

Anisakid parasites of two forkbeards (*Phycis blennoides* and *Phycis phycis*) from the Mediterranean coasts of Andalucía (Southern Spain)

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Abstract

An epidemiological survey of anisakids was carried out on 209 specimens of greater forkbeard (*Phycis blennoides*) and 58 of forkbeard (*Phycis phycis*) captured off the Mediterranean coasts of Andalucía (southern Spain). Four species of nematodes were identified: *Anisakis simplex* s.l., *Anisakis physeteris*, *Hysterothylacium aduncum* and *Hysterothylacium fabri*. The total prevalence was 62.06% in the forkbeard and 58.85% in the greater forkbeard. The highest values of prevalence (56.90%), mean intensity (5.21) and mean abundance (2.96) were all obtained for *H. fabri* in the forkbeard. The most frequent parasite in the greater forkbeard was *H. aduncum* with 51.20% prevalence and values of 3.00 and 1.53 for mean intensity and mean abundance, respectively. The infestation parameters were also analyzed according to the host length, observing a maximum of parasitized fish (91.67%) in samples of *P. blennoides* longer than 40 cm, while in *P. phycis*, the highest prevalence (82.35%), conditioned by *H. fabri* parasitization, was found in fish with lengths between 30 and 35 cm.

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1. Introduction

Anisakidosis is the disease condition in humans caused by infestation by third stage larvae (L₃) of marine nematodes of the Anisakidae family. In humans the *Anisakis simplex* larvae can cause lesions at different points of the digestive tract, usually leading to severe pain [1–3]. These parasites have also been implicated in allergies, from angioedema-urticaria to anaphylaxis [4–6]. Reports of patients affected with these nematodes are increasingly frequent in Western countries [7–9], as are reports of individuals sensitized to them ([10,11], Del Rey (2003) Estudio parasitológico, epidemiológico y clínico de la anisakidosis en la comarca de Antequera. Doctoral thesis, Universidad de Granada, Granada). Contributing

factors may be culinary habits imported from the Orient, and, above all, a greater knowledge of the disease by health professionals, as well as substantial improvements in the diagnostic methods [12–14].

The species of the *A. simplex* complex [15] are the most important from a public health point of view. A high number of commercially important fish harbour these nematodes [1,16–19], both in viscera and in musculature. Since several decades ago, the presence of anisakid larvae in commercially important fish has been considered a public health problem to such an extent that the European Union has drawn up health guidelines related to fish products.

The aim of this paper is to report and discuss anisakid nematodes in the two species of *Phycis*. The present study demonstrates four species of anisakids (*A. simplex* s.l., *Anisakis physeteris*, *Hysterothylacium aduncum* and *Hysterothylacium fabri*) in the fish species under investigation.

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2. Materials and methods

We examined two species of gadids of the genus *Phycis*: 58 specimens of forkbeard (*Phycis phycis*), with a length of between 24 and 58 cm, and 209 of greater forkbeard (*Phycis blennoides*) between 18 and 55 cm, from the Mediterranean coast of Eastern Andalucía (Spain). After identifying the host species following Cohen et al. [20], and measuring its length, it was dissected by making an incision along the ventral line from the anus to the buccal aperture. The parasites found free in the abdominal cavity were then isolated. To obtain the larvae encapsulated in the viscera and the musculature, these were subjected separately to a pepsic digestion with an HCl solution (pH 2–2.3) at 36 °C, for 3–4 h (modified after McGladery [21]). After the digestion, the nematodes were washed with a 0.9% NaCl solution. Subsequently, after preserving in 70% ethanol, they were cleared in lactophenol and identified, using the characteristics previously described [17,22–25]. To analyze the association between fish length and prevalence (Figs. 1 and 2), we used Pearson's chi-square test on the contingency tables, using the Yates' correction when at least one cell of the table has a joint frequency less than 5.

3. Results

Four species of anisakids were identified in each of the two hosts studied: *A. simplex* s.l., *A. physeteris*, *H. aduncum* and *H. fabri*. In both species of forkbeard, the L₃ of the genus *Anisakis* were mainly found encapsulated in the liver, although some larvae were also found free in the body cavity and only three in the musculature of *P. blennoides*. The L₃ larvae of *H. aduncum* were found in the mesentery

and peritoneum and the L₄ larvae and adults in the intestinal tract. However, the larvae of *H. fabri* (L₃ and L₄) were isolated in the mesentery. No adults were found. The values of total prevalence for both hosts were similar (for the forkbeard 62.07% and for the greater forkbeard 58.85%), while the mean intensity was higher in the forkbeard (4.92) than in the greater forkbeard (3.08).

A total of 321 *H. aduncum* were isolated in the greater forkbeard, of which 39.87% were L₃, 31.15% L₄ and 28.97% adults. This was the most frequent anisakid in this host with 51.20% prevalence against 5.17% obtained for the forkbeard ($P < 0.0001$), where only 3 individuals were found (1 L₃ and 2 L₄). However, in the forkbeard, 172 *H. fabri* were found, of which 19.76% were L₃ and 80.23% were found in the fourth larval stage. The prevalence of this parasite in the forkbeard was 56.90%, against 2.39% in the greater forkbeard ($P < 0.0001$). The parasitizing percentage of *A. physeteris* in both hosts (11.00% in *P. blennoides* and 3.45% in *P. phycis*) was higher than that of *A. simplex* (2.39% and 1.72%, respectively). Larvae of *Anisakis* were found in the musculature of three greater forkbeards, one larva per host (1 L₃ of *A. simplex* and 2 L₃ of *A. physeteris*). In the greater forkbeard we observed that the prevalence of anisakids increases with fish length (Figs. 1 and 2), reaching a maximum in the specimens of more than 40 cm. Nevertheless, in the forkbeard the highest prevalence was observed in the fish of between 30 and 35 cm, due to *H. fabri* (Figs. 1 and 2).

4. Discussion

In Spain, human anisakidosis is clearly related to the consumption of fresh anchovies cured in vinegar ([26], Del Rey (2003), loco citato). Nevertheless, other fish have also been implicated ([27,28], Valero et al. (1992) Descripción de un caso de anisakiosis humana. IX Reunión Científica de la APE, p. 69. León, Spain). Although the forkbeard is consumed relatively frequently in Spain, research on parasitizing anisakids is scarce. Pereira Bueno [29] reported an anisakid prevalence of 68.18% and a mean intensity of 20.6 in 22 greater forkbeard commercialized in Bilbao (Northern Spain), compared with 58.85% and 3.08, respectively, found in this paper. The results given by Pereira Bueno [29] for prevalence and intensity in the musculature of greater forkbeard confirm the risk of contracting anisakidosis through consumption of this fish. De la Torre Molina et al. [30] examined 16 eviscerated greater forkbeards, commercialized in the province of Córdoba (Southern Spain), reporting a prevalence of anisakids of 6.2%. However, the greater forkbeards of our geographical environment do not seem to constitute a public health problem due to the low prevalence of anisakids in the musculature of the fish.

In the species of anisakids found in our study, the higher prevalence of *A. physeteris* than *A. simplex* (Table 1), in

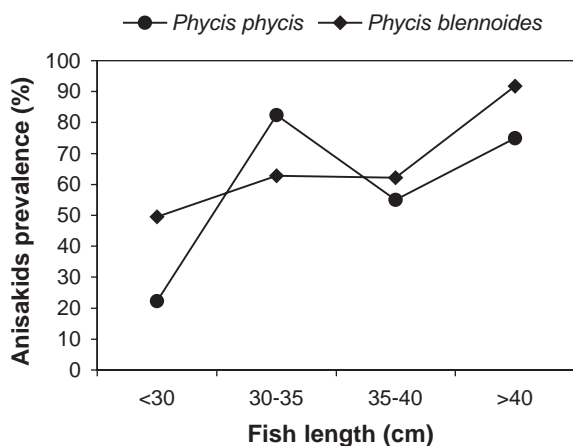


Fig. 1. Anisakids prevalence according to host length. The number of examined hosts per length class was as follows: 119 (<30 cm), 43 (30–35 cm), 23 (35–40 cm) and 24 (>40 cm) for *P. blennoides* and 9 (<30 cm), 17 (30–35 cm), 20 (35–40 cm) and 12 (>40 cm) for *P. phycis*. The anisakids prevalence is significantly associated to host length in *P. blennoides* ($P < 0.002$), but in *P. phycis* the difference is only significant when comparing the prevalence in fish <30 cm with fish >30 cm ($P < 0.02$).

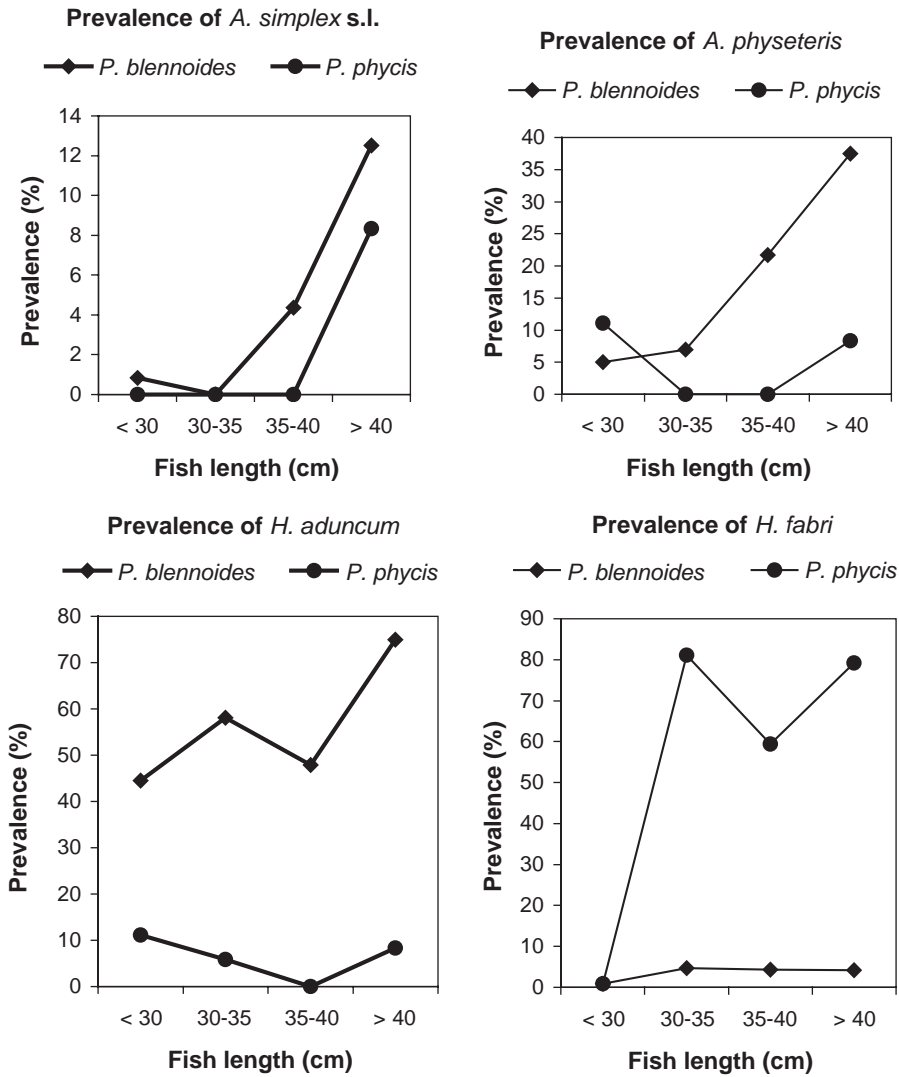


Fig. 2. Prevalence of each anisakid species per fish species according to host length. The number of examined hosts per length class was as in Fig. 1. The anisakids prevalence is significantly associated to host length for all anisakids in *P. blennoides* ($P < 0.0001$ for *A. physeteris* and *H. fabri*, $P < 0.005$ for *A. simplex* and *H. aduncum*), but only for *H. fabri* in *P. phycis* > 30 cm ($P < 0.002$).

both hosts, is worthy of mention. Clavel et al. [28] reported one case of anisakidosis in Spain, due to *A. physeteris*, in a patient who had consumed hake or blue whiting. On the other hand, it should be noted that, although there is no evidence that *Hysterothylacium* larvae parasitizes humans, their importance for public health is a result of the

sensitization that could be produced in the population, due to the fact that some species of this genus, such as those detected in this research, can share antigens with *A. simplex*, which can act as allergens [31].

In the two species of *Phycis* studied, the differences between the prevalences of *H. aduncum* and *H. fabri* are

Table 1
Parasitizing parameters of anisakids in *Phycis*

Infection parameters	<i>A. physeteris</i>		<i>A. simplex</i>		<i>H. aduncum</i>		<i>H. fabri</i>	
	<i>Phycis phycis</i>	<i>Phycis blennoides</i>	<i>Phycis phycis</i>	<i>Phycis blennoides</i>	<i>Phycis phycis</i>	<i>Phycis blennoides</i>	<i>Phycis phycis</i>	<i>Phycis blennoides</i>
Prevalence (N)	3.45 (2)	11.00 (23)	1.72 (1)	2.39 (5)	5.17 (3)	51.20 (107)	56.90 (33)	2.39 (5)
Mean abundance (L)	0.05 (3)	0.19 (39)	0.02 (1)	0.05 (10)	0.05 (3)	1.53 (321)	2.96 (172)	0.04 (9)
Mean intensity (R)	1.50 (1–2)	1.69 (1–5)	1.00 (1)	1.20 (1–5)	1.00 (1)	3.00 (1–22)	5.21 (1–44)	1.80 (1–4)

Prevalence = $100N/F$; mean abundance = L/F ; mean intensity = L/N .

F = number of examined fish; L = number of parasites; N = number of infected fish; R = range.

significant ($P < 0.0001$). This is probably related to the type of prey and habitat of the hosts studied. Macpherson [32] reported that the greater forkbeard mainly feeds on benthic organisms, their principal prey being decapods buried in the mud of the seabed, although, as the fish grows, it also tends to prey on small fish. However, decapods continue to constitute the main part of the diet, by weight. The same author also found that prey size increases with predator size, except in the case of prey which are buried in the mud and are captured randomly. The increase in the prevalence of anisakids with increasing length (Figs. 1 and 2) of the greater forkbeard must be related to the variations in its diet.

According to Papaconstantinou and Caragitsou [33], the forkbeard is piscivorous and feeds more on nektonic animals, consuming mainly decapods and brachyurans. These authors indicated that this gadid is an opportunistic browser and feeds on practically any consumable-sized animal available, reporting an increasing importance of fish in the diet with increasing size of the forkbeard.

Bearing in mind the wide variety of prey animals consumed by both species of *Phycis* and the opportunistic nature of their feeding, especially in the case of *P. phycis*, the presence of different species of anisakids must be due more to habitat than differences in the diet. The forkbeard harbours almost exclusively *H. fabri* and the greater forkbeard almost exclusively *H. aduncum*, the latter acting in some cases as the definitive host. Also, differences occurred in the prevalence of *A. physeteris*, which was 3-fold higher in the greater forkbeard (Table 1; Fig. 2). The fact that cetaceans are the final hosts for the genus *Anisakis* worms may explain why *Anisakis* larvae are so rare in these coastal fish species.

On the other hand, the significant increase in the prevalence of all the anisakids with increasing length of the greater forkbeard (Figs. 1 and 2) could be related, not only to accumulation of parasites in the host during its life, which is why their numbers increase with host age and length ([34,35], Rello (2003) Estudio de los anisakidos parásitos de pescado comercializado en Granada: Faneca, sardina y boquerón. Doctoral thesis. Universidad de Granada, Granada), but also to the change of diet. Macpherson [32], in this host captured in the Spanish Mediterranean (Catalonian coast), observed a certain selection of the prey according to its size, finding small crustaceans with a higher frequency in younger fish, while large decapods and fish were more important in older specimens. However, this increase in the parasitizing percentage with increasing length was not observed in the forkbeard, although the mean intensity of anisakids in *P. phycis* was only significant for *H. fabri* parasitization (Figs. 1 and 2).

Finally, it should be noted that the low prevalence and intensity of the anisakids found in the musculature of the *Phycis* species surveyed mean a low risk for human anisakidosis due to consumption of these fish species in our geographical area.

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