provoke immune responses.

Allergy can occur as Type I immune hypersensitivity, also known as immediate hypersensitivity, and is mediated by antibody of the immunoglobulin E type (IgE). Allergic reactions of this kind can range from mild to severe, of which the most serious (anaphylaxis) can be rapidly fatal if not treated promptly. Although IgE antibody mediates Type I allergic reactions, people having IgE antibody to a particular protein will not necessarily suffer an allergic response to it. Allergens triggering the Type I hypersensitivity are generally proteins. Another medically important immunological hypersensitivity reaction is a Type IV hypersensitivity, also known as delayed or contact sensitivity which is not antibody mediated but is caused by activation of T-lymphocytes at affected sites. Allergens triggering the Type IV hypersensitivity are generally low molecular weight haptenes.

Parasites, especially helminths, produce in infected hosts a Th2 biased immunologic response with production of specific IgE against the parasite. This has to be taken into account when analysing studies on IgE production in relationship with parasite contact. This is especially important, as several studies claim the allergenicity of parasites by finding specific IgE production measured in serum or evidenced by a positive skin prick tests. The production of specific IgE is not to be mistaken as allergy.

A. simplex are, so far, the only fishery-product associated parasite causing clinical allergic responses. The principal clinical allergic responses due to *A. simplex* are:

- Gastro-allergic anisakiasis, in which allergic symptoms are additional symptoms in an acute gastric parasitism after eating raw or undercooked fishery products containing live larvae;
- Allergy to *A. simplex*, resulting from contamination of fishery products with allergens with no necessity for live parasite to elicit the allergic reaction.

Additional allergic responses occur and are:

- Chronic urticaria associated with *A. simplex* sensitization, whose mechanism is not yet clarified;
- Eosinophilic gastroenteritis, rheumatological and dermatological symptoms as well as occupational rhino-conjunctivitis and asthma.

The allergy to *A. simplex* and gastro-allergic anisakiasis is commonly recognised in some regions in Spain (Audicana and Kennedy, 2008); however these are rarely or have never been reported in other parts of Europe. It is not known if this is dues to lack awareness and rare application of diagnostic tests, or to true differences in the incidence of the disease. The remaining allergic diseases associated with *A. simplex* sensitization are very rare.

No other fishery product associated parasites have been clearly implicated with allergic reactions, although studies claim allergenicity of parasite extracts based on positive skin prick tests or specific serum IgE, these are not necessarily associated with clinical allergy.

2.2. Sensitization and exposure to *A. simplex*

There is general agreement that, in most instances, an infection is required to initiate allergic sensitivity to Anisakids. This hypothesis is difficult to establish with certainty, since cases of anisakiasis may go unrecognised or are not medically investigated. The possibility that sensitisation can occur via exposure to antigen alone, in the absence of a live infection, cannot, however, be excluded; there is experimental evidence indicating that nematode materials can generate allergic type (Th2) immune responses without infection (Hewitson et al., 2009), although only a few cases of such autoallergenicity have been reported (Audicana et al., 2002; Cuende et al., 1998; Vidaček, 2009). Once sensitisation has occurred, response to nematode allergens can be highly aggressive and generate severe allergic disease (Audicana and Kennedy, 2008). Through direct clinical observation, some authors have shown that an infection can provoke a concurrent *A. simplex* allergic episode in a sensitised individual, and claim this is the principal mechanism for disease (Daschner et al., 1998; Daschner et al., 2000b). However, others consider that allergic episodes can not only be elicited by infection as described above, but also by exposure to allergen remaining in food fish treated so that no viable larvae remain (Audicana et al., 1997; Audicana et al., 2002; Vidaček, 2009). The relative epidemiological impact for each route of provoking an allergic episode is unknown, however there is general agreement that consumption of fishery products containing viable *A. simplex* larvae presents a greater risk for allergy and initiation of the *A. simplex*-allergic state than consumption of fishery products containing non-viable parasites. Consequently, prevention of sensitisation to *A. simplex* should focus on the prevention of infection.

2.3. Urticaria and anaphylaxis due to *A. simplex* allergy from food

Acute urticaria and angioedema affects 20% of the population at some time in their lives, particularly in young adults (Kaplan, 1992), and although usually self-limiting and not life threatening, the condition is nevertheless unpleasant as a result of the intense itching, inability to sleep and even disfigurement when angioedema is present. Angioedema is associated with urticaria in 30% of cases, and is potentially life threatening because of the risk of oedema of the glottis. Anaphylaxis is a rapid onset and dangerous syndrome characterized by urticaria, angioedema, severe respiratory and gastrointestinal symptoms, collapse and shock. The first signs of an allergic reaction usually appear within 60–120 minutes after ingestion of infected fish but can take up to six hours (Audicana et al., 2002).

In the Basque Country of northern Spain, *A. simplex* is now considered to be the main factor associated with urticaria and angioedema in adults following fish and shellfish consumption and is responsible for 8% of acute urticaria and angioedema cases (Pozo, 1998) and for 27 % of anaphylactic episodes (Audicana, 2002). This constitutes a similar or even higher prevalence compared with other sources of ingested allergens in adult population (fruits, nuts, shellfish and fish flesh). Although the majority of cases were not life threatening, more than 50% of the patients required emergency treatment and there was a single near fatal case of respiratory arrest (Audicana, 2000).

As the awareness about anisakiasis and *Anisakis* as a food allergen increased (SCVPH, 1998), sporadic reports from France (Petithory, 2007), Italy (Foti et al., 2002), Portugal (Falcao et al., 2008), and in regions of Spain other than Basque Country (Añíbarro, 2007; Moreno-Ancillo et al., 1997), demonstrated that this allergy occurred elsewhere in Europe and was not confined to the Basque Country and Japan, where more IgE sensitization to *A. simplex* than to fish proteins has been diagnosed (Kimura et al., 1999). However, Italian and Portuguese cases were similar to those of the Basque Country because they involve true allergy following exposure to cooked fish, whereas reports

from other parts of Spain (Madrid and its surrounding provinces) describe gastroallergic cases caused by consumption of raw or undercooked fish (Daschner et al., 2000b).

General risk factors for serious or fatal food reactions for allergens in foods include: young age, multi-sensitivity, presence of uncontrolled asthma, previous serious food reactions, and eating out of the home. Disease severity will be exacerbated by the lack of immediate availability of epinephrine. Atopic allergic diseases are familiar and have a genetic basis. In contrast, patients allergic to *A. simplex* have a lack of previous atopic dermatitis, asthma, or rhinitis and are generally aged 40–50 years (Audicana et al., 2002). In an Italian study, atopic subjects had a lower risk of *Anisakis* allergy than non-atopic subjects and *A. simplex* allergy was associated with consumption of uncooked seafood (especially anchovies and squid) and an increased risk with age (Foti et al., 2006). In a study of food induced anaphylaxis, logistic regression analysis revealed that age and specific IgE level were the unique risk factors associated with *A. simplex* allergy (Audicana, 2002). In many cases, the patients did not suspect allergy to *A. simplex or* to fishery products because of previous tolerance but attributed allergic disease to analgesic and/or antibiotic treatment. Other characteristics from the clinical histories of patients include the patients and their general practitioners linked the symptoms with medications (although such causes were later rejected and the episodes occurred at night (Audicana et al., 2002). A genetic predisposition to *A. simplex* allergy and the presence of HLA class II alleles has been shown (Sanchez-Velasco et al., 2000). This association, together with the different habits of fish consumption, could explain some differences in allergy rates because the HLA inheritance is a good marker of genetic differences.

2.4. Gastro-allergic anisakiasis (GAA)

As described above, urticaria was initially described as a systemic symptom accompanying human gastric anisakiasis in Japan and was estimated to be present in about 10 % of acute parasitic episodes (Asaishi et al., 1980). Reports from Spain in the late 1990's also described urticaria as the most common allergic reaction, with frequent abdominal symptoms of gastric anisakiasis. This clinical presentation was designated as gastro-allergic anisakiasis (Daschner et al., 1998; Daschner et al., 2000a).

Gastro-allergic anisakiasis is defined as an acute IgE-mediated generalized reaction (urticaria-angioedema-anaphylaxis) after the intake of *A. simplex* infected fish, where the live larva induces the symptoms during penetration of the gastric mucosa (Daschner et al., 2000a). Allergic symptoms are typically accompanied by gastric/abdominal symptoms, but these often remain mild or even absent (Alonso-Gomez et al., 2004; Daschner et al., 1998).

As soon as acute allergic symptoms were recognised as associated with *A. simplex* a series of publications appeared describing a high frequency of urticaria or anaphylaxis in previously "idiopathic" reactions. These reports came initially from different regions of Spain, but progressively reports of cases of allergy related to *A. simplex* from other, predominantly European, countries. In parallel, a series of reports of *A. simplex* gastric or intestinal parasitism without allergic symptoms were also published. It is difficult to evaluate whether the high number of reported cases is due to a real increase of *A. simplex* and associated disorders or a heightened awareness due to the reports in the scientific literature.

The rise in GAA can, at least in part be accounted for by awareness and knowledge of allergic disease presentation. This is due to the peculiar clinical characteristics of GAA, which is difficult to suspect if the attending physician is not aware of this condition. The main clue for a suspicion of an allergic reaction being possibly caused by *A. simplex* is a careful history in the emergency room. The patient's history has to include the question of intake of raw or undercooked fish in the 24 hour period before the onset of urticaria, angioedema or anaphylaxis. True food allergy with an immediate hypersensitivity reaction is usually straightforward to diagnose since the allergic symptoms begin almost immediately after the suspected food agent has been consumed. However, GAA can begin up

to 24 hours after the intake of the parasitized fish and this characteristic seems to be the reason for a high proportion of under diagnosed anisakiasis prior to more widespread recognition of GAA. This entity has been described as a host response to an acute parasitism and should clearly be differentiated from the IgE-mediated acute allergy caused by non-viable *A. simplex* material, which would thus reflect a true food-allergy (Daschner et al., 2002).

GAA is clinically composed by two types of reactions: the first being the local reaction of the gastric mucosa followed by the generalized allergic reaction. The gastric reaction reflects the previously described gastric parasitism where the third stage *A. simplex* larva penetrates the gastric mucosa by action of their enzymatic peptidases and produces the epigastric pain, nausea and vomiting. Early Japanese reports proposed this local reaction to be an IgE-mediated allergic reaction (Kasuya and Koga, 1992). GAA has been described as a simultaneous primary and secondary immunologic reaction as all immunoglobulin isotypes, including specific IgM are present from the first day of parasitism. With the exception of IgM, all other antibody isotypes (IgE, IgA, IgG, IgG_4) show significant elevation after one month, with production of IgE antibodies against additional antigens as demonstrated by serial immunoblotting studies (Daschner et al., 2002). This immunologic polyclonal stimulation is produced by an active live larva, even if it is removed after some hours either by gastroscopic extraction or spontaneously as occurs in the majority of cases. Serial determination of immunoglobulins has gained importance in the diagnosis of allergic reactions for distinguishing between a food-allergy like reaction and that induced by a viable larva (Daschner et al., 1999). It has been proposed that the generalized allergic reaction reflects a similar local reaction which is a protective host reaction against invasion in order to prevent a further penetration of the larva and produces the different forms of chronic reaction (Daschner et al., 2005). There is a clear negative association between cases of GAA and the chronic forms of intestinal anisakiasis, where no allergic reactions are described (Asaishi et al., 1980; González Quijada et al., 2005).

The first allergic symptoms can appear as late as 24 hours or longer after the intake of the viable parasite in a meal containing fishery-products, because it is not the direct contact of the *A. simplex surface* allergens leading to the allergic reaction but the excretory-secretory (E/S) products released by the larva during gastric penetration. Major *A. simplex* allergens like Ani s 1 and Ani s 7 have been identified in the excretory compartment which bears enzymatic activity allowing penetration of mucosal tissue (Anadon et al., 2009; Gomez-Aguado et al., 2003).

Oral challenge tests have been performed with non-viable larvae in patients with previous GAA and patients have not developed any reaction (Daschner et al., 1999; Sastre et al., 2000). Even proteins that remain stable after heat treatment or pepsin digestion have not produced any allergic reaction in such tests, when excretory-secretory proteins were administered orally, supporting the hypothesis that only viable larvae are capable of inducing allergic reactions in these patients (Baeza et al., 2004).

Systemic allergic reactions vary between urticaria/ angioedema and anaphylactic shock, as in other IgE-mediated acute allergic reactions. Some patients with predominantly digestive symptoms display an erythematous reaction in the head, neck and superior thoracic region, sometimes without pruritus. This reaction is not necessarily IgE-mediated and could be due to other non-specific mechanisms, e.g. a vasomotor reaction of the nervous system. The latency of symptoms between fish intake and the allergic reaction can range between minutes to more than 24 hours. Abdominal symptoms appear over the same time period, but with a tendency to a shorter time interval. Some patients have diarrhoea, which could be due to a nervous reaction by the digestive tract or as a distant reaction in the course of anaphylaxis. It has been shown, that GAA can also be diagnosed in patients without abdominal symptoms (Alonso-Gomez et al., 2004).

GAA is an acute reaction with a mainly rapid resolution. Allergic symptoms rarely remain for longer than 24 hours, whereas the remission of abdominal symptoms depends on the extent of the local gastric tissue damage.

Risk factor for GAA, have a clear association with the consumption of raw and minimally processed fish and fishery products (Pozio, 2008; van Thiel and van Houten, 1966). This is also true for allergic reactions as well as for non-allergic gastric or intestinal anisakiasis. For GAA there is an association between increasing risk and age, this is probably a confounding factor associated with fish and fishery product eating habits: previous episodes of atopy are not a risk factor (Falcao et al., 2008). However, patients with allergic symptoms due to *A. simplex* sensitization, displaying no difference in the overall atopy status, but have a higher frequency of allergy against house dust mites and a diminished frequency of allergy against other aeroallergens, mainly pollen (Daschner, 2008). In one study an association of hypersensitivity to *A. simplex* with HLA class II DRB1*1502-DQB1*0601 haplotype has been described and it has been suggested that, the DRB1*1502 being the uncommon Thai allele in Spain region, the pressure of this eating habit selected for an association with this haplotype in two areas as far apart as Japan and Spain (Sanchez-Velasco et al., 2000).

2.5. *A. simplex* sensitization associated chronic urticaria (CU)

Chronic urticaria is a disabling disease with an important impact on the quality of life. Some studies discuss a possible association between chronic urticaria or pruritus and sensitization to *A. simplex* (Daschner et al.; Daschner et al., 2005; Gracia-Bara et al., 2001). The possible pathological mechanism has not been studied, but it has been postulated that *A. simplex* antigens following consumption of fish could be responsible for the perpetuation of hives and has opened a debate of a diet without *A. simplex* antigens (Audicana and Kennedy, 2008; Daschner et al., 2005), i.e. any fish, in at least a subgroup of patients.

In patients with CU in endemic areas, the frequency of *A. simplex* sensitization is much higher than in the general population. This fact led to investigations into a possible causal relationship between *A. simplex* sensitization and CU. With the help of the measurement of specific IgG_4 against *A. simplex,* CU patients were assigned to two groups with a different outcome after a two-month diet without any fish. Patients with detectable specific IgG_4 were more prone to a remission of urticarial symptoms than those without detectable specific IgG_4 or without sensitization to *A. simplex* (Daschner et al., 2005). However it has been reported that patients with GAA mount an immunological response by all immunoglobulin isotypes, including IgG_4. CU patients with high specific IgG_4 levels are likewise supposed to have suffered parasitism by this nematode. Thus, it has been proposed that, contrary to patients with GAA, some patients react with a chronic urticarial reaction after contact with *A. simplex.* If this is correct, a diet excluding non-viable *A. simplex* larvae after the diagnosis of GAA does not lead to new allergic episodes, even after several years, and is not contradicted by the fact that some patients begin a chronic urticarial reaction after an episode of parasitic contact.

In this disease, it is possible that live larvae of *A. simplex* as well as non-viable larvae or related antigens are involved in the pathogenesis of a chronic urticarial reaction although the mechanism for disease has yet to be elucidated.

2.6. Other immunologically induced disorders due to *A. simplex*

Sensitization to *A. simplex* could also explain some cases of eosinophilic gastroenteritis in which the etiological agent has not been identified. In one out of ten such cases reviewed by pathological studies, vestiges of larval nematodes were found. Rheumatological symptoms have been described in association with *A. simplex* infection in patients whose symptoms began with skin manifestations and arthritis (Cuende et al., 1998).

With respect to occupational allergy, there are reports from Spain (Añíbarro and Seoane, 1998; Aníbarro, 1997; Armentia et al., 1998; Armentia et al., 2006; Audicana, 2002; Carretero, 1997), Italy (Purello-D'Ambrosio, 2000; Scala et al., 2001), and South Africa (Nieuwenhuizen et al., 2006). Occupational cases of *A. simplex* allergy have been described in fishmongers (Añíbarro and Seoane, 1998; Armentia et al., 2006; Audicana, 2002), fishermen (Nieuwenhuizen et al., 2006; Purello-D'Ambrosio, 2000), a frozen-fish factory worker (Scala et al., 2001), a female cook (Pulido-Marrero et al., 2000), or were related to exposure (by contact or inhalation) to fish meal in chicken feed

(Armentia et al., 1998). A case of protein-contact-dermatitis as a result of *A. simplex* has been described in a housewife in which both type I (IgE-mediated and immediate) and type IV (delayed) hypersensitivity were demonstrated (Añíbarro and Seoane, 1998).

The fact that there are few cases of rhino conjunctivitis and occupational asthma described due to *A. simplex* suggests that sensitization by inhalation route is unusual, or is rarely diagnosed and it is important to appreciate more than 38 million people work in fish production activities (fishery and aquaculture) aside from related sectors (fishmongers, seafood handlers in restaurants, animal feed production workers etc.) as well as those exposed in domestic environments (Añíbarro and Seoane, 1998; Armentia et al., 1998; Armentia et al., 2006; Audicana, 2002; Nieuwenhuizen et al., 2006; Purello-D'Ambrosio, 2000; Scala et al., 2001). *A. simplex* is a more potent allergen sensitizer than the fish proteins themselves (Kimura et al., 1999), and occupational groups who are exposed to fish and fishery products (i.e. fishermen, fish processing workers, workers who feed fish to animals, together with cooks and others involved with catering of seafood) have an increased risk of *A. simplex* sensitization.

It should also be considered that the individuals belonging to these categories are also the most suitable candidate for ingestion of raw fish while working. Thus despite widespread exposure in certain professions (one study demonstrated that 50% of fishmongers were sensitized to *A. simplex* (Nieuwenhuizen et al., 2006) occupational allergy to *A. simplex* is very rare.

2.7. Allergen cross-reactions

Cross-reactions occur where an allergen or allergens from one biological source elicits a potentially deleterious allergic reaction in an individual that had been sensitised to a different source. The type of reaction under consideration is due to antibody cross-reactivity, the relevant reactions being Type I hypersensitivity reactions as mediated principally by antigen/allergen-specific IgE antibody. There is little current evidence for their importance of Type IV allergic reactions to nematodes in ingested fish (aside from cellular responses such as lymphocytic infiltrations and eosinophilic granuloma around a tissue-penetrating larva), though such reactions should not be ignored in the long term.

There are essentially three potentially deleterious immune reactions due to allergen cross-reactivity. First, cross-reactions can exist between closely related nematode parasites of fish, specifically between those of the genera *Anisakis*, *Pseudoterranova, Phocascaris, and Contracaecum* within the Anisakidae. Secondly, cross-reactions between fish-derived parasites and others in the Order Ascaridida, for other members of which humans can be the definitive (*Ascaris lumbricoides*) or accidental (e.g. *Ascaris suum, Baylisascaris* spp., *Toxocara* spp., *Toxascaris* spp.) hosts. Thirdly, allergen cross-reactions between Anisakid nematodes in fishery product and environmental (e.g. house dust mites, cockroaches) or food allergens from edible crustaceans (e.g. prawns, shrimps).

Cross-reactivity between *A. simplex* and numerous invertebrate species has experimentally been proved by different methods; however the clinical importance of such cross-reactivities is poorly understood and requires further analysis.

2.7.1. Cross-reactions among anisakid nematodes

While there is little if any information on cross-reactions between *A. simplex* and other anisakids at the clinical or experimental levels, it is likely that such cross-reactivities exist given the known antigenic similarities between ascaridid nematodes as a whole (Iglesias and Leiro, 1996 ; Kennedy et al., 1988a; Maldonado and Hita, 2004 ; Perteguer et al., 2003b). *A. simplex* may present the most common and widespread infection risk to humans relative to other anisakids, so it is likely that the *Anisakis* sensitisation will render individuals sensitive to the allergens of other species. While certain defined allergens such as Ani s 7 do not cross-react detectably between *Anisakis* and *Pseudoterranova*

(Anadon et al., 2009), it remains possible that cross-reactions with the allergens of other species ingested dead or alive may elicit deleterious reactions.

2.7.2. Cross-reactions with other groups of nematodes

There is experimental evidence and emerging clinical data that extensive antigenic cross-reactivities between *Anisakis* and other ascarids exist (Iglesias et al., 1996; Kennedy et al., 1988b; Maldonado et al., 2004; Perteguer et al., 2003a). In Europe, people are occasionally infected with the human roundworm *A. lumbricoides* and the very similar pig roundworm *A. suum*, but a considerably more common source of ascarid infection is the dog parasite *Toxocara canis*, which gives rise to a persistent infection in the soft tissues accompanied by several allergic-type immune responses, particularly in children (Pinelli and Dormans, 2005 ; Pinelli et al., 2009). It remains possible, therefore, that sensitisation by infection with these parasites may also sensitise to *Anisakis* allergens. While this possibility remains to be properly investigated, there are currently no consistent clinical data linking exposure to other ascaridid parasites with sensitivity to *Anisakis* allergens, but the possibility requires to be properly investigated. Specifically, studies in some regions of Spain indicate that the high prevalence of *Anisakis* sensitization in subjects who are not otherwise of an allergic disposition are probably not explicable by exposure to other parasitic diseases, the more likely explanation still being previous episodes of anisakiasis (Audicana and Kennedy, 2008; Daschner, 2006).

2.7.3. Cross-reactions with non-nematode environmental, food allergens or transmissible agents

There are recent publications reporting clinical allergen cross-reactions between ascarid nematodes and environmental allergens such as house dust mites (*Dermatophagoides pteronyssinus, D. farinae, Blomia tropicalis*) and also on cross-reactivity with *A. simplex* (Johansson et al., 2001b; Pascual et al., 1997b). False positives are, however, common in ImmunoCAP and IgE-immunoblotting tests, possibly because they use crude or complex antigen mixtures containing carbohydrates that have antigenic determinants that are widespread in nature, including other parasites, crustaceans, insects, mites, bacteria, and plants (Johansson et al., 2001a; Pascual et al., 1997a). Cross-reactions between *Anisakis* and crustaceans (prawns, shrimps), insects and mites, one major allergen identified is the so-called 'panallergen'[*], tropomyosin, which is a muscle protein that is highly conserved amongst invertebrates. Other sources of cross-reactivity include biotinyl-enzymes (Lorenzo et al., 1999b), which stimulate the production of IgE antibodies in some patients. Other potential sources of allergens that cross-react in vitro with *Anisakis* include the cestode parasite *Echinococcus granulosus* (Daschner et al., 2000c) and the common annelid earthworm *Lumbricus terrestris* (De Castro Martinez et al., 2007).

2.8. Diagnosis

2.8.1. Diagnosis of *Anisakis* allergy

The diagnosis of allergy to *A. simplex* is based on the following criteria: (1) a compatible history, such as urticaria, angioedema or anaphylaxis following fishery-product consumption, (2) positive skin-prick test, (3) specific-IgE against *A. simplex* (radioimmunoassay) with values >0.7 kU/l, (4) a lack of reaction to proteins from the host fishery products and/or other possible cross-reacting antigens such as crustaceans, snails, cockroach, dust mites and insects. Other foods and allergens should also be screened in such cases (Audicana and Kennedy, 2008). IgE immunoblotting with sera from allergic

[*] See glossary

patients appears to be specific to *A. simplex* because cross-reactivity was not observed in sera from African patients with other parasitosis (Del Pozo et al., 1996). However, when sera from other control populations (with no seafood consumption and with multiple parasitic diseases) were tested, the specific IgE against *A. simplex* antigens were detected (Moneo et al., 2000). Interestingly, the *A. simplex* antigens recognized by the monoclonal antibody (mAb) UA3 were also detected in all patients with unambiguous *A. simplex* allergy (Lorenzo et al., 1999a). It is hypothesised that the antigen recognized by this mAb could provide a tool for specific diagnosis of *Anisakis* allergy.

Currently, the best confirmation of food allergy is a double-blind challenge-test against a placebo (Audicana et al., 2002; Audicana and Kennedy, 2008), although this may be precluded on ethical considerations. Some authors report surprising results since oral *A. simplex* challenge tests were negative whereas conjunctival tests were positive. These studies could be biased by patients' recruitment (gastro-allergic versus. allergic patients) and the antigen challenge since lyophilized or extracted larvae may not be representative of the natural allergen exposure (Sastre et al., 2000). Somatic and E/S recombinant allergen proteins production can help in the diagnostic techniques (prick tests and in vitro tests) and even in the development of allergen vaccines (immunotherapy) in the future.

It is common to find IgE against *A. simplex in* subjects who do not react allergically to this parasite. The identification of specific IgE against the parasite is not a reliable indicator of allergy, and specific IgE has been detected in 25% of otherwise healthy controls (Del Pozo et al., 1997). Possible explanations for the existence of IgE against *A. simplex* without clinical manifestations are: cross-reactivity with other nematodes because of presence of a panallergen, such as tropomyosin; cross-reactivity with carbohydrates or phosphorylcholine and cross-reactivity with glycans present in glycoproteins of other nematodes; the presence of biotinyl-enzymes that can stimulate the production of IgE in some patients; or a prior episode of anisakiasis which has not been diagnosed (Audicana and Kennedy, 2008).

2.8.2. Diagnosis of gastro-allergic anisakiasis as an acute parasitism

Initial experience with gastroscopic evaluation of GAA was useful for diagnosis and extraction of the nematode, however subsequent clinical experience later showed that gastroscopic intervention is only rarely required because of spontaneous resolution (Alonso-Gomez et al., 2004). Clinical history is fundamental: the intake of raw or undercooked fishery products in the 24 hours previous to the onset of an acute allergic reaction is the main criterion leading to initial clinical suspicion. This probability is heightened if abdominal symptoms like epigastric pain, nausea or vomiting precede or accompany the allergic reaction. A correct clinical history and further allergic evaluation can provide a strong indication of the initial diagnosis.

The skin prick test (SPT) with an extract of *A. simplex* is highly sensitive. Specificity depends on the prevalence of sensitization in the population. Cross-reactivity with other nematodes or arthropods has been described to give false positive results. Although the presence of a positive skin prick test or a specific serum- IgE against *A. simplex* is attributed to a previous acute *A. simplex* infection, the reactivity does not necessarily appear in the clinical context of the actual allergic reaction (Daschner et al., 2005). Thus other diagnostic methods are being studied. A serial follow up of specific (and total) IgE is useful, as the polyclonal stimulation induced by the viable larva produces elevated levels after one month (with respect to the levels at day 0) with decreasing values after 6 months (Daschner et al., 1999).

Other diagnostic methods are still experimental and not commercially available. The most promising is major allergen Ani s 7 which is a component of the excretory-secretory fraction of *A. simplex* and the only allergen recognized by 100% of infected patients (Anadon et al., 2009). No antibody against a single allergen has been identified, that discriminates within the group of parasitized patients with

and without allergic symptoms. Even patients with a history of intestinal anisakiasis show specific IgE against *A. simplex* or against single allergens like Ani s 1 (Caballero and Moneo, 2002).

2.8.3. Diagnosis of *Anisakis* sensitization associated chronic urticaria

In patients with chronic urticaria who are sensitized against *A. simplex* (detectable specific IgE in serum or by skin prick test) and display detectable serum specific IgG_4, the chronic urticaria is likely to be induced by parasite proteins. The response to a temporary diet excluding fishery products can simultaneously be of diagnostic and therapeutic value. Otherwise, most patients that respond with clinical improvement to this temporary diet (Daschner et al., 2005) tolerate fishery products afterwards. If no clinical response is observed after the temporary diet the interpretation will mainly be that chronic urticaria and *A. simplex* sensitization are independent features.

With respect to other serum isotypes, specific IgG_4 is produced in all patients with GAA (Daschner et al., 2002). Its presence in patients with chronic urticaria sensitized against *A. simplex* has been attributed to a previous contact with viable *A. simplex* but it is presently not clear if its absence in *A. simplex* associated chronic urticaria is due to either a longer time interval from the last parasitic episode, a different immunologic response after parasitism, or a marker of an allergic reaction to non-viable *A. simplex* proteins (Daschner et al., 2005).

2.9. Other considerations

2.9.1. Human genetics and the immune repertoire

There is considerable variation in allergic diseases between different individuals. The immune recognition of protein allergens in humans is strongly influenced by genetics. The most important controlling factor, however, is the major histocompatibility complex (MHC), which is genetically highly polymorphic and controls responsiveness by immune (T) cells that in turn control the antibody repertoire; the allergic IgE antibody response is strongly controlled by T cells and examples exist of strong MHC control of IgE responses to nematode allergens (Fraser et al., 1993; Kennedy et al., 1991; Tomlinson et al., 1989). This means that few if any people who are allergic to *Anisakis* are likely to respond to all the potential allergens in the parasites, and individuals who will respond to an identical (sub)set of allergens will be rare, despite the finding that more than 90% of patients with *Anisakis* allergy respond to at least one of the three major allergens (Ani s1, Ani s4 or Ani s 5). There is therefore a high degree of unpredictability in which individuals will respond to any given allergen or antigen combinations.

2.9.2. Development and activation-associated nematode allergens.

Parasitic nematodes in general are known to radically alter their surface, secretion patterns and somatic antigens. This is particularly true for *A. simplex* which triggers a change in its enzyme and secretion profile after invading the host tissue (Hwang et al., 2003). This means that there are differences between the antigens (allergens) of living or dead larvae in fish that are produced or released on entering the human gastro-enteric tract.

2.9.3. Intrinsic or infection-acquired allergenic sensitisation

The balance of opinion is that allergic sensitisation to *A. simplex* is initiated primarily by infection with a larva that invades the tissues of the stomach or intestine. There is, however, evidence in the general parasitology literature that secretory products of nematodes can generate a Th2 type of response without an infection (Hewitson et al., 2009). Thus, it is surprising that most helminths do not generate a clinical allergic reaction, even if a Th2 response with the production of IgE antibodies is often induced. Different clinical allergic entities described so far in relationship with *A. simplex*

cannot exclude that this widespread nematode is an exception. It is therefore theoretically possible for *A. simplex* allergens to initiate an allergic condition through exposure of humans to somatic materials from dead parasites, or from the secretions of living larvae that invade the tissues of the stomach or intestine, or larva that do not invade but persist for a period time before expulsion.

3. EXPOSURE ASSESSMENT

As discussed in the previous chapter, gastro-allergic anisakiasis and allergy to *Anisakis* are two distinct clinical syndromes. If allergy to *Anisakis* is related to a previous acute parasitic episode following exposure to live larvae, the exposure assessment has clearly distinctive features. This is especially important not only of different dietary recommendations, but also for its implications in preventive public health interventions. The exposure assessment is further complicated because of differences in response to *A. simplex* Audicana reported that freezing of fish will not protect against allergenic reactions to ingested *A. simplex* antigens in humans (Audicana et al., 1997) although it has also been described that patients who a sensitised to *A. simplex* tolerated ingestion of dead larvae and remained asymptomatic (Garcia et al., 2001).

3.1. Antigen exposure through fishery products

A. simplex allergens are highly resistant to heat and freezing (Falcao et al., 2008; Fernandez de Corres et al., 1996) therefore treatments which kill Anisakidae in fishery products may not protect the consumers against allergic hazards due to the ingestion. The antigenicity of the larvae is preserved after freezing and may explain why some sensitized patients develop symptoms after ingestion of infested frozen fish (Rodriguez-Mahillo et al., 2008).

A. simplex allergens may be also present in the flesh of fish in areas close to the larvae (Audicana and Kennedy, 2008) so that parasite allergens can be present in the edible fish muscle and might cause allergic symptoms irrespective of the ingestion of larvae (Solas et al., 2008). Edible parts of the fish which contain larvae and larval antigens include the flesh and viscera. No hazard has been identified in edible fish oils.

Hake steaks artificially parasitized with *A. simplex* larvae were subjected to different treatments (chilling, freezing, heating at 86.3°C and microwave cooking) and then examined by scanning electron microscopy (Tejada et al., 2006). No apparent changes were observed in the frozen larvae, no disruptions in the cuticle were perceptible. Indeed another recent study showed that freezing did not greatly alter the larval body. When ruptures were observed, the antigen release to the incubation media was not enhanced, and most of the antigenic content was retained inside the bodies of the larvae.

Further studies are needed in order to elucidate whether the changes observed in the cuticle reduce the resistance of the parasites to the action of gastric enzymes in the gastrointestinal tract and to determine the release of allergens to the flesh by the viable larvae during chilled storage of the fish (Tejada et al., 2006).

Exposure to *A. simplex* allergens may also occur from fish-flesh in areas where an intact larvae is no longer present (Audicana and Kennedy, 2008) or in animals parasitized in non-edible sites. Hence where an allergic reaction is provoked by antigen alone, then parasite allergens present in the edible fish muscle might cause allergic symptoms in the absence of ingestion of intact larvae (Solas et al., 2008).

3.2. Epidemiological data

3.2.1. Anisakiasis and related allergy

Epidemiological data are scarce, even in Spain, where allergy to *A. simplex* is better recognised and more studied than in other Member States within Europe. With respect to the initial study giving rise to the concept of GAA, in 1997, 96 cases of GAA were diagnosed in patients arriving to the main emergency centre of a Hospital ascribed to a region of about 500,000 inhabitants in Madrid (Alonso-Gomez et al., 2004). Extrapolating from that, there is a minimal annual incidence of 20 patients per 100,000 inhabitants. These data can be interpreted as an underestimation, since within this region of 100 km^2, many patients with mild symptoms were unlikely to seek support from emergency medical or local primary health care provision. In this study the ingestion of anchovies in vinegar sauce was the most common risk factor (79/96 patients), but other fish meals were also implicated as there were 9 cases linked with consumption of undercooked hake, 2 cases with raw cod and 6 with other undercooked fish (Alonso-Gomez et al., 2004).

Special emphasis has therefore to be given to raw fish intake. Examples of different specialities in European countries which, if not adequately treated, are of increased hazard for *A. simplex* allergy are sushi, sashimi, smoked herring (or mackerel, sprat, salmon, halibut, haddock, sardine), ceviche, salted herring, marinated anchovies (It. *alici marinate;* Sp. *boquerones en vinagre*), gravlax salmon, *Rollmops* (marinated herring), *nieuwe* (herring), bloater and kipper (whole, salted in brine, cold smoked herring). Individuals at risk will be likely to have repeated exposure to *Anisakis* allergens because of eating habits and the high prevalence of infection in some species of fish (Audicana and Kennedy, 2008)

The prevalence of positive skin prick tests varies widely, even between different regions in Spain. In Central Spain healthy donors have a 15 to 20% probability of having specific IgE antibodies against *A. simplex* (Daschner et al., 1998). Whereas cross-reactivity with other parasites or even arthropods may explain this high frequency, data on parasite infection generating possible cross-reactions with helminths (e.g. *Ascaris* or *Toxocara*) in this region are not available. Thus the high prevalence of specific antibodies against *Anisakis* has been interpreted as a marker of a previous parasitic episode, even if the patient does not remember any fish intake associated symptoms (Daschner et al., 2005). There is insufficient data are available about prevalence of positive skin prick test results from other European countries to allow comparisons with data from Spain.

Following the initial realisation that *A. simplex* is a causative agent of acute allergic reactions, centres in Spain recognised and have published series of reports of *A. simplex* related allergies. In Table 1 reports from single hospitals from Spain and other countries are summarized in Table 1.

Table 1: *A. simplex* allergy reports worldwide with a special mention of anaphylaxis.

Country	Year	Author	Numbers of allergic patients	
			Total	With anaphylaxis
Egypt	2000	(Aly, 2000)	2	
France	2002	(Pecquet et al., 2002)	3	
France	2002	(Magnaval et al., 2002)	1	
Italy	2009	(Asero et al., 2009)	1	1
Italy	2008	(Ventura et al., 2008)	10	3
Italy	2002	(Foti et al., 2002)	43	
Italy	2001	(Scala et al., 2001)	1 (occupational)	
Italy	2000	(Purello-D'Ambrosio, 2000)	14 (occupational)	
Japan	2006	(Kameyama et al., 2006)	1	
Japan	1990	(Kasuya et al., 1990)	1	
Korea	2009	(Choi et al., 2009)	10	3
Portugal	2008	(Falcao et al., 2008)	Epidemiologic study of sensitization and urticaria	
South Africa	2006	(Nieuwenhuizen et al., 2006)	43 (occupational)	
Spain	2007	(Añíbarro, 2007)	54	22
Spain	2006	(Armentia et al., 2006)	8 (occupational sensitization)	
Spain	2002	(Audicana, 2002)	150	39
Spain	2001	(Dominguez-Ortega et al., 2001)	3	3
Spain	2001	(Garcia et al., 2001)	75	12
Spain	2001	(Gracia-Bara et al., 2001)	17	6
Spain	2000	(Audicana, 2000)	67	18
Spain	1999	(Mendizabal-Basagoiti, 1999)	36	
Spain	1998	(Armentia et al., 1998)	2 (occupational)	
Spain	1998	(Añíbarro and Seoane, 1998)	1 (occupational)	
Spain	1998	(Cuende et al., 1998)	3 urticaria and rheumatological disorders	
Spain	1998	(Daschner et al., 1998)	11	
Spain	1998	(Gomez et al., 1998)	8 retrospective diagnosis of possible eosinophilic gastroenteritis	
Spain	1998	(Rosel Rioja et al., 1998)	2	
Spain	1997	(Carretero, 1997)	1 (occupational)	
Spain	1997	(Aníbarro, 1997)	1 (occupational)	
Spain	1997	(Moreno-Ancillo et al., 1997)	23	5
Spain	1996	(Fernandez de Corres et al., 1996)	28	17
Spain	1995	(Audicana et al., 1995)	10	1
Spain	1997	(Montoro et al., 1997)	23	
Spain	2004	(Alonso-Gomez et al., 2004)	96	

Parasitic episodes without allergic symptoms have also been described by different groups in Europe over the same time period (Table 2):

Table 2: Parasitic episodes without allergic symptoms

Region	Year	Diagnosis and number of patients	Author
Madrid	1999	5 gastric anisakiasis 2 intestinal anisakiasis	(Oliveira et al., 1999)
Cordoba (Southern Spain)	2000	13 intestinal anisakiasis	(Lopez Penas et al., 2000)
Pamplona (North Spain)	2002	8 gastric anisakiasis 13 intestinal anisakiasis	(Castán et al., 2002)
Toledo (Central Spain)	2003	10 gastric anisakiasis 15 intestinal anisakiasis	(Repiso Ortega et al., 2003)

Further to fish eating habits, significant differences of sensitization prevalences between regions could be explained not only by different fish eating habits, but also by different parasite prevalences in the fish consumed. A recent study of anisakids in anchovies revealed a higher prevalence of *A. simplex* in fish from the Atlantic than from the Mediterranean, whereas within the Mediterranean a higher prevalence was found in the Ligurian Sea compared with the Catalan sea (Rello et al., 2009), see chapter 5.

4. TREATMENT FOR KILLING PARASITES IN FISHERY PRODUCTS

The critical control points for prevention of consumers' exposure to fishery-parasites are: the quality of the raw material, i.e. the catching or rearing of stock free from the parasites; the application of physicochemical treatments to fishery products to ensure killing of any parasites which may be present; or by the physical separation of parasite contaminated fishery products during processing. All three of these options are potential control measures for control of allergic diseases, and the second option (physical-chemical treatments to kill parasites) will also be effective at preventing infections. As previously discussed in section 3.3, *A. simplex allergens* are highly resistant to heat and freezing (Falcao et al., 2008; Fernandez de Corres et al., 1996) therefore treatments which kill Anisakidae in fishery products may not protect the consumers against allergic hazards due to the ingestion: these aspects are not further considered in this chapter.

There is more information on the resistance to physical and chemical treatments by *A. simplex* than for other fishery parasites. The properties of *A. simplex* are likely to be similar to that of other multicellular parasites (although trematode metacercariae are considerably more heat resistant), and information on other genera and species of organism will be given where available.

4.1. Assessing viability

Viability is here defined as the ability of individual anisakid larvae to survive various chemical and physical treatments or processing procedures. A viable larva is defined as that physically intact and motile, as demonstrated by spontaneous movements following stimulating by bending with forceps and a needle. It is not currently possible however to confirm that intact and motile larvae are capable of successfully infecting a human. It may be important in the future for the establishment of methods to assess whether viable larvae, as here defined, from different sources are capable to human infection.

4.2. Treatments defined by legislation

One of the first countries in Europe that applied freezing as preventive treatment for anisakiasis was the Netherlands in 1968, with the so called "Green Herring Laws" which stated that fresh herring should be frozen in such a manner as to reach a temperature of at least -20°C within 12 hours and stored for a period of 24 hours prior to being released to the public. This resulted in a decrease of 40-50 human cases per year to less than 10 cases per year after the legislative action was implemented (Sakanari, 1995). In 1987 the EEC subsequently implemented legislation/recommendations for the similar freezing requirements (-20°C for 24h) as were implemented in the regulation of Netherlands (Eurofish-Report, 1987).

The current EC Regulation 853/2004 requires freezing to a temperature of not more than –20°C in all parts of the product for not less than 24 hours to the following:

- fishery products to be consumed raw or almost raw;
- fishery products from herring, mackerel, sprat, (wild) Atlantic and Pacific salmon , if they are to undergo a cold smoking process in which the internal temperature of the fishery product is not more than 60°C;
- marinated and/or salted fishery products, if the processing is insufficient to destroy nematode larvae.

The freezing treatment must be applied to either the raw or the finished product. The EC Regulation also states that food business operators need not carry out the freezing treatment if epidemiological

data are available indicating that the fishing grounds of origin do not present a health hazard with regard to the presence of parasites and the competent authority so authorises. Moreover, this regulation requires that a document from the manufacturer stating the type of process they have undergone must accompany fishery products referred to in point 1 when placed on the market, except when supplied to the final consumer (Chapter III, Section VIII, Annex III of Reg. 853/2004). Under the chapter V "Health standards for fishery products " in the same section, the Regulation also states that "food business operators must ensure that fishery products have been subjected to a visual examination for the purpose of detecting visible parasites before being placed on the market. They must not place fishery products that are obviously contaminated with parasites on the market for human consumption".

In the USA, the FDA (Food and Drug Administration) requires that all fish and shellfish intended for raw or semi-raw (e.g. marinated or partly cooked) consumption should be blast frozen to -35°C (-31°F) or below for 15 hours, or be completely frozen to -20°C (-4°F) or below for 7 days (FDA, 1998). The same freezing treatment is required in Canada (Weir, 2005). The temperature and time difference between the EU and US regulations reflects either the total storage time (FDA) or the time the product core achieves the critical temperature (AFSSA, 2007). These preventive measures have been adopted by the fish industry in Europe and North America as part of their Hazard Analysis and Critical Control Points (HACCP) systems (Audicana and Kennedy, 2008).

The CODEX standard for salted Atlantic herring and salted sprat (CODEX, 2004) states that the viability of nematodes shall be examined after artificial digestion with magnetic stirring treatment. If living nematodes are detected, products must not be placed on the market for human consumption unless they are treated by freezing to -20ºC for not less than 24 h in all parts of the product, or adequate combination of salt content and storage time or by other processes with the equivalent effect.

4.3. Chemical treatment

Salting and marinating are the chemical treatments most commonly used to inactivate viable Anisakidae larvae.

4.3.1. Salting

Anisakidae larvae are sensitive to salt only under certain conditions. It has been estimated that 28 days of storage in brine with 6.3% salt and 3.7% acetic acid in the aqueous phase of the fish was the maximum survival time for herring (Karl, 1995). A study conducted by the French CEVPM showed that under industrial production condition for dry salted herring, the total necessary time for the parasites to be killed was 20 days (CEVPM, 2005). A study by the Spanish AESAN indicated that when NaCl concentration up to 8-9% was reached for at least 6 weeks, no further freezing treatment would be needed (AESAN, 2007).

Opisthorchis metacercariae in fish are killed at 13.6% NaCl after 24 hours (Kruatrachue et al., 1982).

4.3.2. Marinating

Marinating, is the process of soaking foods in a seasoned, often acidic, liquids with or without cooking. The aim of marinating is not only to inhibit the action of bacteria and enzymes, but also tenderize the connective tissue and change the taste, textural and structural properties of the raw material, resulting in a product with a characteristic flavour and an extended shelf life (Duyar, 2009). The active ingredients of the marinade can include vinegar, lemon juice, wine, soy sauce, or brine.

Early studies showed that *A. simplex* larvae are resistant to traditionally conditions of marinating and can survive 25 days in a mixture of salt and vinegar (Kuipers et al., 1960). Depending on the salt concentrations, the survival of larvae reaches 35 to 119 days (Karl, 1995). Thus the traditional marinating procedure for anchovies in vinegar has been one of the most important sources of human *A. simplex* disease, both with and without allergic symptoms. Previous German and Danish procedures for marinating herring fillets require acetic acid concentrations of 5 to 7%, but require long treatment times to kill *A. simplex* larvae: 5 weeks by the German method with additional use of hydrogen peroxide, currently banned in several EU countries, and 6 weeks by the Danish method (Karl, 1995). Some preparations with short treatments for marinating are adopted due to economic and organoleptic reasons, one recent study describes a marinating procedure for anchovies with the use of 10% acetic acid (vol/vol) plus 12% salt which guaranteed destruction of *A. simplex* larvae within 5 days (Sanchez-Monsalvez et al., 2005): the sensory characteristics of the product were shown to be satisfactory.

Arcangeli (1996) found that a marinade of vinegar (6% acetic acid) and 10% sodium chloride applied for 24 h to sardines, followed by the addition of sunflower seed oil and refrigeration for 13 days, inactivates all *A. simplex* larvae.

In the following tables, the conditions for successful killing of *A. simplex* larvae (Table 3) and parasites other than anisakids (Table 4) in fishery products are reported.

Table 3: Conditions for successful killing of *A. simplex* in fishery products

Fish	Treatment	Parameters	Reference
Herring	salting	5% NaCl, > 17 weeks	(Karl, 1995)
		6-7% NaCl, 10-12 weeks[8]	
		8-9% NaCl, 6 weeks	AESAN, 2007
	dry salting	20 days	CEVPM, 2005
Anchovies	marinating	10% acetic acid plus 12% salt for a minimum of 5 days	Sanchez-Monsalvez, 2005
		2.4% of acetic acid and 6% of NaCl for 35 days	AESAN, 2007
		10% acetic acid, 12% NaCl for 5 days	Sanchez-Monsalvez et al., 2005
Sardines	marinating	6% acetic acid, 10% NaCl for 24h + 4°C for 13 days	(Arcangeli et al., 1996)
Herring	marinating	28 days in brine with 6.3% NaCl and 3.7% acetic acid	Karl, 1995
Sockeye salmon and canary rockfish	freezing	-35°C for 15h, followed by -18° for 24h	Deardorff and Throm, 1998
Arrowtooth flounder	freezing	-15°C for 96h; -20°C for 60h; -30°C for 20h; -40°C for 9h	(Adams et al., 2005)
In vitro larvae	freezing	L3 survived at -10°Cfor up to 4 h and at -5°C for 5 h. No survival at -15°C for few minutes	(Wharton and Aalders, 2002)
	heating	60°C for > 15 minutes	(Sanchez-Monsalvez et al., 2006)
	heating	>60°C (core temperature) for 1 min;	(Bier, 1976)
	heating	74° for 15 sec	Audicana and Kennedy, 2008
	heating	60°C for 10 min (3 cm thick fillet)	Wootten and Cann, 2001
	plant extract	[6]-shogaol at 62.5 µg/ml ; [6]-gingerol at 250 µg/ml	(Goto et al., 1990)
King salmon and Arrowtooth flounder	high pressure	414 MPa for 30-60 seconds	(Molina-Garcia and Sanz, 2002)
		276 MPa for 90-180 seconds	
		207 MPa for 180 seconds	
Herring	irradiation	6-10 kGy	(Van Mameren and Houwing, 1968)
Sea eel	irradiation	>1 kGy	Seo et al., 2006

8 A reduction of either the salt or the acetic acid concentration to one half will at least double the storage time

Table 4: Conditions reported for successful killing of parasites other than anisakids in fishery products

Parasites	Treatment	Parameters	Reference
Diphyllobothrium spp.	freezing	-18°C for 24h	Salminen, 1970
Diphyllobothrium spp.	heating	>56°C	
Opisthorchis spp. *Clonorchis* spp.	freezing	-10°C for 5 days	WHO, 1979
Opisthorchis spp.	salting	13.6% NaCl, 24 hours	Kruatrachue et al., 1982
Trematode metacercariae	heating	50°C for 5 h; 70°C for 30 min	Waigakul, 1974
Trematode metacercariae	irradiation	0.15-0.5 kGy	Bhaibulaya, 1985; Chai et al., 1993.

Considering the above presented data, many traditional marinating methods are not sufficient to kill *A. simplex* larvae. Chemical treatment, if not combined with freezing, must be optimised for each individual fish preparation, as survival of *A. simplex* larvae depends on various factors (fish size, fat content, and the active ingredients). Thus, studies on viability of larvae with marinating preparations for herring differ from those of anchovies. With respect to anchovies in vinegar sauce, the published data show effectiveness in killing *A. simplex* larvae if anchovies are treated with 10% acetic acid plus 12% salt for a minimum of 5 days.

According to the *Agencia Espanola de Seguridad Alimentaria* (AESAN, 2007), to kill *A. simplex* larvae, 35 days treatment together with 2.4% of acetic acid and 6% of NaCl are required. Thus, it can be concluded that vinegar and salt can reduce the hazard associated with *A. simplex*, but do not eliminate it nor do they reduce it to an acceptable level. Because of that, it is necessary to freeze products prior to marinating (for example Spanish *escabechar*).

4.3.2.1. Other chemical procedures:

Vegetable products have been studied for their possible usefulness to kill *A. simplex* larvae under experimental conditions: shogaol and gingerol extracted from *Zingiber officinale*, as well as components of *Perilla* leaves are able to kill *A. simplex* under specific conditions (Goto et al., 1990; Hierro et al., 2006; Hierro et al., 2004).

Other in vivo studies have been conducted about the activity against *A. simplex* larvae of chemical compounds, in particular monoterpenic derivatives obtained from different essential oils, such as alpha-pinene, beta-pinene, ocimene, myrcene, geranyl acetate, and cineole (Navarro et al., 2008). The most active compound was alpha-pinene. Further in vivo studies are required to investigate whether addition of these compounds to food could have a killing effects alone or in synergy with other compounds and treatments) on *A. simplex*. These in vitro studies should be extended in order to evaluate their usefulness in food processing.

4.4. Physical treatment

4.4.1.1. Freezing treatment

Factors affecting the efficacy of freezing for inactivating anisakid larvae include the temperature, time needed for reaching the final temperature in core fish tissues, maintenance time and fat contents of fish (AFSSA 2007).

One of the first study about effects of freezing on *A. simplex* larvae was conducted in 1953 (Gustafson, 1953), before the first legislative requirement about freezing fish for public health reasons

(Green Herring Law) was adopted in the Netherland. In this initial study freezing and storage at -5°C or -10°C, even for several days, did not kill all the larvae. After 12 days at -10°C, 4% of larvae recovered were still alive. On storage at -17°C (internal temperature of -14°C), 5.5% of the larvae (33 out 600) were alive. After 24 hours and longer, the core temperatures had reached the freezer temperature and no live nematodes were recovered (Gustafson, 1953).

A. simplex larvae has been reported to survive freezing at -10°C for up to 4h which may be aided by the production of trehalose by the parasite which can act as a cryoprotectant (Wharton and Aalders, 2002). Monitoring of fish freezing in commercial blast freezers and under conditions which simulate those of a domestic freezer, indicate that it may take several days for all parts of the fish to reach a temperature that will kill the larvae (for instance it was observed that 20 kg container of fish did not reach -35°C until 28 hours;(Wharton and Aalders, 2002). This aspect, and the moderate freezing tolerance of larvae, emphasize the need for fish to be frozen at a sufficiently low temperature (at least -15°C) for a sufficient time to ensure that fish are safe for consumption (Wharton and Aalders, 2002).

A study was conducted on fish fillets at -35° C for 15 hours to determine the effects of commercial blast-freezing on the viability of third-stage larvae of *A. simplex* encapsulated in the muscle and viscera of sockeye salmon (*Oncorhynchus nerka*) and canary rockfish (*Sebastes pinniger*). The frozen fish were subsequently stored at -18°C, and samples taken after 1, 24, 48, and 72 hours of storage. Four live but comatose larvae were found out of 1671 larvae recovered after blast freezing and 1 hour storage at -18°C, but no viable larvae were recovered from fish stored for the longer periods (Deardorff and Throm, 1988).

The EU Hygiene Regulation (Reg. 853/2004) requires that frozen fishery products must be kept at a temperature of not more than -18°C in all parts of the product. Some studies demonstrate that there is a direct correlation between time and temperature in order to kill *A. simplex* larvae (Adams et al, 2005) and that 100% of *A. simplex* larvae in fish muscle are killed at a temperature of -15°C for 96h, so this minimum period should be recommended for the storage at -18°C, to ensure successful parasite killing.

For cestode larvae, the *Diphyllobothrium* plerocercoid is inactivated if the fish is kept in household freeze at -18ºC for at least one day (Salminen, 1970).

Freezing of fish at -10°C (5 days) will kill *Clonorchis* and *Opisthorchis* metacercariae (World Health Organization, 1979)

It should be noted that 1-star and 2-stars freezers can reach temperature of -6°C and -12°C respectively, and only 3-star and 4-star domestic freezers may operate at a temperature of -18°C or less, thus the fish need to be frozen in all parts of the products for a time longer than 24 hours to ensure that nematode parasites are inactivated (Wharton and Aalders, 2002).

It is to be expected that the lethality of freezing to nematode larvae will be a function of temperature and dwell time. Even -5°C shows some lethality, but the results of the Gustafson experiments suggests the critical temperature to ensure a high proportion of nematodes are killed within several hours (>24hours) is at least -17°C. It is also apparent from the results of the investigations reported that freezing of itself may not kill all larvae. A very small proportion of larvae may survive, but they are moribund, that is, the larvae do not show spontaneous movement, but will move when stimulated. It is not known, but has been suggested that such moribund larvae are not be capable of infecting humans. However, assuming motile larvae are infectious provides a greater margin of safety for example in HACCP analyses. Therefore freezing should be followed by a period of storage in the frozen state to ensure complete elimination of the infectious hazard.

4.4.1.2. Heat treatment

Studies showed that a core temperature of 60°C for 1 minute is sufficient to kill any larva present in the fishery product (Bier, 1976). However, reaching such a core temperature depends on the product thickness and composition. It has been estimated that a 3 cm thick fillet should be heated at 60°C for 10 minutes to ensure all larvae are destroyed (Wootten and Cann, 2001).

Heating temperatures of ≥60°C for at least 1 min when cooking or smoking fish (Bier, 1976), or heating temperatures up to ≥74°C for at least 15 seconds when microwave cooking (Adams et al., 1999) of fish to be eaten raw have been recommended to kill the parasites and prevent infections (Audicana and Kennedy, 2008).

For *Diphyllobothrium* spp., plerocercoids do not survive temperatures above 56ºC. Thus, the infection risk is eliminated if the fish is fried, boiled, or adequately smoked (Salminen, 1970).

Heating and cooking also inhibits infectivity of trematode metacercariae in fish. Heating of 50°C (5 hours) or 70°C (30 min) inactivates free *O. viverrini* metacercariae (Waigakul, 1974).

Thus freezing and cooking remain the reference processes guaranteeing the destruction of larvae, under well defined conditions. It should nevertheless be recalled that these treatments may not inactivate allergens. Treatments which provide an equivalent level of protection as freezing at a temperature of not more than -20ºC for not less than 24 hours in all parts of the product for the killing of *A. simplex* larvae include:

- Freezing at -35ºC for 15 hours or at -15°C for at least 96 hours;
- Cooking at > 60ºC for at least 1 minute (core temperature)

4.4.1.3. High hydrostatic pressure

High hydrostatic pressure has been demonstrated to be an effective technique for treating food to reduce the number of pathogenic microorganisms and to extend shelf life (Knorr, 1999). A pressure of 200 MPa for 10 minutes at 0-15°C kills *A. simplex* larvae, as well as pressures down to 140 MPa when the treatment time is increased up to 60 minutes. In addition, cycles of compression and decompression applied for a specific time were found to be more effective at killing larvae than a single pressure treatment for a similar time (Molina-Garcia and Sanz, 2002). It should be noted that such long treatment times would be impractical for the food industry. A pilot study was performed to determine the effect of high hydrostatic pressure on the viability of *A. simplex* larvae in raw fillets of king salmon and arrowtooth flounder, and to evaluate the effects of the treatment on the colour and texture of the fillets. Different pressure and time combinations were required to kill 100% of the larvae, and were as follows: 414 MPa for 30-60 seconds, 276 for 90-180 seconds, and 207 MPa for 180 seconds. For 100% killing, however, a significant increase in the whiteness of the flesh was observed: this effect on the colour and appearance of the fillet may limit its application to the processing of fish for raw consumption (Dong et al., 2003). However, pressure treatment could be applicable to processed fish, e.g. marinated and cold-smoked fish, where the tissues are already substantially modified. In these processes, the pressure needed to kill parasites could be lower when combined with other treatments (Molina-Garcia and Sanz, 2002). In a recent study, the application of a pressure of 300 MPa for 5 minutes has resulted in the inactivation of *A. simplex* larvae in the tissues of mackerel (*Scomber scombrus*), and a similar procedure has been suggested for the treatment of other fatty fish such as sardines and anchovies (Brutti et al., 2009). These experimental studies should be extended in order to evaluate their usefulness in food processing.

4.4.1.4. Drying

No specific data was found in the literature on the efficacy of drying for inactivating parasitic larvae in fishery products, thus drying cannot be considered an effective treatment for that purpose.

4.4.1.5. Irradiation

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

Irradiation doses that kill *A. simplex* larvae in salted herring were reported to be higher than 6–10 kGy (Loaharanu, 1997a; Van Mameren and Houwing, 1968). Similarly, another study found *A. simplex* larvae to be highly resistant to irradiation doses of 2 kGy or 10 kGy (FAO/IAEA 1992). Another recent study based on an *in vivo* experiment in rats demonstrates that *A. simplex* third-stage larvae in the sea eel (*Anago anago)* are not inactivated up to 1 kGy (Seo et al., 2006). Irradiation is therefore not effective in inactivating *A. simplex* larvae, since they appear to be highly resistant to the irradiation doses which are normally recommended.

For liver flukes, investigations in Thailand demonstrated that low dose irradiation of freshwater fish can prevent infectivity of metacercariae of *O. viverrini* when such fish are prepared in local dishes made from raw or semi processed fish (Bhaibulaya, 1985). At 0.5 kGy, the metacercariae could not develop in hamsters and caused no infection in their livers. The effective inactivation of *Opisthorchis* metacercariae through irradiation has also been recently reported (Naz'mov et al., 2001), although high doses were used (12.5-25 kGy), much above the recommended levels.

Irradiation with 0.15 KGy inactivates metacercariae of *O. viverrini* and *C. sinensis* in fish (Sornmani et al., 1993; Chai et al., 1993). These experimental studies should be extended in order to evaluate their usefulness in food processing.

4.4.1.6. Low voltage current

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

4.4.1.7. Smoking treatment

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

Hot smoking exposes foods to smoke and heat in a controlled environment; products are subjected to temperatures >60°C (average reference parameters: 70°C-80°C for 3-8 hours approximately). *A. simplex* larvae are unable to withstand such conditions (FDA/CFSAN, 2001; Sainclivier, 1985). Cold smoking can be used as a flavour enhancer for example to salmon or scallops, and smokehouse temperatures for this process are maintained below 38 C: the process lasts from a few hours to a several days. During cold smoking, temperatures are insufficiently high for killing parasite larvae (Khalil, 1969; Szostakowska et al., 2005), thus the products must undergo an initial inactivation treatment.

Gardiner reported that neither cold smoking for 12 h at 25.6°C nor refrigeration for 27 days killed *A. simplex* larvae in salmon (Gardiner, 1990). This analysis indicated that fresh salmon and cold-smoked salmon had 1-3 and 1-5 *A. simplex* viable larvae per 200 g of fish, respectively. A similar result was found in whole Pacific herring (*Clupea harengus pallasi*), where *A. simplex* larval remained viability after brining and smoking at an average temperature of 19°C for 24 h was 100% and 87.5%, respectively (Hauck, 1977). Thus during hot smoking, products are treated at >60°C for some hours, and *A. simplex* larvae are unable to withstand such conditions. During cold smoking, instead, the temperature are too low (<38°C) in order to kill the parasitic larvae.

In summary, many traditional marinating and cold smoking methods are not sufficient to kill *A. simplex* larvae. Such treatment, if not combined with freezing, must be optimised for each individual fishery-product preparation, as survival of *A. simplex* larvae depends on various factors (fish size, fat content, and the active ingredients). Freezing raw fishery products prior to smoking remains the most effective way to ensure that viable parasites are killed in cold-smoked products to be consumed by the public. There is insufficient information to show that alternative treatments, including high hydrostatic pressure, drying, irradiation, and low voltage currents, are effective at killing anisakid larvae under conditions that preserve the products' organoleptic qualities.

5. CRITERIA FOR ASSESSING HEALTH HAZARD RELATED TO THE PRESENCE OF PARASITES FROM FISHERY PRODUCTS OF DIFFERENT ORIGINS AND PRODUCTION METHODS

Fishery products are of either wild or aquacultural origin. The purpose of this section is to evaluate criteria that can be used to determine if information on fishing grounds or production methods can indicate a health hazard related to the presence of parasites in fishery products which are intended to be eaten raw or almost raw.

5.1. WILD CATCH FISHERY PRODUCTS

For wild caught fishery products eaten raw or almost raw, information on the prevalence, abundance, as well as species and geographical distributions of the parasites and their hosts together with monitoring systems and trends in parasite presence and abundance are important.

5.1.1. Saltwater fishery products

Fish parasite communities are dynamic across the geographical range of host species. For example, there are significant differences in relative proportions of different *Anisakis* sibling species between horse mackerel populations caught in the NE Atlantic and Mediterranean Sea, respectively. Thus, *A. simplex* (sensu stricto) is the dominant species detected in horse mackerel from the Bay of Biscay, the south and west coast of Ireland, North Sea and Norwegian Seas. In contrast *A. pegreffii* is more prevalent with decreasing latitude, reaching 87% relative to *A. simplex* (sensu stricto), in horse mackerel caught off southern Portugal. However, *A. pegreffii* is the main species occurring in horse mackerel from the eastern and western Mediterranean, while both sibling species show mixed infections in almost equal proportions in horse mackerel from the Gibraltar area of Mediterranean Sea (Mattiucci et al. 2007).

The prevalence of *A. simplex* (not discriminated between sibling species) in fish varies considerably between different geographical areas/fishing grounds and fish host species. Thus, the *Anisakis* prevalence in horse mackerel is 100% in Atlantic waters off western Ireland, the northern North Sea, the Mediterranean off southern Sicily, the Adriatic and Tyrrhenian Sea, while of much lower prevalence (0 – 2%) in horse mackerel from South East France and around the Balearic Islands (MacKenzie, 2008). In general, both prevalence and abundance of *A. simplex* seem to be considerably lower in fishes from western areas of the Mediterranean Sea compared to the same fish host species caught from Atlantic waters. Along the Turkish Black Sea coast however, anisakid species, except of *H. aduncum*, seem to be absent in whiting (Ismen and Bingel, 1999). The apparent absence of *A. simplex* in the Black Sea might be due to the unusual physical, chemical and ecological characteristics of this area, which is known to be poor for krill, which in other waters act as the main intermediate host of *Anisakis* (Tomczak and Godfrey, 1994; Victoria Herreras et al., 1997).

5.1.1.1. Pelagic and coastal ecology of parasites

The factors and mechanisms that determine the occurrence and distribution of *A. simplex* in the NE Atlantic ecosystem are still largely unknown. In these waters *A. simplex* occurs in fish in at least two ecologically different environments, i.e. the pelagic/oceanic and the benthic/coastal ecosystem. The habitats of these two ecosystems are characterized by different physical conditions and different key species with respect to zooplankton, teleost fishes and marine mammals. For example, krill and the minke whale (*Balaenoptera acutorostrata*) seem to act as main intermediate and definitive hosts, respectively, for *Anisakis* in the pelagic/oceanic environment, while various non-krill crustaceans and more stationary cetacean species such as porpoise (*Phocoena phocoena*) and killer whale (*Orcinus orca)*, may play these roles in the NE Atlantic benthic/coastal habitats. Additionally, the potential of

various coastal seal species to act as definitive host for *Anisakis* in different areas around the NE Atlantic is still largely unknown.

Variations of this general pattern however occur. For example, Klimpel et al. (2004) investigated the life cycle of *A. simplex* in the Norwegian Deep (northern North Sea) and found that the pearl side acts as obligatory 2nd intermediate host to *A. simplex*, apparently crucial for transmitting the 3rd stage larvae from the crustacean 1st intermediate host to a suitable transport host such as saithe, before final transfer to the cetacean definitive host.

A. simplex commonly occur in various ecologically and economically important fish species such as herring, mackerel, blue whiting, cod and monkfish, from NE Atlantic pelagic/oceanic and benthic/coastal habitats, respectively. The previously outlined *Anisakis* sibling species show ecological differences with respect to their geographical distribution, macro-habitat and host preferences. Such differences may have favoured development of area- and habitat dependent life cycle strategies and adaptations within geographically and ecologically defined *Anisakis* populations. This again may be reflected (and indirectly measurable) by, for example, different prevalence patterns in seasonality, growth, DNA sequences, fecundity and, probably, virulence between different hosts.

Within Scottish waters there are significant geographical differences in the infection of demersal fish with larval *A. simplex* and *Pseudoterranova* spp. in the muscle. In cod there is a general trend for *Pseudoterranova* to be more abundant in coastal areas, particularly in the Minch and off the North Coast, and much less common in the offshore northern North Sea (Petrie, 2009; Wootten and Waddell, 1977). Conversely, *A. simplex* is far more common in offshore areas than in coastal waters. A similar pattern of infection is detected in *A. simplex* infection of whiting (Wootten and Waddell, 1977). In monkfish the differences are less marked but the same trend is evident (Petrie, 2009).

These observed differences in geographical distribution are likely to reflect variations in the abundance of different hosts of *Anisakis* and *Pseudoterranova*. Thus, pinnipeds, the final hosts of *Pseudoterranova* are found in coastal areas, for example, to the North and West of Scotland whilst cetaceans, the major final hosts of *Anisakis* are also abundant in offshore areas (Mead and R.L., 2005; Rice, 1998; Wootten and Waddell, 1977; Young, 1972). The major invertebrate hosts of *A. simplex*, especially euphausiids, are also abundant in offshore areas (Smith and Wootten, 1978).

The life-cycles of *Anisakis* and *Pseudoterranova* are complex, involving many different potential hosts, and the factors governing the geographical abundance of the life-cycles are not fully understood. The distribution of these parasites is further complicated by migrations of infected fish (Petrie, 2009). Nonetheless, the general division between coastal and offshore areas for *Anisakis* and *Pseudoterranova* would appear to be correct. There is insufficient information to determine if there is any distinction in this distribution between different species of *Anisakis* and *Pseudoterranova*.

Anisakis and *Pseudoterranova* are generally most abundant in European NE Atlantic waters. These are traditionally some of the most productive fishing areas in Europe and the abundance of different hosts at all trophic levels presumably accounts for the overall abundance of the parasites.

5.1.1.2. Monitoring and surveillance systems in fishing grounds

In Norway, a long-term surveillance program was launched in 2006 to monitor the occurrence and distribution of quality reducing or potentially consumer hazardous anisakid nematodes in the commercially most important pelagic fish species (blue whiting, herring, Atlantic mackerel and capelin) commonly exported to various international markets for consumption. The surveillance program aims to examine statistically and demographically (sample size, fish size/age, sex) representative numbers of fish caught during the main annual fishing seasons from the most common fishing grounds in the North, Norwegian and Barents Sea. The investigations are regularly conducted on board Norwegian fishing vessels thus reflecting normal fishing practices, as well as on-board

storage, transport and delivery to processing plants. Furthermore, this surveillance data is designed to reveal any space and time trends in the occurrence of these parasites, along with possible changes in stock composition and migration pattern of the fish hosts. The program is performed by the National Institute of Nutrition and Seafood Research (NIFES, www.nifes.no) in Norway, and up-to-date surveillance data are, whenever required, readily accessible to the Norwegian Food Safety Authority (NFSA, www.mattilsynet.no).

There is currently no available systematic monitoring and surveillance system for *Anisakis* larvae in other European waters. Studies of varying size and complexity have been performed recently including in Scottish waters (Petrie, 2009), and coastal regions of Galicia (Abollo et al 2001), Madeira (Costa et al., 2003; Costa et al., 2008) and the eastern Atlantic and western Mediterranean (Rello et al., 2009), as well as fish purchased in Western France from various fisheries (Chord-Auger et al., 1995). Given the wide range of fish hosts for anisakid larvae and the potential costs of performing monitoring, it is only feasible for such programmes to target a restricted number of host species, as in the Norwegian programme described above.

5.1.1.3. Trends

The lack of long term sampling programmes for anisakids in European waters means that there is little information on temporal trends in population of these parasites. Studies by Rae (Rae, 1963, 1972) showed that the abundance of larval anisakids in the flesh of cod increased dramatically between 1958 and 1970. Unfortunately Rae did not distinguish between *Anisakis* and *Pseudoterranova,* so was not able to ascribe the increase to either or both of these genera. Wootten and Waddell (1977) suggested that in offshore areas of the northern North Sea this increase was likely to have been due to *A. simplex* because this genus was by far the most abundant in those waters. In more inshore waters either genus or *Pseudoterranova* alone were likely to be present. Further evidence for an increase in larval *Anisakis* infection in Scottish waters over this period comes from the levels of infection in the musculature of whiting (Wootten and Waddell 1977). These authors highlighted that sampling during 1960-1963 showed a prevalence of infection of whiting of 1.5% whereas in 1971-74 the prevalence was at least 33% in any area, apart from the Clyde estuary. Even assuming an under-reporting in the earlier sampling periods it appears that the prevalence has considerably increased in the later years of the study.

Further sampling of cod in 2005-06 by Petrie (Petrie, 2009) suggests that there was a further increase in the abundance of both *Anisakis* and *Pseudoterranova* in cod in both inshore and offshore Scottish waters. However, differences in sampling areas and the sizes of fish examined means that this data must be interpreted with caution.

The reasons for these increases in larval anisakid populations are uncertain. Too little is known about the dynamics of *Anisakis* and *Pseudoterranova* populations in all their hosts, as well as the population dynamics of the host populations themselves, to be able to draw any firm conclusions on the causes of changes in anisakid abundance. Grey seals are the major final host of *Pseudoterranova* in Scottish waters and the population size has grown very considerably since the 1960s (Special-Committee-on-Seals, 2008). Increases in *Pseudoterranova* have been ascribed to this rise in seal numbers (Rae 1972) However, without more knowledge of the population dynamics in definitive as well as intermediate host populations, and the relative importance of different hosts in the transmission of the parasite, clear explanations are not possible. For *Anisakis,* the reasons for population changes are even less evident.

Each anisakid species infecting fishery products is genetically diverse. Feeding and/or spawning migrations of many fish host species as well as definitive hosts lead to a considerable level of gene flow between subpopulations of these parasites, and the distribution of each species can change. There is, for instance, already some evidence that natural or anthropogenic ecological changes can affect the distribution of anisakid species, e.g. changes in sea temperature and currents, over-fishing

with depletion, recovery and geographic expansion of fish populations. Furthermore, temporal or spatial variations in distribution and population size of some important definitive mammalian host species, several of which undertake extensive seasonal migrations, may also affect parasite abundance and hence patterns of infection in fishery products. It is therefore important that surveillance of fishery products for infections by anisakids is increased.

Finally, it has been speculated that the currently prevailing regulatory measures regarding the exploitation of marine mammals has led to an increasing population of some mammalian definitive hosts to *Anisakis* and *Pseudoterranova* (Audicana, 2002; Audicana and Kennedy, 2008; Audicana et al., 2003; McCarthy, 2000; Oshima, 1987; Sakanari and McKerrow, 1989). Several observations indicate that relationship exists between *A. simplex* population size and definitive host population size; however these relationships are likely to be complex. For example, the abundance of *P. decipiens* in cod from the eastern outer Oslofjord, southern Norway, markedly declined from 1990 to 1991, which appeared to be related to the phocine distemper virus (PDV) epizootic which killed about 70% of the common seal (*Phoca vitulina*) population in 1988 (Des Clers and Andersen, 1995). Additionally, Hauksson (Hauksson, 2002) recorded a decrease in *Pseudoterranova* spp. abundance in sea scorpion (*Myoxocephalus scorpius*) in shallow waters off western Iceland following a decline in size of local populations of grey seal (*Halichoerus grypus*) caused mainly by over-exploitation and accidental catch in fishing gear. For *A. simplex* however, information is scarce as to the possible relationship between parasite abundance in fish and the population size of actual definitive host species in given areas. Strømnes and Andersen (2000) explained, at least in part, the marked "spring rise" of *A. simplex* prevalence in three fish species from central coastal Norway by the increasing number of whales, especially minke (*Balaenoptera acutorostrata*) and fin whale (*B. physalus*) when migrating through the area during March and April on their way to summer feeding grounds in the Barents Sea. However, neither scientific nor empirical data exist to date regarding the long-term effects of strict hunting regulations for marine mammals on the total biomass of anisakid nematodes in any waters.

In the Baltic Sea, harbouring several brackish water localities (e.g. the Bay of Bothnia), the anisakid species *A. simplex* (s.s.), *Contracaecum osculatum* (s.s.) and *H. aduncum* frequently occur in various fish species including herring and cod, from the south-western and southern fishing grounds, while *P. decipiens* occurs only sporadically (Szostakowska et al., 2005). An investigation of *A. simplex* in the harbour seal (*Phoca vitulina*) from the Skagerrak, Kattegat and the Baltic revealed that *P. decipiens* was the most prevalent nematode species. The abundance of the species was highest in the northernmost areas (16.8-22.9 parasite per animal) and lowest in the southern Kattegat and the Baltic (3.3 and 4.0 parasites per animal respectively) (Lunneryd, 1991). In the Bay of Bothnia, *C. osculatum* reaches prevalences of 20% and 15% in Atlantic salmon and cod, respectively. The grey seal (*Halichoerus grypus*) seems to act as main definitive host for the parasite in this area (Valtonen et al., 1988). No scientific reports exist regarding occurrence of *Anisakis* larvae in fish in the Bay of Bothnia.

Several authors have attempted to summarize biotic and abiotic influences on the distribution and abundance of these parasites (Marcogliese, 2001; McClelland, 2002b). The distribution of definitive hosts seems to influence the abundance of this parasite; however, some unexpected changes have been observed, such as decline in abundance of Anisakid associated with increases of definitive host populations. *Pseudoterranova* abundance in marine mammals may vary with season, host age, geographical origin, host diet and presence of potential competitors such as *Contracaecum*. Moreover, data on long-term variation of Anisakid abundance in seals are often inconclusive or insufficient. Both Marcogliese (2001) and McClelland (2002b) estimate that the relationship between definitive host population size and Anisakid abundance in fish is complex and may be mitigated by other factors such as water temperature and oceanic currents.

5.1.1.4. Geographical distribution

Table 5 shows examples of prevalence and intensity data of *Anisakis* in various fish host species from different geographical areas and illustrates that, despite the lack of data on, systematic monitoring, the size of fish, and anatomical sites of infection, together with the dynamic nature of host populations and infections, there are no areas where fishery products are consistently free of *Anisakis* larvae.

Table 5. Examples of prevalence and intensity data of *A. simplex* in various fish host species from different geographical area**.**

Area	Fish species	Number of samples	Prevalence; intensity (mean±SD, range where available)	Literature source
Barents Sea	Cod	212 (oceanic); 207 (coastal)	96%; 4.3 and 6.1 (oceanic and coastal, respectively)	(Aspholm, 1995)
Northern Norway	Pearl side and saithe		49.6% and 100.0% respectively; 1.1 and 2.6	(Klimpel et al., 2004)
Portuguese coast	Horse mackerel	58	76%; 6.2±10.2 (1-46)	(Silva and Eiras, 2002)
	Atlantic mackerel	45	96%; 12.7±14.8 (1-80)	
	Hake	3	100%; 51.3±5.7 (45-59)	
	Blue whiting	65	94%; 14.3±18.9 (1-89)	
Off Madeira, Portugal	Chub mackerel	154	70%; 2.2±0.12 (1-6)	Costa et al. 2003
Mediterranean coast of Spain	Blue whiting (17-24 cm)	224	12% 1.19	Valero et al. 2000
	Blue whiting (> 25 cm)	77	17%; 1.5	
	Hake		41%; 1.7 (in muscle: 6.4% prevalence; 1 intensity)	Valero et al. 2006
Mediterranean Sea (Italy)	common scad and the blue scad	822	80–100%; 19.3–36.8 (common scad); 18.2–70.7 (blue scad)	(Manfredi et al., 2000)
Galicia	hake, blue whiting, ling, gar pike, monkfish, Atlantic mackerel, black bream and horse mackerel		> 70%; > 14	Abollo et al. (2001)
France	Cod	304	1.97 %; 1.5 ±0.8 (1-3)	(Angot, 1993)
	Whiting	169	3.55 %; 1.14±0.5 (1-2)	

Apart from infection from *A. simplex*, there have been reports of *Diphyllobothrium nihonkaiense* infection contracted in France, at least six cases, through the consumption of raw Pacific salmon imported from Canada (Paugam et al., 2009; Yera et al., 2006): the parasite species identification was made by molecular analysis of two mitochondrial genes. This case is rather unusual since *D. nihonkaiense* has never been reported along the Pacific coast of North America (Paugam et al., 2009; Yera et al., 2006). Thus, *Diphyllobothrium* should also be considered as potentially present in imported wild catch saltwater fishery products.

In conclusion, because of the complexity and variation in distribution of parasites, all wild caught seawater fishery products must therefore be considered at risk of containing parasites and should not be eaten raw or almost raw without further treatment.

5.1.2. Freshwater fishery products

As outlined in Chapter 1, the presence of appropriate habitats with intermediate (snail, freshwater fish) and definitive hosts (mammals, including human) is essential for the transmission, and these combinations must be sustainable for the parasites to remains endemic in a region.

With regard to the important parasitic diseases such as clonorchiasis and opisthorchiasis, more than 100 species of freshwater fish (belonging to 13 families, especially the Cyprinidae) and three species of freshwater shrimp occur in many regions of the world (e.g. North America, Europe, Asia) which can serve as infection sources for humans (Chai et al., 2005a). The susceptibility to acquire parasites from the first intermediate host is variable, and the infection rate (e.g. with metacercariae) varies greatly between fish and other fishery-product species. Seasonal variations are observed in respective intermediate hosts. In more temperate latitudes, the maximum discharge of cercariae from snails and infection of second intermediate hosts with metacercariae is observed in summer (June to August in the northern hemisphere). In sub-tropical latitudes, maximum infections are observed in spring and autumn. Conversely in the tropics, where seasonal variations of temperature are less pronounced, aridity in the dry season may reduce the infection level in intermediate host (Chubb, 1979). Furthermore, metacercariae in the second intermediate host can survive for at least one year or even for the life span of the fish (up to 5-6 years).

Estimations on the prevalence of most relevant parasitic diseases acquired from fishery products are available from national surveys of humans living in endemic regions e.g. with clonorchiasis in China (Xu et al., 1995) or Vietnam (De et al., 2003). Conversely, data are scarce on distribution of fishery product-borne parasites which cause mild illness in humans which are consequently reported to health authorities less commonly (e.g. diphyllobothriasis). There are no systematic studies on parasitic stages transmitted by the first intermediate host (snails, copepods), second intermediate host (freshwater fish) or definitive host (carnivores).

Prevention of contamination of freshwater fish harbouring parasitic stages depends on environmental control of surface waters where fish are caught. Improvement of aquaculture may be possible with the cooperation and participation of local populations (Edwards, 1992). This includes the protection of fish ponds from pollution with sewage and other wastes, the proper disposal of waste material from fish and shellfish processing, as well as the systematic monitoring and control of large bodies of surface water. However, these measures present many difficulties and production of freshwater fish with a negligible risk for parasitic infections may be impracticable especially in countries where the hygiene of traditional fish ponds and fish feed is poorly managed, untreated human excreta or wastewater frequently contaminates fish ponds which are open to fouling by intermediate hosts and animal reservoirs (Abdussalam, 1995). Measures for the control of the first intermediate host (i.e. elimination of snails by molluscicides) are not recommended due to their effects on fish foods and residues in fish. It is therefore important to concentrate on preventive and control measures aimed at inactivation of parasitic stages in food (WHO, 1995).

In conclusion, because of the complexity and variation in distribution of parasites, all wild caught freshwater fishery products must therefore be considered at risk of containing parasites and should not be eaten raw or almost raw without further treatment. Nevertheless there is no evidence for allergic reactions caused by freshwater fish parasites.

5.2. AQUACULTURE FISHERY PRODUCTS

Similarly to wild caught fishery products, for aquacultural fishery products eaten raw or almost raw, information on the prevalence, abundance, as well as species and geographical distributions of the parasites and their hosts together with monitoring systems and trends in parasite presence and abundance are important. In addition, the following aspects are also important to consider: information on the fish species and susceptibility to parasites; origin of the stock (cultured from embryos, wild capture and ranched); production system (on or off-shore open cages, ponds, flowthrough, closed tanks with recirculation systems); type of feed and feeding methods (dry pellet produced by extrusion and high temperature, live food (rotifers, *Brachionus* spp. and *Artemia*) or fresh food such as fresh fish (e.g. anchovies); time span for growth; and processing method.

Twelve farmed fish species will be considered here which are farmed in Europe or elsewhere and commonly consumed in the EU. All these fish are potential hosts of anisakids or other parasites and aquacultural practices which potentially increase exposure to parasites together with the availability of systematic monitoring and the risk for specific parasites are outlines in Table 6. Apart from farmed Atlantic salmon, and to a lesser extent Pacific salmon, there are no sufficient data from systematic surveys for parasites of public health importance in these marine or freshwater farmed fish species. Many of these seawater fish species are known to be parasitized by anisakids and other parasites in the wild, including cod, rainbow trout, sea bass, sea bream, tuna, and halibut (Petrie, 2009; Platt, 1975; Wootten and Waddell, 1977).

There are two farming practices which do pose an increased risk of infection with anisakids. The first is if farmed fish are fed unprocessed marine fish waste which, depending on their origin, are likely to be infected with larval anisakids. Within Europe this type of feed is not commonly practiced but may be used in cod and tuna farming. *Anisakis* can be readily transferred between fish hosts (Smith 1974; Wooten and Smith 1975). Wootten and Smith (1975) found cultured trout in fresh water infected with *Anisakis* larvae that were apparently acquired through the feeding of marine fish waste.

The second practice that increases the risk of infection in farmed fish is the capture of juvenile wild fish for subsequent on growing in captivity. This occurs in both cod and tuna culture in Europe. In Norway cod for ongrowing are captured at sizes of 3-5g juveniles and 1-2 kg adults. The former are unlikely to be infected with anisakids (Wootten 1978) but the latter, depending on when they are caught, are very likely to be infected with *Anisakis* and/or *Pseudoterranova*.

The available experience suggests that where Atlantic salmon is reared in floating cages or onshore tanks, and fed on artificial diet the risk of infection with larval anisakids is negligible unless changes in farming practices occur. However, monitoring data is not available for all other farmed fish therefore it is not possible to identify which farmed fish species do not present a health hazard with respect to the presence of parasites. There is a risk of infection, although unquantifiable, if fish are captured from the wild for ongrowing or are fed unprocessed trash fish.

5.2.1. Farmed seawater fishery products

5.2.1.1. Farmed Atlantic salmon, Pacific salmon and rainbow trout

The production system of Atlantic salmon is quite complex due to its anadromous biology (Fig.4). Broodstock are selected from sea site production stocks, and normally moved into freshwater tanks or

cages prior to eggs production. Eggs are stripped dry, fertilized with milt and laid down in trays or silo systems.

Hatching takes place in hatchery trays or following transfer to tanks. Following yolk sac absorption, alevins will swim up in the water column, indicating readiness to first feed. "Feeding fry" can be grown on in tanks, either using flow-through or various recirculation systems, or subsequently in lake cage systems.

Fish are usually maintained at ambient temperature and light regimes to produce "S1" smolts* in the spring of the year following hatch. Fish are transferred to sea sites following the determination that they have smolted and are adapted for seawater survival. Ongrowing at sea normally takes place in cages consisting of large nets suspended from various floating "walkway" systems anchored to the seabed.

Sea sites are selected on their suitability with regard to water temperature, salinity, flow and exchange rates, proximity to other farms and/or wild fisheries, and in compliance with local licensing regulations. Atlantic salmon grows best in sites where water temperature extremes are in the range 6-16°C, and salinities are close to oceanic levels (33-34‰). Water flows need to be sufficient to eliminate waste and to supply well oxygenated water (approximately 8 ppm). Atlantic salmon are ongrown in sea sites for up to 2 years with harvesting of fish from 2 kg upwards.

The bulk of salmon feeds are produced by three or four large companies. Fish meal and fish oil, derived largely from the huge industrial fisheries in South America, still form the basis of salmon diets, although increasing pressure on these sources have led to increased research into the substitution of fish products with vegetable protein and oil sources. Salmon grower diets contain high levels of fish oil, which is efficiently converted by the salmon, often at food conversion ratios of close to 1:1. Feeding methods and technology have advanced in recent years, and many sea farms use computerized systems to drive automated feeding systems, with feedback mechanisms to detect when fish have finished feeding. This allows fish to be fed to satiation without overfeeding and consequent feed wastage.

In order to produce fish with the flesh colour demanded by the market, carotenoid pigments are added to the diet during the seawater growing phase of the production cycle despite their high cost. Methods of harvesting vary but fish are generally starved for up to 3 days beforehand.

* Smoltification, a process triggered by changing photoperiod

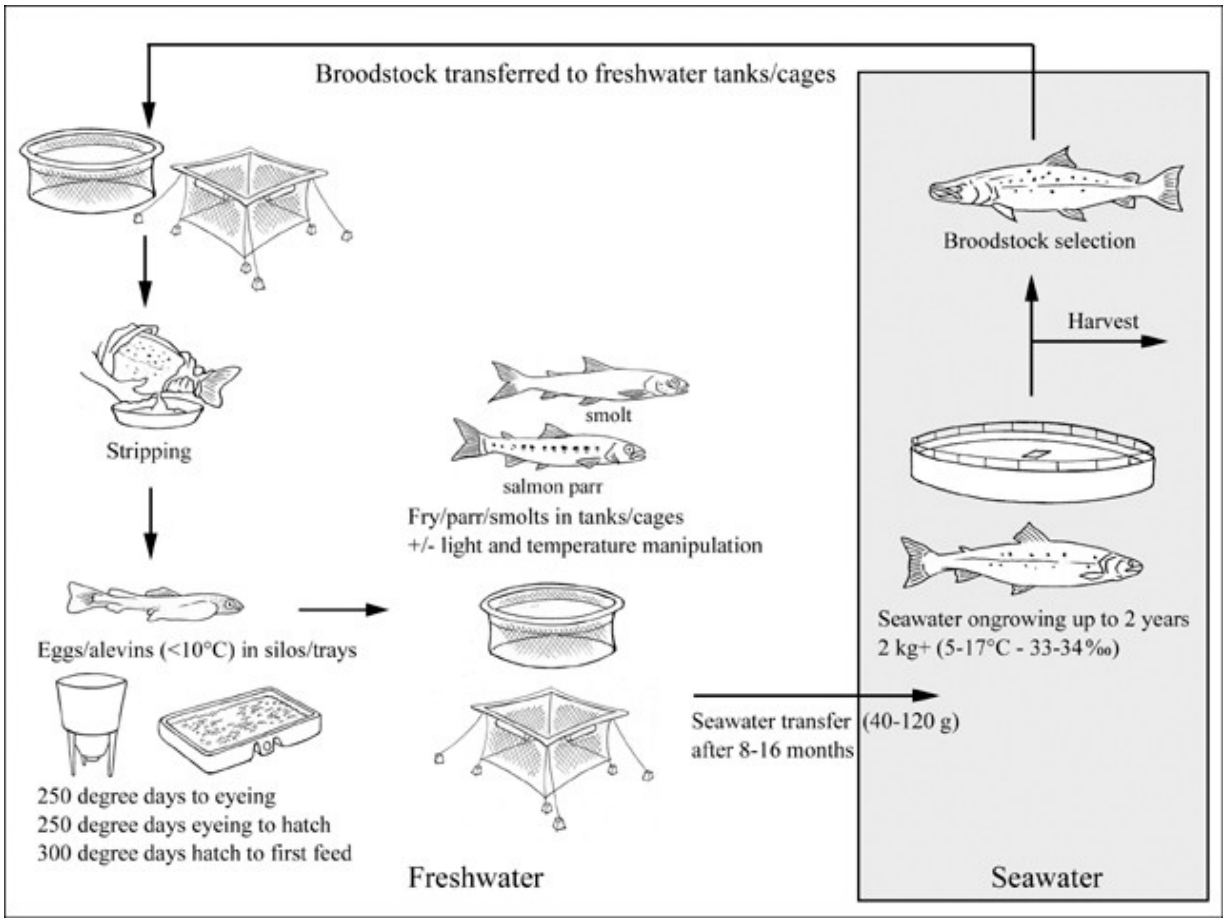

Figure 4: Production cycle of *Salmo salar* (FAO, 2010)

In northern Europe there is a considerable culturing of rainbow trout in floating sea cages in coastal waters. The technology used is broadly similar to that used for salmon culture. Fish are fed a commercial pelleted diet throughout the period of culture.

Farmed fish have not generally been regarded as infected with larval anisakids. In this respect the most studied of the European farmed species is the Atlantic salmon with major surveys completed in Norway (Lunestad, 2003), Pacific North America (Deardorff and Kent, 1989; Marty, 2008) France (Angot and Brasseur, 1993), Scotland (Wootten et al., 2010), and Chile (Sepulveda et al 2004). Inoue et al (2000) surveyed Pacific salmon in Japan. Several thousands of fish were examined in all these surveys but no anisakids were detected in the flesh. Marty (2008) recorded a single anisakid penetrating the intestinal caecum of an Atlantic salmon from British Columbia, but a species identification was not performed.

Wootten (2010) examined 720 farmed salmon from 12 marine sites in Scotland covering most salmon farming areas of the country, including areas such as the Shetland Islands, where anisakids are abundant in wild fish, but found no infected fish. Skov (Skov et al., 2009) carried out a survey of 166 sea-reared rainbow trout from Western Zealand, Denmark but found no fish infected with larval anisakid nematodes, even though a variety of wild fish from the same area were parasitized. That wild Atlantic salmon are commonly parasitized by *Anisakis* has been shown by a number of authors, from Norwegian (Bristow and Berland, 1991) and from Scotland waters (Wootten et al., 2010). The latter study reported that 100% of 55 wild salmon from the East and North coasts of Scotland were parasitized by *Anisakis* in the flesh with up to 172 worms per fish. In addition Beck (2008) and

Noguera (2009) described high numbers of *Anisakis* larvae in the abdominal musculature of wild Atlantic salmon with "red vent syndrome" from UK waters.

The reasons for the absence of anisakids in farmed fish is probably due to the nature of the farming methods, since, as described above, fish are often farmed in areas where anisakids are known to be abundant, and farmed species are susceptible to the parasites. Since these fish are reared in floating cages raised off the sea bed or in tanks on-shore, and are fed an artificial pelleted diet, there is relatively little opportunity to ingest infective anisakid larvae. Thus, although *P. decipiens* is very abundant in many coastal salmon farming areas (Petrie, 2009; Wootten and Waddell, 1977) raising cages off the sea bed means that the farmed fish are very unlikely to come into contact with infected benthic invertebrate hosts. Pelagic invertebrates will pass through cages and thus will be available for fish to eat, as shown by the presence of *H. aduncum* in farmed salmon. However, major invertebrate hosts of *Anisakis*, such as euphausiids, are not abundant in inshore waters. The use of artificial diets reduces the risk of opportunist feeding on wild prey.

Skov (2009) found that only two out of the 166 sea-reared rainbow trout they examined contained remains of small fish in the stomach. This constitutes one potential route of infection with larval anisakids for farmed salmon, although the lack of infection in the latter indicates it is very low. It may be noteworthy that Wootten (1978) in a survey of small and juvenile gadoids, species of which might be expected to enter fish cages, were not infected with *Anisakis* or *Pseudoterranova.*

5.2.1.2. Cod

There are two major cod farming methods used. The first is based on capturing wild cod, both juveniles (3-5 g) and 1-2 kg cod for on-growing, while the other focuses on the production of cod from hatching to market size.

In Figure 5 the farming methods of Atlantic cod are shown.

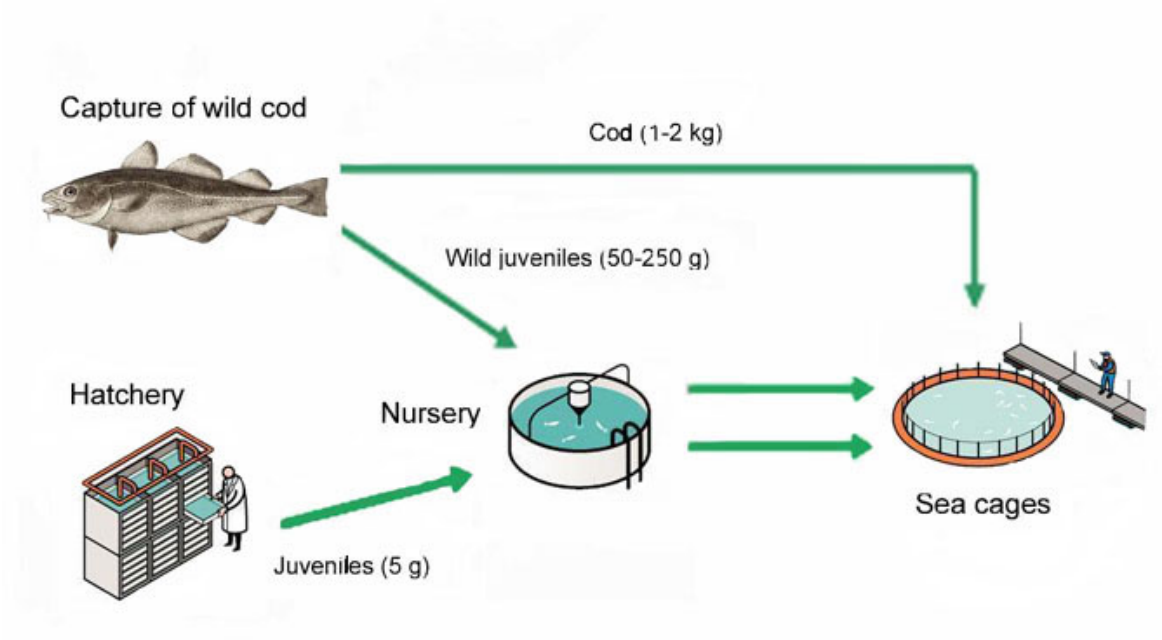

Figure 5: The currently applied farming methods of Atlantic cod (Levsen, 2009)

The culturing method based on on-growing of wild cod, especially larger fish (1-2 kg), has an inherent risk that wild-caught specimens may be infected with anisakid nematode larvae, including long-lived and flesh-migrating species such as *A. simplex* and *P. decipiens*. Moreover, whenever feeding net-pen

reared cod with fresh, unprocessed marine fish or offal, the risk exists that nematodes and other parasites may be transferred to, and establish in, the cultured fish. There is no information available on the presence of parasites in farmed cod.

5.2.1.3. Sea bass and sea bream

Production system for sea bass and sea bream are quite similar. Although seabass are farmed in seawater ponds and lagoons, the bulk of production comes from sea cage farming.

The Italian *'vallicoltura'* or the Egyptian 'hosha' are extensive fish rearing systems that act like natural fish traps, taking advantage of the natural trophic migration of juveniles from the sea into coastal lagoons.

The traditional method of lagoon management places special barriers in appropriate lagoon sites to capture fish during their autumn migration to the open sea. Barriers made of reeds, nets or cement stay open from February until May for the lagoon to be naturally stocked with fry. In this system seabass is usually cultured in polyculture with sea bream, mullets and eels. The limiting factor is in the natural feeding behaviour of the seabass that, as predators, may drastically reduce the natural resources of the lagoon ecosystem.

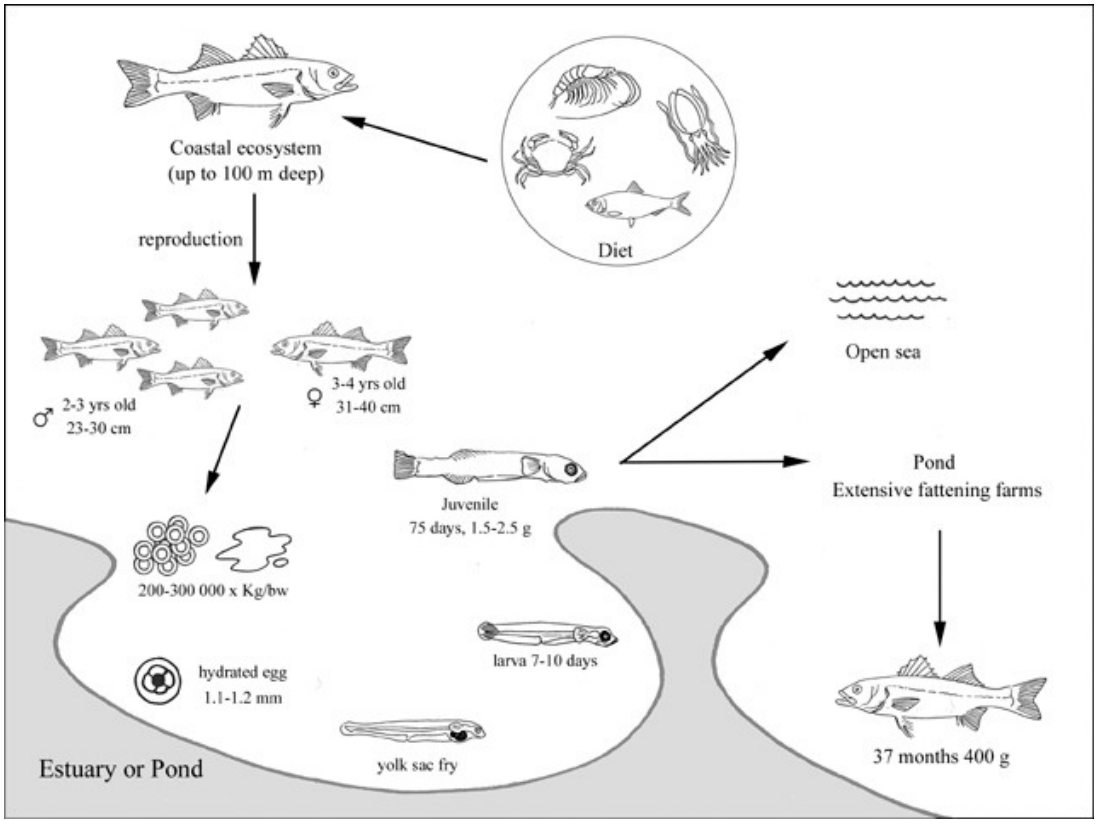

Figure 6: Sea bass production cycle – extensive system (FAO, 2010).

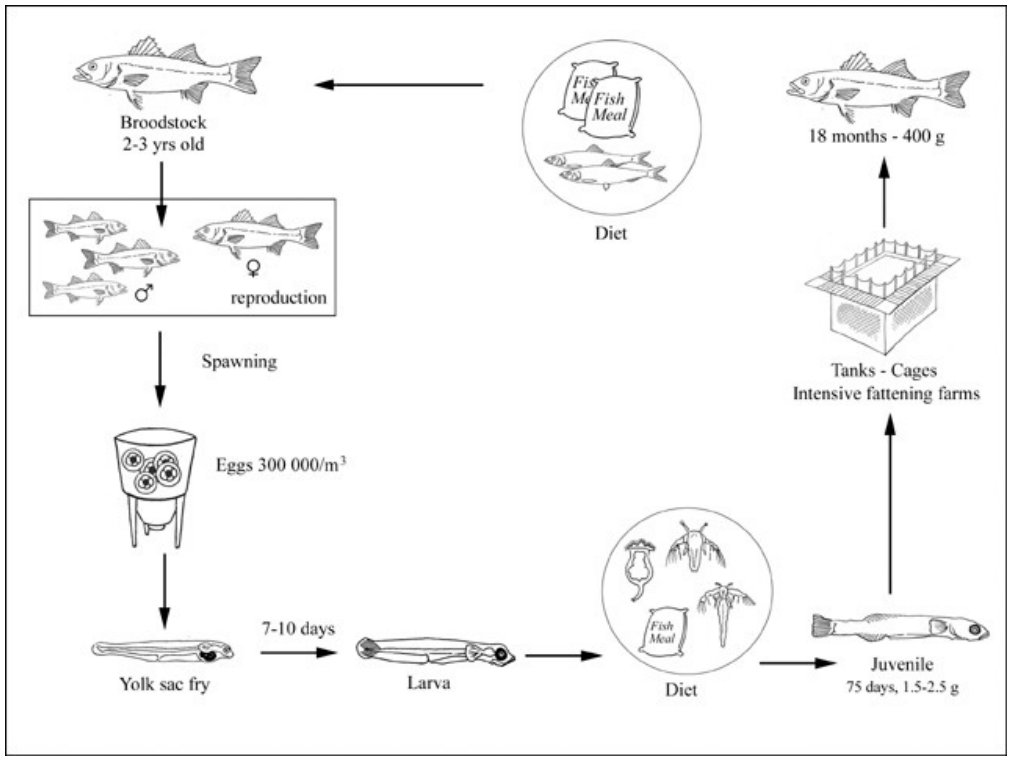

Figure 7: Sea bass production cycle – intensive system – (FAO, 2010)

Figure 8: Sea cages (FAO, 2010)

Figure 9: Flow-through (FAO, 2010)

In intensive farming systems, juveniles are sold to farmers as ongrowing stock. Feeds are distributed by automatic feeders every 10-15 minutes for small fish or by hand for larger fish. Grading is necessary at least two or three times per cycle, in order to avoid growth differentiation and cannibalism. Fattening can occur in tanks or in cages system.

Net-pens (cages) can be of different kinds but the principle is the same; all types are based on a natural exchange of water through pens. Some farms are anchored close to the land and can be served from a landing. Others are located in the open sea or in the middle of a protected bay and can only be served by boat.

Tanks are usually supplied with seawater maintained in a continuous flow-through system under ambient temperature. Alternatively, brackish water pumped from adjacent lagoons may be used. High stocking densities are used which means that accurate control of water quality and careful observations of fish health are essential.

In intensive production, ongrowing units are supplied with fry from hatcheries and controlled diet is provided. In order to fulfil the nutritional requirements of larval stages of sea bass and sea bream, first feeding uses live foods. For these reasons, dedicated live feed production facilities are an integral part of bass and bream hatcheries. Seabass are fed on enriched *Artemia nauplii* and weaning on microdiets occurs by day 40 (out of 80 days in total). Sea bream larvae also require live food, both rotifer (*Brachionus* spp.) and *Artemia nauplii* (EFSA, 2008).

No information is available on infection of farmed sea bream and sea bass with larval anisakids. When these species are reared in floating cages or on shore tanks there would appear to be a low risk of infection for the same reasons suggested for Atlantic salmon. Where sea bass and sea bream are reared in natural lagoon systems there may be a risk of infection, but the extent of this is unknown. However the probable infection status of the wild juvenile fish used as basis for this culture method is unknown. It is also uncertain whether fish can acquire parasites whilst feeding within shallow lagoon systems, although such areas do not appear to be very favourable for the development of anisakids.

5.2.1.4. Tuna

Tuna farming started in the 1990s and bluefin tunas are the main species farmed. The countries involved include Australia, Japan, Mexico and several Mediterranean countries (particularly Croatia, Italy, Malta, Morocco, Spain and Turkey).

Tuna-farming in the Mediterranean is not true aquaculture but an additional final step of a standard fishery which relies on the already overexploited wild tuna stock. Tuna farming in the Mediterranean is a phenomenon driven mainly by Japanese market demands. Farmed tuna is higher in oil content, which makes it particularly desirable for sushi.

Tuna are captured at sea by purse seine netting and transferred to a specialised towing sea-cage. The cages are then slowly towed, sometimes large distances, to grow-out sites. Once the fish are transferred into the grow-out cages, they continue to be fed a diet of fresh bait fish, squid, pellets, or a combination of these feeds. When fish are fat and the market in Japan is favourable, harvesting is generally carried out by net-crowding some fish and removal by gaff or diver.

The risk of infection of farmed tuna is with larval anisakids. Wild fish which are captured as the basis of the culture system may be infected although no information is available. After capture the feeding of tuna with fresh trash fish might also increase the risk of infection with larval anisakids, again, no information is currently available.

5.2.1.5. Turbot

Larvae are usually cultured in fish farms. The newly hatched larvae feed from their vitelline reserves, then after mouth opening, feeding is based on rotifers and *Artemia*. Phytoplankton is added to the culture medium. Weaning is in round-cornered square tanks with open-circuit pumped seawater. Various commercial feeds are used at the weaning stage.

In the on-growing phase, turbot are either reared in on-shore tanks (the most common technique for this species) or in flat-bottomed cages. The square or circular cement tanks are used with open-circuit pumped seawater. Feeding consists of extruded pellets, introduced manually or automatically. The elements that determine productivity are temperature and fry quality. The square or circular tanks (10-30 m³) are open-circuit pumped with seawater. Juveniles are fed with dry pelleted feed, introduced manually or automatically. Cages submerged at various levels, or floating cages, in both cases flat-bottomed, are used. The frames are metal, with a metal or netting bottom. Extruded pelleted feeds are manually fed.

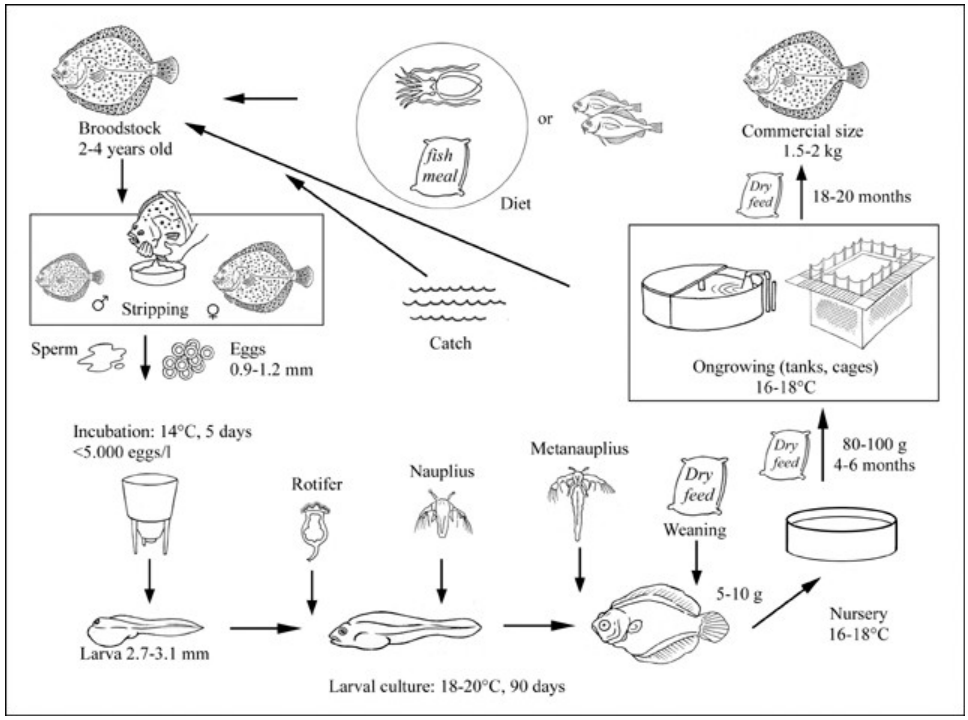

Figure 10: Production cycle of turbot (FAO, 2010)

The risk of infection of cultured turbot with larval anisakids would appear to be low, although there is no information on their infection status. Fish reared in floating cages or onshore tanks are unlikely to become exposed to infective larvae. Turbot are also fed a pelleted diet which will not be a source of infection.

5.2.2. Farmed freshwater fish

5.2.2.1. Trout

Monoculture is the most common practice for trout, and intensive systems are considered necessary in most situations to make the operation economically attractive.

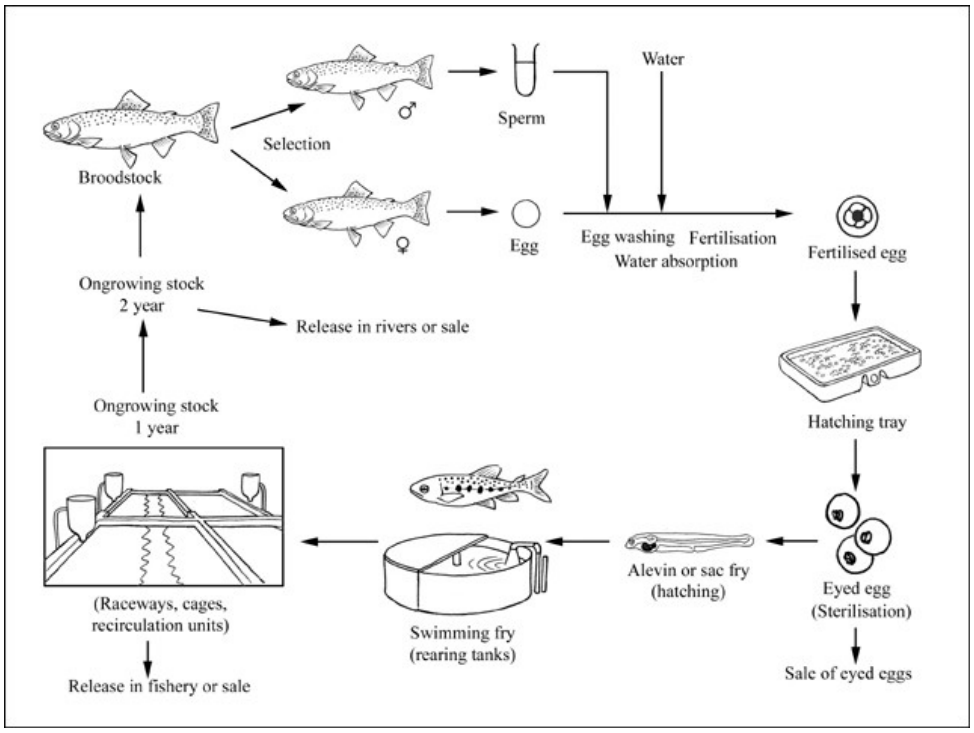

Figure 11: Production cycle of trout (FAO, 2010)

Trout may be reared in fresh water in ponds, raceways, tanks or floating cages. In all instances fish are fed throughout the period of culture on commercial pelleted diets which are not a source of infection. There is some opportunity for feeding on live prey in ponds and especially in floating cages. All fish are bred in captivity. Water supply may be from ground water or surface water. The latter may be filtered. Trout can also be reared in re-circulation systems.

Trout reared in freshwater tanks, ponds or raceways are very rarely parasitized by helminths that present a risk to humans. In cases where water used for culture is drawn from lakes and reservoirs without filtration there may be a risk of infection with *Diphyllobothrium* (Wootten and Smith, 1975) since copepods containing infective stages may enter the system and be preyed upon by trout. Fish reared in cages in still water bodies may be at more risk of infection if they feed on infected copepods.

5.2.2.2. Eel

Production of eels is based on wild catches of glass eels (elvers) used for further ongrowing. When eels reach marketable size they are transferred to larger ponds (1000-1500 m²). The ponds may be static or flow-through.

Extensive culture of European eels under natural conditions occurred in Italy, Germany and Denmark up to the 1970s in ponds. The use of indoor intensive production systems started experimentally in Europe in the 1970s with the use of recirculation technology and become the most common production method in northern Europe, mainly in the early 1980s in The Netherlands, Denmark, Italy and Germany. European eel farming is now mainly associated with recirculation systems. These techniques have resulted in highly intensive farming conditions.

Glass eels are captured around the shores of France, Portugal, Spain and the United Kingdom and either used nationally or exported to eel farmers in other countries. Some glass eel fishing in Spain and Portugal uses scoop nets and traps. Initially the glass eels are kept in small tanks for quarantine purposes. The eels are weaned to artificial diets with cod roe and, later on, dry starter feed. When the

eels reach approximately 5 g they are transferred to a juvenile production unit with larger tanks. At this point the eels can digest dry feed pellets.

The intensive culture in recirculation systems consist of square or circular tanks. The eels are stocked at a size of 50 g. Extruded dry feed is fed automatically several times a day. Individual growth rates are very different, and grading every 6 weeks is necessary in order to reach a high overall growth performance.

Eels are also extensively cultured in marine and brackish waters within a form of aquaculture known as *valliculture*, such as in Italy, in the north Adriatic. The elvers are mainly imported from France but also from Denmark, the Netherlands and Sweden.

Almost all forms of intensive culture use formulated feeds in the form of a moist paste for glass eels and steam-pressed or extruded pellets for the later stages.

The risk of infection of eels reared in intensive recirculation systems with parasites of human health significance would appear to be very low, although no information is available on their infection status. Infective stages of relevant parasites will not enter such systems and eels are fed on artificial diets. Although wild glass eels are used to stock culture systems there are no published records of potential human parasites, including larval anisakids, in such fish.

5.2.2.3. Carp

Breeding of carp is carried out in cement tanks or small ponds.

The hatched fry are kept in large conical tanks for 1 to 3 days, and are usually stocked at the stage of 'swim-up' or 'feeding fry' into properly prepared ponds.

Shallow, aquatic weed-free drainable ponds are the most suitable for carp nursing. Nursery ponds must be prepared before stocking to encourage the development of a rotifer population, since this constitutes the first food of feeding fry. The ponds should be inoculated with *Moina* or *Daphnia* after stocking. Supplementary feeds, such as soybean meal, cereals meals, meat meal, or mixtures of these materials are used. Rice bran or rice polishings can also be used for feeding fry.

If there are predatory insects, snakes, frogs, birds or wild fish in the area where ponds are situated, tank nursing of carp can be applied. By applying hay and manure, dense populations of *Paramecium* and rotifers can be established in these tanks. Collected zooplankton and fine particle size meals, or complete starter foods can be used. Industrial type systems, such as raceways, or water recirculation systems are also suitable for nursing.

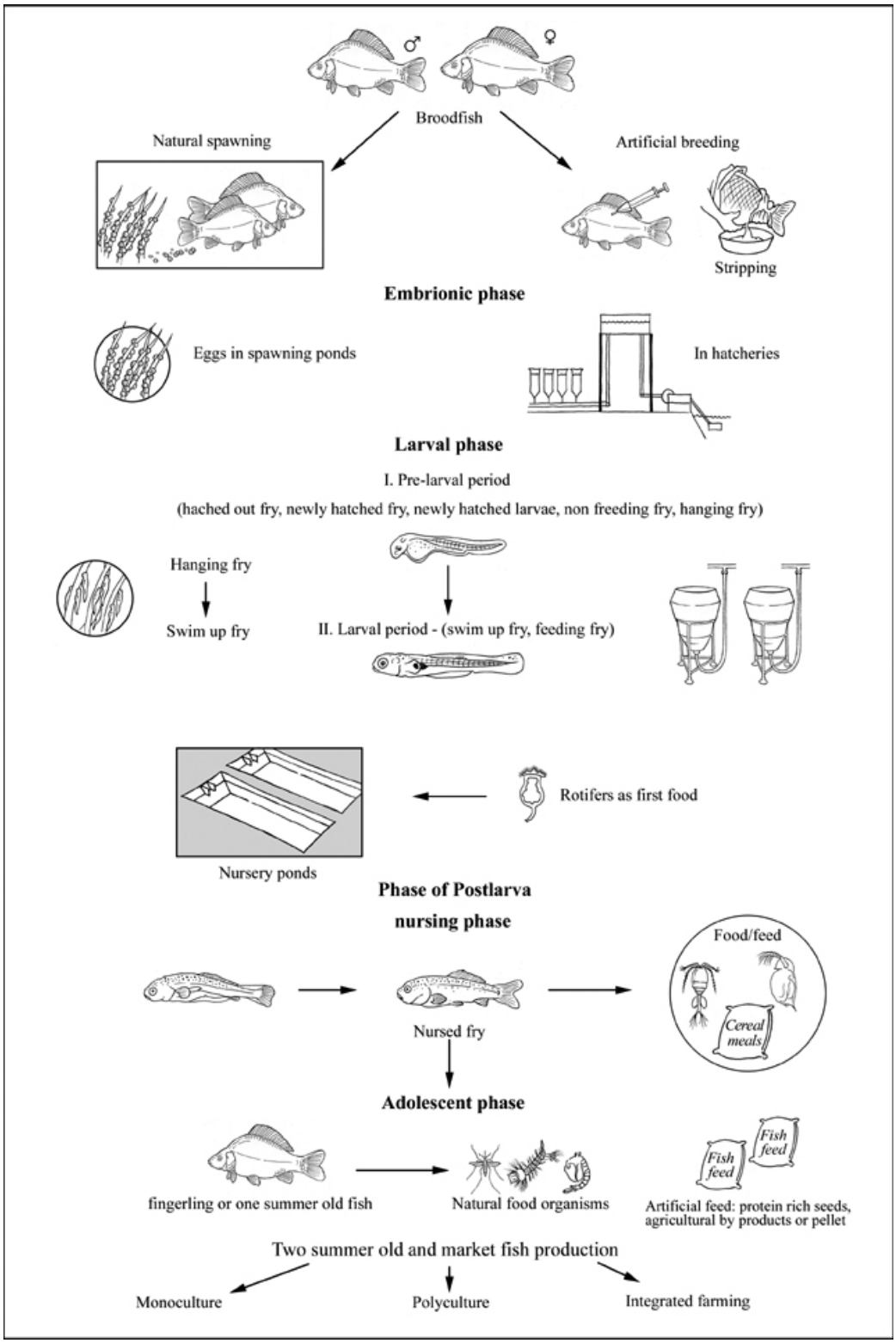

Figure 12: Production cycle of carps (FAO, 2010)

The production of carp fingerlings normally takes place in semi-intensive ponds, based on manure/fertilizer-generated natural food and supplementary feeding.

Frequent application of manure is necessary to maintain the plankton population. The feeding is based mainly on agricultural by-products in subtropical areas, or on cereals and/or pellets in temperate zones.

Common carp can be produced in extensive, natural food and supplementary feed-based monocultural production systems, in stagnant water ponds. Artificial feed-based intensive monocultural production can be carried out in cages, irrigation reservoirs, and running water ponds and tanks, or in recirculation systems.

Common carp are also stocked with Chinese carps, tilapia, mullet, etc., in polycultural systems. This constitutes a natural food and supplementary feed-based production method, in which fish are stocked into the same ponds that have different feeding habits and occupy different ecological niches.

Common carp can also be stocked into natural waters, reservoirs, and temporarily inundated areas, in order to utilize the natural food production of these waters for enhanced capture fisheries. Because carp are almost exclusively produced in semi-intensive ponds on farms or under more intensive conditions, feeding is only practised to complement the energy and protein requirements provided by pond primary production.

There is no evidence of Opisthorchiidae infection in farmed common carps in Europe, but in South-East Asia many carp species, grass carps and bighead carps and to a lesser extent common carp, may be at risk of infection with *Clonorchis sinensis.* There are also sporadic reports from Asia of humans infected with parasitic trematodes (including *Clonorchis sinensis, O.felineus, Metagonimus takahashii, Haplorchis taichui, Echinochasmus fujianensis)* after consumption of raw or undercooked flesh of common carp (Chai et al., 2005a) A risk for European consumers may be posed if these fishery products are traded to Europe.

Table 6. Risk profile for aquacultural practices in farmed fish species with potential for infection by parasites of public health importance.

	Fish species	Production system				Larval feeding	Adult feeding		Larval/juvenile stages		Grow-out time span	Processing		Monitoring data available	Susceptible for parasitic infection in wild	Overall risk of parasite infection
		Open			Closed	Live food	Fresh food[9]	pellet	Wild	Farmed	months					
		Cages	ponds	Flow-through tanks	Recirculation tanks	Rotifers, *Artemia*	fish (anchovies, sardines)					gutted	ungutted			
Seawater	Atlantic salmon	X						X		X	>24	X		Y	*A. simplex; P. decipiens; Metagonimus spp.;*	*Negligible*
	Pacific salmon and rainbow trout	X						X		X	12	X		N	*A. simplex; P. decipiens, Diphyllobothrium* spp.	*Not known*
	Sea bass	X		X		X		X		X[10]	14-18	X		N	*A. simplex; P. decipiens*	*Not known*
	Sea bream	X				X	X	X		X	12-16	X		N	*A. simplex; P. decipiens*	*Not known*
	Tuna	X							X		12	X		N	*A. simplex; P. decipiens*	*Not known*
	Turbot	X				X		X		X	>24	X		N	*A. simplex; P. decipiens*	*Not known*
	Cod	X				X	X	X	X	X	>24	X		N	*A. simplex; P. decipiens; Cryptocotile spp.*	*Not known*
Fresh water	Trout	X	X	X	X			X		X	12	X		N	*Diphyllobothrium* spp	*Not known*
	Eel		X		X			X	X		>24	X	X	N	-	*Not known*

9 Negligible risk if frozen for more than 24 hours
10 In some extensive systems (valliculture), larvae/juvenile stages could be wild

	Common carp		**X**			**X**	**X**				**>24**		**X**	**N**	*C.sinensis; O.felineus; Metagonimus takahashii; Haplorchis taichui; Echinochasmus fujianensis*	*Not known*
	Grass carp and bighead carp		**X**			**X**	**X**				**>24**		**X**	**N**	*C. sinensis; Haplorchis taichui*	*Not known*

Practice with increased potential for parasite infection of fish	Y	N

CONCLUSIONS

To assess the food safety concerns due to possible allergic reactions in consumers to parasites that may be present in fishery products

1. There is a lack of adequate data on exposure and susceptibility to allergic reactions to parasites of public health importance in the fishery products.

2. No fishery product associated parasites apart from *Anisakis simplex* have been clearly implicated with allergic reactions.

3. The likelihood is that the primary initiator of the different forms of allergy to anisakid nematodes in humans is infection by live *A. simplex* larvae. Once sensitisation has occurred, response to nematode allergens can be highly aggressive and generate severe allergic disease.

4. Infection can provoke a concurrent *A. simplex* allergic episode in a sensitised individual. Allergic episodes can be also elicited by exposure to allergen alone in a sensitised individual. The relative epidemiological impact for each is unknown.

5. The principal clinical allergic responses due to *A. simplex* are:
    a. Gastro-allergic anisakiasis, in which allergic symptoms are additional to an acute gastric parasitism after eating fishery products containing live larvae;
    b. Allergy to *A. simplex*, resulting from contamination of fishery products with allergens with no necessity for live parasite to elicit the allergic reaction.

6. The allergy to *A. simplex* and gastro-allergic anisakiasis is relatively common in some regions in Spain and there is considerable variation in clinical observations between regions. However this allergy has rarely been reported in other parts of Europe. It is not known if this is due to lack of awareness and infrequent application of investigation and diagnostic tests, or to true differences in the incidence of the disease.

7. Consumption of fishery products containing viable *A. simplex* larvae presents a greater risk for allergy than consumption of fishery products containing non-viable parasites. However it is not possible to assess the risk for allergy of consumption of fishery products where the parasite is only present in parts not consumed.

8. Prevention of sensitisation is most likely to be effective by control of *A. simplex* infection. .

Identification of alternative treatments for killing viable parasites in fishery products and evaluate their effectiveness compared to the freezing method described in the hygiene Regulations

1. There is more information on the resistance to physical and chemical treatments by *A. simplex* than for other parasites in fishery products. The properties of *A. simplex* are likely to be similar to that of other multicellular parasites (although trematode metacercariae are considerably more heat resistant).

2. Freezing or heat treatments remain the most effective processes guaranteeing the killing of parasitic larvae, under well defined conditions.

3. Many traditional marinating and cold smoking methods are not sufficient to kill *A. simplex* larvae.
4. There is insufficient information to show whether alternative treatments, including high hydrostatic pressure, irradiation, drying, and low voltage currents, are effective for killing Anisakidae larvae as an alternative to freezing.
5. Treatments which provide an equivalent level of protection as freezing at a temperature of not more than -20ºC for not less than 24 hours for the killing of *A. simplex* larvae include:
    a. Freezing at -35ºC for at least 15 hours or at -15°C for at least 96 hours, at the core of the fishery products;
    b. Heat treatment at >60ºC at the core of the fishery products for at least 1 minute.
6. Treatments used to kill viable parasites are equally applicable to all fishery products.

Criteria for when fishing grounds for wild catch and fishery products from aquaculture do not present a health hazard with regard to the presence of parasites, and an assessment of available documentation for farmed Atlantic salmon

1. Criteria to determine whether fishery products from fishing ground is likely to present a health hazard take into account information on the prevalence, abundance, as well as species and geographical distributions of the parasites and their hosts together with results from monitoring systems and trends in parasite presence and abundance.
2. There is a lack of adequate data on the geographical distribution, prevalence, intensity, and anatomical distribution of parasites of public health importance in fishery products.
3. No sea fishing grounds can be considered free of *A. simplex* larvae.
4. All wild caught seawater and freshwater fish must be considered at risk of containing viable parasites of human health hazard if these products are to be eaten raw or almost raw.
5. For fishery products from aquaculture, information on the prevalence, abundance, as well as species and geographical distributions of the parasites and their hosts together with monitoring systems and trends in parasite presence and abundance are important. In addition, the following aspects are also important to consider: information on the fish species and susceptibility to parasites; origin of the stock; production system; type of feed and feeding methods; time span for growth; and processing method.
6. The available evidence suggests that where farmed Atlantic salmon is reared in floating cages or onshore tanks, and fed compound feedstuffs, which are unlikely to contain live parasites, the risk of infection with larval anisakids is negligible unless changes in farming practices occur.
7. Apart from farmed Atlantic salmon, sufficient monitoring data are not available for any other farmed fish therefore it is not possible to identify which farmed fish species do not present a health hazard with respect to the presence of parasites if these products are to be eaten raw or almost raw.

RECOMMENDATIONS

1. Co-ordinated studies to improve surveillance and diagnostic awareness of allergic reactions to parasites in fishery products should be encouraged.

2. Collection of more epidemiological data on the disease in the EU should be encouraged to provide better insights into the impact of *A. simplex* parasitized fish on human disease.

3. In regions where allergy to parasites in fishery products is detected, prospective monitoring and risk assessments should be performed to assess the effectiveness of the implementation of public health interventions.

4. There should be clear and practical information to clinicians, fishery product handlers and the general public for risk reduction.

5. Research should be encouraged to clarify:

   - The mechanisms of allergic sensitisation and exposure to *A. simplex* in fishery products.

   - Differences between geographical regions in the exposure to parasites and the incidence and presentation of allergic diseases due to *A. simplex*.

   - Infectivity as well as inactivation of parasites in fishery products in relation to treatments, host fish species, and effects of passage through transport hosts.

   - The effects of different farming practices on the prevalence of anisakids (and other parasites of public health importance) in farmed fish.

   - The complete life cycle, geographical and seasonal distribution, prevalence, intensity, and anatomical location of parasites of public health importance in fishery products.

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APPENDICES

APPENDIX A

Table 8: Fish-borne parasitic zoonoses in humans occurring in Europe and associated biological and medical parameters

Parasites	Intermediate hosts	Definitive hosts	Geographic distribution of parasites in host species	Symptoms and disease in humans	Allergy	Prepatency	Infective form	Method of diagnosis	Treatment (excluding preventive measures)
Nematodes									
Anisakis simplex sensu lato	Almost all sea water fish and cephalopods, including salmon, tuna, herring, mackerel, squid, anchovies, etc.	Mostly cetaceans	Global	Sporadic stomach distress, abdominal pain, blood in stool if intestinal, allergy	Yes	1-12 hours, up to 5 days	Third-stage larva	Endoscopy, radiology, surgery, regurgita-tion. Allergic test	Surgical removal, allergy treatment
Pseudoterranova decipiens	"	Mostly pinnipeds	"	Pharyngeal	No	1-12 hours, up to 5 days	Third-stage larva	Endoscopy, radiology, surgery, regurgitation	Surgical removal
Cestodes									
Diphyllobothrium spp.	Salmon, pike, perch, turbot	Dogs, Bears	Scandinavian countries, western Russia, North and South America; reported from Cuba, Korea, Japan, Africa, Australia	Abdominal pain, weight loss, anaemia; mild cases asymptomatic	No	16-42 days	Plerocercoid in fish	coproscopy	Praziquantel 5-10mg/kg for 1 day

Parasites	Intermediate hosts	Definitive hosts	Geographic distribution of parasites in host species	Symptoms and disease in humans	Allergy	Prepatency	Infective form	Method of diagnosis	Treatment (excluding preventive measures)
Trematodes									
Opisthorchis viverrini	Freshwater fish and snails	Dogs, cats, rats, pigs	Europe, Turkey, Caribbean Islands, Southeast Asia, southern Russia, India, Japan	Liver enlargement, fibrosis, bile ducts thickening, anorexia	No	1 month	metacercaria	Serological and stool	Praziquantel 25-75mg/kg
O.felineus	"	Dogs, foxes, cats, rats, pigs, rabbits, seals, lions, wolverines, martens, polecats	Spain, Italy, Albania, Greece, France, Macedonia, Switzerland, Germany, Poland, Russia, Turkey	"	No	"	"	coproscopy	"
Metorchis bilis	Mostly cyprinids and freshwater snails		"	"	No	"	"	"	"
Heterophyes heterophyes	Mullet, tilapia, brackish water snail	Cats, dogs, foxes, wolves pelicans	Southeast Europe, USA, Africa, Israel, Philippines	Mucoid diarrhoea, Migration of the eggs to extra-intestinal sites (heart, brain	No	Several weeks	metacercaria	coproscopy	Praziquantel 75 mg/kg 3 doses x for 2 day
Metagonimus yokogawai	Salmon, mullet, cyprinids	Dogs, cats, rats	Far East, Siberia, Balkans, Israel, and Spain	diarrhoea and colicky abdominal pain	No	Several weeks	metacercaria	coproscopy	Praziquantel 75 mg/kg 3 doses x for 2 day
Cryptocotile lingua	Charr, cod, flatfishes even from aquaculture		Norway, Canada, Alaska	Abdominal discomfort	No	Several weeks	metacercaria	coproscopy	Praziquantel 75 mg/kg 3 doses x for 2 day
Echinocyasmus perfoliatus	Freshwater snails and fish (e.g. *Carassius*)	Foxes, rats, wild boars, dogs	Japan, China, Taiwan, Hungary, Italy, Rumania, Russia	generally mild, but ulcerations in the stomach or duodenum	No		metacercaria	coproscopy	Praziquantel 75 mg/kg 3 doses x for 2 day
Paragonimus westermani	crab or crayfish	pigs, dogs, and a variety of feline	South-east Asia and Japan	diarrhoea, abdominal pain, fever, cough, urticaria, hepatosplenomegaly, pulmonary abnormalities, and eosinophilia	No	2-3 months	metacercaria	Coproscopy or microscopy of sputum; biopsy;serological	Praziquantel 75 mg/kg 3 doses x for 2 day

APPENDIX B

Table 7: Fish species name and common names in the main five European languages

Latin names	*French*	*English*	*German*	*Italian*	*Spanish*
Anguilla anguilla	anguille d'Europe	European eel	Aal	anguilla	anguila
Atheresthes stomia	flétan du Pacifique	arrowtooth flounder	Pazifischer Heilbutt	halibut del pacifico	halibut del pacifico
Euthynnus pelamis	bonite à ventre rayé	skipjack tuna	Echter Bonito	tombarello	listado
Belone belone	orphie	gar pike	Hornhecht	aguglia	aguja
Clupea harengus	hareng	herring	Herring	aringa	arenque
Conger conger	congre	conger eel	Congeraal, Meeraal	grongo	congrio
Ctenopharyngodon idella	carpe herbivore, carpe amour	grass carp	Graskarpfen	amur, carpa erbivora	carpa herbívora
Cyprinus carpio	carpe	common carp	Karpfen	carpa	carpa
Dicentrarchus labrax	bar européen	European seabass	Seebarsch	spigola; branzino	lubina
Engraulis encrasicolus	anchois	anchovy	Sardelle	acciuga, alice	anchoa, boqueron
Gadus morhua	morue commune, cabillaud	Atlantic cod	Kabeljau, Dorsch	merluzzo bianco	bacalao
Hippoglossus hippoglossus	flétan de l'Atlantique	Atlantic halibut	Atlantischer Heilbutt	halibut atlantico	Fletán, halibut atlantico
Hypophthalmichthys nobilis	carpe marbrée	bighead carps	Marmorkarpfen	carpa a testa grossa	carpa cabezona
Lepidopus caudatus	coutelas, sabre d'argent	frostfish; silver scabbard	Degenfisch	sciabola	pez cinto, espadilla
Loligo spp	calmar, encornet	squid	Kalmar	calamaro	calamar
Lophius piscatorius	baudroie, lotte	monkfish	Seeteufel	rana pescatrice	rape
Maurolicus muelleri	marguerite perlée	pearl side	Lachshering	maurolico	anchoa de fondo
Meriangius merlangus	merlan	whiting	Wittling, Merlan	molo, merlano,	merlan, plegonero
Merluccius merluccius	merlu	hake	Seehecht	nasello	merluza
Micromesistius poutassou	merlan bleu	blue whiting	Blauer Wittling	melù, potassolo	bacaladilla
Molva molva	lingue	ling	Lengfisch	molva	maruca
Octopus spp.	poulpe	octopus	Tintenfisch	polpo	pulpo
Oncorhynchus kisutch[*]	saumon argenté	coho (=silver) salmon	Silberlachs	salmone argentato	salmón plateado

[*]These are some of the Pacific salmon species. Pacific salmon species belong to the genus *Oncorhynchus*, they include many species such as chum salmon (*O. keta*), chinook (*O. tshawytscha*), and pink (*O. gorbuscha*) among others.

Latin names	*French*	*English*	*German*	*Italian*	*Spanish*
Oncorhynchus mykiss	truite arc-en-ciel	rainbow trout	Regenbogenforelle	trota iridea	trucha arco iris
*Oncorhynchus nerka**	saumon rouge	sockeye salmon	Rotlachs	salmone rosso	salmón rojo
Perca fluviatilis	perche	perch	Flussbarsch	pesce persico	perca
Pollachius pollachius	lieu jaune	pollock	Pollack	merluzzo giallo	abadejo
Pollachius virens	lieu noir	saithe	Seelachs	merluzzo carbonaro	carbonero, palero
Scophthalmus maximus	turbot	turbot	Steinbutt	rombo chiodato	rodaballo
Salmo salar	saumon de l'atlantique	Atlantic salmon	Lachs	salmone	salmón
Salmo trutta	Truite fario	trout	Forelle	trota	trucha
Sarda sarda	bonite	bonito	Bonito	palamita	bonito
Sardina pilchardus	sardine, pilchard	sardine	Sardine	sardina	sardina
Scomber japonicus	maquereau espagnol	chub mackerel,	Pazifische Makrele	lanzardo	estornino
Scomber scombrus	maquereau	mackerel	Makrele	sgombro	caballa
Scorpaena spp	rascasse, scorpène	scorpionfish	Drachenköpfe	scorfano	rascacio, cabracho, escorporas
Sebastes pinniger	sébaste citron	Canary rockfish	Kanarische Stachelköpfe	pesce pietra canario	rocote canario
Sepia spp.	seiche commune	common cuttlefish	Sepia	seppia	sepia
Sparus aurata	dorade royale	gilthead sea bream	Dorade	orata	dorada
Thunnus alalunga	germon	albacore	Weisser Thunfisch	alalunga	bonito del norte, atún blanco ó albacora
Thunnus thynnus	Thon rouge	tuna	Thunfisch	tonno rosso	atún rojo
Trachurus picturatus	chinchard jurel	blue scad; blue jack mackerel	Dunkler Stöcker	sugarello pittato	chicharro
Trachurus trachurus	chinchard	common scad, horse mackerel	Bastardmakrele, Heller Stöcker	suro, surgarello	jurel

GLOSSARY / ABBREVIATIONS

- **Abundance**: is the number of individuals of a particular parasite in a single host, regardless of whether the host is infected or not. Consequently, **mean abundance** denotes the total number of individuals of a particular parasite in a sample of a particular host divided by the total number of host specimens examined, including both infected and uninfected hosts.

- **Accidental host:** are those that are not part of the natural chain of infection and do not normally lead to infection of the definitive hosts, but are accidentally infected and dead ends in the life cycle of the parasite.

- **Allergic urticaria:** a skin reaction with hives (raised, itchy areas of skin), which are changing and do normally not persist at the same location more than 24 hours. Acute, short-lived urticaria (less than 24 or 48 hours) is allergic and mediated by specific IgE against foods, drugs, insects, gastro-allergic anisakiasis etc.

- **Anadromous:** fish that live mostly in the ocean, and breed in fresh water (Greek: 'Ana' is up; The noun is "anadromy")

- **Angioedema:** see allergic urticaria, like hives, but affects deeper skin layers.

- **Anisakiasis:** human disease caused by an infection with a live *Anisakis* larva.

- **Belly flaps:** see hypaxial muscles.

- **Cartilaginous fish:** jawed fish with paired fins, paired nares, scales, two-chambered hearts, and skeletons made of cartilage rather than bone. They are divided into two subclasses: Elasmobranchii (sharks, rays and skates) and Holocephali (chimaera, sometimes called ghost sharks, which are sometimes separated into their own class

- **Catadromous:** fish that live in fresh water, and breed in the ocean.

- **Cercaria:** the larval stage of a trematode worm having a tail that disappears in the adult stage.

- **Copepods:** a group of small crustaceans found in the sea and nearly every freshwater habitat. Many species are **planktonic** (drifting in sea waters), but more are **benthic** (living on the ocean floor). Copepods are sometimes used as bio-indicators and they are usually the dominant members of the zooplankton, and are major food organisms for small fish, whales, seabirds and other crustaceans such as krill in the ocean and in fresh water. They represent the intermediate host for many fish parasites of public health importance (e.g. *Anisakis* spp., *Diphyllobothrium* spp.)

- **Coproantigen:** antigen present in the faeces, may be useful for detection in diagnostic procedures.

- **Coracidium:** the larval stage after egg hatching of pseudophyllidian cestodes such as *Diphyllobothrium* and *Spirometra* spp. This ciliated free-swimming larval stage contains six hooks like those in the oncospheres of other tapeworms.

- **Definitive host:** an animal in which the sexually reproducing adult form of the parasite occurs and from which the offspring is shed.

- **Demersal fish:** fish that live on or near the bottom of the sea or lakes, which usually consist of mud, sand, gravel or rocks. In coastal waters they are found on or near the continental

shelf, and in deep waters they are found on or near the continental slope or along the continental rise. They are not generally found in the deepest waters, such as abyssal depths or on the abyssal plain. The word demersal comes from the Latin *demergere*, which means to sink. Demersal fish are bottom feeders. They can be contrasted with pelagic fish which live and feed away from the bottom in the open water column.

- **Eosinophilic gastroenteritis:** a rare and heterogeneous condition characterized by patchy or diffuse eosinophilic infiltration of gastrointestinal tissue. Peripheral blood eosinophilia and elevated serum IgE are usual. The damage to the gastrointestinal tract wall is caused by eosinophilic infiltration and degranulation. Typically presents with a combination of chronic nonspecific symptoms which include abdominal pain, nausea, vomiting, diarrhoea, weight loss, and abdominal distension.

- **Epaxial muscles:** the dorsal ('upper') part of the body musculature of fish, 'trimmed fillet'.

- **Fishery products:** all seawater or freshwater animals [except for live bivalve molluscs, live echinoderms, live tunicates and live marine gastropods, and all mammals, reptiles and frogs] whether wild or farmed and including all edible forms, parts and products of such animals

- **Gastroallergic anisakiasis:** an acute allergic reaction (urticaria/ angioedema or anaphylaxis) which is produced in the context of an acute gastric parasitism by *A. simplex*, when the parasite attempts to penetrate the gastric mucosa.

- **Hypaxial muscles:** the part of the body musculature of fish that encloses the visceral cavity on either body side, commonly called 'belly flaps'

- **Intensity** (of infection): the number of individuals of a particular parasite in a single infected host.

- **Intermediate hosts:** those that harbour juvenile stages of the parasite (their larvae), and allow the parasite to moult one or more times.

- **Mean intensity:** the average intensity of a particular parasite among the positive individuals in a host sample, i.e. the total number of a particular parasite in a host sample divided by the number of hosts infected with that parasite.

- **Metacercaria:** the encysted resting or maturing stage of a trematode parasite in the tissues of an intermediate host or on vegetation.

- **Microsporidia:** unicellular fungal-like parasites, several groups are known pathogens of fish in both sea- and freshwater fish as well as an opportunistic pathogen of humans.

- **Miracidium:** the first larval stage of trematodes, released from the egg which is usually shed in the faeces of the definitive vertebrate host. When the egg is immersed in the water environment, the free-swimming miracidium infects the intermediate host (mollusc).

- **Panallergen:** an allergen widely distributed in nature. Panallergen may explain why some people previously sensitized with pneumoallergens (aerial allergen) could have an allergic reaction after eating foods. They are responsible for many IgE cross-reactions even between taxonomy unrelated allergen sources.

- **Parasitic diseases:** those caused by eukaryotic organisms (both unicellular of multicellular).

- **Paratenic host (or transport host):** a host of a parasite where survival but no larval development occurs. This stage may be crucial for successful transfer of the parasite to the next host level, e.g. another transport host upwards the food chain, or the definitive host

- **Pelagic fish**: fish which spend all or most of their adult life in the water column of coastal, oceanic or lake waters. Typically, many pelagic fish species show extensive shoaling behavior: e.g. herring, mackerel, blue whiting.

- **Plerocercoid:** the second larval stage of pseudophyllidean cestodes which infects a wide range of vertebrate hosts including fish, amphibia, reptiles, mammals, and birds (second intermediate hosts – the first one is a crustacean copepod). The definitive host becomes infected by eating the tissues of the second intermediate host.

- **Prevalence** (of a disease): the proportion of diseased individuals in the whole population.

- **Procercoid:** the first larval stage of pseudophyllidean tapeworms that develops after ingestion by the first intermediate host (e.g. copepod).

- **Redia:** the larval stage of certain trematodes that is produced within the sporocyst after infection of the first intermediate host (snail) and that can give rise to additional rediae or to cercariae.

- **Sporocyst:** the larval stage of certain Trematodes, formed in the first intermediate host (snail); it gives rise to rediae or cercariae.

- **Teleost fish** (also called bony fish): a taxonomic group of fish that includes the ray-finned fish (Actinopterygii) and lobe-finned fish (Sarcopterygii). Most bony fish belong to the ray-finned fish.