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Nematode larvae in fish on the German market 20 years of consumer related research

Nematodenlarven in Fischen auf dem deutschen Markt 20 Jahre Forschung für den Verbraucher

Horst Karl

Summary

In commercially exploited marine fish from the North Atlantic mainly 2 species of nematode larvae are of public interest: *Anisakis* sp. (herring or whale worm) and *Pseudoterranova decipiens* (cod or seal worm). The paper summarizes the German research to reduce the number of nematode larvae in fishery products after the nematode crisis in 1987 and to introduce safe processing conditions.

Information is given on the distribution of nematodes in fish species at capture, on the effect of possible migration due to delayed gutting and on the prevalence and abundance of *Anisakis* in herring from different stocks.

The paper also summarizes the investigations on preventive measures to kill nematodes during commercial processing and household preparations.

An overview on the current EU and Codex regulations is given concerning parasites in fish.

Keywords: *Anisakis* prevalence, *Anisakis* migration, *Anisakis* safety measures, *Anisakis* regulations

Zusammenfassung

In Konsumfischen aus dem Nordatlantik sind vor allem zwei Parasitenarten von öffentlichem Interesse: *Anisakis* sp. (Heringswurm) und *Pseudoterranova decipiens* (Kabeljauwurm).

Die vorliegende Arbeit gibt einen Überblick über Forschungsarbeiten, die seit der Nematodenkrise in 1987 durchgeführt wurden, um den Nematodenbefall in Fischereierzeugnissen zu minimieren und eine sichere Verarbeitung zu gewährleisten.

Diskutiert werden die Nematodenverteilung in fangfrischen Fischen, die Auswirkungen eines verzögerten Ausnehmens und die Befallsrate und -intensität in Heringen aus verschiedenen Fanggebieten.

Weiterhin werden die Untersuchungsergebnisse zur sicheren Abtötung von Nematodenlarven bei der kommerziellen Verarbeitung und im Haushalt zusammengefasst und wichtige EU Verordnungen und Codexstandards hinsichtlich des Parasitenbefalls von Fischen vorgestellt.

Schlüsselwörter: *Anisakis*-Vorkommen, *Anisakis* Wanderungsverhalten, *Anisakis* sichere Verarbeitung, *Anisakis* Gesetze

Introduction

The presence of *Anisakis* sp. and other nematode larvae in fish as food has been known for a long time. Agersborg (1918) reported that people in some villages of northern Norway rejected to buy freshly caught cod due to heavy infestation of the muscle meat with nematodes. Kahl reported in 1936 that visible *Pseudoterranova* larvae embedded under the skin of smelt disgusted German housewives along the Elbe region and they often refused to use smelt in their kitchen. But it was not until the late fifties that nematodes in fish were recognised as a public health problem in the Netherlands (Thiel et al., 1960). After consumption of lightly salted Maatjes herring, several people were hospitalised due to acute abdominal pain caused by live nematodes, genus *Anisakis*. If live nematode larvae are ingested by man they may penetrate into the wall of the gastrointestinal tract and cause acute inflammations. This human disease is named *Anisakidosis*. In 1969, the Dutch authorities implemented regulations regarding safe processing procedures of herring (salting, freezing, heating and marinating) in order to kill all larvae in their herring products (Anon., 1969).

The relevance of a similar regulation for German fishery products was discussed by Priebe in 1971. He pointed out that several German herring products were not safe in respect of nematodes and corresponding measures should be introduced.

Aware of the possible risk the West German fish trade organisation together with German herring processing companies adopted the Dutch regulation and established recommendations for safe processing conditions of German herring products. The herring industry followed these recommendations for years, but during the mid 80s a few enterprises importing herring products to Germany did no longer adhere to this voluntary agreement which finally led to the nematode crisis in 1987. A TV team (Monitor, 1987) picked up this problem and broadcasted on the TV screens enlarged worms (*Anisakis* sp.) crawling out of fish fillets, which shocked the German consumer. The fish sales dropped 80 % and fish workers lost their jobs.

Since 1987 a lot of efforts were undertaken by the German government, scientists and the fish industry to avoid nematode larvae in fish products and to regain the confidence of the consumer in fish products. A new regulation on health requirements for fish and shell fish (fish regulation) came into force in 1988 (BMJFFG, 1988), covering all aspects of handling and processing of fish referring to nematodes and a maximum allowable number of nematodes in fishery products was laid down (BGA, 1988).

However, the occasional occurrence of nematode larvae in fish as food when entering the market is inevitable as the results of the present paper will demonstrate.

Information is given on the distribution of nematodes in fish species at capture, on the effect of possible migration due to delayed gutting and on the prevalence and abundance of *Anisakis* in herring from different stocks.

The paper also discusses the investigations undertaken at the institute (MRI) on preventive measures to kill nematodes during commercial processing and household preparations.

Additionally, an overview on the current EU and Codex regulations concerning parasites in fish will be given.

General aspects

Parasites in fish are very common. Some species of helminthic parasites are transmitted from fish and shellfish to humans and can cause serious diseases. A list of important pathogenic parasites transmitted by fish and shellfish is given in Table 1.

In commercially exploited marine fish species from the North Atlantic primarily 2 species (Fig. 1) are of public interest (Berland, 2003):

Anisakis sp. (herring or whale worm) and *Pseudoterranova decipiens* (cod or seal worm).

Problems encountered are health and aesthetic aspects. When eaten alive both nematode larvae can cause intestinal *Anisakidosis*. In Japan, approximately 2000 cases occur each year due to the consumption of raw fish (Sushi and Sashimi) (Chai et al., 2005). Most of the *Anisakidosis* cases reported are caused by *Anisakis simplex* (Ishikura et al., 1992).

Anisakis larvae also have an allergenic potential, as studies from Spain and Japan showed (Valls et al., 2005; Moneo et al., 2007).

Beside the health related aspects also the aesthetic aspect has to be considered. A consumer who finds live nematodes in fish products may never eat fish again.

Anisakis is found world wide in more than 100 fish species, e.g. herring, mackerel, cod, saithe, plaice, haddock, blue whiting, halibut, hake, Alaska pollock, wild salmon, tuna, barracouta, orange roughy etc., in cephalopods, e.g. different squid species, octopods, and in crustaceans, e.g. wild shrimps. *Pseudoterranova decipiens* is reported in more than 75 fish species, e.g. cod, saithe, turbot, haddock, torsk, Greenland halibut, Scottish dab, smelt.

TABLE 1: Important pathogenic parasites transmitted by fish and shell fish (Huss et al., 2004)

Parasites	Distribution	Host
Nematodes		
<i>Anisakis simplex</i>	North Atlantic, Asia	marine species
<i>Pseudoterranova decipiens</i>	North Atlantic, Asia	marine species
<i>Gnathostoma</i> sp.	Asia	freshwater species, frog
<i>Capillaria</i> sp.	Asia	freshwater species
<i>Angiostrongylus</i> sp.	Asia, Africa, South America	freshwater prawns, fish, snails
Cestodes		
<i>Diphyllbothrium latum</i>	Northern hemisphere	freshwater species
<i>D. pacificum</i>	Peru, Chile, Japan	seawater species
Trematodes		
<i>Clonorchis</i> sp.	Asia	freshwater species, snails
<i>Opisthorchis</i> sp.	Asia	freshwater species
<i>Paragonimus</i> sp.	Asia, America, Africa	snails, crustaceans, fishes
<i>Echinostoma</i> sp.	Asia	clams, snails, freshwater fish

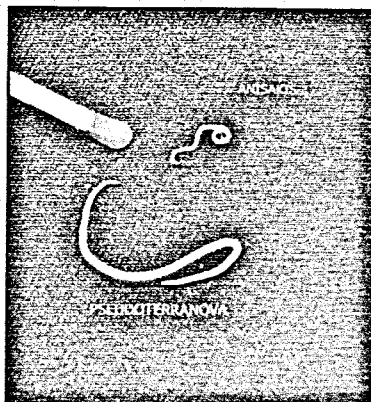


FIGURE 1: *Anisakis* and *Pseudoterranova* larvae.

Migration studies

The investigations on fish at capture showed that the occurrence of some nematode larvae in the edible part of fresh caught fish seems to be inevitable, but there was and still is some controversy as to whether immediate gutting can prevent an additional migration of nematodes into the flesh. Earlier studies observed some migration in fat fish species like herring (*Clupea harengus*) and mackerel (*Scomber scombrus*) but not in lean fish species like whiting (*Merlangius merlangus*), and Alaska Pollock (*Theragra chalcogramma*) (Smith, 1984; Arthur et al., 1982; Smith and Wotten, 1975). Other authors found no migration in barracouta (*Thyristes atun*), red cod (*Pseudophycis bachus*) and red gurnard (*Chelidonichthys kumu*) (Wharton et al., 1999).

Within the last 15 years several new migration studies applying commercial fish handling practices were undertaken at our institute in cooperation with other research institutions.

The migration behaviour in fat summer and lean winter herring from the North Sea was thoroughly studied within an EU-project (FAR, 1992) using various conditions which included ice, RSW/CSW, sea water of +10 °C and delayed ice storage (Roepstorff et al., 1993). Other ice storage experiments were performed with saithe (*Polachius virens*), haddock (*Melanogrammus aeglefinus*), red fish (*Sebastes* sp.) (Karl et al., 2002) and mackerel (*Scomber scombrus*).

A brief summary of the results is compiled in Table 3. Detailed information on the experimental design and a full discussion of the results can be found in the cited publications.

Except for mackerel where a slight but not significant increase of the abundance of nematode larvae in the belly flaps was detected, no migration of nematodes from the guts into the belly flaps and / or the fillets was observed for all other species and all conditions listed in Table 3.

A reduction of the consumer risk from *Anisakis* sp. and *Pseudoterranova decipiens* can only be achieved by leaving as little as possible of the belly flaps attached to the fillets and strictly keeping to processing conditions intended to kill non visible larvae.

On the other hand, early gutting can improve the quality of the fishes, especially when intended for ice storage over a longer period (Karl and Meyer, 2007).

Infestation of herring from different stocks and fishing grounds with *Anisakis* sp.

The occurrence of *Anisakis* larvae in the flesh and viscera of herring from different fishing grounds of the North Sea, west of the British Isles, the Celtic Sea and the Baltic Sea was examined during the periods 1993 and 2002. The application of the same sampling and detection methods (digestion and UV-press-method [Karl and Leinemann, 1993]) allows a comparison of the data for all the years and fishing grounds.

This study was undertaken to obtain an overview on the infestation of different herring stocks important for the supply of the German market. The approximate locations of the sampling places are given in Figure 4.

The prevalence (percentage of infected herring) of nematodes in herring from all fishing grounds except for

herring from the fishing ground of the Baltic Sea east of Bornholm ranged between 81 and 100 %.

The low infection rate of eastern Baltic herring is known (Lang et al., 1990) and has been used to discriminate between different Baltic herring populations (Podolska et al., 2006).

Similar high prevalences of nematodes in North Sea herring and the slightly lower infection rates in herring from Ireland and St. Kilda were already reported by Davey (1972) and by Banning and Becker (1978) which shows that the situation has not changed significantly within the last 30 years.

The abundance (mean number of nematodes per fish, based on all fishes analysed) of nematodes in the viscera differs considerably between fishing grounds. The intestines of North Sea herring (II, III, IV) and herring from the western Baltic Sea (VIII) were more infected than those from fishes of the Celtic Sea (VII) and from waters west of the British Isles (I). The lower abundance in the viscera of herring from the Dogger Bank (VI) and the Skagerrak area (V) compared to other fishing grounds of the North Sea can probably be explained by the different length of the herring sampled. Roepstorff et al. (1993) showed that the worm burden in the viscera increased with the length of the fish host.

Of more interest for the consumer is the abundance of nematodes in the musculature. To determine the number of nematodes in the muscle meat the fishes were manually filleted and deskinning. Before analysis most of the belly flaps were removed as should be realised by mechanically filleting under good commercial practice. Both trimmed fillets together are referred to as flesh in Table 4.

The abundance of nematode larvae in the North Sea herring muscle ranged between 0.1 and 0.53 and was on average higher than in herring from Ireland and St. Kilda.

TABLE 3: Results of various migration studies

Species	Storage time (d)	Storage condition	Migration of nematodes
Herring lean	5	ice	No
Herring fat	5	ice	No
Herring fat	5	sea water 10 °C	No
Saithe	6	ice	No
Saithe	12 h	on deck	No
Haddock	10	ice	No
Haddock	6	ice	No
Red fish	8	ice	No
Mackerel	9 h	on deck	?

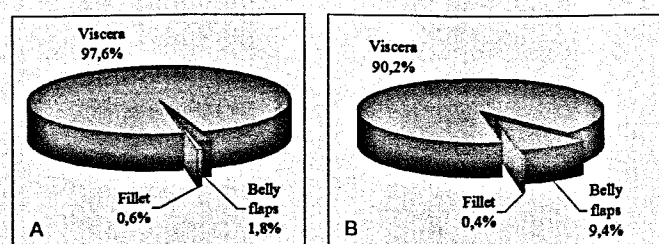


FIGURE 3: Distribution of *Anisakis* in living fish: viscera, belly flaps, fillets (A: North Sea herring, B: Saithe).

The flesh of herring from the eastern Baltic Sea was practically free of *Anisakis* sp..

Yet, also apparently less infected herring stocks cannot guarantee nematode free products. Consequently, safety measures during processing and household preparation have to be implemented.

Preventive measures to kill nematodes during commercial processing and household preparation

As follow up of the nematode crisis in 1987 the different German processing conditions for herring products were studied in a range of experiments to establish safe conditions regarding the mortality of *Anisakis* larvae.

Additional temperature regimes of typical household cooking and frying procedures were assessed for safe preparation as part of the EU-project (FAR, 1992) using fresh saithe fillets as experimental material. These data have not been published yet.

Commercial processing

Freezing

Different commercial freezing practices have been examined for their effectiveness to kill nematodes (Karl and Leinemann, 1989; Karl and Priebe, 1991).

Freezing equipment tested included: horizontal plate freezer, air blast freezer, carbon dioxide shock freezer, cold storage cabinets.

Viable larvae of nematodes in herring and herring fillets were inactivated by active freezing of the fish within 2 to 3 hours to a core temperature of -18 °C and -20 °C with additional cold storage of the frozen product for 24 hours at -18 °C or -20 °C, respectively.

These conditions are also found in the EU regulation No. 853/2004 (European Parliament and Council, 2004). The freezing conditions are valid for horizontal plate freezers and air blast freezers.

Freezing of herring by carbon dioxide shock freezer at -60 °C to a core temperature of -20 °C without additional frozen storage for 24 hours is sufficient to kill all nematodes.

TABLE 4: Prevalence and abundance of *Anisakis* sp. in herring from different fishing grounds

	Catch date	Fishing area	Position	No. of herring	Mean length (cm)	Prevalence (%)	Abundance	
							Flesh	Viscera
I	Oct 02	St. Kilda WBI	57°38'N 08°34'W	100	26.9	93	0.09	7.9
II	Mar 94	Unst Bank NS	61°03'N 01°45'W	90	30.0	100	0.53	38.1
III	Jan 96	Utsira B. NS	59°41'N 03°12'E	99	25.5	99	0.16	16.6
IV	Oct 02	Fladen NS	57°51'N 01°18'E	100	27.8	100	0.46	54.1
V	Apr 00	Skagerrak NS	57°28'N 08°36'E	100	23.0	90	0.10	6.8
VI	May 96	Dogger NS	54°08'N 02°12'E	100	24.0	81	0.18	9.9
VII	Mar 98	Celtic Sea Irl.	51°56'N 08°01'W	100	25.5	92	0.10	14.2
VIII	Feb 99	Western Baltic Sea	54°12'N 11°50'E	94	26.6	98	0.22	18.4
IX	Sep 99	Central Baltic Sea	54°56'N 15°24'E	98	22.1	3	0.01	0.14

NS: North Sea, WBI: West of British Isles, Irl.: Ireland.

TABLE 5: Salting conditions of herring to kill nematodes

Product	Salt content in the water phase (%)	Minimum Storage time (d)
Heavily salted fish	20	21
Salted fish	15	28
Spice salted fish („Kräuterhering, Matjes nach nordischer Art“)	12–13	35
Herring fillets – Matjes type (Fast ripening mixtures)	10	Recommended ripening time of 4 d not sufficient

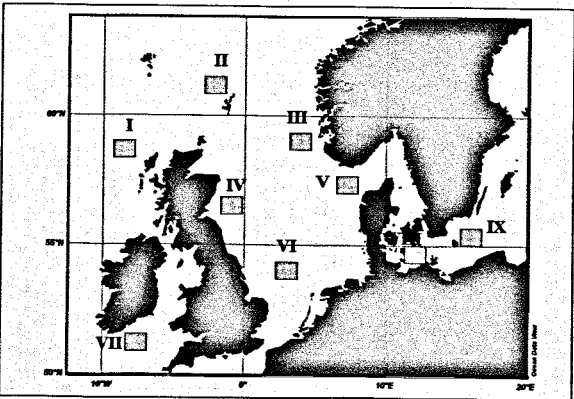


FIGURE 4: Sampling locations of herring from different fishing grounds.

Slow freezing processes by placing fishes in cold storage cabinets at -20°C prolong the inactivation time of nematodes considerably.

When herring is packed in 20 kg plastic containers and the containers are placed in cold storage cabinets at -20°C it took at least 2–3 days to reach a core temperature of -20°C . Applying these conditions, an additional cold storage of 24 hours at the given temperature was not sufficient to kill all nematodes embedded in the packed herrings. It is therefore recommended to keep the 20 kg containers at a core temperature of -20°C for a longer time before thawing.

Similarly long freezing times can be expected to bring fish packed in a 1–2 kg plastic bag to a core temperature of -20°C , when placing them in the freezer unit of a household refrigerator. This has to be considered in case of deep freezing fish before serving as Sushi at home.

Marinating

The marination of herring has a long tradition in northern Europe, especially in Scandinavia and in Germany. A large variety of recipes exists in the different countries, but all include the preservation of herring fillets by means of salt (NaCl) and acetic acid.

The survival of *Anisakis* sp. larvae in herring fillets marinated in brines depends mainly on the concentration of salt in the aqueous phase of the fish tissue (Priebe et al., 1973).

The traditional German procedure for marinating herring in brine consisting of 14 % (w/w) salt, 7 % (w/w) acetic acid and 33.3 ml of 30 % H_2O_2 , with a ratio herring to brine of 1.5 : 1 requires a storage time of at least 5 weeks to kill all nematodes.

Other concentrations of salt and acetic acid were also studied (Karl et al., 1995). A reduction of the salt content in the water phase of the tissue increased the survival time of the nematodes considerably.

Salting

Different salting conditions to produce salted, spice salted and Maatjes type herring products have been examined regarding the survival of nematodes. The viability of nematode larvae in salted herring products depends on the salt concentration in the aqueous phase of the fish tissue and on the storage time (Karl and Leinemann, 1989a; Karl, 1987). The results of the respective studies are summarized in Table 5.

Most of the conditions were included in the former German Fish Regulation from 1988.

Heat smoking

The effect of heat smoking on the survival of nematodes in round herring (German: Bückling) was studied using an automatic smoking kiln with external smoke generator and a typical herring smoking program. The fish was heated to a core temperature of 70°C and then immediately cooled. None of the 500 nematodes isolated survived these conditions (Karl, 1987a).

Further heating experiments were performed within the FAR EU-project. The results are discussed under the topic household preparations.

Household preparations

The heat resistance of *Anisakis* larvae naturally embedded in muscle was studied with fresh belly flaps of saithe

TABLE 6: Heat resistance of nematodes in saithe

Core temperature ($^{\circ}\text{C}$)	Number of experiments	Number and condition of nematodes	
		dead	alive
< 50	5	21	10 (32 %)
50–54	16	92	6 (7 %)
55–59	7	433	0

TABLE 7: Temperatures obtained by various household cooking conditions

Cooking method	Cooking time (min)	Temperature obtained ($^{\circ}\text{C}$)	Portion weight (g)
Teflon frying pan, unbattered	7	57–70	132–148
Steel frying pan, unbattered	7	54–58	132–176
Glass mould in oven	25	70–80	162–182
Stainless steel cooking pot	10	62–65	200–212
Microwave oven	> 3	83	approx. 140

heated in plastic bags in a water bath at 80°C to a given core temperature. For each experiment three belly flaps were tied together to a thickness of 3 cm and additionally live nematodes isolated from herring intestines were placed between the layers.

The results are shown in Table 6. No nematode survived heat treatment, when the core temperature of the fish product reached $> 55^{\circ}\text{C}$.

Home cooking of fish includes various preparations. The fish is either steamed or cooked in cooking pots, baked in glass moulds in the oven, fried in a frying pan or heated in a microwave oven.

Various experiments were conducted to assess the final core temperature during different home cooking procedures by heating portions of saithe (130–200 g) according to recipes of different cooking books. Temperatures obtained varied with the thickness of the portions, the recipe used and the heating technique applied. Most home cooking procedures were safe but some cooking times specified in cooking books were not sufficient to reach core temperatures of $> 55^{\circ}\text{C}$. Typical temperatures obtained are compiled in Table 7.

Current regulations

EU regulations

The EU has implemented common regulations concerning parasites in fish and fishery products.

Regulation (EC) No. 853/2004

Regulation (EC) No. 853/2004 (European Parliament and Council, 2004) lays down requirements for handling fishery products concerning parasites (Chapter III D) as well as health standards for parasites in fishery products (Chapter V D).

No 853/2004 Chapter III D. Requirements concerning parasites

1. The following fishery 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; this treatment must be applied to the raw product or the finished product:
 - a) fishery products to be consumed raw or almost raw
 - b) fishery products from the following species, if they are to undergo a cold smoking process in which the internal temperature of the fishery product is not more than 60°C :
 - (i) herring, (ii) mackerel; (iii) sprat; (iv) wild Atlantic and Pacific salmon and
 - c) marinated and / or salted fishery products, if the processing is insufficient to destroy nematode larvae.
2. Food business operators need not carry out the treatment required under point 1 if
 - a) 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.
3. 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.

No 853/2004 Chapter V D. Parasites

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.

Regulation No 2074/2005

Regulation No 2074/2005 (Commission Regulation, 2005) set out in Annex II the detailed rules for visual inspection of parasites. Generally, food business operators have to carry out their own checks at all stages in the production of fishery products.

Chapter I of Annex II gives definitions of visible parasites, visual inspection and candling. Chapter II describes the rules to detect parasites in fishery products.

No 2074/2005 Annex II Chapter I

1. "Visible parasite" means a parasite or a group of parasites which has a dimension, colour or texture which is clearly distinguishable from the fish tissue.
2. "Visual inspection" means non-destructive examination of fish or fishery products with or without optical means of magnifying and under good light conditions for human vision, including, if necessary, candling.
3. "Candling" means, in respect of flat fish or fish fillets, holding up fish to a light in a darkened room to detect parasites.

No 2074/2005 Annex II Chapter II

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

consumption. Depending on the system of gutting used, the visual inspection must be carried out:

- a) in the case of manual evisceration, in a continuous manner by the handler at the time of evisceration and washing;
 - b) in case of mechanical evisceration, by sampling carried out on a representative number of samples being not less than 10 fish per batch.
2. The visual inspection of fish fillets or fish slices must be carried out by qualified persons during trimming and after filleting or slicing. Where an individual examination is not possible because of the size of the fillets or the filleting operations, a sampling plan must be drawn up and kept available for competent authority in accordance with Chapter II(4) of Section VIII to Regulation (EC) No 853/2004. Where candling of fillets is necessary from technical viewpoint, it must be included in the sampling plan.

Codex Alimentarius Commission

The handling of parasites in fish products is regulated in several Codex standards.

The Codex standards for quick frozen fish (Codex STAN 190, 1995) and for quick frozen blocks of fish fillet, minced fish and mixtures of fillets and minced fish flesh (Codex STAN 165, 1995) lay down detailed requirements for the detection of parasites by candling, define a parasite and give allowable figures for parasites in a product.

Candling has to be done by placing a thawed sample unit on a 5 mm thick acryl sheet with 45 % translucency, using a light source giving 1500 Lux 30 cm above the sheet. These conditions have been first published by Valdimarsson et al. (1985).

A sample unit shall be considered as defective, if two or more parasites per kg are detected by the candling method with a capsular diameter greater than 3 mm or a parasite not encapsulated and greater than 10 mm in length.

Defect means a condition found in a product which fails to meet essential quality, composition and / or labelling provisions of the appropriate Codex product standards.

The Codex standard for salted Atlantic herring and salted sprat (Codex STAN 244, 2004) gives in Annex I a method for the determination of the viability of nematodes.

Nematodes are isolated by digestion and transferred into a new digestion solution and inspected visually for viability. Reanimation time can last more than two hours (Grabda and Bier, 1988).

Conclusions

Since the nematode crisis in 1987 extensive measures were undertaken by the German authorities, scientists and the fish industry to reduce the number of nematode larvae in fishery products and to introduce safe processing conditions.

However, the occasional occurrence of dead nematode larvae in fish as food when entering the market is inevitable. Neither immediate gutting nor exploitation of less infected stocks can guarantee nematode free products. *Anisakis* and *Pseudoterranova* larvae can be embedded in the flesh of marine fish species already at capture. A reduction of nematodes can only be achieved in most fishery products by consequent removal of the belly flaps. Current regulations and processing conditions as well as

most household cooking procedures protect the German consumer effectively against infection with live nematodes.

The growing popularity of raw fish dishes may increase the risk of infection with live nematode larvae if deep freezing is not consequently applied by restaurants and consumers.

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