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Larval anisakids (Nematoda: Ascaridoidea) in horse mackerel (*Trachurus trachurus*) from the fish market in Granada (Spain)

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Abstract Horse mackerel (*Trachurus trachurus*) from the fish market in Granada, South Spain, were surveyed for anisakid nematodes. The fish came from fishing ports all over the country. Larval anisakids were found in 39.4% of the fish examined. In all, 26.1% were infected with third-stage larvae (L3s) of *Anisakis simplex;* 0.3%, with *A. physeteris* L3s; 31.1%, with *Hysterothylacium aduncum* L3s; and 1.7%, with fourth-stage larvae (L4s) of *Hysterothylacium* sp. Horse mackerel from Mediterranean Sea coast ports (South and Southeast Spain) had the lowest levels of infection and those from Cantabrian Sea coast ports (North Spain) had the highest levels. Variations in infection levels with host size (age) and season of capture were surveyed.

Introduction

The Anisakidae are ascaridoid nematodes that parasitize fish, mammals, birds, and, rarely, reptiles (Hartwich 1974). Although the life cycles of many anisakid species are not completely understood, it is known that marine fish can act as intermediate, paratenic, or definitive hosts. Although larval anisakids commonly infect commercially important marine fish species, there are few records of their occurrence in marine fish from Southwest Europe (Carvalho-Varela and Cunha-Ferreira 1984; Sanmartín et al. 1989).

In the present study we surveyed anisakids in horse mackerel, *Trachurus trachurus*, from the fish market in Granada, South Spain. Spain, especially in the coastal area, has a traditional gastronomy based on fish, and horse mackerel is frequently consumed. The fish market of Granada was considered suitable for the survey because it is an important fish-distribution center where 1,121 metric tons of horse mackerel were sold during 1992.

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Materials and methods

A total of 360 *Trachurus trachurus* were randomly collected from the Granada fish market from January to December 1990, with the port of origin being noted. Each fish was measured for its length and dissected. Viscera and musculature were digested separately in a pepsin HCl solution at 37°C; viscera were digested for 60–75 min and the musculature, for 2–4 h. The parasites were collected from digested material. After being washed in a 0.9% NaCl solution and observed under a light microscope, the parasites were fixed in a solution comprising 9 parts 70% ethanol to 1 part glycerin and then cleared in glycerin (Neveu-Lemaire 1936). Larvae were then identified (Ruiz-Valero et al. 1992) according to structural features (Hartwich 1974; Yoshinaga et al. 1987; Petter and Maillard 1988) but were not analyzed as to the possible genetic variation of *Anisakis simplex* for the differentiation of A and B populations (Nascetti et al. 1986).

Results

Larval anisakids were found in 142 (39.4%) of 360 horse mackerel examined in 1990. Of the 6,075 anisakids recovered, 432 were *Anisakis simplex* L3s, 1 was an L3 of *A. physeteris*, 5,631 were *Hysterothylacium aduncum* L3s, and 11 were *Hysterothylacium* sp. L4s. The respective prevalence of *A. simplex*, *A. physeteris*, *H. aduncum*, and *Hysterothylacium* sp. was 26.1%, 0.3%, 31.1%, and 1.7%.

Geographic variation in infection levels

When horse-mackerel samples were partitioned according to geographic origin, it became apparent that *Hyster-*

Table 1 Geographic variations in the prevalence of larval anisakids in horse mackerel (*ND* Not detected)

	Anisakis simplex	*Hysterothylacium aduncum*	*Hysterothylacium* sp.
Cantabrian Sea	49.5%	82.3%	ND
Atlantic Ocean	36.0%	22.0%	ND
Mediterranean Sea	6.3%	5.6%	4.2%

Table 2 Length of parasitized and nonparasitized horse mackerel[a] (*NS* Nonsignificant difference according to students's *t*-test)

	Length of fish (cm)	
	Parasitized	Nonparasitized
Jan	25.0±4.5 (16)	19.5±4.2 (14)*
Feb	28.3±2.8 (9)	23.2±5.4 (13)*
Mar	29.3±3.1 (11)	21.4±9.1 (8)*
Apr	23.6±1.4 (7)	16.0±5.5 (21)*
May	25.6±3.3 (20)	19.6±6.3 (38)*
Jun	27.4±2.2 (11)	16.0±4.6 (9)*
Jul	24.9±4.8 (11)	14.1±2.6 (20)*
Aug	27.1±5.9 (7)	15.7±3.1 (24)*
Sep	26.3±4.7 (17)	16.3±1.2 (19)*
Oct	27.1±3.0 (15)	18.9±4.0 (28)*
Nov	28.7±4.8 (12)	19.6±4.2 (10)*
Dec	22.8±4.3 (6)	19.0±3.7 (14)[NS]
Mean[b]	26.3±1.9 (12)	18.3±2.6 (12)*

([a]) Results are expressed as mean values ± SD (*n*)
([b]) Mean of the mean value for every month ± SD (12 months)
(*) $P<0.05$ according to Student's *t*-test

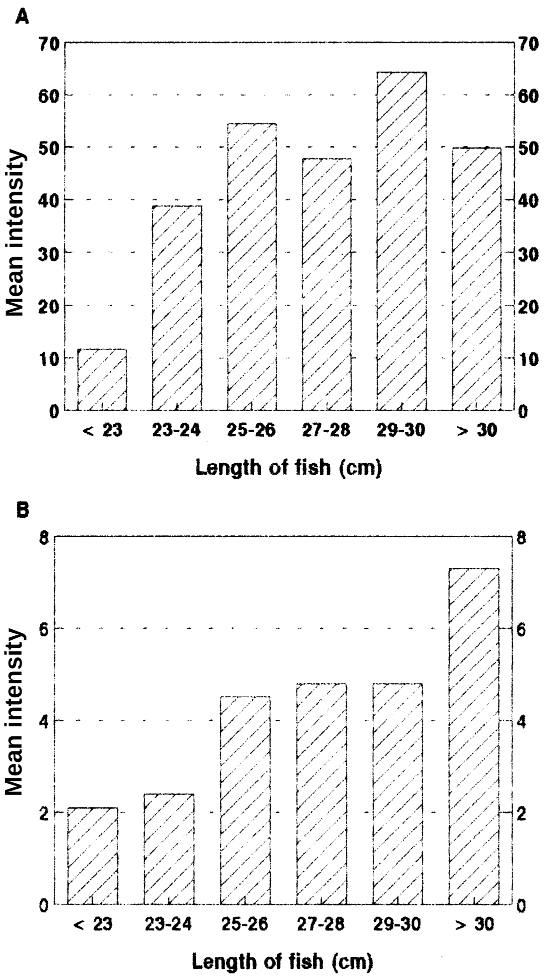

Fig. 1A,B Variation in the mean intensity of larval anisakids with the length of horse mackerel. **A** *Hysterothylacium aduncum*. **B** *Anisakis simplex*

Table 3 Variation in the prevalence of larval anisakids in horse mackerel with host length[a] (*ND* Not detected)

Parasite	Length of fish (cm)					
	<23	23–24	25–26	27–28	29–30	>30
Anisakids	9.0	60.7	71.8	73.6	73.3	90.5
Hysterothylacium sp.	3.2	*ND*	*ND*	*ND*	*ND*	*ND*
H. aduncum	2.6	39.3	61.5	58.5	73.3	90.5
A. simplex	4.2	42.9	48.7	58.5	43.3	52.4
Two species[b]	1.1	21.4	38.5	43.4	43.3	52.4

([a]) Results are expressed as percentages of the parasitized fish
([b]) Parasitized by *H. aduncum* and *A. simplex* simultaneously

othylacium sp. were found only in horse mackerel from Mediterranean harbors but that fish from Cantabrian ports had the highest level of infection overall (Table 1).

Variation in infection levels with host length

Table 2 compares the lengths of parasitized and nonparasitized fish, whereas Fig. 1 shows the variation in parasite mean intensity with host length. The intensity of *H. aduncum* was greatest in fish measuring 29–30 cm in length (mean intensity, 64.2; range, 1–300); the mean intensity of *A. simplex* larvae was 4.8 (range, 1–25). Parasitized fish measuring >30 cm in length were infected with 7.3 (range, 2–22) larvae of *A. simplex* and with 49.9 (range, 1–174) *H. aduncum* larvae.

The prevalence of *H. aduncum* and *A. simplex* in fish measuring >30 cm in length was 90.5% and 52.4%, respectively (Table 3). However, all fish parasitized by L4s of *Hysterothylacium* sp. were <20 (range, 14–20) cm long. Finally, all fish measuring <13 cm in length were uninfected.

Seasonal variation in infection levels

Anisakid mean intensity varied throughout the year (Fig. 2). *Hysterothylacium* sp. occurred in horse mackerel only from September to January. The highest levels of anisakid infection were found in large fish marketed in March and June (Fig. 3).

Distribution of anisakids in fish tissues

The distribution of larval anisakids in horse-mackerel tissues is illustrated in Table 4. Whereas *A. simplex* occurred mainly in the abdominal cavity of fish parasitized by this worm, *H. aduncum* and *Hysterothylacium* sp. showed a similar distribution both in the body cavity and in visceral organs. One *A. physeteris* L3 and five *Hysterothylacium* sp. L4s were found in the body cavity, whereas six *Hysterothylacium* sp. L4s occurred in visceral organs.

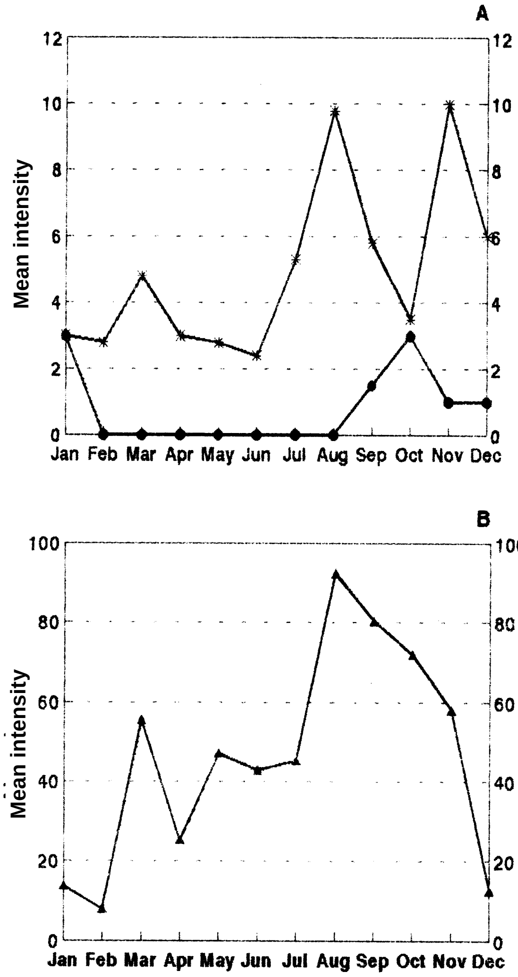

Fig. 2A,B Seasonal variations in the mean intensity of larval anisakids. **A** *Hysterothylacium* sp. (*circles*). *A.simplex* (*asterisks*). **B** *H. aduncum*

Table 4 Distribution of larval anisakids in horse-mackerel tissues[a] (*ND* Not detected)

	A. simplex	*H. aduncum*	*Hysterothylacium* sp.
Body cavity	61.2	48.3	45.5
Organs	37.0	51.7	54.5
Muscular tissue	1.8	*ND*	*ND*

[a] Data express the percentage of larvae in each location

Prevalence of anisakid infection according to the location of the parasite in fish

From Table 5 it can be seen that both *A. simplex* and *H. aduncum* occurred mainly in the body cavity of horsemackerel. Moreover, approximately equal proportions of infected fish presented parasites in their visceral organs. However, the muscular tissue of fish was generally not parasitized.

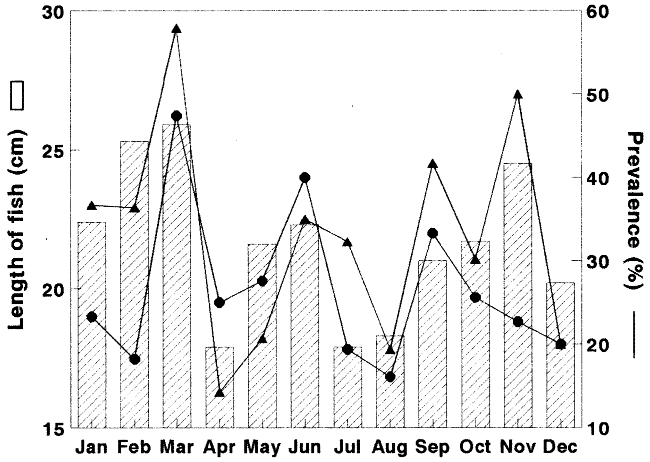

Fig. 3 Seasonal variations in the length of horse mackerel (*bars*) and in the prevalence of larval anisakids (*triangles* Prevalence of *H. aduncum*, and *circles* prevalence of *A. simplex*)

Table 5 Prevalence of anisakid infection according to the location of the parasite in horse mackerel[a] (*ND* Not detected)

	A. simplex	*H. aduncum*	*Hysterothylacium* sp.
Body cavity	83.0%	81.2%	50.0%
Organs	45.6%	58.9%	50.0%
Muscular tissue	3.3%	*ND*	*ND*

[a] Data express the percentage of fish with larvae in each location

Discussion

Horse mackerel are pelagic fish that eat small fish and planktonic crustaceans (Muus and Dahlstrøm 1966) and may therefore become infected with *Anisakis simplex* and *Hysterothylacium aduncum* larvae by consuming euphausiids, which are intermediate hosts of these nematodes (Smith 1983).

Sanmartín et al. (1989), who previously surveyed anisakids in *Trachurus trachurus* from Northwest Spain, found that the prevalence of *A. simplex* and *H. aduncum* was 43.9% and 78.0%, respectively. These records are similar to those we found in fish from the Cantabrian Sea coast (North coast of Spain; prevalence: *A. simplex*, 49.5%; *H. aduncum*, 82.3%; Table 1). In Portugal, Carvalho-Varela and Cunha-Ferreira (1984) have reported an anisakid prevalence of 51.4% in horse mackerel from Lisbon fish markets, which is higher than that we found in fish from the Atlantic Ocean coast (West and Southwest coast of Spain; Table 1). Similarly, Huang (1988) observed an anisakid prevalence of 62.5% in *T. trachurus* from Paris fish markets. Our results indicate that horse mackerels from the western Mediterranean Sea have lower levels of anisakid infection than do fish from other coasts of Spain (Table 1).

We observed that infected fish were significantly larger than uninfected fish (Table 2) and that variations in the length of fish sampled each month coincided directly with variations in *H. aduncum* prevalence throughout the year, whereas in *A. simplex* they coincided especially between March and September (Fig. 3). Furthermore, anisakid mean intensity was higher in the second half of the year (Fig. 2). We also observed that parasite mean intensity varied directly with the length (age) of horse mackerel, at least in *A. simplex* (Fig. 1). According to Smith and Wootten (1978), seasonal variations in infection levels are probably due to changes in the population of infected euphausiids in the zooplankton.

Grabda (1974) studied variations in the prevalence of *Anisakis* with host size in herrings. She found that herring measuring >30 cm in length were infected more frequently than those measuring <30 cm in length and that herring that were <20 cm long were not infected. Similarly, in our study the anisakid prevalence in horse mackerels measuring <23 cm in length was only 9% as opposed to 90.5% in those measuring >30 cm in length (Table 3).

Given the high level of infection, horse mackerel serve as a good host for anisakids, especially for *H. aduncum* (prevalence, 31.1%; mean intensity, 50.3). Larvae were found in the body cavity and viscera but were seldom observed in the skeletal musculature (Table 4). The prevalence of *A. simplex* was 26.1% and its mean intensity was 4.6. Although some larvae were found in muscles, most occurred in the body cavity (61.2%; Table 4). These results are similar to those reported by Huang (1988). In addition, most of the infected fish harbored anisakid larvae in their body cavity (Table 5).

In summary, 39.4% of the horse mackerel surveyed in the present study were parasitized by anisakid larvae. There is no evidence that *Hysterothylacium* larvae are pathogenic in humans or that these nematodes can even survive at human body temperature (Huang 1988). Adroher et al. (1991) reported that the optimal temperature for the survival of *H. aduncum* in vitro was 16°C and that this nematode survived for only a few hours at 37°C. However, the pathogenicity of *Anisakis* larvae in humans is well known. A human infection with *A. simplex* L3s was recently reported in southern Spain (Valero et al. 1992). The patient had eaten marinated horse mackerel. Hence, horse mackerel, a fish frequently consumed by people of our geographic area, can be regarded as a potential source of anisakiasis.

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