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The occurrence and infection dynamics of *Anisakis* larvae in the black-scabbard fish, *Aphanopus carbo*, chub mackerel, *Scomber japonicus*, and oceanic horse mackerel, *Trachurus picturatus* from Madeira, Portugal

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Abstract

Larval stages of *Anisakis* spp. (Nematoda: Anisakidae) were found encapsulated or free in the viscera and abdominal cavity of the black-scabbard fish, *Aphanopus carbo*, chub mackerel, *Scomber japonicus* and oceanic horse mackerel, *Trachurus picturatus* in Madeiran waters. The prevalence of infection reached 97.2% (n = 142) for *A. carbo*, 69.5% (n = 154) for *S. japonicus* and 62.5% (n = 40) for *T. picturatus*. Considerable differences in parasite intensities between *A. carbo* and both *S. japonicus* and *T. picturatus* were found, with mean intensities up to 69.6 in *A. carbo*, while in the other two fish hosts the intensity reached only a maximum of 2.6. These differences were probably due to different feeding behaviours of the hosts. Intensities of *Anisakis* sp. in *A. carbo* were high irrespective of sex and season. No relationship between host length and prevalence of infection was observed for *A. carbo*, while for *S. japonicus* a weak positive significant relationship was found.

Introduction

Larval stages of anisakid nematodes of the genus *Anisakis* Dujardin, 1845 are commonly found in the viscera and musculature of many species of teleost fish. The fish act as intermediate or paratenic hosts, whereas marine mammals, definitive hosts, harbour the adult stages (Davey, 1971; Anderson, 1992). Although Davey (1971) considered only three valid species within the genus *Anisakis* (*A. simplex* Rudolphi, 1809, *A. physeteris* Baylis, 1923 and *A. typica* Diesing, 1860), it is now known that the genus includes more than six species genetically and morphologically differentiated (Nascetti *et al.*, 1986; Mattiucci *et al.*, 1997; Paggi *et al.*, 1998) which can occur in the same host as mixed infections. Infection by anisakids can affect the commercial value of fish, particularly when larvae are located in the musculature, and thus represent some economical loss for the fisheries industry (Smith & Wootten, 1975; Rohde, 1984; Angot & Brasseur, 1995). Accidental human infections with anisakid larvae can occur, following the consumption of poorly cooked infected fish. The resulting disease, anisakiasis, is well documented by several authors (Carvajal & Rego, 1985; Möller, 1991; Smith, 1999). The present work reports on the occurrence and infection dynamics of larval *Anisakis* spp. in three commercial fish species in Madeira: the black-scabbard fish, *Aphanopus carbo*, chub mackerel, *Scomber japonicus* and oceanic horse-mackerel, *Trachurus picturatus*. In Madeira, fish is a common food item, and in particular the three species under study (especially the

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black-scabbard fish) are routinely used in the diet of the local population.

Materials and methods

One hundred and forty two *A. carbo*, 154 *S. japonicus* and 40 *T. picturatus* from the Atlantic Ocean, Madeira (33°7′30″-32°22′20″N and 16°16′30″-17°16′38″W) were examined for nematodes. Samples of *A. carbo* were obtained from the Fisheries Department of Funchal, from October 1993 to June 1995. Samples of both *S. japonicus* and *T. picturatus* were purchased at a local fish market during October 1999 to May 2000. Fish length, weight, sex and date of purchase were recorded. For morphological studies, larvae were relaxed in tap-water, fixed in 70% ethanol at 60°C and stored in fresh 70% ethanol (Berland, 1984). Larvae were cleared in lactophenol and examined with a Zeiss Axioplan Photomicroscope, using DIC optics. For genetic analysis, live nematode larvae collected from the above mentioned fish species, were deep-frozen (−80°C) and kept so until electrophoretic analysis was made possible. Single worms were defrosted on ice, and crushed in distilled water. Homogenates were analysed by horizontal starch gel electrophoresis according to procedures previously described by Nascetti *et al.* (1986) and Mattiucci *et al.* (1997). Nineteen enzymes encoding for 24 putative loci were tested (see Mattiucci *et al.*, 2002). Procedures for the molecular characterization of anisakid larvae were described by Pontes (2001). Comparisons of host length, sex, prevalence and intensity of infection with *Anisakis* spp. were analysed, where appropriate, by simple linear regression compared to an F-test and a one-way ANOVA. Prevalence, intensity and abundance indices were calculated after Bush *et al.* (1997), and data were expressed as *Anisakis* spp. without separation of species.

Results

Larval *Anisakis* species were found free in the visceral cavity, or encapsulated on the external walls of the stomach, intestine, liver and gonads of fish. In the black-scabbard fish some larvae penetrated the musculature of the abdominal laps. Larvae were classified as L3 stages of *Anisakis* sp. belonging to Type I and Type II larvae (*sensu* Berland, 1961). Further identification of larvae using both multilocus electrophoresis and rDNA intergenic spacers (ITS-1 and ITS-2), assigned species to *Anisakis simplex* s.s., *A. pegreffii*, Campana Rouget & Biocca, 1955, *A. ziphidarum*, Paggi *et al.*, 1998, *A. physeteris* and *A. brevispiculata* Dollfus, 1968, (in *A. carbo*), while in both *S. japonicus* and *T. picturatus*, *A. typica*, *A. simplex* s.s., *A. pegreffii*, *A. ziphidarum*, and *A. physeteris* were identified. Additionally, in both *A. carbo* and *S. japonicus* two unknown *Anisakis* species were found, which were provisionally named as *Anisakis* sp. I and sp. II. Results on the genetic and molecular characterization will be published at a later stage. In *A. carbo*, the prevalence of infection of *Anisakis* spp. reached 97.2% (n = 142) (table 1), whilst in the other two fish species prevalence was 69.5% (n = 154) and 62.5% (n = 40) for *S. japonicus* (table 2) and *T. picturatus* respectively. Juveniles of *A. carbo* showed lower prevalence values (71.43%) compared with adults. The prevalence in the adult group remained at 100% except for the length group >125 cm where a slight decrease was observed (table 1). In *A carbo* the intensity of infection showed no significant variations between seasons (ANOVA, $F = 1.042$, d.f. = 3, $P = 0.377$) and there were no differences in intensity between males and females (ANOVA, $F = 0.929$, d.f. = 1, $P = 0.337$). The number of larvae per fish ranged from 1 to 230 (table 1). The relationship between host length and intensity was positive and not significant ($r = 0.736$, $r^2 = 0.592$, $F = 4.371$, $P = 0.095$). For *S. japonicus* the prevalence of infection showed an increasing tendency with fish length, from 58.6 to a maximum of 80.8% (table 2). In this fish host the intensity of *Anisakis* spp. ranged from 1 to 6 larvae (table 2). Positive but insignificant relationships were found between the prevalence of infection and fish length ($r = 0.143$, $r^2 = 0.021$, $F = 3.194$, $P = 0.08$), but a weak significant difference was found between intensity of infection and fish length ($r = 0.224$, $r^2 = 0.05$, $F = 8.004$, $P = 0.0053$).

In *T. picturatus* the nematode load ranged from 1 to 7 but no statistical analysis was performed in this case, as only 40 fish ranging in length from 17 to 28 cm were examined. The total number of nematodes recovered from these fish was 66 with a mean intensity of 2.64 ± 0.329 (SE) and a mean abundance of 1.65 ± 0.329 (SE).

Discussion

The black-scabbard fish, *A. carbo*, is a deep-water fish, reaching up to 150 cm in length and feeding mainly on cephalopods and fish (Freitas, 1998), whereas both the chub mackerel, *S. japonicus* and the oceanic horse

Table 1. The prevalence, intensity and abundance of larval *Anisakis* spp. in *Aphanopus carbo*, relative to fish length, from October 1993 to June 1995.

Length class of fish (cm)	No. of fish	No. of infected fish	Prevalence (%)	No. of parasites (range)	Mean intensity ± S.E.	Abundance ± S.E.
60–82	7	5	71.43	24 (1–14)	4.80 ± 2.33	3.43 ± 1.84
105–110	12	12	100	550 (17–131)	55.0 ± 10.79	55 ± 10.79
110–115	45	45	100	1755 (1–230)	47.43 ± 6.63	47.43 ± 6.63
115–120	35	35	100	1637 (6–218)	51.16 ± 7.82	51.16 ± 7.82
120–125	29	29	100	1288 (8–131)	46.0 ± 5.84	46.0 ± 5.84
>125	14	12	85.71	766 (23–220)	69.64 ± 18.98	58.92 ± 17.51
Total	142	138	97.20	6020	48.94 ± 3.75	47.40 ± 3.71

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Table 2. The prevalence, intensity and abundance of larval *Anisakis* spp. in *Scomber japonicus*, relative to fish length, from October 99 to May 2000.

Length class of fish (cm)	No. of fish	No. of infected fish	Prevalence (%)	No. of parasites (range)	Mean intensity ± S.E.	Abundance ± S.E.
<23	29	17	58.62	26 (1–3)	1.53 ± 0.15	0.90 ± 0.17
24–25	30	17	56.67	37 (1–6)	2.18 ± 0.40	1.23 ± 0.30
25–26	26	21	80.77	55 (1–6)	2.62 ± 0.33	2.12 ± 0.34
26–27	25	19	76.0	46 (1–4)	2.42 ± 0.21	1.84 ± 0.26
>27	44	33	75.0	75 (1–6)	2.27 ± 0.18	1.71 ± 0.20
Total	154	33	69.50	239	2.23 ± 0.12	1.55 ± 0.12

mackerel, *T. picturatus* are epipelagic fish species feeding on small crustaceans and fish (Whitehead *et al.*, 1986). The large number of *Anisakis* larvae found in *A. carbo* (see table 1) compared with the number of larvae found in the other two hosts examined (see table 2) can be explained by the predatory voracity of *A. carbo* and its feeding strategy being non-selective and generalist (Freitas, 1998). This fish host accumulates large numbers of *Anisakis* larvae, which will be transmitted to several different whale hosts, thus participating in the *Anisakis* deep-water life cycle. In *A. carbo* the *Anisakis* species present were predominantly those maturing in whales. Other studies showed that ascaridoid larvae accumulate in marine fish with age, predatory behaviour and increased feeding (Smith, 1984) although some variation can occur as observed by Hemmingsen *et al.* (1993) for cod, *Gadus morhua*. On the contrary *S. japonicus* and *T. picturatus,* although with relative high prevalence of infection (69.5 and 62.5%), harboured low numbers of *Anisakis* larvae (maximum 7 per fish). This is probably a consequence of their smaller size and feeding on small planktonic crustaceans. The level of infection of planktonic crustaceans, euphausiids in particular, with *Anisakis simplex* larvae as demonstrated by Smith (1983) was generally no more than 4%. Additionally, as both *S. japonicus* and *T. picturatus* are inhabitants of the epipelagic ecosystem, where a number of other fish species act as paratenic hosts for *Anisakis* spp. the need to carry heavy burdens of *Anisakis* larvae is negligible. On the other hand, the deep-water ecosystem is a more hostile environment, with fewer fish species presumably susceptible to transport larval stages of the nematodes. A combination of the feeding ecology of the fish species, i.e. predominantly zooplankton feeders versus a voracious predatory feeder, and the habitat characteristics may explain the differences in the intensity of infection with the anisakids. A relatively low intensity of infection with *A. simplex* (4.8) in the epipelagic fish *T. trachurus* was previously reported from southern Spanish waters (Adroher *et al.*, 1996). In the present case, mean intensities with anisakids were even lower (2.23 and 2.64 for *S. japonicus* and *T. picturatus* respectively). In addition, in these two epipelagic fish species a typical epipelagic dwelling *Anisakis* species, *A. typica* was found which was not seen in *A. carbo*. The prevalence values of *A. simplex* in *T. trachurus* from the Cantabrian Sea and Spanish Atlantic coast were 49.5% and 36.0%, for the length classes <23 up to >30 cm long (Adroher *et al.*, 1996), which is in accordance with present results. Similarly Sanmartin-Duran *et al.* (1989) found a prevalence of 43.9% for *T. trachurus* from northwestern Spain. A slightly higher prevalence was found in the same fish host examined from a Lisbon market (51.4%, Carvalho-Varela & Cunha-Ferreira, 1984). *Trachurus picturatus* from the Ligurian Sea showed remarkably higher values of prevalence and intensity of *A. simplex* (Manfredi *et al.*, 2000). These differences in both the prevalence and intensity of *Anisakis* sp. in the same species, *T. picturatus*, could be interpretated as an indication of the existence of non-mixing populations of the same fish species.

Investigations on the prevalence and intensities of *Anisakis* spp. in the black-scabbard fish were only done for the Madeiran samples. The high loads of *Anisakis* found in this fish host, and the fact that anisakid larvae were seen penetrating muscles, may point to a possible health problem for consumers.

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