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

F.J. Rello†, A. Valero* and F.J. Adroher
Department of Parasitology, Faculty of Pharmacy, University of Granada, E-18071 Granada, Spain

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 $\geq$ 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 anisakidosis 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. Koie (1993) reported the presence of A. simplex, Contraacucum 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.
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 benthiopelagic 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 (Rodriguez-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 *Anakis* 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. ziphidium* 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 (Grädna, 1974; Bussmann & Ehrich, 1979; McGladdery & Burt, 1985; Takao, 1990; McClelland & Marcogliese, 1994; Adroher et al., 1996; Hemmingsen et al., 2000; Valero et al., 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 Rodriguez-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 Rodriguez-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) |
| *Anisakis*                               | 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) |
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 (Rodrı´guez-Merayo & Villegas, 1993), and similar parasitization levels were found over the course of a year (Rodrı´guez-Merayo, 1991, cited by Rodrı ´guez-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).

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 (Rodrı´guez-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.

**References**


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