

SCIENTIA MARINA 81(2)
June 2017, 179-185, Barcelona (Spain)
ISSN-L: 0214-8358
doi: <http://dx.doi.org/10.3989/scimar.04552.26A>

Comparison of anisakid infection levels between two species of Atlantic mackerel (*Scomber colias* and *S. scombrus*) off the Atlantic Portuguese coast

Maria João Santos^{1,2}, Ricardo Castro^{1,2}, Francisca Cavaleiro^{1,2}, Luis Rangel^{1,2}, Harry Wilhelm Palm³

¹ Faculty of Sciences of University of Porto, Biology Department, Animal Pathology Laboratory, Rua do Campo Alegre, s/n, FC4, 4169-007 Porto, Portugal.

(MJS) (Corresponding author) E-mail: mjsantos@fc.up.pt. ORCID-ID: <http://orcid.org/0000-0001-6655-491X>

(RC) E-mail: sr.ricardocastro@gmail.com. ORCID-ID: <http://orcid.org/0000-0002-4381-3605>

(FC) E-mail: fcavaleiro@fc.up.pt. ORCID-ID: <http://orcid.org/0000-0001-9978-3401>

(LR) E-mail: luisfiliperangel@sapo.pt. ORCID-ID: <http://orcid.org/0000-0002-8503-7763>

² CIIMAR/CIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal.

³ Rostock University, Faculty of Agricultural and Environmental Sciences, Aquaculture and Sea Ranching, Justus-von-Liebig-Weg 6, 18059 Rostock, Germany.

(HP) E-mail: harry.palm@uni-rostock.de. ORCID-ID: <http://orcid.org/0000-0003-2918-3253>

Summary: Anisakiasis is a problematic zoonotic infection associated with the consumption of raw or undercooked fish. Atlantic mackerel (*Scomber colias*) is of high commercial interest in Portugal and has been reported as a common host of *Anisakis* spp. In this study, the occurrence of anisakids is evaluated in *S. colias* and also *Scomber scombrus*, and the potential zoonotic risk associated with consumption of these two fishes is evaluated according to the recorded infection levels. These were found to be high for both fish species: a mean intensity and prevalence of 21.7 worms/fish and 85% for *S. colias*, and 16.4 worms/fish and 83.3% for *S. scombrus*, respectively. No correlation was detected between anisakid intensity and host total length, total weight, condition factor, and hepatosomatic and gonadosomatic indices for both fish species, but significantly higher intensity values were detected for more mature *S. scombrus*, i.e. fish recording a higher gonadosomatic index. Molecular tools allowed the identification of two species of *Anisakis*, *A. simplex* (s.s.) and *A. pegreffii*. They differed in their occurrence: in *S. colias* the prevalence of *A. simplex* (s.s.) was 18% and that of *A. pegreffii* was 82%, whereas in *S. scombrus* the prevalence of *A. simplex* (s.s.) was 73% and that of *A. pegreffii* was 27%. Occasionally, worms of *Hysterothylacium aduncum* were identified for both fish. The different infection levels of the two *Anisakis* species in both hosts off the Portuguese coast raise the hypothesis of a different life cycle at the level of the invertebrate intermediate host. *S. colias* lives in deeper waters than *S. scombrus*, and the differences found in infection levels suggest that *A. pegreffii* main first intermediate host also live in deeper waters, compared with *A. simplex* (s.s.) main first intermediate host. The higher infection levels of *A. simplex* (s.s.) (most infectious to humans) in *S. scombrus* suggest that its consumption when slightly cooked, as in grilled fish (so popular in Portugal), could be more problematic for the development of anisakiasis in humans than the consumption of *S. colias* and thus be of potential public health concern.

Keywords: anisakids; *Anisakis pegreffii*; *Anisakis simplex* (s.s.); Atlantic mackerels; Portuguese coast; molecular identification; food safety.

Comparación de los niveles de infección de anisákidos entre dos especies de caballa del Atlántico (*Scomber colias* y *S. scombrus*), de la costa atlántica portuguesa

Resumen: La anisakiasis es una zoonosis problemática asociada con el consumo de pescado crudo o poco cocinado. La caballa del Atlántico (*Scomber colias*) es un pez de mayor interés comercial en Portugal y se ha informado que es un hospedador común de *Anisakis* spp. En este estudio, la incidencia de anisákidos se evalúa en *S. colias* y *Scomber scombrus*, y el potencial riesgo zoonótico asociado con el consumo de estos dos peces se evalúa de acuerdo a los niveles de infección registrados. Estos se encontraron altos para ambas especies de peces - intensidad media y la prevalencia de 21.7 gusanos/pescado y 85% para *S. colias*, y 16.4 gusanos/pescado y 83.3% para *S. scombrus*, respectivamente. No se detectó ninguna correlación entre la intensidad de anisákidos y datos del hospedador, longitud total, peso total, factor de condición, y los índices hepatosomáticos y gonadosomáticos para ambas especies de peces, pero sí se detectaron valores de intensidad significativamente más altos para los pescados más maduros en *S. scombrus*, es decir, de pescado con mayor índice gonadosomático. Las herramientas moleculares permitieron la identificación de dos especies de *Anisakis simplex*, es decir, *A. simplex* (s.s.) y *A. pegreffii*. Ambos diferían en su ocurrencia - la prevalencia de *A. simplex* (s.s.) fue del 18% y del 82% *A. pegreffii* en *S. colias*, mientras que la prevalencia de *A. simplex* (ss) fue del 73% y del 27% *A. pegreffii* en *S. scombrus*. De vez en cuando, se identificaron los gusanos de *Hysterothylacium aduncum* para los dos peces. Los diferentes niveles de infección de las dos especies de *Anisakis* en ambos hospedadores, frente a la costa portuguesa, plantean la hipótesis de un ciclo de vida diferente en el nivel del huésped intermediario invertebrado. *S. colias* vive en aguas profundas frente a *S. scombrus*, que podría ser también cierto para

primer hospedador intermediario principal de *A. pegreffii* en comparación con lo de *A. simplex* (s.s.). Los mayores niveles de infección de *A. simplex* (s.s.) (más infecciosos para los humanos) en *S. scombrus* sugieren que su consumo ligeramente cocinados, como en el pescado a la parrilla (tan popular en Portugal), podría ser más problemático en relación con el desarrollo de la anisakiasis en los seres humanos, en comparación con el consumo de *S. colias* y por lo tanto ser de posible preocupación de salud pública.

Palabras clave: anisakidos; *Anisakis pegreffii*; *Anisakis simplex* (s.s.); caballas del Atlántico; costa portuguesa; identificación molecular; seguridad alimenticia.

Citation/Como citar este artículo: Santos M.J., Castro R., Cavaleiro F., Rangel L., Palm H.W. 2017. Comparison of anisakid infection levels between two species of Atlantic mackerel (*Scomber colias* and *S. scombrus*) off the Atlantic Portuguese coast. *Sci. Mar.* 81(2): 179-185. doi: <http://dx.doi.org/10.3989/scimar.04552.26A>

Editor: J. Lloret.

Received: September 19, 2016. **Accepted:** January 18, 2017. **Published:** April 17, 2017.

Copyright: © 2017 CSIC. This is an open-access article distributed under the terms of the Creative Commons Attribution (CC-by) Spain 3.0 License.

INTRODUCTION

Portugal has a long tradition of fishing and is the leading country in fish consumption in the European Union (EUMOFA 2016), with 55.3 kg of sea food per capita per year in 2014. For this reason, zoonotic infections such as anisakiasis are a potential risk and a case of major public health concern in this country. Fish dishes, such as grilled (slightly cooked) fish, are one of the most popular food specialties in Portugal, not only among the local people but also among the many tourists who visit the country each year. Atlantic mackerels *Scomber colias* Gmelin, 1789 and *Scomber scombrus* L., 1758 are pelagic-neritic scombrids of great commercial interest that are consumed lightly grilled. According to the Portuguese Institution for Statistical Data, 29543 t of *S. colias* and 588 t of *S. scombrus* were landed in 2014 (DGRM 2015), with *S. colias* being one of the top four fish sold in the country.

Anisakid nematodes are the most abundant parasites of marine fishes worldwide (Mattiucci and Nascetti 2008). They are parasites of zoonotic potential, having the ability to infect humans through the consumption of raw or lightly cooked fish, causing the emerging infection anisakiasis, and thus being a food safety concern (MacCarthy and Moore 2000). Associated symptoms of anisakiasis include severe gastric or intestinal disease or, in the mild version, allergies (Audicana et al. 2002, 2003). In Portugal, no reports of severe disease due to *Anisakis* infections have been recorded so far. However, in a survey conducted among the population in a fishery town located in the south of the country, it was found that 8% of the people were allergic to anisakids (Nunes et al. 2003). In Spain, France, Italy, Germany, the Netherlands and Japan, severe episodes of anisakiasis have already been recorded (Arizono et al. 2012, Mattiucci et al. 2013, Ubeira et al. 2000).

Both *S. colias* and *S. scombrus* are common hosts of anisakids in different geographic areas, particularly in Europe (Abollo et al. 2001, Mattiucci and Nascetti 2008, Pontes et al. 2005). There are also several records from African countries (Abattouy et al. 2011, Farjallah et al. 2008, Kijewska et al. 2009). So far, no updated information is available for Atlantic mackerels fished

off continental Portugal, and no attempt has been made to identify the anisakids isolated from *S. scombrus* to the species level (Rego et al. 1985).

The main purpose of this study was to determine and compare the infection levels (prevalence and intensity) of anisakid nematodes in *S. colias* and *S. scombrus*, to correlate parasite and host data, and to evaluate whether the fish species can represent a danger when consumed raw or undercooked, taking into account (i) the infecting species and (ii) the recorded infection levels.

MATERIALS AND METHODS

Host sampling

Several samples of Atlantic chub mackerel, *S. colias* (n=40 in total), and Atlantic mackerel, *S. scombrus* (n=42 in total), were collected from October to December 2009 (n=19 and 21, respectively) and from January to June 2010 (n=21 for both species). The Atlantic mackerels were fished in the FAO area Atlantic Northeast 27, subarea Portuguese waters IX, by trawling fisheries operated by local boats. The fish were all purchased at the harbour fish market and then freshly dissected or frozen for subsequent parasitological analyses. Host identification was done according to Collette (1986), based on the external and internal features. Fish length was also evaluated [mean±standard deviation (range)]: 32.6±3.6 (25.4-39.2) cm for the Atlantic chub mackerel; and 31.9±2.3 (21.8-36.4) cm for the Atlantic mackerel (see Table 1). As some parasites might accumulate in older, i.e. larger fishes, and thus bias our sample, a set of hosts with similar lengths were used for the analysis. Also, fish weight (g), Fulton's condition factor ($K = \text{weight}/\text{length}^3 \text{ g cm}^{-3}$), hepatosomatic index ($\text{HI} = \text{liver weight (g)} / \text{total weight (g)} \times 100$), gonadosomatic index ($\text{GI} = \text{gonadal weight (g)} / \text{total weight (g)} \times 100$) and sex ratio were recorded for each fish species (see Table 1).

Parasitological survey

During the parasitological survey for anisakids, the following infection sites were analysed under the

Table 1. – Comparison of host data (total weight, total length, condition factor, hepatosomatic index, gonadosomatic index and sex ratio) and anisakid infection levels (prevalence and intensity) in the Atlantic mackerels, *Scomber colias* and *S. scombrus*, off the Portuguese continental coast. * mean±SD (minimum-maximum)

	<i>Scomber colias</i> (n=40)	<i>Scomber scombrus</i> (n=42)
Host Data		
Total weight (g)*	346.3±128.4 (135.3-602.4)	288.9±73.0 (62.5-474.5)
Total length (cm)*	32.6±3.6 (25.4-39.2)	31.9±2.4 (21.8-36.4)
Condition factor (g cm ⁻³)*	0.010±0.001 (0.007-0.011)	0.009±0.001 (0.006-0.012)
Hepatosomatic index	1.0±0.4 (1.0-3.0)	1.1±0.3 (1.0-2.0)
Gonadosomatic index*	1.1±1.5 (0.0-8.0)	3.6±3.6 (0.0-18.0)
Sex ratio (male/female/immature)	11/24/5	5/35/2
Parasite Data		
Prevalence (%) (number of infected hosts)	85.0 (34)	83.3 (35)
Intensity *	21.7±20.2 (1-87)	16.4±40.9 (1-245)

stereo-microscope (magnification 30×): digestive tract, gonads, heart and muscle. The muscle portion examined was cut off from the ventral part of the fish, and also from the belly flaps, as this is considered one of the most infected muscular regions in the fish according to Mehrdana et al. (2014). The isolated worms were cleaned in saline solution (0.9% NaCl) and fixed and preserved in 70% ethanol. For morphological identification purposes, anisakids were cleared and mounted in glycerine, following Moravec (1998). Since around 700 worms were sampled from each fish species, a subsample of worms found around the viscera (and morphologically identified as *Anisakis simplex* s.l., type I) was identified to the species level using molecular tools. Moreover, around 19 anisakids were taken from about 19 specimens of each species of scombrids (i.e. one worm/fish at random). A minimum sample size of 15 worms was chosen as recommended for calculating reliable prevalence levels (Jovani and Tella 2006). Specimens of *Hysterothylacium*, occasionally found in both fish hosts, were also characterized using the same molecular methods.

Molecular characterization of anisakids

This procedure follows closely the one described in Kuhn et al. (2011). Eighteen and 20 anisakids (*Anisakis* sp. and *Hysterothylacium* sp.) from *S. colias* and *S. scombrus*, respectively, were analysed molecularly. DNA extraction was done for each larva, cut into pieces, using a genomic DNA extraction kit (peqGOLD MicroSpin Tissue, PEQLAB Biotechnology GmbH, Germany) and following the instructions of the manufacturer. The region of the rDNA included the ITS-1, 5.8S, ITS-2 and flanking sequences (=ITS+), and was amplified using the primers Forward TK1 (5'-GGC-AAA-AGT-CGT-AAC-AAG-GT-3') and Reverse NC2 (5'-TTA-GTT-TCT-TTT-CCT-CCG-CT-3'). PCR reaction (50 µl) included 25 µl Master Mix (18.75 µl ddH₂O, 5 µl reaction buffer Y, 1 µl dNTP, 0.25 µl Taq-DNA-polymerase), 14 µl ddH₂O, 3 µl of each primer and 5 µl of genomic DNA. Each PCR reaction was performed in a PCR thermocycler (GeneTouch, Biozym scientific, Germany) under the following conditions: an initial denaturation at 95°C for 1 min, 40 cycles of 94°C for 45 sec (denaturation), 55°C for 45 sec (annealing), 72°C for 45 sec (extension), and a final extension at 72°C for 10 min. Samples with no DNA were included for negative control. PCR products were

checked on 1% agarose gel that ran for 1 h 15 min at 80V provided by an electrophoresis power supply (EV 243, Consort, Belgium) inside an electrophoresis system (MSMIDI, MULTISUB, Biozym, Germany). The gel was then analysed with a BiodocAnalyse (Biometra, AnalytikJena, Germany) associated with a photographic camera (Canon EOS 1100D) and BioDoc Analyse software version 2.66.3.22 (Biometra, AnalytikJena, Germany). A GelRed 100 bp DNA ladder marker (peqGold Leader Plus, PEQLAB Biotechnologie GmbH, Germany) was used to estimate the size of the PCR products. The agarose gel products were afterwards purified with a Cycle-Pure Kit (peqGold, PEQLAB Biotechnology GmbH, Germany).

Twenty µl of each DNA sample and 2.5 µl of each primer per sample was sent to the laboratory (GATC Biotec AG, European Custom Sequencing Centre, Cologne, Germany) for sequencing. In total, 38 worms were sequenced. The species were identified by comparison with sequences previously deposited in GenBank, using the BLASTn algorithm and the BioEdit software version 7.1.3.0 (Hall 1999) to previously aligned sequences forward and reverse provided by the sequence laboratory.

Infection levels of anisakids

Prevalence and intensity [mean±SD (minimum-maximum)] were calculated for each identified taxon according to Bush et al. (1997) and considering each of the two species of host.

Binary Pearson correlations among anisakid intensity and host total weight, total length, condition factor, hepatosomatic index and gonadosomatic index were conducted. Moreover, the anisakid intensities were analysed in smaller versus larger fish for host total weight, total length, condition factor, hepatosomatic index, gonadosomatic index and sex (male versus female), using the Mann-Whitney's U test. In the later analysis, the two classes of fish were separated by the median value for each parameter. In the case of detecting significant differences, the anisakid intensities [mean±SD (minimum-maximum)] for the smaller and bigger hosts were determined. Non-parametric tests were chosen for the statistical analysis because the study of the normality using the Kolmogorov-Smirnov's test for some variables, such as anisakid intensity, showed that they did not follow a normal distribution (needed in parametric tests), as for instance.

Occurrence of *A. simplex* s.s. and *A. pegreffii* was compared between the two species of host using the chi-squared test.

All statistical analyses were conducted in SPSS for Windows, version 23 (level of significance: $P < 0.05$).

RESULTS

Anisakid infection levels

Both Atlantic mackerels recorded very high infection levels – a prevalence of anisakids of 85.0% and 83.3% for *S. colias* and *S. scombrus*, respectively. In total, 1312 anisakid worms were sampled from both fish, 737 from *S. colias* and 575 from *S. scombrus*. Mean intensity was higher for *S. colias*, with 21.7 worms/host, than for *S. scombrus*, with the 16.4 worms/host. These infection values were recorded for worms mainly found around the viscera, because the muscular tissue was found infected only once, with a single anisakid recorded in *S. scombrus* (prevalence of 2.4%) and none in *S. colias* (prevalence of 0%).

Binary correlations between anisakid intensity and host total weight, total length, condition factor, hepatosomatic index and gonadosomatic index were found to lack statistical significance.

The comparison of anisakid intensities among bigger and smaller hosts, separated by the median value, for host total weight, total length, condition factor, hepatosomatic index, gonadosomatic index and sex (males versus females) were in most cases non-significant, with probability values higher than 0.306, except for the gonadosomatic index, which was significant and recorded a probability value of 0.001. The intensities [mean±SD (range)] for the immature and mature hosts, according to the gonadosomatic index weight, were 6.0±8.6 (1-34) and 27.5±56.8 (2-245) worms per fish, respectively.

Anisakid molecular identification

Among the 36 *Anisakis* worms sequenced, several sequences were deposited in GenBank (accession numbers KF923929 and KF923930 for *A. simplex* (s.s.) and KF923927 for *A. pegreffii* from *S. scombrus*; and KF923928 for *A. simplex* (s.s.), and KF914636 and KF923926 for *A. pegreffii* from *S. colias*). The distribution for each *Anisakis* species for both fish hosts is given in Table 2. *A. simplex* (s.s.) was significantly more frequent in *S. scombrus* than in *S. colias*, whereas the opposite trend was reported for *A. pegreffii* ($\chi^2=9.03$, $P < 0.01$).

Hysterothylacium aduncum specimens were also occasionally recorded, and two worms were sequenced,

one worm per fish host species (GenBank accession numbers KF923932 from *S. scombrus* and KF923931 from *S. colias*, both showing 99% similarity to the *H. aduncum* sequences HM598666.1 and JQ934883.1 (Smrzlić et al. 2012)).

DISCUSSION

In this study, infection levels for anisakids were evaluated considering two species of Atlantic mackerel, *S. colias* and *S. scombrus*, sampled at the continental Portuguese coast. The high infection levels (83%-85% prevalence) recorded for both fish are greater than the 10% reported for *S. scombrus* off continental Portugal by Rego et al. (1985), and the 69.5% reported for *S. colias* off the Madeira Islands, Portugal, by Costa et al. (2003). However, they are lower than the 100% reported for *S. colias* off the Azores Islands, Portugal, by Shukhgalter (2004). High prevalence values were also recorded in other geographic regions: 87% for *S. colias* in El Rincon, Argentina (Cremonte and Sardella 1997) and 92% for *S. scombrus* and 100% for *S. colias* in the Adriatic Sea off Croatia (Mladineo 2003, Mladineo and Poljak 2014). In contrast, in Moroccan waters, prevalence of anisakids in *S. colias* varied from 57% to 67.9% (Abattouy et al. 2011). High prevalence values were also recorded for anisakids of other fish species from the Portuguese mainland, such as the blue whiting (*Micromesistius poutassou*, 77.7%), the black scabbardfish (*Aphanopus carbo*, 100%) (Cruz et al. 2007, 2009) and the blackspot seabream (*Pagellus bogaraveo*, 100%) (Hermida et al. 2012). Moreover, Costa et al. (2004) also reported a high prevalence (89.6%) for the blackspot seabream off Madeira Islands, Portugal.

In our samples, not only the overall prevalence but also the mean intensity of anisakids was high, with 21.7 worms per host for *S. colias* and 16.4 worms per host for *S. scombrus*. These values were similar to the ones recorded from the Azores Islands (Hyer Bank), with 17.1 worms per host for *S. colias* (Shukhgalter 2004).

The correlation analysis of anisakid intensity and host total weight, total length, condition factor, hepatosomatic index and gonadosomatic index for *S. colias* and *S. scombrus* was found not to be significant. Moreover, the comparison analysis of anisakid intensity among smaller and bigger fish according to host total weight, total length, condition factor, hepatosomatic index, gonadosomatic index and sex (males versus females) showed mostly the same behaviour: the results were non-significant, except for the gonadosomatic index in *S. scombrus*. In the latter, more mature fish, with higher index values, recorded significantly higher intensities of anisakids than fish with lighter gonads, with mean values of 6.0 and 27.5, respectively. These findings can be interpreted as a conspicuous high preference for the first intermediate host by the more mature hosts. The lack of significant values for correlation between intensity of anisakids and host data was also recorded by Mladineo and Poljak (2014) for *S. colias* and *Sardina pilchardus* in the Adriatic Sea, and by Costa et al. (2004) for *Pagellus bogaraveo* off Madeira Island. By contrast, Cruz et al. (2009)

Table 2. – *Anisakis* species distribution in *Scomber colias* and *S. scombrus* off the Portuguese continental coast, recorded around the fish viscera.

	<i>Scomber colias</i> (n=17)	<i>Scomber scombrus</i> (n=19)
<i>A. simplex</i> (s.s.)	4 (18%)	14 (73%)
<i>A. pegreffii</i>	13 (82%)	5 (27%)

recorded a significant increase in anisakid intensity according to the host length for *Aphanopus carbo* off Sesimbra (mainland) and Madeira Islands, Portugal. Additionally, Chou et al. (2011) found significantly higher intensity of *Anisakis* larvae in larger and older spotted mackerel (*Scomber australasicus*) off the Taiwanese coast. Furthermore, Mladineo and Poljak (2014) recorded significant values for host length and abundance of *Anisakis* from anchovies (*Engraulis encrasicolus*), European hake (*Merluccius merluccius*) and whiting (*Merlangius merlangus*) in the Adriatic Sea. The correlation significance between either intensity or abundance of *Anisakis* and host length, seems to be dependent on the fish species, and for *S. colias* the present work is at least the second time that it has been measured (Mladineo and Poljak 2014), but with no significance.

In our study, the edible portion of the fishes, the muscle, recorded low or absent infection values, with 2.4% for *S. scombrus* and 0% for *S. colias*. Nevertheless, a potential risk of infection should not be discarded, because anisakid larvae can migrate from the viscera to the muscle after death of the host (Smith and Wootten 1975, Hauck 1977). These results suggest that fish caught from Portuguese northern Atlantic waters are highly susceptible to carry infections with anisakid nematodes and thus represent a potential human health problem. Taking into account these infection values, we advise that Atlantic mackerels caught off Portugal be ingested with care, and only after being properly frozen or cooked. The European Union (2011) recommends that for the safe consumption of fishery products, raw or undercooked, a previous period of freezing and storage at a core temperature of -20°C or below for not less than 24 h, or of -35°C or below for not less than 15 hours should be applied. In addition, the recommendation of fish evisceration and low temperatures on board just after the catch should be followed more closely. These procedures will avoid as much as possible the migration of the worm to the muscle after the host dies.

The present study identifies for the first time the anisakid species found in mainland Portuguese Atlantic mackerels, *S. scombrus*, and recognizes the presence of mixed infections with both *A. simplex* s.s. and *A. pegreffii* in the two scombrid fish species. For *S. colias* off Madeira Island, Pontes et al. (2005) recorded six different *Anisakis* species for *S. colias*, namely *A. simplex* s.s., *A. pegreffii*, *A. nascetti*, *A. typica*, *A. ziphidarum* and *A. physeteris*. The detection of *A. simplex* (s.s.) and *A. pegreffii* mixed infections have also been recorded for *S. scombrus* in Galicia from the northwestern coast of Spain (Abollo et al. 2001), and for *S. colias* and *S. scombrus* in the Alboran Sea off the southern coast of Spain (Abollo et al. 2003). These findings corroborate the statement that the Portuguese and Spanish coasts are a sympatric area for both parasite species (Abollo et al. 2001, Marques et al. 2006).

Taking into account the distribution of the different *Anisakis* species in our fish sample, we further hypothesize that this pattern may be related to differences in the anisakid life cycle hosts, since the two fish

mainly occur at different depths. *A. pegreffii* larvae are probably found in deeper waters, as are their main first host, than *A. simplex* (s.s.) larvae in less deeper waters, because *A. pegreffii* larvae were more frequent in *S. colias* (a deeper fish living at 50-300 m) than in *S. scombrus* (living at 0-200 m depth). However, this subject is controversial. According to Mattiucci et al. (1997), *A. simplex* (s.s.) is found mainly in benthic or demersal fishes, while *A. pegreffii* is found mainly in pelagic fishes, based on the marine mammal final host distribution. Abollo et al. (2001) confirmed the occurrence of *A. simplex* (s.s.) in benthic and demersal fishes but could not confirm the occurrence of *A. pegreffii* in pelagic fishes, having analysed a range of different fishes and cephalopods. Kuhn et al. (2013) found that both *Anisakis* species have similar proportions of fish hosts that live in pelagic, benthopelagic and demersal environments. However, after a second analysis of this author's data, we may see that *A. pegreffii* occurs in a higher number of reef-associated fish species than *A. simplex* (s.s.), thus supporting our hypothesis. Also, the high abundance of *A. pegreffii* in *Trachurus trachurus* (Abattouy et al. 2013), a benthopelagic fish reaching depths of 0 to 1050 m (Lloris and Moreno 1995), supports our hypotheses. The geographic distribution of a parasite will be the conjunction of the distributions of its different hosts belonging to its life cycle. And a parasite with high host specificity will have a smaller distribution, probably limited to that of its hosts, than a non-specific parasite, which is expected to have a broad distribution.

We are aware that further data on the distribution within the *Anisakis simplex* complex at the invertebrate host level is needed in order to prove our hypothesis. Smith (1983) proposed that euphausiids were the major intermediate host of *A. simplex* s.l., in the northeast Atlantic and northern North Sea, and perhaps universally. Recently, Gregori et al. (2015) detected the occurrence of larvae of *A. simplex* (s.s.) and *A. pegreffii* in the euphausiid *Nyctiphanes couchii*, justifying the co-occurrence of both species in the same fish species, and its sympatry in the northeast Atlantic. However, other invertebrate species were also indicated as host candidates for *Anisakis* species, such as copepods (eg. *Acartia tonsa*, and *Oitona similis*) (Koeie 2001). The low infection levels recorded for these first intermediate hosts to date in the ocean do not allow us to fully understand what species are more important for the life cycle of each *Anisakis* species, but further research studies will certainly solve this problem in the short term.

According to earlier studies, the two recorded *Anisakis* species have a different potential zoonotic level in humans: *A. simplex* (s.s.) is more prone to migrate into the muscle than the other species, and also withstands better the gut juices of the human stomach, with high survival rates (Arizono et al. 2012). These differences in parasite behaviour explain why more human infections have been reported due to *A. simplex* (s.s.) than to *A. pegreffii* in Japan, where both species co-occur and the ingestion of raw fish is particularly common (Umehara et al. 2007). Moreover, the average number

of *Anisakis* muscle larvae in *Scomber japonicus* can be 12 times higher for *A. simplex* s.s. than for *A. pegreffii*, (Suzuki et al. 2010). Taking into account that our Atlantic mackerels have significantly different infections with these two *Anisakis* species and likewise a similar anisakid load, we may conclude that it is less safe to eat *S. scombrus*, since it harbours more *A. simplex* (s.s.) than *S. colias*, unless it is appropriately frozen or well cooked.

ACKNOWLEDGEMENTS

The authors would like to acknowledge all the helpful suggestions provided by one of the anonymous reviewers. This research was partially supported by the Research Line INSEAFOD Innovation and valorization of seafood products: meeting local challenges and opportunities, within the R&D Institution CIIMAR (Interdisciplinary Centre of Marine and Environmental Research) funded by the Northern Regional Operational Programme (NORTE2020) through the European Regional Development Fund (ERDF). It was also supported by FCT grants for M.J.S. (Sabbatical leave, n° BSAB-1239/2012), A.S. and R.C. (CIIMAR/C2008/BII-11/2010). We also thank Ana Silva for helping with the anisakid sampling.

REFERENCES

- Abattouy N., Valero A., Benajiba M.H., et al. 2011. *Anisakis simplex* s.l. parasitization in mackerel (*Scomber japonicus*) caught in the North of Morocco - Prevalence and analysis of risk factors. *Int. J. Food Microbiol.* 150: 136-139. <https://doi.org/10.1016/j.ijfoodmicro.2011.07.026>
- Abattouy N., Lopez A.V., Maldonado J.L., et al. 2013. Epidemiology and molecular identification of *Anisakis pegreffii* (Nematoda: Anisakidae) in the horse mackerel *Trachurus trachurus* from northern Morocco. *J. Helminthol.* 88: 257-263. <https://doi.org/10.1017/S0022149X13000102>
- 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. <https://doi.org/10.1007/s004360100389>
- Abollo E., Paggi L., Pascual S., et al. 2003. Occurrence of recombinant genotypes of *Anisakis simplex* s.s. and *Anisakis pegreffii* (Nematoda: Anisakidae) in an area of sympatry. *Infect. Genet. Evol.* 3: 175-181. [https://doi.org/10.1016/S1567-1348\(03\)00073-X](https://doi.org/10.1016/S1567-1348(03)00073-X)
- Arizono N., Yamada M., Tegoshi T., et al. 2012. *Anisakis simplex* sensu stricto and *Anisakis pegreffii*: biological characteristics and pathogenetic potential in Human Anisakiasis. *Foodborne Pathog. Dis.* 9: 517-521. <https://doi.org/10.1089/fpd.2011.1076>
- Audicana M.T., Ansotegui I.J., Fernández de Corres L. et al. 2002. *Anisakis simplex*: dangerous – dead and alive? *Trends Parasitol.* 18: 20-25. [https://doi.org/10.1016/S1471-4922\(01\)02152-3](https://doi.org/10.1016/S1471-4922(01)02152-3)
- Audicana M.T., del Pozo M.D., Iglesias R., et al. 2003. *Anisakis simplex* and *Pseudoterranova decipiens*. In: Miliotis M.D., Bier J.V. International Handbook of Foodborne Pathogens. CRC Press, pp. 613-636. <https://doi.org/10.1201/9780203912065.ch38>
- Bush A.O., Lafferty K.D., Lotz, J.M., et al. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol.* 83: 575-583. <https://doi.org/10.2307/3284227>
- Collette B.B. 1986. Scombridae. In: Whitehead P.J.P., Bauchot M.-L., Hureau J.-C., et al. (eds) Fishes of the North-eastern Atlantic and the Mediterranean. vol 2. Unesco, United Kingdom, pp. 981-997.
- Chou Y.-Y., Wang C.-S., Chen H.-G., et al. 2011. Parasitism between *Anisakis simplex* (Nematoda: Anisakidae) third-stage larvae and the spotted mackerel *Scomber australasicus* with regard to the application of stock identification. *Vet. Parasitol.* 177: 324-331. <https://doi.org/10.1016/j.vetpar.2010.12.003>
- Costa G., Pontes T., Mattiucci S., et al. 2003. The occurrence and infection dynamics of *Anisakis* larvae in the black-scabbard fish, *Aphanopus carbo*, chub mackerel, *Scomber japonicus*, and oceanic horse mackerel, *Trachurus picturatus* from Madeira, Portugal. *J. Helminthol.* 77: 163-166. <https://doi.org/10.1079/JOH2002156>
- Costa G., Madeira A., Pontes T. et al. 2004. Anisakid nematodes of the blackspot seabream, *Pagellus bogaraveo*, from Madeiran waters, Portugal. *Acta Parasitologica.* 49: 156-161.
- Cremonte F., Sardella N.H. 1997. The parasite fauna of *Scomber japonicus* Houttuyn, 1782 (Pisces: Scombridae) in two zones of the Argentine Sea. *Fish. Res.* 31: 1-9. [https://doi.org/10.1016/S0165-7836\(97\)00024-6](https://doi.org/10.1016/S0165-7836(97)00024-6)
- Cruz C., Barbosa C., Saraiva A. 2007. Distribution of larval anisakids in blue whiting off Portuguese fish market. *Helminthol.* 44: 21-24. <https://doi.org/10.2478/s11687-006-0051-8>
- Cruz C., Saraiva A., Santos M.J., et al. 2009. Parasitic infection levels by *Anisakis* spp. larvae (Nematoda: Anisakidae) in black scabbardfish *Aphanopus carbo* (Osteichthyes: Trichiuridae) from Portuguese waters. *Sci. Mar.* 73S2: 115-120. <https://doi.org/10.3989/scimar.2009.73s2115>
- Direcção Geral de Recursos Naturais, Segurança e Serviços Marítimos (DGRM). 2015. Estatística da Pesca 2014. Instituto Nacional de Estatística, I.N.E.I.P., Lisboa.
- European Union (E.U.). 9 December 2011. Regulation no 1276/2011 of the European Parliament and of the Council amending annex III to regulation (EC) no 853/2004 of the European Parliament and of the Council as regards the treatment to kill viable parasites in fishery products for human consumption. *Eur. Lex* 54: 39. <http://eur-lex.europa.eu/eli/reg/2011/1276/oj>
- EUMOPA. 2016. The EU fish market. Accessed on 18/10/2016 at <https://www.eumofa.eu/documents/20178/77960/The+EU+fish+market+-+2016+Edition.pdf>
- Farjallah S., Slimane B.B., Busi M., et al. 2008. Occurrence and molecular identification of *Anisakis* spp. from the North African coasts of Mediterranean Sea. *Parasitol. Res.* 102: 371-379. <https://doi.org/10.1007/s00436-007-0771-9>
- Gregori M., Roura A., Abollo E., et al. 2015. *Anisakis simplex* complex (Nematoda: Anisakidae) in zooplankton communities from temperate NE Atlantic waters. *J. Nat. Hist.* 49: 13-14. <https://doi.org/10.1080/00222933.2014.979260>
- Hall T.A. 1999. BioEdit a user-friendly biology sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41: 95-98.
- Hauck A. K. 1977. Occurrence and survival of the larval nematode *Anisakis* sp. in the flesh of fresh, frozen, brined, and smoked Pacific Herring, *Clupea harengus pallasii*. *J. Parasitol.* 63: 515-519. <https://doi.org/10.2307/3280014>
- Hermida M., Mota R., Pacheco C.C., et al. 2012. Infection levels and diversity of anisakid nematodes in blackspot seabream, *Pagellus bogaraveo*, from Portuguese waters. *Parasitol. Res.* 110: 1919-1928. <https://doi.org/10.1007/s00436-011-2718-4>
- Jovani R., Tella J.L. 2006. Parasite prevalence and sample size: misconceptions and solutions. *Trends Parasitol.* 22: 214-218. <https://doi.org/10.1016/j.pt.2006.02.011>
- Koie M. 2001. Experimental infections of copepods and sticklebacks *Gasterosteus aculeatus* with small ensheated and large third-stage larvae of *Anisakis simplex* (Nematoda, Ascaridoidea, Anisakidae). *Parasitol. Res.* 87: 32-36. <https://doi.org/10.1007/s004360000288>
- Kijewska A., Dzido J., Shukhgalter O., et al. 2009. Anisakid parasites of fishes caught on the African shelf. *J. Parasitol.* 95: 639-645. <https://doi.org/10.1645/GE-1796.1>
- Kuhn T., García-Márquez J., Klimpel S. 2011. Adaptive Radiation within Marine Anisakid Nematodes: A Zoogeographical Modeling of Cosmopolitan, Zoonotic Parasites. *PLoS ONE* 6: e28642. <https://doi.org/10.1371/journal.pone.0028642>
- Kuhn T., Hailer F., Palm H.W., et al. 2013. Global assessment of molecularly identified *Anisakis* Dujardin, 1845 (Nematoda: Anisakidae) in their teleost intermediate hosts. *Folia Parasitol.* 60: 123-134.

- <https://doi.org/10.14411/fp.2013.013>
Lloris D., Moreno T. 1995. Distribution model and association in three pelagic congeneric species (*Trachurus* spp.) present in the Mediterranean Iberic Sea. *Sci. Mar.* 59: 399-403.
- MacCarthy J., Moore T.A. 2000. Emerging helminth zoonoses. *Int. J. Parasitol.* 30: 1351-1360.
[https://doi.org/10.1016/S0020-7519\(00\)00122-3](https://doi.org/10.1016/S0020-7519(00)00122-3)
- Marques J.M., Cabral H.N., Busi M., et al. 2006. Molecular identification of *Anisakis* species from Pleuronectiformes off the Portuguese coast. *J. Helminthol.* 80: 47-51.
<https://doi.org/10.1079/JOH2005325>
- Mattiucci M., Nascetti G. 2008. Advances and trends in the molecular systematics of Anisakid Nematodes, with implications for their evolutionary ecology and host-parasite co-evolutionary processes. *Adv. Parasitol.* 66: 47-148.
[https://doi.org/10.1016/S0065-308X\(08\)00202-9](https://doi.org/10.1016/S0065-308X(08)00202-9)
- Mattiucci M., Nascetti G., Gianchi R., et al. 1997. Genetic and ecological data on the *Anisakis simplex* complex, with evidence for a new species (Nematoda, Ascaridoidea, Anisakidae). *J. Parasitol.* 83: 401-406.
<https://doi.org/10.2307/3284402>
- Mattiucci S., Fazio P., De Rosa A., et al. 2013. Anisakiasis and gastroallergic reaction associated with *Anisakis pegreffii* infection, Italy. *Emerg. Infect. Dis.* 19: 496-499.
<https://doi.org/10.3201/eid1903.121017>
- Mehrdana F., Bahlool Q., Skov J., et al. 2014. Occurrence of zoonotic nematodes *Pseudoterranova decipiens*, *Contracaecum osculatatum* and *Anisakis simplex* in cod (*Gadus morhua*) from the Baltic Sea. *Vet. Parasitol.* 205: 581-587.
<https://doi.org/10.1016/j.vetpar.2014.08.027>
- Mladineo I. 2003. *Anisakis simplex* in the Adriatic Sea. *Periodicum. Biologorum* 105: 389-392.
- Mladineo I., Poljak V. 2014. Ecology and Genetic Structure of Zoonotic *Anisakis* spp. from Adriatic. *Appl. Environ. Microb.* 80: 1281-1290.
<https://doi.org/10.1128/AEM.03561-13>
- Moravec F. 1998. Nematodes of freshwater fishes of the neotropical region. Academia Praha, Institute of Parasitology, Academy of Sciences of the Czech Republic, pp. 381-383.
- Nunes C., Ladeira S., Mergulhão A. 2003. Allergy to *Anisakis simplex* in the Portuguese Population. *Rev. Port. Imunoalergologia* XI: 30-40.
[https://doi.org/10.1016/s0091-6749\(03\)80911-9](https://doi.org/10.1016/s0091-6749(03)80911-9)
- Pontes T., D'Amelio S., Costa G., et al. 2005. Molecular characterization of larval Anisakid nematodes from marine fishes of Madeira by PCR-based approach, with evidence for a new species. *J. Parasitol.* 91: 1430-1434.
<https://doi.org/10.1645/GE-565R1.1>
- Rego A.A., Carvalho-Varela M., Mendonça M.M., et al. 1985. Helminthofauna da sarda (*Scomber scombrus* L.) peixe da costa continental portuguesa. *Mem. I. Oswaldo Cruz* 80: 97-100.
<https://doi.org/10.1590/S0074-02761985000100015>
- Shukhgalter O.A. 2004. The parasite fauna of the chub mackerel (*Scomberidae: Scomber japonicus* Houttuyn, 1782) in the central-eastern Atlantic (Atlantic coast of Northern Africa and the Azores Archipelago banks). *Parazitol.* 38: 160-170.
- Smith J. 1983. *Anisakis simplex*: Morphology and morphometry of larvae from euphausiids and fish, and a review of the life-history and ecology. *J. Helminthol.* 57: 205-224.
<https://doi.org/10.1017/S0022149X00009512>
- 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.
[https://doi.org/10.1016/0020-7519\(75\)90019-3](https://doi.org/10.1016/0020-7519(75)90019-3)
- Smrzlić V., Valić D., Kapetanović D., et al. 2012. Molecular characterisation of Anisakidae larvae from fish in Adriatic Sea. *Parasitol. Res.* 111: 2385-2391.
<https://doi.org/10.1007/s00436-012-3094-4>
- Suzuki J., Murata R., Hosaka M., et al. 2010. Risk factors for human *Anisakis* infection and association between the geographic origins of *Scomber japonicus* and anisakid nematodes. *Int. J. Food Microbiol.* 137: 88-93.
<https://doi.org/10.1016/j.ijfoodmicro.2009.10.001>
- Ubeira F.M., Valinas B., Lorenzo S., et al. 2000. Anisakioidosis y alergia. Un estudio seroepidemiológico en la Comunidad Autónoma Gallega. Documentos Técnicos de Salud Pública, Serie B, 24. Ed. Consellería de Sanidade e Servizos sociais (Xunta de Galicia, España).
- Umehara A., Kawakami Y., Araki J., et al. 2007. Molecular identification of the etiological agent of the human anisakiasis in Japan. *Parasitol. Int.* 56: 211-215.
<https://doi.org/10.1016/j.parint.2007.02.005>