HELMINTHOLOGIA, 42, 1: 3 – 7, 2005

Larval anisakids from horse mackerel in Portugal

C. CRUZ, A. VAZ, A. SARAIVA

Departamento de Zoologia e Antropologia, Faculdade de Ciências, Universidade do Porto. Praça Gomes Teixeira, 4099-002 Porto, Portugal, E-mail: *cfcruz@fc.up.pt*

Summary

Horse mackerel (*Trachurus trachurus*) obtained monthly in a fish market of Oporto from October to August were surveyed for anisakid nematods (Nematoda: Anisakidae) in muscle and body cavity. The larval anisakids (*Anisakis* sp. L_3 and *Hysterothylacium* sp. L_3) were found in body cavity but not in muscle. The infection levels of total larval anisakids (prevalence = 38.3 %; mean intensity = 2.2; mean abundance = 0.84), *Anisakis* sp. (prevalence = 18.7 %; mean intensity = 2.0; mean abundance = 0.37) and *Hysterothylacium* sp. (prevalence = 22.0 %; mean intensity = 2.0; mean abundance = 0.44) were low. Significant positive correlations were found between host length and *Anisakis* sp. occurrence, *Anisakis* sp. abundance, total larval anisakids occurrence and total larval anisakids abundance. The variation observed in infection levels throughout the year was discussed.

Key words: *Anisakis* sp.; *Hysterothylacium* sp.; horse mackerel; Portugal

Introduction

Anisakid nematodes (Nematoda: Anisakidae) are common parasites of marine fish that are used as definitive, intermediate or paratenic hosts. The definitive hosts are usually the top predators of the food web like marine mammals, piscivorous birds and piscivorous fishes. Numerous fish species are paratenic hosts in these life cycles (Dick & Choudhury, 1999). Larvae of some of these nematodes, like *Anisakis simplex* and *Pseudoterranova decipiens*, can infect humans after ingestion of raw or undercooked parasited fish (Ubeira, 2000).

Horse mackerel, *Trachurus trachurus* L., is a fish species where the larval anisakids *Anisakis simplex* L_3 and *Hysterothylacium aduncum* L_3 have been described (Sanmartin *et al*., 1994; Abaunza *et al*., 1995; Adroher *et al*., 1996; Manfredi *et al*., 2000; Abollo *et al*., 2001). The definitive hosts for these parasite species are respectively marine mammals and piscivorous fishes. Horse mackerel are pelagic fish that eat planktonic crustaceans, squids and small fish (Whitehead *et al*., 1986) and may therefore become infected with *Anisakis* larvae by feeding on euphausiids, squids or small fish (Smith,1974; Smith, 1983a; Sanmartin *et al*., 1994; Abollo *et al*., 2001) and with *Hysterothylacium* larvae by consuming euphausiids and copepods (Smith,1983a; Balbuena *et al*., 2000).

Although Portuguese population commonly consumes horse mackerel, records about anisakids from this host in Portugal are very scarce (Carvalho Varela & Cunha Ferreira, 1984; Silva & Eiras, 2003).

In the present study larval anisakids were surveyed in horse mackerel from a fish market of Oporto, Portugal, during eleven months.

Material and Methods

A total of 209 specimens of *Trachurus trachurus*, obtained monthly in a fish market of Oporto from October 1998 to August 1999, were examined approximately 12 to 36 h after capture, for the presence of anisakids in muscle and body cavity. Each specimen was measured, dissected and the body cavity was examined for the presence of anisakids. The larvae were removed, killed in hot 70 % ethanol, fixed in 70 % ethanol and cleared in glycerine. Portions of perivisceral musculature of approximately 4 g were digested in a pepsin HCl solution at 37ºC for 7 hours (Sanmartin *et al*., 1994) and observed by stereomicroscope for the presence of larvae. Additionally, portions of musculature were pressed between two glasses and examined by light microscopy. The generic identification of the larvae was based on the morphologic characteristics according to Grabda (1991) and Moravec (1994). The prevalence, mean intensity and mean abundance of *Anisakis* sp., *Hysterothylacium* sp. and total larval anisakids were determined. The above mentioned parameters were also determined for each host size class (15.0 – 17.9 cm; 18.0 – 20.9 cm; 21.0 – 23.9 cm; 24.0 – 26.9 cm; 27.0 – 29.9 cm) and for each year season. The significance of differences between *Anisakis* sp. and

Hysterothylacium sp. occurrence, intensity and abundance were analysed by a Chi-square test (the intensity and abundance values ≥ 4 were combined in the same category). The correlation between host length and occurrence, intensity and abundance of *Anisakis* sp., *Hysterothylacium* sp. and total larval anisakids were analysed by Gamma correlation (Siegel & Castellan, 1989) using the above defined host size classes. The Chi-square test was used to compare the occurrence of *Anisakis* sp., *Hysterothylacium* sp. and total larval anisakids among the year seasons. No statistical method was used to compare the intensity and abundance among the year seasons, because the frequencies of a great number of classes were too small.

Results

Larval anisakids were found in 80 (38.3 %) out of 209 horse mackerel. All the larvae were found in the body cavity or at the surface of visceral organs and no larvae were observed in the muscle. 78 out of 175 larvae were *Anisakis* sp. L_3, 91 *Hysterothylacium* sp. L_3 and 6 larvae were not identified. Only 6 specimens were infected simultaneously by *Anisakis* sp. and *Hysterothylacium* sp. larvae.

Table 1 shows the prevalence, mean intensity and mean abundance of *Anisakis* sp., *Hysterothylacium* sp. and total larval anisakids in the overall sample and in each host size class. No significant differences were found between *Anisakis* sp. and *Hysterothylacium* sp. occurrence ($\chi^2 = 0.531$, d.f. = 1, $P > 0.05$), intensity ($\chi^2 = 4.541$, d.f. = 3, $P > 0.05$) and abundance ($\chi^2 = 5.234$, d.f. = 4, $P > 0.05$) in the overall sample. When horse mackerel samples were partitioned according to host length, significant positive correlations were found between host length and *Anisakis* sp. occurrence (G = 0.5232, z = 6.1908, $P < 0.05$), *Anisakis* sp. abundance (G = 0.5563, z = 6.2389, $P < 0.05$), total larval anisakids occurrence (G = 0.2767, z = 3.6590, $P < 0.05$) and total larval anisakids abundance (G = 0.2367, z = 3.4200, $P < 0.05$). No significant correlations were found between host length and *Hysterothylacium* sp. occurrence (G = -0.0437, z = -0.4856, $P > 0.05$), *Hysterothylacium* sp. intensity (G = 0.0734, z = 0.5065, $P > 0.05$) and *Hysterothylacium* sp. abundance (G = -0.0342, z = -0.3962, $P > 0.05$), as well as between host length and *Anisakis* sp. intensity (G = 0.3074, z = 1.5956, $P > 0.05$) and total larval anisakids intensity (G = 0.0325, z = 0.3026, $P > 0.05$). The highest values of *Hysterothylacium* sp. mean intensity, *Hysterothylacium* sp. mean abundance and total larval anisakids mean intensity were found in the intermediate host size class (21.0 – 23.9 cm).

Table 2 shows the prevalence, mean intensity and mean

Table 1. Prevalence, mean intensity and mean abundance of larval anisakids infection in *Trachurus trachurus* (S.D. – standard deviation, min. – minimum, max. – maximum) related to host size

	Number of fish Infected/Examined	Prevalence (%)	Mean intensity ± S.D. (min. – max.)	Mean abundance ± S.D. (min. – max.)
Anisakis sp.				
Host size class (cm)				
15.0 – 17.9	1/48	2.1	1.0	0.02 ± 0.14 (0 – 1)
18.0 – 20.9	4/39	10.3	1.0 ± 0 (1 – 1)	0.10 ± 0.31 (0 – 1)
21.0 – 23.9	10/41	24.4	2.0 ± 1.89 (1 – 6)	0.49 ± 1.25 (0 – 6)
24.0 – 26.9	12/58	20.7	2.5 ± 3.28 (1 – 10)	0.52 ± 1.77 (0 – 10)
27.0 – 29.9	12/23	52.2	1.9 ± 1.73 (1 – 7)	1.00 ± 1.57 (0 – 7)
Total	39/209	18.7	2.0 ± 2.25 (1 – 10)	0.37 ± 1.24 (0 – 10)
Hysterothylacium sp.				
Host size class (cm)				
15.0 – 17.9	11/48	22.9	1.7 ± 1.01 (1 – 4)	0.40 ± 0.87 (0 – 4)
18.0 – 20.9	9/39	23.1	1.6 ± 0.88 (1 – 3)	0.36 ± 0.78 (0 – 3)
21.0 – 23.9	8/41	19.5	2.9 ± 2.64 (1 – 7)	0.56 ± 1.60 (0 – 7)
24.0 – 26.9	15/58	25.9	2.1 ± 1.53 (1 – 7)	0.53 ± 1.19 (0 – 7)
27.0 – 29.9	3/23	13.0	1.3 ± 0.58 (1 – 2)	0.17 ± 0.48 (0 – 2)
Total	46/209	22.0	2.0 ± 1.56 (1 – 7)	0.44 ± 1.09 (0 – 7)
Total larval anisakids				
Host size class (cm)				
15.0 – 17.9	13 / 48	27.1	1.8 ± 1.07 (1 – 4)	0.50 ± 0.99 (0 – 4)
18.0 – 20.9	12 / 39	30.8	1.5 ± 0.80 (1 – 3)	0.46 ± 0.82 (0 – 3)
21.0 – 23.9	16 / 41	39.0	2.8 ± 2.35 (1 – 7)	1.07 ± 1.98 (0 – 7)
24.0 – 26.9	26 / 58	44.8	2.4 ± 2.70 (1 – 11)	1.07 ± 2.15 (0 – 11)
27.0 – 29.9	13 / 23	56.5	2.1 ± 1.75 (1 – 7)	1.17 ± 1.67 (0 – 7)
Total	80 / 209	38.3	2.2 ± 2.06 (1 – 11)	0.84 ± 1.66 (0 – 11)

abundance of *Anisakis* sp., *Hysterothylacium* sp. and total larval anisakids as well as the host length in each year season. The occurrence of *Anisakis* sp. (χ^2 = 9.657, d.f. = 3, P < 0.05), *Hysterothylacium* sp. (χ^2 = 10.306, d.f. = 3, P < 0.05), and total larval anisakids (χ^2 = 14.077, d.f. = 3, P < 0.05) was significantly different throughout the year. The highest values of mean abundance and prevalence were recorded in spring (except for *Hysterothylacium* sp. prevalence which the highest value was found in summer) and the lowest values in autumn.

Table 2. Prevalence, mean intensity and mean abundance of larval anisakids infection in *Trachurus trachurus* and host length (mean ± standard deviation, minimum – maximum) during the sampling period

	Host length (cm)	Number of fish examined	Prevalence (%)	Mean intensity	Mean abundance
Anisakis sp.					
Sampling season					
Autumn	21.3 ± 2.8 (17.3 – 26.2)	49	8.2	1.0	0.08
Winter	24.4 ± 2.9 (17.8 – 29.4)	60	25.0	1.5	0.38
Spring	22.1 ± 4.1 (15.1 – 29.5)	60	26.7	2.5	0.67
Summer	19.0 ± 4.6 (15.6 – 29.9)	40	10.0	2.8	0.28
Total	22.0 ± 4.1 (15.1 – 29.9)	209	18.7	2.0	0.37
Hysterothylacium sp.					
Sampling season					
Autumn	21.3 ± 2.8 (17.3 – 26.2)	49	10.2	2.8	0.29
Winter	24.4 ± 2.9 (17.8 – 29.4)	60	16.7	2.0	0.33
Spring	22.1 ± 4.1 (15.1 – 29.5)	60	28.3	1.9	0.53
Summer	19.0 ± 4.6 (15.6 – 29.9)	40	35.0	1.8	0.42
Total	22.0 ± 4.1 (15.1 – 29.9)	209	22.0	2.0	0.44
Total larval anisakids					
Sampling season					
Autumn	21.3 ± 2.8 (17.3 – 26.2)	49	16.3	2.2	0.37
Winter	24.4 ± 2.9 (17.8 – 29.4)	60	40.0	1.8	0.72
Spring	22.1 ± 4.1 (15.1 – 29.5)	60	48.3	2.6	1.23
Summer	19.0 ± 4.6 (15.6 – 29.9)	40	47.5	2.1	0.67
Total	22.0 ± 4.1 (15.1 – 29.9)	209	38.3	2.2	0.84

Discussion

The presence of larvae of *Anisakis* sp. and *Hysterothylacium* sp. in horse mackerel is in accordance to the reported presence of *Anisakis* sp. (Silva & Eiras, 2003), *Anisakis simplex* (Sanmartin *et al*., 1994; Abaunza *et al*., 1995; Adroher *et al*., 1996; Manfredi *et al*., 2000; Abollo *et al*., 2001) and *Hysterothylacium aduncum* (Sanmartin *et al*., 1994; Adroher *et al*., 1996) in this fish species.

Hysterothylacium sp. and *Anisakis* sp. were found in the body cavity but not in muscle. Adroher *et al*. (1996) reported absence of *Hysterothylacium aduncum* in horse mackerel muscle but Sanmartin *et al*. (1994) found a high percentage (38 %) of these larvae in this tissue. *Anisakis simplex* and *Anisakis* sp. has been reported in horse mackerel muscle, but the percentage of larvae found in this tissue varied from very low (1.8 %) to high (41.8 %) values (Sanmartin *et al*., 1994; Adroher *et al*., 1996; Abollo *et al*., 2001; Silva & Eiras, 2003). It must be stressed that very low percentages of larvae in muscle may result in a non detection of the parasite in this tissue in moderate size samples showing very low abundances (in present work only 78 *Anisakis* sp. larvae and 91 *Hysterothylacium* sp. larvae were detected in 209 fish).

The reasons for these variations are not clear. It has been reported post-mortem migration of *A. simplex* into the flesh of some host species (Smith & Wootten, 1975; Smith, 1984) and therefore the time interval between capture and observation (or gutting) may be responsible for the relative amount of larvae in muscle. It has been also suggested that the occurrence of migration into muscle may depend on the amounts of lipids in this tissue, since migration has been described in "fatty" species and is absent in "non fatty" hosts (Smith & Wootten, 1975; Cattan & Carvajal, 1984; Smith, 1984), but as far as we know no correlation study has been done between post-mortem migration and the fat content in specimens belong to the same fish species. Smith (1984) suggested that the feeding habits of the host may affect the preferences of the larvae for a microhabitat (body cavity and visceral organs or muscle) before the death of the host since species which feed on euphausiids presented consistently low proportions of larvae in muscle and piscivorous fishes had larvae more widely distributed.

The relationship between host length and *A. simplex* infestation level was previously described for *T. trachurus* by Abaunza *et al*. (1995), Adroher *et al*. (1996) and Manfredi *et al.* (2000), with higher infestation being obtained as length and age increases. In the present work prevalence and abundance are significantly correlated to host length and although intensity does not present a significant correlation, the two lower length classes showed a very low mean intensities and the three higher length classes higher mean intensities. As length classes higher than 30 cm (not inspected in present work, but observed in the above reported works) showed a marked increase in mean intensity, a positive significant correlation can be expected with the inclusion of longer fish in the sample.

In contrast, no correlations were found between host length and *Hysterothylacium* sp. prevalence, intensity and abundance. Furthermore, the lowest mean intensity was found in the largest fishes (27.0 – 29.9 cm) and the highest mean intensity in the intermediate host size class (21.0 – 23.9 cm). This situation contrasts with that recorded by Adroher *et al.* (1996) who found an increase of *H. aduncum* prevalence related to the increase of *T. trachurus* length, and the lowest mean intensity in the smallest host size class (< 23 cm) and the highest mean intensity in fish measuring 29 – 30 cm.

The total larval anisakid prevalence and abundance showed a relationship to host length similar to those described for *Anisakis* sp. in the present work. The increase of total larval anisakid prevalence with the increase of host length was previously described in *T. trachurus* by Adroher *et al.* (1996). The highest mean intensity was found in the intermediate host size class (21.0 – 23.9 cm), which is similar to that observed for *Hysterothylacium* sp. in the present work.

It must be stressed that a relationship between infection level and age or length of the host can be expected for parasites like *A. simplex* which produce a long term infestation (Koie, 2001). However changes in defence mechanisms or in diet of the host associated with age may disturb this relationship, since these factors can affect the mortality rate of the parasite and the recruitment of larvae by ingestion of the preceding infected host. The absence of relationship between *Hysterothylacium* sp. level of infection and host length suggest that these factors may be involved.

The seasonal variation in the infection levels is difficult to analyse due to the different mean host length in the sampling seasons. However, as the occurrence and abundance of *Anisakis* sp. and total larval anisakids are positive correlated to host length, it seems clear that the prevalence and mean abundance of *Anisakis* sp. and total larval anisakids observed during spring and autumn do not present the expected values for fishes of those sizes. Spring presents higher values than expected (the mean host length is similar to the overall mean and prevalences and mean abundances are higher than those observed in the total sample) and autumn lower values than expected (host length is similar and prevalences and mean abundances lower). An abundance peak of *Anisakis simplex* L_3 during spring was previously reported for cod and redfish by Stromnes & Andersen (2000). These authors suggested that an increase of euphausiids associated to an increase in the supply of eggs by definitive hosts (northward migrating whales) might be responsible for this pattern of infection. On the other hand, Smith (1983b) suggested that seasonality might not be expected because *Anisakis* eggs shed from final hosts possibly throughout the year may develop and hatch at any time. In the present work it seems premature to discuss the causes of the observed variation without additional data.

The level of *Anisakis* sp. and *Hysterothylacium* sp. infections observed in present work was generally much lower than the level of *Anisakis simplex* and *Hysterothylacium aduncum* infections in *T. trachurus* previously reported in Spain and Italy (Sanmartin *et al.*, 1994; Abaunza *et al.*, 1995; Adroher *et al.*, 1996; Manfredi *et al.*, 2000; Abollo *et al.*, 2001). Geographical differences and size of the fish inspected may be responsible for this variability. The level of infections detected in the present work is also lower than the level of infections previously reported in Portugal for horse mackerel. Carvalho Varela & Cunha Ferreira (1984) reported an anisakid prevalence of 51.4 % from Lisbon fish markets and Silva & Eiras (2003) reported a prevalence of 76 % and a mean intensity of 6.2 for *Anisakis* sp. infection from West Portuguese coast specimens with similar length (mean length = 22.9 cm) to the fish inspected in the present work. Different feeding areas associated to different diets composition and therefore distinctive recruitment of the larvae may explain this discrepancy.

References

ABAUNZA, P., VILLAMOR, B., PÉREZ, J. R. (1995): Infestation by larvae of *Anisakis simplex* (Nematoda: Ascaridata) in horse mackerel, *Trachurus trachurus*, and Atlantic mackerel, *Scomber scombrus*, in ICES Divisions VIIIb, VIIIc and Ixa (N-NW of Spain). *Sci. Mar.*, 59: 223 – 233

ABOLLO, E., GESTAL, C., PASCUAL, S. (2001): *Anisakis* infestation in marine fish and cephalopods from Galician waters: an updated perspective. *Parasitol. Res.*, 87: 492 – 499

ADROHER, F. J., VALERO, A., RUIZ VALERO, J., IGLESIAS, L. (1996): Larval anisakids (Nematoda: Ascaridoidea) in horse mackerel (*Trachurus trachurus*) from the fish market in Granada (Spain). *Parasitol. Res.*, 82: 253 – 256

BALBUENA, J. A., KARLSBAKK, E., KEVENSETH, A. M., SAKSVIK, M., NYLUND, A. (2000): Growth and emigration of third-stage larvae of *Hysterothylacium aduncum* (Nematoda: Anisakidae) in larval herring *Clupea harengus*. *J. Parasitol.*, 86: 1271 – 1275

CARVALHO VARELA, M., CUNHA FERREIRA, V. (1984): Larva migrans visceral por *Anisakis* e outros Ascarídeos: helmintozoonoses potenciais por consumo de peixes marinhos em Portugal. *Rev. Port. Ciênc. Veter.*, 79: 299 – 309

CATTAN, P. E., CARVAJAL, J. (1984): A study of the migration of larval *Anisakis simplex* (Nematoda: Ascaridida) in the Chilean hake, *Merluccius gayi* (Guichenot). *J. Fish Biol.*, 24: 649 – 654

DICK, T. A., CHOUDHURY, A. (1999): Phylum Nematoda. In WOO, P. T. K. (Ed.): *Fish Diseases and Disorders. Vol.I*

– *Protozoan and Metazoan Infections.* CABI Publishing, New York
GRABDA, J. (1991): *Marine Fish Parasitology. An Outline.* PWN – Polish Scientific Publishers, Warszawa
KOIE, M. (2001): Experimental infections of copepods and sticklebacks *Gasterosteus aculeatus* with small ensheathed and large third-stage larvae of *Anisakis simplex* (Nematoda, Ascaridoidea, Anisakidae). *Parasitol. Res.*, 87: 32 – 36
MANFREDI, M. T., CROSA, G., GALLI, P., GANDUGLIA, S. (2000): Distribution of *Anisakis simplex* in fish caught in the Ligurian Sea. *Parasitol. Res.*, 86: 551 – 553
MORAVEC, F. (1994): *Parasitic Nematodes of Freshwater Fishes of Europe*. Academia, Prague and Klumer Academic Publisher, Dordrecht
SANMARTIN, M. L., QUINTERO, P., IGLESIAS, R., SANTAMARINA, M. T., LEIRO, J., UBEIRA, F. M. (1994): *Nematodos parasitos en peces de las costas gallegas*. Diaz de Santos, S.A., Madrid
SIEGEL, S., CASTELLAN JR., N. J. (1989): *Nonparametric Statistics for the Behavioural Sciences*. McGraw-Hill Book Company, New York
SILVA, M. E. R., EIRAS, J. C. (2003): Occurrence of *Anisakis* sp. in fishes off the Portuguese West coast and evaluation of its zoonotic potencial. *Bull. Eur. Ass. Fish Pathol.*, 23: 13 – 17
SMITH, J. W. (1974): Experimental transfer of *Anisakis* sp. larvae (Nematoda: Ascaridida) from one fish host to another. *J. Helminthol.*, 48: 229 – 234
SMITH, J. W., WOOTTEN, R. (1975): Experimental studies on the migration of *Anisakis* sp. larvae (Nematoda: Ascaridida) into the flesh of herring, *Clupea harengus* L. *Int. J. Parasitol.*, 5: 133 – 136
SMITH, J. W. (1983a): Larval *Anisakis simplex* (Rudolphi, 1809, det. Krabbe, 1878) and larval *Hysterothylacium* sp. (Nematoda: Ascaridoidea) in euphausiids (Crustacea: Malacostraca) in the North-East Atlantic and northern North Sea. *J. Helminthol.*, 57: 167 – 177
SMITH, J. W. (1983b): *Anisakis simplex* (Rudolphi, 1809, det. Krabbe, 1878) (Nematoda: Ascaridoidea): morphology and morphometry of larvae from euphausiids and fish, and a review of the life-history and ecology. *J. Helminthol.*, 57: 205 – 224
SMITH, J. W. (1984): The abundance of *Anisakis simplex* L_3 in the body cavity and flesh of marine teleosts. *Int. J. Parasitol.*, 14: 491 – 495
STROMNES, E., ANDERSEN, K. (2000): "Spring rise" of whaleworm (*Anisakis simplex*; Nematoda, Ascaridoidea) third-stage larvae in some fish species from Norwegian waters. *Parasitol. Res.*, 86: 619 – 624
UBEIRA, F. M., VALINAS, B., LORENZO, S., IGLESIAS R., FIGUEIRAS, A., GARCIA-VILLAESCUSA, R. (2000): *Anisaquiosis y alergia. Un estudio seroepidemiológico en la Comunidad Autónoma Gallega*. Documentos Técnicos de Salud Pública, Serie B, nº 24. Ed. Conselleria de Sanidade e Servicios sociais (Xunta de Galicia, Espana)
WHITEHEAD, P. J. P., BAUCHOT, M-L., HUREAU, J. C., NIELSEN, J., TORTONESE, E. (1986): *Fishes of the North-eastern Atlantic and Mediterranean*. Unesco, Paris

RECEIVED JUNE 18, 2003

ACCEPTED JANUARY 23, 2004