Parasitol Res (2001) 87: 492–499
DOI 10.1007/s004360100389

ORIGINAL PAPER

E. Abollo · C. Gestal · S. Pascual

Anisakis infestation in marine fish and cephalopods from Galician waters: an updated perspective

Received: 24 January 2001 / Accepted: 29 January 2001 / Published online: 4 April 2001

Abstract A total of 2,673 fresh specimens of cephalopod and fish representing 35 species were obtained from commercial local fisheries in Galician waters (NW Spain). They were examined for anisakid nematodes by digestion of the muscle and elution of the viscera and whole body cavity. All larval nematodes recovered were identified by light microscopy and multilocus electrophoresis as belonging to the species *Anisakis simplex* sensu stricto and *A. pegreffii*. Encysted larvae mostly occurred in the viscera but were also found in the flesh of squid and fish. Demographic values for larval nematodes are discussed in relation to host preferences and the ecological niche of both anisakid species at the sampling area. Primary recommendations are also expressed concerning the effects of current fishing and aquaculture practices on the *Anisakis* problem.

Introduction

The 'nematode problems' in North Atlantic fisheries have traditionally been of general concern to scientists (Möller 1989). In the last 50 years, extensive evidence has been supplied to recognize that the presence of anisakid larvae on and in the viscera and flesh or free in the body cavity of many economically important cephalopod and fish species may affect fish processing and also has public health implications. The nematode larvae have always been well known to fishermen, private companies, the fish trade, seafood surveillance and administrative organisations. The presence of nematodes in fish hosts was recognized as early as the thirteenth century (Myers 1976) but due to recent media coverage nematodes are nowadays identified as an aesthetic problem by the general public which has led to notable adverse effects on marketing. Removing parasites adds appreciably to the costs of packaging, reducing the value of the product and representing a financial loss in marketing value. The magnitude of the problem has been increasing since the 1960s as the parasite has become more prevalent in North Atlantic fisheries (Smith and Wootten 1979; McClelland et al. 1985).

Apart from their economic significance, in the mid-1950s anisakids became medically important as the aetiological agent of a painful disease for fish consumers. Third-stage larvae of *Anisakis simplex* sensu lato are pathogenic to humans when raw, undercooked, or lightly marinated fish are ingested (Nagasawa 1990; Ishikura et al. 1992). In such cases the larvae may penetrate into the gastrointestinal mucosa, causing gastric or intestinal anisakiasis (Sakanari and McKerow 1989; Kikuchi et al. 1990). Although most cases have been described in Japan (Ikeda et al. 1989; Kagei and Isogaki 1992; Matsumoto et al. 1992) in the last few years they have increased world-wide even in many countries where less fish is consumed (Petithory and Marty 1988). Moreover, allergic and anaphylactoid reactions caused by *A. simplex* s.l. have been described (Kasuya et al. 1989, 1990), emphasizing the need to consider it as an aetiological factor in urticaria related to the consumption of fish (Audicana et al. 1995; Ardusso-Lovera et al. 1996; Del Pozo et al. 1997; Montoro et al. 1997). In fact, a recent study of seroprevalence with crude larval extracts of *A. simplex* using sera from Spanish people showing no clinical symptoms of anisakiasis was positive in 12.0% of an examined random population (García-Palacios et al. 1996).

The success of control and legislation measures against any parasitic disease is dependent on a knowledge of its aetiology (causative agent) and natural history (epidemiology) (Molyneux 1998). Whatever the method of control employed, the use of accurate diagnostic tools and studies on the population ecology of parasites can help when elaborating methods of control for fish nematodes. The aim of the present study, which

E. Abollo · C. Gestal · S. Pascual (✉)
Área de Parasitología, Facultad de Ciencias,
Universidad de Vigo, Aptdo. 874-36200 Vigo, Spain
E-mail: spascual@correo.uvigo.es

was undertaken in response to growing awareness in society following recent reports in the Spanish media regarding marketing of fish containing anisakids in local fisheries, was twofold: to obtain the specific identity of *Anisakis* larvae; and to establish their prevalence pattern in local fishery products landed at Galician ports.

Materials and methods

Sampling

Samples were collected during 1997–1998 from commercial landings by fishermen operating with otter and pair trawlers and traditional gear at the Galician coast (NW Spain: 42°05′–45°15′N, 07°00′–09°20′W). All specimens, including 988 fishes (28 species) and 1,685 cephalopods (seven species), were placed on flake ice until gutting and/or digestion at the laboratory, where they were examined on arrival while still fresh. Each host individual was weighed (BW, total body weight, to the nearest g) and measured (BL, total body length for fish and dorsal mantle length for cephalopods, to the nearest mm). The whole body cavity and the viscera of each individual were carefully dissected and thoroughly examined for anisakids. They were removed from surrounding host tissue with the aid of a stereomicroscope, counted and the site of infestation noted. Worms in the fish flesh were recovered and counted using Smith and Wootten's (1975) pepsin-HCl digest method.

Taxonomy

To identify the nematodes by light microscopy (LM), they were liberated from their capsules, fixed in hot 70% ethanol, cleared in glycerine or lactophenol and temporarily placed in mounts. Identification of the specimens was based on the morphology of the digestive tract, the position of the boring tooth in relation to the excretory pore and the morphology of the postanal tail with a typical terminal mucron (Berland 1989). For genetic specific identification of larvae within the *A. simplex* sibling complex, a total of 85 and 323 individuals comprising 18 subsamples from infested cephalopod and fish species, respectively, were repeatedly washed in 0.9% saline solution and frozen in individual plastic vials at –80 °C. Identification was done by multilocus electrophoresis in standard horizontal starch gel performed on single specimens. Details of the electrophoretic procedure and diagnostic loci were as described by Mattiucci et al. (1997).

Demographic values

In a fish-processing industry, accurate electrophoretic identification of each larvae is time-consuming and expensive and involves the purchase and maintenance of expensive equipment. Similarly, diagnosis by microscopy, even by a well-trained microscopist, is extremely labour intensive, especially when a large number of samples needs to be screened in a relatively short time, such as during routine checks by fish inspectors. In order to overcome this problem and to avoid the traditional lack of comparable studies in the past, demographic values of parasitic infestation are referred to *A. simplex* s.l. and calculated according to Bush et al. (1997). Based on prevalence values, anisakids were classified into component ($P > 10\%$) and secondary species ($10\% \geq P > 1\%$) (Bush et al. 1990).

Results

Cephalopods and fish examined for anisakid parasites are listed in Tables 1 and 2. The general morphology of all larval specimens examined by LM closely resembled that of *A. simplex* (Rudolphi, 1809) larva (I) of Berland. All the *A. simplex* s.l. larvae found in four cephalopod species and 14 fish species were genetically assigned to *A. simplex* s.str., whereas all larvae assigned to *A. pegreffii* were found in seven fish species but not in cephalopods (Tables 1, 2). Of the infested fish species, 50% were shared by *A. simplex* s.str. and *A. pegreffii*. These include *Prionace glauca, Belone belone, Micromesistius poutassou, Scomber scombrus, Trachurus trachurus, Lepidorhombus boscii* and *Scorpaena scrofa*.

A. simplex s.l. larvae were usually found encapsulated in tight flat coils on or in the viscera of fish and cephalopods, sometimes aggregated in enormous numbers. Specimens of third-stage larvae from cephalopods are mainly found free in the body cavity and encapsulated in flat spirals in the sheath of connective tissue surrounding the mantle muscle, mesenteries, gonads and the internal wall of the stomach. *A. simplex* s.l. larvae were also found encapsulated on the fish's liver, stomach, mesenteries, gonads and musculature, mainly in the belly flaps and rarely in the dorsal parts. *M. poutassou, S. scombrus, Merluccius merluccius, Lophius piscatorius* and *T. trachurus* often harboured muscle-encapsulated *Anisakis* larvae (3.2–41.1% of the total worm burden), while the flesh of other fish species was not infested. However, in all fishes, the majority of *Anisakis* larvae were found in the viscera (≥58.9% of the total worm burden). In *S. scombrus* and *T. trachurus* but mostly in *M. poutassou* we noted post-mortem migrations of *Anisakis* larvae from the body cavity to the skin, without encysting in the fish flesh.

In cephalopods, the highest prevalence of *Anisakis* larvae occurred in *Todarodes sagittatus* (34.28%; 23–45) and *T. eblanae* (23.50%; 20–27). Prevalence values of 100% were recorded in five fish species: *P. glauca, B. belone, M. merluccius, L. piscatorius* and *S. scrofa*.

The overall mean intensity and abundance of herringworm varied considerably among the host species sampled. The lowest values were found in Loliginidae and Sepiidae among the cephalopods, and Aterinidae, Clupeidae and Anguillidae among the fishes. The highest herringworm mean intensities and abundance were found in ommastrephid squids, *P. glauca*, *M. merluccius*, *L. piscatorius*, *S. scombrus*, *T. trachurus* and *S. scrofa*.

The nematode *A. simplex* s.l. was classified as a component species in all the infested specimens of paratenic host examined, except for the common cuttlefish *S. officinalis* (3.42%; 0.7–6). Three cephalopod and 14 fish species were free of anisakid larvae.

Discussion

The third-stage larvae of *A. simplex* s.l. have been recorded world-wide in approximately 200 fish species (Smith and Wootten 1978; McClelland et al. 1990; Koie 1993a) and in 25 cephalopod species (Hochberg 1990). The use of paratenic hosts opens up immense possibilities

Table 1 Samples of cephalopod examined for nematodes of the *Anisakis simplex* complex. Sample size (*N*), standard body length (*BL*: mean ± SD; range) and total body weight (*BW*: mean ± SD; range). Site of infestation: *S* stomach, *G* gonads, *BC* body cavity, *MM* mantle muscle. *P* Prevalence (%; 95% CI). *I* Mean intensity (mean ± SE; range). *A* Abundance (mean ± SE)

Host	Parasite							
	N	BL (mm)	BW (g)	Species	Site	P	I	A
Cephalopods	1,685							
Sepiidae	240					2.50 (0.3–5)		
Sepia officinalis[b], common cuttlefish	175	231.60 ± 24.30 (198–255)	1,264.80 ± 328.6 (877–1,693)	*A. simplex* s.str.	S	3.42 (0.7–6)	2.00 ± 0.41 (1–3)	0.06 ± 0.03
Sepia elegans, elegant cuttlefish	15	119.20 ± 9.93 (105–133)	247.60 ± 64.4 (174–346)	–		0	–	–
Sepia orbignyana, pink cuttlefish	50	60.38 ± 11.15 (45–80)	46.46 ± 67.44 (12–268)	–		0	–	–
Loliginidae	75					0		
Alloteuthis subulata, european common squid	75	6.90 ± 2.6 (3–12)	82.9 ± 23.9 (45–120)	–		0	–	–
Ommastrephidae	1,370					15.81 (14–18)		
Todaropsis eblanae[a], broadtailed short-finned squid	650	166.80 ± 2.73 (11–25)	231.33 ± 65.01 (108–389)	*A. simplex* s.str.	S,G,BC,MM	23.50 (20–27)	7.34 ± 12.28 (1–68)	5.87 ± 11.34
Illex coindetii[a], lesser flying squid	650	173.00 ± 2.30 (11.3–32.0)	202.03 ± 62.80 (100–380)	*A. simplex* s.str.	S,G,BC	11.07 (9–13)	5.56 ± 1.14 (1–150)	1.01 ± 0.59
Todarodes sagittatus[a], european flying squid	70	333.75 ± 66.13 (255–400)	1,115.25 ± 662.2 (389–1,800)	*A. simplex* s.str.	S,BC	34.28 (23–45)	7.55 ± 1.19 (1–22)	2.55 ± 0.60

[a] Component species, [b] secondary species

Table 2 Samples of fish examined for nematodes of the *A. simplex* complex. Sample size (*N*), standard body length (*BL*: mean ± SD; range) and total body weight (*BW*: mean ± SD; range). Site of infestation: *M* mesenteries, *L* liver, *S* stomach, *G* gonads, *Mu* musculature, *BC* body cavity. *P* Prevalence (%; 95% CI). *I* Mean intensity (mean ± SE; range). *A* Abundance (mean ± SE). *% Flesh* Percentage of worms in the flesh

Host	Parasite								
	N	BL(mm)	BW(g)	Species	Site	P	I	A	% Flesh
Fish	988								
Pleurotremata	50					50.00 (32–67)			
Prionace glauca[a], blue shark	25	110.25 ± 9.03 (100–122)	3,910 ± 169.4 (3,700–4,086)	*A. simplex* s.str. *A. pegreffii*	M,L,G,BC	100	25.25 ± 16.68 (5–44)	25.25 ± 16.68	0
Scyliorhinus canicula, lesser-spotted dog-fish	25	48.38 ± 2.10 (45–50)	317.75 ± 53.90 (237–347)	–		0	–	–	
Anguiliformes	25					33.33 (9–57)			
Conger conger[a], conger eel	25	33.52 ± 6.04 (28–43)	398 ± 32.16 (299–416)	*A. simplex* s.str.	M,L,G	33.33 (9–57)	2 ± 1 (1–3)	0.67 ± 1.11	0
Ateriniformes	25					100			
Belone belone[a], garpike	25	87.22 ± 5.66 (80–95)	796 ± 143.75 (569–983)	*A. simplex* s.str. *A. pegreffii*	M,L,G	100	3 ± 1.41 (2–4)	3 ± 1.41	0
Clupeiformes	50					0			
Sardina pilchardus, sardine	50	20.12 ± 2.66 (15–31)	77.35 ± 24.07 (16–102)	–		0	–	–	
Gadiiformes	247					77.73 (72–82)			
Merluccius merluccius[a], hake	25	39.90 ± 10.06 (25–53)	475 ± 215.35 (256–812)	*A. simplex* s.str.	M,L,G,S,Mu,	100	14.25 ± 10.75 (1–23)	11.40 ± 11.28	41.1
Micromesistius poutassou[a], blue whiting	147	21.36 ± 1.83 (27–18)	58.09 ± 16.06 (117–34)	*A. simplex* s.str. *A. pegreffii*	M,L,G,Mu	91.16 (86–95)	5.06 ± 4.92 (1–40)	4.61 ± 4.91	6.4
Molva dypterygia[a], ling	25	66.50 ± 2.12 (62–67)	879.5 ± 108.19 (813–976)	*A. simplex* s.str.	M,L,G	100	4.50 ± 4.95 (1–8)	4.50 ± 4.95	0
Pollachius pollachius, pollack	25	22.08 ± 0.66 (62–67)	90.00 ± 11.17 (71–105)	–		0	–		
Trisopterus luscus[a], whiting pout	25	28.00 ± 1.50 (26–29)	263.33 ± 39.00 (236–308)	*A. simplex* s.str.	M,L,G	32.00 (14–50)	2.67 ± 1.53 (1–4)	1.0 ± 1.73	0
Lophiiformes	25					100			
Lophius piscatorius[a], frog fish	25	44.38 ± 2.17 (41–46)	977.25 ± 184.38 (830–1,247)	*A. simplex* s.str.	M,L,G,S,Mu	100	19.00 ± 11.49 (6–34)	19.00 ± 11.49	24.0
Perciformes	491					30.34 (26–34)			
Ammodytes tobianus, lesser saneel	61	18.38 ± 1.53 (14–21)	14.64 ± 3.18 (8–20)	–		0	–	–	
Brama brama, ray's bream	25	48.02 ± 5.26 (33–58)	1,272 ± 112.60 (988–1,513)	–		0	–	–	

Table 2 (Contd.)

Host	Parasite								
	N	BL(mm)	BW(g)	Species	Site	P	I	A	% Flesh
Callionimus lyra, gragonet	25	23.83 ± 3.22 (18–28)	66.33 ± 29.17 (35–117)	–		0	–	–	
Coris julis, rainbow wrasse	25	26.20 ± 1.97 (16–23)	139.5 ± 55.91 (55–216)	–		0	–	–	
Diplodus sargus, white bream	25	20.95 ± 1.97 (16–23)	139.5 ± 55.91 (55–216)	–		0	–	–	
Hyperoplus lanceolatus, greater sandeel	25	28.70 ± 3.77 (24–34)	50.40 ± 13.28 (36–63)	–		0	–	–	
Labrus bergylta, ballan wrasse	25	26.88 ± 3.25 (22–30)	361.5 ± 118.36 (186–436)	–		0	–	–	
Mugil labeo, grey mullet	25	18.50 ± 2.7 (15–22)	77.02 ± 10.32 (22–98)	–		0	–	–	
Mullus surmuletus, mullet	25	24.75 ± 1.55 (22–26)	193.75 ± 39.05 (139–229)	–		0	–	–	
Scomber scombrus[a], Atlantic mackerel	55	26.63 ± 5.62 (23–37)	147.32 ± 80.97 (88–361)	*A. simplex* s.str. *A. pegreffii*	M,L,G,S,Mu	74.54 (63–86)	6.15 ± 5.72 (1–22)	4.61 ± 5.62	4.3
Sparus aurata, gilthead	25	20.00 ± 4.26 (14–26)	200.16 ± 39.52 (154–245)	–		0	–		
Spondyliosoma cantharus[a], black bream	25	20.04 ± 1.24 (18–23)	66.12 ± 12.06 (43–82)	*A. simplex* s.str.	M,L	80.00 (60–100)	2.63 ± 1.60 (1–6)	2.10 ± 1.79	0
Symphodus melops, corkwing wrasse	25	16.50 ± 3.20 (12–21)	71.60 ± 33.16 (25–113)	–		0	–	–	
Trachurus trachurus[a], horse mackerel	100	30.67 ± 3.81 (23–41)	254.68 ± 117.34 (84–652)	*A. simplex* s.str. *A. pegreffii*	M,L,S,G,Mu	88.00 (82–94)	15.51 ± 22.58 (1–126)	13.57 ± 21.73	3.2
Pleuronectiformes	25					100			
Lepidorhombus boscii[a], four-spotted scaldfish	25	32.50 ± 2.48 (30–35)	298.25 ± 69.14 (197–345)	*A. simplex* s.str. *A. pegreffii*	M,L	100	5.25 ± 1.26 (4–7)	5.25 ± 1.26	0
Scorpaenifromes	50					84.00 (74–94)			
Eutrigla gurnardus[a], gurnard	25	32.17 ± 2.11 (28–35)	333 ± 88.82 (230–437)	*A. simplex* s.str.	M,L	68.00 (50–86)	1.80 ± 1.79 (1–5)	1.50 ± 1.76	0
Scorpaena scrofa[a], scorpion fish	25	30.50 ± 3.94 (25–38)	581 ± 126.32 (319–675)	*A. simplex* s.str. *A. pegreffii*	M,L	100	6.47 ± 6.13 (1–24)	6.47 ± 6.13	0

[a] Component species, [b] secondary species

of dispersion for third-stage larvae of *A. simplex* s.l. and the completion of its life-cycle. Among the large variety of nematode larvae occurring in fish and cephalopods, only large worms of the genus *Anisakis* Dujardin, 1845 and *Pseudoterranova* Mozgovoi, 1950 are of concern as regards public health. The importance of properly identifying the larvae during fish processing has obviously been exemplified in relation to those species with public health implications. In recent years, the use of genetic methods, particularly multilocus electrophoresis, has proved quite successful in the study and detection of anisakid sibling species (Mattiucci et al. 1997, 1998). Genetic studies on the taxonomy of the *A. simplex* complex revealed the existence of three sibling species: *A. simplex* s. str., previously named *A. simplex* B, which is widespread between 30°N and the Antarctic Polar Circle; *A. pegreffii*, previously named *A. simplex* A, which appears widely distributed in the austral region between 35°S and 55°S, as well as in the Mediterranean Sea; and *A. simplex* C, which shows a discontinuous range, including Pacific Canada and the region south of 35°S (Mattiucci et al. 1997). The present survey states that *A. pegreffii* and *A. simplex* s. str. are component species in 17 paratenic host species from Galician waters. This finding widens the host range of third-stage larvae *A. simplex* s.str. and *A. pegreffii* to 12 and 6 paratenic host species, respectively, in temperate waters off the north-eastern Atlantic coast.

Mattiucci et al. (1997) showed that the paratenic hosts of *A. simplex* s.str. are mainly benthic or demersal, whereas those of *A. pegreffii* are mainly pelagic. These authors argued that the hosts with mixed infestations of both species are meso- or benthopelagic. Such differences may be related to different feeding habitats of the definitive hosts of anisakids. Based on this, the predominant use of pelagic fish hosts by *A. pegreffii* and of benthic or demersal fish hosts by *A. simplex* s.str. suggests differences in their life-cycles, which follow either a pelagic or a benthic food chain, respectively. Our data showed that 80% of the fish hosts for *A. simplex* s.str. are nektobenthic or demersal. Surprisingly, no infestation by *A. pegreffii* was found in large pelagic cephalopods. Furthermore, 57% and 43% of the fish species with mixed infestations (*A. simplex* s.str. and *A. pegreffii*) are pelagic and demersal or nektobenthic, respectively. We note that the infestation pattern of *A. simplex* s. str. in Galician waters is highly similar to those presented by Mattiucci et al. (1997) although somewhat different for *A. pegreffii*. Moreover, though *Anisakis* larvae exhibit little specificity for paratenic hosts (Holmes 1990; McClelland et al. 1990), the former finding suggests that transmission of *Anisakis* may be regulated by host preferences (Inglis 1979; Martell and McClelland 1995; Mattiucci et al. 1997). For example, Cannon (1977) showed that in southern Queensland the anisakines are not randomly distributed in the marine environment. Natural transmission occurs in specific habitats and in relation to characteristic host diets (diet and habitat being interrelated). Recently, Højgaard (1998) supported the hypothesis that *A. simplex* s.l. is better adapted to the offshore regions than to the coastal or the estuarine environment. Oshima (1972), Smith (1983a) and Nagasawa and Nakata (1984) noted that *Anisakis* larvae are 'open-water types' typically found in predators of nekton and adapted to oceanic environments. Similarly, in Galician waters all larval worms from cephalopods were identified as *A. simplex* s. str. This is not surprising considering that in the sampling area the nektonic ommastrephid squids are an important component of the diet of the long-finned pilot whale *Globicephala melas* in offshore waters close to the shelf edge (Gonzalez et al. 1994), and the adult worms collected from this final host were all identified as *A. simplex* s.str. (Abollo 1999).

In Ibero-Atlantic temperate waters, anisakid parasites are a natural part of the trophic web of marine ecosystems (Abollo et al. 1998a). In fact, encapsulated third-stage larvae of the *A. simplex* complex have previously been reported in commercially exploited cephalopods (Pascual et al. 1996a) and marine fish stocks (Sanmartin et al. 1989; Abaunza et al. 1994). Toothed whales acquire the nematodes by preying on fish and squids (Pascual 1996b) and serve as final hosts, harbouring third- and fourth- stage larvae and the adults, including sexually mature individuals (Abollo et al. 1998b). The eggs are passed into the faeces of cetaceans and embryonate in the seawater (Nagasawa 1990). Recently, Køie (1993b), Køie and Fagerholm (1993) and Køie et al. (1995) found that the larva prior to hatching is surrounded by two cuticles and thus should be considered the third-stage larva. Fish and squid, as paratenic hosts, acquire *A. simplex* s.l. by feeding on crustaceans (mainly euphausiids; Smith 1971, 1983b) that are harbouring the third-stage larvae. If small fish or squid are preyed on by larger fish or squid, the larvae are capable of re-establishing in the latter without a moult. Very large carnivorous fishes and cephalopods may thus accumulate enormous numbers of larvae. In fact, in Galician waters it was noted that larger carnivorous host species are those with higher values of prevalence, mean intensity and abundance of infestation by *A. simplex* s. l. This finding, together with the above argument on host preferences, suggests that the life-cycle of *A. simplex* s.l. in Galician waters takes place with a limited number of host species in which the accumulation effects through predatory relationships between sympatric hosts are considerable.

An important question that remains to be addressed by fisheries and aquaculture management is that widespread customs in commercial fishing and aquaculture practices may aggravate the *Anisakis* problem in Galicianlocal fish products. Firstly, commercial fishing practices may aggravate the *Anisakis* prevalence in the fishing areas. Thus, fresh evisceration of fish and disposal of heavily infested viscera at sea may result in increased abundance of the parasite in fish which feed on discarded viscera (McClelland et al. 1990). Secondly, it is well known that when some ungutted fish species are stored on ice for periods of more than a few hours, some

A. simplex larvae migrate from the viscera to the flesh (Smith and Wootten 1978; Smith 1983a). Thus, precautions should be taken on board directly after capture, particularly when eviscerating larger fish specimens. This may largely reduce the presence of anisakids in edible parts of fish which are commercially important. Nevertheless, the present study noted post-mortem migrations of *A. simplex* third-stage larvae into the flesh of some species (*S. scombrus*, *T. trachurus* and *M. poutassou*) from the fish market in Galicia, as was also stated by Smith (1984). Finally, infestation by *Anisakis* larvae has also been noted in populations of the common octopus, *Octopus vulgaris*, in cultured systems in Galician waters (authors' unpublished data), the source of infestation in these cases of intensive aquaculture being untreated, infested marine fishes often provided as food.

Acknowledgements We especially thank Prof. Lia Paggi and Simonetta Mattiucci (Istituto di Parassitologia, Universitá degli Studi di Roma, La Sapienza, Rome, Italy) for help with electrophoretic identification of anisakids. Thanks are also due to Xunta de Galicia for financial support under project XUGA30110A97.

References

Abaunza P, Villamor B, Pérez JR (1994) Infestation by *Anisakis simplex* (Nematoda: Ascaridata) in horse mackerel, *Trachurus trachurus*, and Atlantic mackerel, *Scomber scombrus*, in ICES divisions VIIIb, VIIIc, and IXa (N-NW of Spain). Sci Mar 59:223–233

Abollo E (1999) Anisáquidos en aguas de Galicia: del gen al ecosistema. PhD thesis, Universidade de Vigo

Abollo E, Gestal C, López A, González AF, Guerra A, Pascual S (1998a) Squid as trophic bridges for parasite flow within marine ecosystems: the case of *Anisakis simplex* (Nematoda: Anisakidae), or when the wrong way can be right. In: Payne AIL, Lipinski MR, Clarke MR, Roeleveld MAC (eds) Cephalopod biodiversity, ecology and evolution. S Afr J Mar Sci 20:223–232

Abollo E, López A, Gestal C, Benavente P, Pascual S (1998b) Macroparasites in cetaceans stranded on the northwestern Spanish Atlantic coast. Dis Aquat Org 32: 227–231

Ardusso Lovera DD, Quirce Gancedo S, Díez Gómez ML, Cuevas Agustín M, Eiras Martínez P, Sánchez Cano M, Sanz Antón S, Losada Cosmes E (1996) Hipersensibilidad inmediata al parásito del pescado *Anisakis simplex*. Estudio de reactividad cruzada. Rev Esp Alergol Immunol Clin 11:280–286

Audicana M, Fernández de Corres L, Muñoz D, Fernández E, Navarro JA, Del Pozo MD (1995) Recurrent anaphylasis caused by *Anisakis simplex* parasitizing fish. J Allergy Clin Immunol 96:558–560

Berland B (1989) Identification of larval nematodes from fish. In: Möller H (ed) Nematode problems in North Atlantic fish. Report from a workshop in Kiel 3–4 April 1989. Int Counc Explor Sea CM/F:6

Bush AO, Aho JM, Kennedy CR (1990) Ecological versus phylogenetic determinants of helminth parasites community richness. Evol Ecol 4:1–20

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83:575–583

Cannon LRG (1977) Some larval ascaridoids from south-eastern Queensland marine fishes. Int J Parasitol 7:233–243

Del Pozo MD, Audicana M, Díez JM, Muñoz D, Ansotegui IJ, Fernández E, García M, Etxenagusia M, Moneo I, Fernández De Corres L (1997) *Anisakis simplex* a relevant etiologic factor in acute urticaria. Allergy 52:576–579

García-Palacios L, González ML, Esteban MI, Mirabent E, Perteguer MJ, Cuéllar C (1996) Enzyme-linked immunosorbent assay, immunoblot analysis and RAST fluoroimmunoassay analysis of serum responses against crude larval antigens of *Anisakis simplex* in a Spanish random population. J Helminthol 70:281–289

González AF, López A, Guerra A, Barreiro A (1994) Diets of marine mammals stranded on the northwestern Spanish Atlantic coast with special reference to Cephalopoda. Fish Res 21:179–191

Hochberg FG (1990) Diseases of Mollusca: Cephalopoda. In: Kinne O (ed) Diseases of marine animals, vol III. Cephalopoda to Urochordata. Biologische Anstalt Helgoland, Hamburg, pp 47–227

Højgaard DP (1998) Impact of temperature, salinity and light on hatching of eggs of *Anisakis simplex* (Nematoda, Anisakidae), isolated by a new method, and some remarks on survival of larvae. Sarsia 83:21–28

Holmes JC (1990) Helminth communities in marine fishes. In: Esch GW, Bush AO, Aho JM (eds) Parasite communities: patterns and processes. Chapman and Hall, New York pp 101–130

Ikeda K, Kumashiro R, Kifune T (1989) Nine cases of acute gastric anisakiasis. Gastrointest Endosc 35:304–308

Inglis WG (1979) Patterns of evolution in parasitic nematodes. In: Taylor AER (ed) Evolution of parasites. Blackwell, Oxford, pp 79–124

Ishikura H, Kikuchi K, Nagasawa K, Ooiwa T, Takamiya H, Sato N, Sugane K (1992) Anisakidae and anisakidosis. In: Sun T (ed) Progress in clinical parasitology, vol III. Springer, Berlin Heidelberg New York, pp 43–102

Kagei N, Isogaki H (1992) A case of abdominal syndrome caused by the presence of a large number of *Anisakis* larvae. Int J Parasitol 22:251–253

Kasuya S, Hamano H, Izumi S (1989) Gastric anisakiasis with anaphylactoid reactions. ACI News 1:13–14

Kasuya S, Hamano H, Izumi S (1990) Mackerel-induced urticaria and *Anisakis*. Lancet 335: 665

Kikuchi Y, Ishikura H, Kikuchi K (1990) In: Ishikura H, Kikuchi K (eds) Intestinal anisakiasis in Japan. Infected fish, seroimmunology, diagnosis, and prevention. Springer, Tokyo Berlin Heidelberg, pp 129–143

Køie M (1993a) Nematode parasites in teleosts from 0 to 1540 m depth off Faroe Islands (The North Atlantic). Ophelia 38:217–243

Køie M (1993b) Aspects of the morphology and life cycle of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda, Ascaridoidea, Anisakidae). Can J Fish Aquat Sci 52:134–139

Køie M, Fagerholm H-P (1993) Third stage larvae emerge from eggs of *Contracaecum osculatum* (Nematoda, Anisakidae). J Parasitol 19:777–780

Køie M, Berland B, Burt MDB (1995) Development to third-stage larva occurs in eggs *Anisakis simplex* and *Pseudoterranova decipiens* (Nematoda, Ascaridoidea, Anisakidae). Can J Fish Aquat Sci 52:134–139

Martell DJ, McClelland G (1995) Transmission of *Pseudoterranova decipiens* (Nematoda: Ascaridoidea) via benthic macrofauna to sympatric flatfhishes (*Hippoglossoides platessoides, Pleuronectes ferrugineus, P. americanus*) on Sable Island Bank, Canada. Mar Biol 122:129–135

Matsumoto T, Iida M, Kimura Y, Tanaka K, Kitada T, Fujishima M (1992) Anisakiasis of the colon: radiologic and endoscopic features in six patients. Radiology 183:97–99

Mattiucci M, Nascetti G, Cianchi R, Paggi L, Arduino P, Margolis L, Brattey J, Webb S, D'Amelio S, Orecchia P, Bullini L (1997) Genetic and ecological data on the *Anisakis simplex* complex, with evidence for a new species (Nematoda, Ascaridoidea, Anisakidae). J Parasitol 83:401–416

Mattiucci S, Paggi L, Nascetti G, Ishikura H, Kikuchi K, Sato N, Cianchi R, Bullini L (1998) Allozyme and morphological identification of *Anisakis*, *Contracaecum* and *Pseudoterranova* from Japanese waters (Nematoda, Ascaridoidea). Syst Parasitol 40:81–92

McClelland G, Misra RK, Martell DJ (1985) Variations in abundance of larval anisakines, sealworm (*Pseudoterranova decipiens*) and related species, in eastern Canadian cod and flatfish. Can Tech Rep Fish Aquat Sci 1392:ix + 57p

McClelland G, Misra RK, Martell DJ (1990) Larval anisakine nematodes in various fish species from Sable Island Bank and vicinity. In: Bowen WR (ed) Population biology of sealworm (*Pseudoterranova decipiens*) in relation to its intermediate and seal hosts. Can Bull Fish Aquat Sci 222:83–118

Möller H (ed) (1989) Nematode problems in North Atlantic fish. Report from a workshop in Kiel 3–4 April 1989. Int Counc Explor Sea CM/F:6

Molyneux DH (1988) Control. In: FEG Cox (ed) Modern parasitology. A textbook of parasitology, 2nd edn. Blackwell, Oxford, pp 243–265

Montoro A, Perteguer MJ, Chivato T, Laguna R, Cuéllar C (1997) Recidivous acute urticaria caused by *Anisakis simplex*. Allergy 52:174–176

Myers BJ (1976) Research then and now on the *Anisakis* nematodes. Trans Am Microsc Soc 95:137–142

Nagasawa K (1990) The life cycle of *Anisakis simplex*: a review. In: Ishikura H, Kikuchi K (eds) Intestinal anisakiasis in Japan. Infected fish, sero-immunology, diagnosis, and prevention. Springer, Tokyo Berlin Heidelberg, pp 31–40

Nagasawa K, Nakata J (1984) Parasite fauna of oceanic squids from the northern North Pacific. Fish Res Jpn Sea Block 2:83–89

Oshima T (1972) *Anisakis* and anisakiasis in Japan and adjacent area. In: Morishita K, Komiya Y, Matsubayashi H (eds) Progress of medical parasitology in Japan, vol IV. Meguro Parasitological Museum, Tokyo, pp 301–393

Pascual S, Gestal C, Estévez JM, Rodríguez H, Soto M, Abollo E, Arias C (1996a) Parasites in commercially-exploited cephalopods (Mollusca, Cephalopoda) in Spain: an updated perspective. Aquaculture 142:1–10

Pascual S, González AF, Arias C, Guerra A (1996b) Biotic relationships of *Illex coindetii* and *Todaropsis eblanae* (Cephalopoda, Ommastrephidae) in the Northeast Atlantic: evidence from parasites. Sarsia 81:265–274

Petithory JC, Marty B (1988) L'anisakiase en France. Lett Infectiol 2:96–99

Sakanari JA, McKerrow JH (1989) Anisakiasis. Clin Microbiol Rev 2:278–284

Sanmartín ML, Quinteiro P, Ubeira FM (1989) Nematodes parasites of commercially important fish in NW Spain. Dis Aquat Org 7:75–77

Smith JW (1971) *Thysanoessa inermis* and *T. longicaudata* (Euphaussidae) as first intermediate hosts of *Anisakis* sp. (Nematoda: Ascaridata) in the northern North Sea, to the North of Scotland and at Faroe. Nature 234:478

Smith JW (1983a) *Anisakis simplex* (Rudolphi, 1809, det Krabbe, 1878) (Nematoda: Ascaridoidea): morphology and morphometry of larvae from euphausiids and fish, and a review of the life history and ecology. J Helminthol 57:205–224

Smith JW (1983b) Larval *Anisakis simplex* (Rudolphi, 1809, det Krabbe, 1878) and larval *Hysterothylacium* sp (Nematoda: Ascaridoidea) in euphausiids (Crustacea: Malacostraca) in the North-East Atlantic and northern North Sea. J Helminthol 57:167–177

Smith JW (1984) Larval ascaridoid nematodes in myopsid and oegopsid cephalopods from around Scotland and in the northern North Sea. J Mar Biol Assoc UK 64:563–572

Smith JW, Wootten R (1975) Experimental studies on the migration of *Anisakis* sp. larvae (Nematoda: Ascaridida) in herring, *Clupea harengus* L. Int J Parasitol 5:133–136

Smith JW, Wootten R (1978) *Anisakis* and Anisakiasis. Adv Parasitol 16:93–163

Smith JW, Wootten R (1979) Recent surveys of larval anisakine nematodes in gadoids from Scottish waters. Int Counc Explor Sea CM/G:46