Parasitic infection levels by *Anisakis* spp. larvae (Nematoda: Anisakidae) in the black scabbardfish *Aphanopus carbo* (Osteichthyes: Trichiuridae) from Portuguese waters

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Summary: *Aphanopus carbo*, black scabbardfish, specimens from 3 Portuguese regions (the mainland, Madeira and the Azores) during four (mainland and Madeira) or two (Azores) seasons of the year were examined for the presence of *Anisakis*. All the fish examined (n=287) were infected with *Anisakis* L₃ larvae (prevalence = 100%). Significant geographical differences in intensity were found: the Azores showed the lowest mean intensity value (53.7) and Madeira the highest one (253.9). The intensity of infection was positively correlated with the host length in specimens of Sesimbra and Madeira. Significant seasonal differences in intensity were found in the studied regions. The very high values in prevalence and intensity strongly suggest that the consumption of raw or undercooked black scabbard fish is a potential risk for human health.

Keywords: *Anisakis* larvae, *Aphanopus carbo*, fish, prevalence, intensity, geographical differences, Portugal.

Introduction

Nematode larvae of the genus *Anisakis* Dujardin, 1845 (Nematoda: Anisakidae) are common parasites of marine fish and a potential risk for human health since these larvae can infect humans after the ingestion of raw or undercooked fish (Ubeira et al., 2000). The larvae, which occur in the body cavity, visceral organs and muscles in live fish, can migrate into flesh after the death of the host (Smith and Wootten, 1975; Silva and Eiras, 2003) and therefore humans are at risk of becoming infected.
The fish are the intermediate or paratenic hosts of the parasites, whereas marine mammals are the definitive ones. Fish harbouring $L_3$ larvae acquire the infection by feeding on intermediate or paratenic hosts such as crustaceans (euphausiids, copepods and amphipods), fish and cephalopods (Moravec, 1994; Anderson, 2000; Kliment et al., 2008).

The black scabbardfish, Aphanopus carbo Lowe, 1893 (Osteichthyes: Trichiuridae) is economically a very important fish species that is commonly consumed in Portugal and is highly infected by Anisakis larvae (Costa et al., 1996, 2003; Santos et al., 2009).

In the present work the Anisakis larvae infection of A. carbo sampled in 3 different Portuguese regions (Sesimbra (mainland), and the Madeira and Azores Islands) was studied and the possible geographical and seasonal differences in the parameters of infection and the relationship with fish length were analysed.

MATERIALS AND METHODS

A total of 287 specimens of Aphanopus carbo were examined for the presence of metazoan parasites. The fish were captured by fishermen in Portuguese waters of the Atlantic Ocean from autumn 2005 to summer 2006. The 3 regions analysed were Sesimbra (Portuguese west continental coast) and the Madeira and Azores Islands (Fig. 1). Samples from Sesimbra and Madeira were obtained in autumn, winter, spring and summer and those from the Azores in autumn and spring.

The fish were transported in ice boxes to the laboratory, where they were placed in individual plastic bags and frozen until further observation. After being defrosted, specimens were identified to confirm that they belonged to A. carbo and not to A. intermedius, measured (fork length) and dissected. The body cavity, visceral organs and a small portion of musculature were examined under a stereomicroscope. Any anisakid larvae found were removed, counted and conserved in 70% alcohol. Larvae identification was made by light microscopy according to structural features after being cleared in glycerine (Berland, 1961; Grabda, 1991; Moravec, 1994).

The prevalence and mean intensity of Anisakis infection were determined for the three sampling sites according to Bush et al. (1997). In addition the mean intensity was determined for each length class of the host and for each season in the three regions studied.

The significance of the differences in host length among regions and seasons and of the differences in intensity of infection were analysed by Kruskal-Wallis tests (for 3 or more samples) or Mann-Whitney tests (for 2 samples) (Siegel and Castellan, 1989). Whenever significant differences were detected among the 3 regions, analyses between the different pairs of cases were performed by the Mann-Whitney test. The relationship between the intensity of infection and the host length were analysed by Spearman rank correlation (Siegel and Castellan, 1989).

RESULTS

Anisakis $L_3$ larvae were found in all the fish examined. Their morphological features showed that they belonged to Type I and Type II larvae, as defined by Berland (1961). Some larvae were free in the body cavity, but most of them were encapsulated in all the organs examined (oesophagus, stomach, intestine, intestinal cecae, liver, spleen, gall bladder, swimbladder, gonads and kidney) the stomach being the most infected organ. It is worth mentioning that musculature, although not extensively inspected, also contained some larvae.

The length of fish captured in the 3 regions was significantly different (Table 1). The highest mean length value was found in specimens from Madeira and the lowest in specimens from Sesimbra. There were no significant seasonal variations in fish length from Sesimbra and from Madeira, but significant differences were observed between the seasons in Azores.
All the fish examined in the present study were infected (prevalence = 100%), but significantly different intensities were found in the 3 regions. Madeira showed the highest value and the azores the lowest (Table 2).

The analysis of intensity related to host length (Table 3) showed significant positive correlations for Madeira and Sesimbra, and significant negative correlation for the Azores. When the correlation analysis was carried out for each season (not shown in tables), significant positive correlations were again found for Sesimbra (in autumn, winter and summer) and Madeira (in autumn and summer), and no significant negative correlations were found. The comparison among intensities from the 3 regions for each length class again showed the highest and lowest intensity values in specimens from Madeira and the Azores, respectively (Table 3).

The seasonal analysis (Table 4) showed that intensity of the infection is significantly different throughout the year in the 3 regions. Summer (or spring in the Azores) is the season with the highest values, and winter (or autumn in Azores) is the one with the lowest. For each season, the highest and lowest intensity values were found in specimens from Madeira and the Azores, respectively.

**DISCUSSION**

The identification to species level of the nematodes infecting *A. carbo* is only possible using genetic molecular methodologies. The existence of two main clades was demonstrated by genetic studies. They included species showing Type I and Type II larval stages respectively that were closed in relation...
to their definitive hosts, and presented different geographical distributions (see Mattiucci and Nascetti 2006, 2008). The presence of Type I and Type II larvae and preliminary results obtained by the authors of this paper (Saraiva et al., 2007) showed that the larvae collected in this study belong to several Anisakis species from the two groups (Anisakis simplex s.s. and A. ziphidarum and Anisakis sp., Type I lar- vae; A. physeteris, A. brevispiculata and A. paggiae, Type II larvae).

The high prevalence and intensity of infestation by Anisakis larvae observed in A. carbo are in accordance with previous reports (Costa et al., 1996, 2003; Santos et al., 2009). They can be explained

### Table 3.

<table>
<thead>
<tr>
<th>Host length (cm)</th>
<th>Sesimbra</th>
<th>Madeira</th>
<th>Azores</th>
<th>Significance among the 3 regions</th>
<th>Significance between regions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Intensity ± s.d. (number of fish infected)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(minimum–maximum)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>&lt;90.0</td>
<td>66.9 ± 49.9 (20 – 204) (22)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>90.0 - 99.9</td>
<td>97.7 ± 56.2 (12 – 222) (29)</td>
<td>120.5 ± 55.7 (45 – 190) (6)</td>
<td>74.6 ± 22.4 (52 – 112) (8)</td>
<td>χ²= 1.537; df=2 p=0.464</td>
<td>Not Sig</td>
</tr>
<tr>
<td>100.0 - 109.9</td>
<td>215.3 ± 353.9 (16 – 1904) (51)</td>
<td>246.9 ± 256.9 (13 – 1128) (38)</td>
<td>55.7 ± 28.1 (13 – 144) (25)</td>
<td>χ²= 31.826; df=2 p=0.000</td>
<td>Not Sig</td>
</tr>
<tr>
<td>110.0 - 119.9</td>
<td>185.2 ± 197.2 (38 – 813) (14)</td>
<td>251.6 ± 181.9 (52 – 913) (67)</td>
<td>41.7 ± 14.8 (23 – 82) (15)</td>
<td>χ²= 37.755; df=2 p=0.000</td>
<td>Not Sig</td>
</tr>
<tr>
<td>≥119.9</td>
<td>–</td>
<td>389.7 ± 320.6 (41 – 1116) (9)</td>
<td>41.0 ± 44.2 (13 – 92) (3)</td>
<td>–</td>
<td>Sig</td>
</tr>
</tbody>
</table>

### Table 4.

<table>
<thead>
<tr>
<th>Sampling Season</th>
<th>Mean Intensity ± s.d. (number of fish infected)</th>
<th>Significance among the 3 regions</th>
<th>Significance between regions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(minimum–maximum)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td>112.6 ± 91.4 (16 – 390) (30)</td>
<td>173.3 ± 159.7 (13 – 913) (30)</td>
<td>42.6 ± 18.8 (13 – 92) (26)</td>
</tr>
<tr>
<td>Winter</td>
<td>98.3 ± 74.9 (12 – 367) (30)</td>
<td>159.4 ± 95.2 (41 – 400) (30)</td>
<td>–</td>
</tr>
<tr>
<td>Spring</td>
<td>128.4 ± 142.8 (22 – 813) (29)</td>
<td>244.9 ± 162.5 (43 – 638) (30)</td>
<td>65.2 ± 29.2 (13 – 144) (25)</td>
</tr>
<tr>
<td>Summer</td>
<td>290.1 ± 469.6 (23 – 1904) (27)</td>
<td>437.9 ± 292.9 (41 – 1128) (30)</td>
<td>–</td>
</tr>
</tbody>
</table>

### Significance among seasons

χ²= 12.361 df=3; p=0.006

χ²= 28.653 df=3; p=0.000

χ²= 3.016 df=3; p=0.003

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by the predatory voracity of this fish, which probably acquires the infection by feeding on infected cephalopods and fish. Cephalopods and fish, namely Scomber scombrus and Micromesistius poutassou, are noted as the main food items of A. carbo (Froese and Pauly, 2008) and are well-known paratenic hosts of Anisakis (Abauenza et al., 1995; Abollo et al., 2001; Fernández et al., 2005; Cruz et al., 2007).

The different intensities obtained in the three regions must be analysed carefully because significant differences in the host length were observed among the regions. The increase in the infection level of Anisakis (measured by prevalence, intensity and abundance) with the age or length of the host reported for several fish species (Abauenza et al., 1995; Adroher et al., 1996; Manfredi et al., 2000; Valero et al., 2000; Fernández et al., 2005; Cruz et al., 2007) has often been explained by the accumulation of the parasite throughout the host life and by the increased amount of food ingested by larger fish. In the present study this relationship between intensity and host length was found in specimens from Madeira and Sesimbra, as can be seen in the significant positive correlations that were found. As the sample from the Azores showed a high degree of heterogeneity (significant differences in host length between seasons) we cannot discriminate the influence of host length and seasonality on the intensity of infection in the overall sample. However, it must be stressed that the unexpected negative correlation found in specimens from the Azores in the overall sample disappeared when the analysis was carried out for each season.

Although the highest intensity value in Madeira can be partially attributed to host length, a consistent geographical effect in intensity levels is easily visible when the intensities among the three regions are compared for each host length class, with Madeira showing the highest levels and the Azores the lowest ones. The same geographical pattern was observed in each season. It would be interesting in future studies to compare the black scabbard fish diet in the three regions and the Anisakis infection levels of the preys, which may help to explain the geographical differences observed in the present work.

A seasonal variation in intensity of infection was evident in samples from Sesimbra and Madeira, with the highest values in summer. In the Azores, sampling was possible only in two seasons and, as discussed above, it is not possible to discriminate the influence of seasonality from the influence of host length without further sampling. The highest intensity values obtained in summer from Sesimbra and Madeira suggest an increased recruitment of the parasite larvae in this season. Without additional data it is not possible to attribute this seasonality to an increase in abundance of infected preys, to the presence of preys more intensively infected, to a more active feeding in warmer months or, alternatively, to seasonal changes in diet. Varying degrees of seasonality have been reported for anisakid infections in several hosts from different geographical areas (Adroher et al., 1996; Stromes and Anderson, 2000; Valero et al., 2000; Cruz et al., 2005; Farjallah et al., 2006) and this has been attributed to some of the factors presented above.

Finally, the high levels of Anisakis larvae, especially in Madeira specimens, and the fact that parasite larvae were also observed in the muscle strongly suggest that the consumption of raw or undercooked black scabbard fish is a potential health hazard.

ACKNOWLEDGEMENTS

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