Distribution of larval anisakids in blue whiting off Portuguese fish market

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Summary

Specimens of *Micromesistius poutassou* (n=238) obtained in a fish market of Oporto, Portugal, were examined for the presence of larval anisakids. *Anisakis* sp. L₃ larvae (prevalence = 77.7 %; mean intensity = 5.8; mean abundance = 4.5) and *Hysterothylacium* sp. L₃ larvae (prevalence = 5 %; mean intensity = 4.1; mean abundance = 0.2) were found in body cavity, mesenteries, liver and muscles. The *Anisakis* sp. intensity and abundance were significantly related to the host body length. The percentage of *Anisakis* sp. in muscle was inversely related to the host length.

Key words: *Anisakis*; *Hysterothylacium*; blue whiting; 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: adults of the genus *Anisakis* Dujardin, 1845 and *Pseudoterranova* Mozgovoy, 1953 are found in marine mammals, *Contracaecum* Raillet et Henry, 1912 in marine mammals and piscivorous birds and *Hysterothylacium* Ward et Magath, 1917 in piscivorous fishes. Numerous fish species, harbouring L₃ larvae, are paratenic hosts in these life cycles and acquire the infection by feeding on the intermediate or paratenic hosts like crustaceans (euphausiids, copepods, amphipods), fish and cephalopods (Anderson, 2000; Moravec, 1994).

The consumption of fish containing L₃ larvae constitutes a potential risk for public health since the larvae of some species, like *Anisakis simplex* Rudolphi, 1809 and *Pseudoterranova decipiens* Krabbe, 1878, can infect humans after ingestion of raw or undercooked parasited fish (Ubeira *et al.*, 2000). The economic and medical implications of the presence of the larvae, mainly in the edible parts of fish, justify the need of study of larval anisakids infections in commercial species.

The larvae can occur in body cavity, visceral organs and muscles. Reports on the distribution of *Anisakis simplex* larvae in the tissues of some fish species showed a great variation in percentage of larvae in musculature (Abollo *et al.*, 2001; Cattán & Carvajal, 1984; Silva & Eiras, 2003; Smith, 1984; Smith & Wooten, 1975; Stromnes & Andersen, 1998) and factors like the feeding habits of the host or the migratory distance for the infecting larvae within the host have been used to explain the preferences of larvae to muscles or visceral organs.

Blue whiting, *Micromesistius poutassou* Risso, 1926, is a fish species where L₃ larva of *Anisakis simplex* and *Hysterothylacium aduncum*, Rudolphi, 1802 have been reported (Abollo *et al.*, 2001; Fernández *et al.*, 2005; Manfredi *et al.*, 2000; Petter & Maillard, 1988; Sanmartín *et al.*, 1994; Smith, 1984; Valero *et al.*, 2000). Although Portuguese population commonly consumes blue whiting, records of anisakids from this host in Portugal are scarce (Silva & Eiras, 2003).

In the present work, anisakid infection in blue whiting from a fish market of Oporto, Portugal, was surveyed and the distribution of the larvae in fish organs was studied.

Materials and Methods

A total of 238 specimens of *Micromesistius poutassou* were obtained in all the year seasons (52 in summer, 69 in autumn, 64 in winter and 53 in spring) in a fish market of Oporto from July 2003 to May 2004. These specimens came from the fishing port of Matosinhos (Oporto). The fish were examined approximately 24h after capture, for the presence of anisakids in muscles, body cavity and visceral organs. Each specimen was measured and the body cavity, visceral organs and the whole muscle after dissection were examined under a stereomicroscope for the presence of anisakids. In each specimen the larvae were removed, counted and the site of infection noted. The larvae were killed in hot 70 % ethanol, fixed in 70 % ethanol and clear-

ed in glycerine. The generic identification of the larvae was based on the morphologic characteristics according to Grabda (1991) and Moravec (1994), using a light microscope. The prevalence, mean intensity and mean abundance of infection as well as the larvae distribution in the organs, were determined. The correlation between host length and occurrence, intensity and abundance of *Anisakis* sp. were analysed by Gamma correlation (Siegel & Castellan, 1989) using host length classes (16.0 – 17.9 cm; 18.0 – 19.9 cm; 20.0 – 21.9 cm; 22.0 – 23.9 cm; ≥ 24 cm) and intensity and abundance classes (0 larvae; 1 – 2 larvae; 3 – 4 larvae; 5 – 6 larvae; ≥ 7 larvae). The relation between percentage of *Anisakis* sp. larvae in muscles and host length was analysed by Gamma correlation, using the number of *Anisakis* sp. larvae present in muscle versus number of *Anisakis* sp. larvae present in other organs in each host

length classes defined above.

No statistical method was used to analyse *Hysterothylacium* sp. infection due to the low number of fish infected and larvae isolated.

Results

Larval anisakids were found in 187 (78.6 %) out of 238 blue whiting examined (length: 20.0 ± 2.1 cm). Of the 1167 larvae isolated, 1078 (92.4 %) were identified as *Anisakis* sp. larvae, 49 (4.2 %) as *Hysterothylacium* sp. larvae and 40 larvae were not identified.

Table 1 shows the prevalences, mean intensities and mean abundances of larval anisakids. *Anisakis* sp. presented a much higher prevalence and mean abundance and a slightly higher mean intensity than *Hysterothylacium* sp.

Table 1. Prevalence, mean intensity and mean abundance of anisakids larvae in *Micromesistius poutassou*

	Number of fish Infected/Examined	Prevalence (%)	Mean Intensity $\pm$ S.D. (min. – max.)	Mean Abundance $\pm$ S.D. (min. – max.)
<i>Anisakis</i> sp.	185 / 238	77.7	5.8 ± 13.9 (1 – 122)	4.5 ± 12.5 (0 – 122)
<i>Hysterothylacium</i> sp.	12 / 238	5.0	4.1 ± 6.3 (1 – 21)	0.2 ± 1.6 (0 – 21)
Total Larval Anisakids	187 / 238	78.6	6.2 ± 16.1 (1 – 152)	4.9 ± 14.5 (0 – 152)

S.D. – standard deviation, min. – minimum, max. – maximum

Table 2. Prevalence, mean intensity and mean abundance of *Anisakis* sp. larvae in *Micromesistius poutassou* related to host length

Host length class (cm)	Number of fish Infected/Examined	Prevalence (%)	Mean Intensity $\pm$ S.D. (min. – max.)	Mean Abundance $\pm$ S.D. (min. – max.)
16.0 – 17.9	18 / 29	62.1	2.0 ± 1.3 (1 – 6)	1.2 ± 1.4 (0 – 6)
18.0 – 19.9	95 / 117	81.2	2.6 ± 1.8 (1 – 9)	2.1 ± 1.9 (0 – 9)
20.0 – 21.9	38 / 51	74.5	3.9 ± 2.7 (1 – 11)	2.9 ± 2.9 (0 – 11)
22.0 – 23.9	22 / 29	75.9	11.3 ± 20.4 (1 – 88)	8.6 ± 18.3 (0 – 88)
≥ 24	12 / 12	100	33.5 ± 37.0 (2 – 122)	33.5 ± 37.0 (2 – 122)

S.D. – standard deviation, min. – minimum, max. – maximum

Table 3. Distribution of *Anisakis* sp. larvae in *Micromesistius poutassou* organs

	Body Cavity	Mesenteries	Liver	Musculature	Total
Number of fish infected	113	63	82	66	185
Prevalence (%)	47.5	26.5	34.5	27.7	77.7
Number of larvae isolated	267	175	502	134	1078
Percentage of larvae in the organ	24.8	16.2	46.6	12.4	

Table 4. Percentage of *Anisakis* sp. larvae in *Micromesistius poutassou* musculature related to host length

Host length class (cm)	Number of fish	Number of larvae in muscle/ Total number of larvae	% of larvae in musculature
16.0 – 17.9	18	11 / 36	30.6
18.0 – 19.9	95	46 / 244	18.9
20.0 – 21.9	38	19 / 148	12.8
22.0 – 23.9	22	29 / 248	11.7
≥ 24	12	29 / 402	7.2

Table 2 shows the prevalence, mean intensity and mean abundance of *Anisakis* sp. larvae in each host length class. Significant positive correlations were found between host length and *Anisakis* sp. intensity ($G = 0.494$, $z = 6.969$, $p < 0.05$) and abundance ($G = 0.333$, $z = 5.603$, $p < 0.05$). Although no significant correlations were found between host length and *Anisakis* sp. occurrence ($G = 0.149$, $z = 1.713$, $p > 0.05$) the highest and the lowest prevalence were found respectively in the higher and lowest length classes.

Table 3 shows the distribution of *Anisakis* sp. larvae in the fish organs. *Anisakis* sp. larvae were found in body cavity, mesenteries, liver and musculature. The liver and musculature were respectively the most and the least infected organ.

Table 4 shows the percentage of *Anisakis* sp. larvae in the musculature according to host length. The inverse relationship between host length and the percentage of *Anisakis* sp. larvae in muscle, suggested by the results, was confirmed by the negative significant correlation found between these variables ($G = -0.340$, $z = -7.012$, $p < 0.05$).

The low number of fish infected by *Hysterothylacium* sp. as well as the low number of larvae isolated did not allow a consistent analysis of the larvae distribution in fish organs and the study of the relationship between the infection and the host size. However it must be referred that the larvae were found in mesenteries (44.9 %), body cavity (28.6 %), liver (18.3 %) and musculature (8.2 %).

Discussion

The presence of larvae of *Anisakis* sp. and *Hysterothylacium* sp. in blue whiting is in accordance to the reported presence of *Anisakis* sp. (Silva & Eiras, 2003), *A. simplex* (Fernández *et al.*, 2005; Manfredi *et al.*, 2000; Petter & Maillard, 1988; Sanmartín *et al.*, 1994; Smith, 1984), *A. simplex* s.str. (Abollo *et al.*, 2001), *A. pegreffii* (Abollo *et al.*, 2001; Valero *et al.*, 2000), *A. physeteris* (Fernández *et al.*, 2005; Valero *et al.*, 2000) and *Hysterothylacium aduncum* (Fernández *et al.*, 2005; Sanmartín *et al.*, 1994; Valero *et al.*, 2000) larvae in this fish species.

The infection level of *Anisakis* sp. larvae reported in the present study is included in the range of values (prevalence = 63 – 99.5 %; mean intensity = 5.1 – 14.3; mean abundance = 3.7 – 14.4) reported for *Anisakis* spp. in blue whiting in Atlantic coast of Portugal and Spain (Abollo *et al.*, 2001; Fernández *et al.*, 2005; Sanmartín *et al.*, 1994; Silva & Eiras, 2003) and it is much higher than that one (prevalence = 2.66 – 65.5 %; mean intensity = 1.2 – 2.3; mean abundance = 0.03 – 1.5) reported for this host in Mediterranean coast of Spain and Italy (Manfredi *et al.*, 2000; Valero *et al.*, 2000). These results are in accordance to the successful presence of *Anisakis simplex* in Ibero-Atlantic waters reported to numerous fish host species (Abaunza *et al.*, 1995; Abollo *et al.*, 2001; Fernández *et al.*, 2005; Sanmartín *et al.*, 1994; Silva & Eiras, 2003). Probably, in Portuguese waters, blue whiting acquires *Anisakis* sp. larvae by feeding on euphausiids, which are a well known intermediate host of *Anisakis simplex*, since

the study of blue whiting diet in Portuguese coast (Cabral & Murta, 2002) showed that euphausiids are one of the most important preys of this fish species and fish and cephalopods represent only a small percentage of the food items in all ages and in all the year seasons.

Hysterothylacium sp. infection level in the present study is higher than *H. aduncum* infection level observed for blue whiting in Mediterranean coast of Spain by Valero *et al.* (2000), lower than that one observed in Galician waters by Sanmartín *et al.* (1994) and similar to that one observed also in Galician waters by Fernández *et al.* (2005).

The direct relationship between infection level (measured by prevalence, intensity or abundance) and age or length of the host can be expected for parasites like larval anisakids which cause a long term infection. However, changes in defence mechanisms or in the diet of the host (affecting the mortality rate of the parasite and the recruitment of the larvae) associated with age may disturb this relationship. Increase of anisakids infection level with age or length of the host, as well as decrease of infection in higher length classes, have been reported for several host species (Abaunza *et al.*, 1995; Adroher *et al.*, 1996; Cruz *et al.*, 2005; Fernández *et al.*, 2005; Grabda, 1976; Manfredi *et al.*, 2000; Valero *et al.*, 2000). In the present work, the significant correlations obtained between host length and *Anisakis* sp. intensity and abundance seems to reflect the accumulation of the parasite throughout the blue whiting life.

The distribution of *Anisakis* sp. observed in the blue whiting organs is in accordance to the reported preference of these larvae for visceral organs (mostly for the liver) and body cavity and consequently low percentage in the muscle of this host species. The percentage of *Anisakis* sp. larvae observed in the muscle (12.4 %) is included in the range of values (6.4 – 20 %) reported for this host (Abollo *et al.*, 2001; Sanmartín *et al.*, 1994; Silva & Eiras, 2003; Smith, 1984). 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) since species which feed on euphausiids presented consistently low proportions of larvae in muscle and piscivorous fishes had larvae more widely distributed. As referred above, the diet of blue whiting of all ages and in all the year seasons is based in euphausiids and therefore the low value observed is expected to this fish species.

The existence of an optimal migratory distance for the infecting larvae from their point of entry is a hypothesis used to explain variations in percentage of larvae in muscle according to fish size. Under this hypothesis, the percentage of larvae in muscle should be inversely related to fish size since the migratory distance to the muscle increase as the fish get large. Stromnes and Andersen (1998) tested this hypothesis in 3 host species (*Pollachius virens*, *Gadus morhua*, *Sebastes marinus*), but no relationship was found. Therefore, these authors suggested that the larvae distributions are not determined by an optimal migratory distance but possibly by the availability of nutrients within host tissues. In contrast, we found a negative significant correla-

tion between the percentage of *Anisakis* sp. larvae in muscle and fish length and therefore the percentage of larvae in blue whiting muscle may be explained by an optimal migratory distance.

Hysterothylacium larvae distribution in blue whiting organs observed in the present work is in accordance to the preference of *H. aduncum* larvae for mesenteries reported by Fagerholm (1982).

Finally, the present data concerning *Anisakis* sp. shows that the consumption of raw or undercooked blue whiting constitutes a risk for human health in this geographical area.

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