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Anisakid parasites of the pouting (*Trisopterus luscus*) from the Cantabrian Sea coast, Bay of Biscay, Spain

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Abstract

An epidemiological survey was undertaken of anisakids in 139 specimens (length: 13.2–24.5 cm) of pouting or bib (*Trisopterus luscus*) captured off the coast of northern Spain in the Cantabrian Sea. Third-stage larvae of two species of nematodes, *Anisakis* larvae type I and *Hysterothylacium aduncum*, were isolated. One adult female *H. aduncum* was also detected in the intestine of one pouting. Total prevalence of anisakids was 88.5%. *Hysterothylacium aduncum* and *Anisakis* showed, respectively, prevalence of 87.8% and 22.3%, mean intensity of 19.7 and 3.5, and mean abundance of 17.3 and 0.8. Analysis of infection parameters as a function of host length revealed a much higher prevalence in pouting specimens with length <20 cm (94.4% for *H. aduncum*; 28.0% for *Anisakis*) than in those with length ≥ 20 cm (65.6% for *H. aduncum*; 3.1% for *Anisakis*). The high mean intensity of *Anisakis* in muscle of parasitized pouting (5.9) may pose human health risks, although these are minimized by eating only thoroughly cooked pouting, as is the custom in Spain.

Introduction

Anisakids are present as third-stage larvae (L3), in a wide variety of fish and aquatic invertebrates, which act as intermediate/paratenic hosts in the life cycle of these nematode parasites. The fish appear to act by storing the parasites and transporting them to their final hosts, fish-eating aquatic vertebrates. However, humans can intervene in the life cycle of some anisakids as an accidental host. Thus, *Anisakis simplex*, *A. physeteris* and *Pseudoterranova decipiens* have been found in the human body, causing different disorders given the general designations of anisakidosis, anisakiosis or anisakiasis (Clavel *et al.*, 1993; Mercado *et al.*, 2001; Umehara *et al.*, 2007). Allergic phenomena have also been related to the intake of fish infected with *A. simplex* or *Hysterothylacium aduncum* (Fernández-Caldas *et al.*, 1998; Valero *et al.*, 2003; Del Rey Moreno *et al.*, 2006). Therefore, commercial fish destined for human consumption must be tested for the presence of anisakids as part of a preventive approach to reduce the increasing incidence of anisakiasis and related allergies. At our laboratory, parasitization studies are carried out on the more common varieties of fish sold in Spain. We report here the results of a study of *Trisopterus luscus* (common names: pouting, bib, whiting pout, pout whiting or pout) in which *Anisakis* larvae type I (*sensu* Berland, 1961) and *H. aduncum* were detected in both viscera and muscle. There have only been a few reports on parasitization by anisakids in the *Trisopterus* genus. Køie (1993) reported the presence of *A. simplex*, *Contracaecum* sp., *C. osculatum*, *H. aduncum* and *H. rigidum* larvae in *T. minutus* and of *H. aduncum* larvae in *T. esmarkii*. Only Rodríguez-Merayo & Villegas (1993), Abollo *et al.* (2001) and Silva & Eiras (2003) appear to have studied *T. luscus*, demonstrating the presence of *Hysterothylacium* and *A. simplex* in pouting.

Materials and methods

We purchased, in our local public fish market (Granada, Spain), 139 specimens of pouting from the port of Ondarroa (43°19.5′N, 02°25.4′W) caught in 1999

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on the Spanish coast of the Bay of Biscay. They were identified as *Trisopterus luscus* L. (Pisces, Gadidae) by their morphological features (Cohen *et al.*, 1990). In the laboratory, the fish were measured to the nearest 0.1 cm and dissected to separate viscera and muscle, which were then subjected to pepsin digestion (Huss & Drewes, 1989). Larvae were gathered from the liquid resulting from pepsin digestion and washed with 0.9% NaCl solution. Subsequently, after preservation in 70% ethanol, they were cleared in lactophenol and mounted for microscopic observation and identification according to the compatibility of their morphological features with descriptions by Berland (1961), Petter & Maillard (1988) and Petter & Cabaret (1995).

The association between fish length and prevalence was analysed by using the Fisher's exact test for comparing prevalences and the bootstrap two-sample *t*-test for comparing mean intensities and mean abundances. Free QP 3.0 computer software was used for these analyses. It was developed by Reiczigel & Rózsa (Quantitative parasitology 3.0, 2005, Budapest; http://www.behav.org/qp/qp.htm) to deal with the notoriously left-biased frequency distributions of parasites, based on the theoretical background published by Rózsa *et al.* (2000).

Results

The study group comprised 139 pouting with lengths ranging from 13.2 to 24.5 cm (mean ± SD = 18.6 ± 2.4 cm). Anisakids were found in 88.49% of the fish, with a mean intensity of 20.44 and mean abundance of 18.09 (table 1). Detected species were *Anisakis* larvae type I (*sensu* Berland, 1961) and *Hysterothylacium aduncum.* All individuals were in L3 stage except for one, identified as an adult female *H. aduncum aduncum* by morphobiometric study (Petter & Cabaret, 1995). Infection parameters were much higher for *H. aduncum* than for *Anisakis*, with 87.77% of fish parasitized by the former versus 22.30% by the latter (table 1).

L3 were gathered from both viscera and muscles of these fish. Table 2 shows the localization of the parasites. *Anisakis* larvae were found in the muscle of 5.04% of fish and *H. aduncum* larvae in the muscle of 51.08% of fish.

Analysis of prevalence as a function of host length revealed that parasitization by both *Anisakis* and *H. aduncum* significantly decreased with an increase in the length of the fish (fig. 1). However, the data did not show a significant correlation between parasitic intensity and host length.

Discussion

Age–growth studies on *T. luscus* off the Cantabrian Asturian coast (Merayo & Villegas, 1994) and off the Aquitaine French coast (Puente, 1988), both in the Bay of Biscay, yielded comparable results and were applied to the fish in the present study. Hence, based on the global equation published by Merayo & Villegas (1994), the fish in this study are estimated to be in their first or second year of life, ranging in age from 10 to 24 months. Pouting mature sexually during their second year of life (Puente, 1986, cited by Puente, 1988; Foucher, 2001), therefore the longest fish in this study (>20 cm or >17 months) could be considered mature. Pouting is a benthopelagic fish that can live up to 4–5 years.

In the present study, *Anisakis* L3 larvae type I (*sensu* Berland, 1961) and L3 of *H. aduncum* were detected at higher infection rates than previously described in pouting captured in northern Spain (Rodríguez-Merayo & Villegas, 1993; Abollo *et al.*, 2001). Our detection of an adult female *H. aduncum* is the first reported finding of an adult of this species in pouting, indicating that, at least occasionally, this fish can act as its final host.

Both *Anisakis* spp. and *H. aduncum* were previously isolated in fish (e.g. hake, mackerel, horse mackerel, bogue) from this geographical area. Studies reported that >96% of *Anisakis* type I *sensu* Berland, 1961 larvae recovered from the fish were genetically identified as *A. simplex* s.s. and <4% as *A. pegreffii* (Mattiucci *et al.*, 2004, 2008). Other *Anisakis* species with type I *sensu* Berland, 1961 L3 (i.e. *A. typica, A. ziphidarum* and *A. simplex* C) have not been reported in the Atlantic Ocean above a latitude of 40°N (Davey, 1971; Mattiucci *et al.*, 2002, 2007; Marques *et al.*, 2006). Therefore, the *Anisakis* larvae collected in the present survey can be assumed to be *A. simplex* s.l., predominantly *A. simplex* s.s.

Analysis of anisakid prevalence as a function of host length (fig. 1) showed that parasitization by *Anisakis* and *H. aduncum* was lower in fish of longer length, contrasting with reports on other host fish (Grabda, 1974; Bussmann & Ehrich, 1979; McGladdery & Burt, 1985; Takao, 1990; McClelland & Marcogliese, 1994; Adroher *et al.*, 1996; Hemmingsen *et al.*, 2000; Valero *et al.*, 2000, 2006). We detected a markedly higher prevalence of parasitization by *H. aduncum* (65–97%) in pouting with lengths of 14.5–23.5 cm than was found by Rodríguez-Merayo & Villegas in 1993 (3–30% *Hysterothylacium* sp., possibly *H. aduncum*). Moreover, fish with lengths of 14.5–17.5 cm showed maximum parasitization (>92%) in our study and minimal parasitization (3%) in their study, which reported highest parasitization levels (35–37%) in the longest fish (32.6–41.5 cm). Likewise, the prevalence of *Anisakis* larvae was highest (22–36%) in the shortest fish (<20 cm) in the present study and lowest (3.1%) in fish with lengths of 20–24.5 cm. Although Rodríguez-Merayo & Villegas (1993) reported prevalences of *Anisakis* larvae (probably *A. simplex* s.l.) of 7% in fish with lengths of 17.6–23.5 cm versus 29% in fish with lengths of

Table 1. Parasitic infection levels by anisakid nematodes in *Trisopterus luscus*.

	Prevalence (%)	Mean abundance ± SE	Mean intensity ± SE (range)
Anisakids	88.49	18.09 ± 19.54	20.44 ± 19.58 (1–105)
Anisakis larvae type I	22.30	0.78 ± 3.48	3.52 ± 6.76 (1–28)
Hysterothylacium aduncum	87.77	17.30 ± 19.49	19.71 ± 19.63 (1–105)

Table 2. Parasitic infection levels by anisakid nematodes in muscle and viscera of *Trisopterus luscus*.

	Prevalence (%)	Mean abundance ± SE	Mean intensity ± SE (range)
Anisakids			
Viscera	87.05	16.53 ± 18.26	18.98 ± 18.34 (1–93)
Muscle	52.52	1.56 ± 2.98	2.97 ± 3.57 (1–19)
Anisakis larvae type I			
Viscera	21.58	0.48 ± 1.63	2.27 ± 2.92 (1–13)
Muscle	5.04	0.29 ± 2.00	5.86 ± 7.34 (1–18)
Hysterothylacium aduncum			
Viscera	87.05	16.04 ± 18.30	18.42 ± 18.46 (1–93)
Muscle	51.08	1.27 ± 2.08	2.48 ± 2.35 (1–12)

38.6–41.5 cm, prevalence was not significantly correlated with length. However, they found a significant association between intensity and length, indicating a possible accumulation of parasites with higher age (Bussmann & Ehrich, 1979).

Taken together with previous data, the present findings indicate a progressive increase in the parasitization of pouting by anisakids in the Cantabrian Sea. In 1971, Quadros Benvegnu (1971) had detected a low parasitization (4.8%) by 'nematodes' (probably anisakids) in pouting from these waters. Variations in observations do not appear to be influenced by seasonal changes (Rodríguez-Merayo & Villegas, 1993), and similar parasitization levels were found over the course of a year (Rodríguez-Merayo, 1991, cited by Rodríguez-Merayo & Villegas, 1993). However, changes in parasitization were observed as a function of the age of pouting, which showed a clear shift in diet from calanoids to mysids when a length of 50 mm was reached and a second shift to shrimp at a length of 130 mm, although fish of this size continued to feed largely on mysids (Hostens & Mees, 1999).

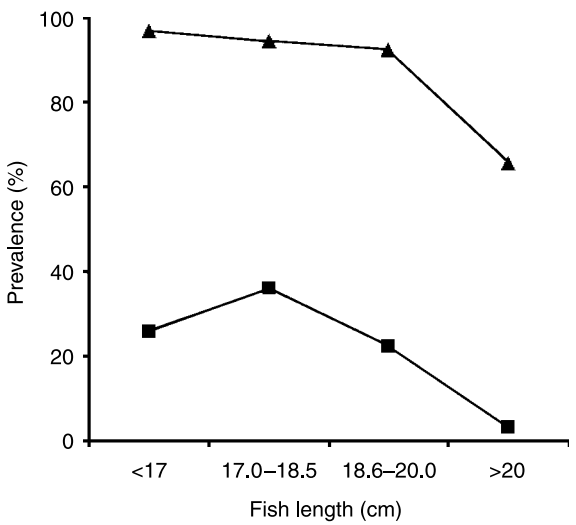

Fig. 1. Prevalence of each anisakid species in pouting as a function of fish length. The number of examined hosts per length class was: 31 (<17 cm), 36 (17.0–18.5 cm), 40 (18.5–20.0 cm) and 32 (>20 cm). Anisakid prevalence was significantly associated with host length for all anisakids in *Trisopterus luscus* [Fisher's exact test; $P < 0.005$ for *Anisakis* larvae type I (■); $P < 0.001$ for *Hysterothylacium aduncum* (▲)].

It remains to be investigated whether this apparent increase in the parasitization of pouting by anisakids (especially *Hysterothylacium*) is influenced by the local setting (e.g. specific littoral ecosystems) or produced by factors related to the Cantabrian Sea as a whole (e.g. increase in parasite abundance, fishing-induced decrease in host abundance), or by other factors affecting the stability of the marine ecosystem in this area. Thus, Klimpel & Rückert (2005) showed that the abundance of *H. aduncum* in haddock and whiting from the North Sea is related to the abundance of hyperiids (intermediate hosts of this worm) in the surveyed area and in the stomach of the fish. Moreover, very high numbers of hyperiids were observed in stratified waters. The possibility of a successful transfer of *H. aduncum* is therefore much higher in these stratified areas because there is greater availability of food for intermediate and final hosts.

With regard to the public health risk posed by the consumption of this species, we found a low prevalence of *Anisakis* larvae in the muscle but at a high mean intensity of around 6 (table 2). Previous studies detected no *Anisakis* larvae in the muscle of pouting (Rodríguez-Merayo & Villegas, 1993; Abollo *et al.*, 2001). A high prevalence of *H. aduncum* (51.08%) was found in muscle, but the intensity was only 2.48. Consumers must be informed of the need to thoroughly cook these fish, destroying all anisakid larvae, as has always been common practice in our country, to avoid the possibility of anisakiasis.

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