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The fishing area as a possible indicator of the infection by anisakids in anchovies (*Engraulis encrasicolus*) from southwestern Europe

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ABSTRACT

A study was conducted on the parasitization by anisakids of European anchovies (*Engraulis encrasicolus*) from the Eastern Atlantic (Gulf of Cádiz and Strait of Gibraltar) and Western Mediterranean (Ligurian Sea, Gulf of Lion, Catalonia coast and NW Alborán Sea) throughout 1998 and 1999. The anisakids detected were identified as third larval stages of *Anisakis* larva type I and *Hysterothylacium aduncum*. Global prevalence was 9.4% for *Anisakis* and 24.5% for *H. aduncum*. Analysis of the origin of the anchovies revealed a higher prevalence of *Anisakis* than *H. aduncum* in fish from the Atlantic and *vice-versa* in fish from the Mediterranean. Analysis of various fishing areas in the Western Mediterranean revealed a prevalence of *Anisakis* in fish from the Ligurian Sea that was 5-fold or more than in the other three areas, with a significantly greater prevalence of *H. aduncum* in fish from the NW Mediterranean than from the Spanish Mediterranean coast. The prevalence of infection was found to be significantly related to anchovy length for both *Anisakis* and *H. aduncum*. More than 55% of *Anisakis* larvae were found in the muscle. According to these data, the risk of acquiring anisakiasis/anisakidosis from the consumption of raw or under-cooked anchovies may depend upon the area in which they were caught.

1. Introduction

Anisakids are parasitic aquatic nematodes with life cycles involving crustaceans, cephalopods, fish, marine mammals and sea birds. The accidental intake of these nematodes, generally after the consumption of raw or inadequately-cooked parasitized fish, can cause digestive disorders and/or allergies in humans (Audícana and Kennedy, 2008; Daschner et al., 2000). Anisakids are frequently detected in fish eaten by humans, including herring, hake, horse mackerel and cod, among others (Mattiucci and Nascetti, 2008).

Anisakids (generally *Anisakis* spp. and *Hysterothylacium aduncum*) have been found in parasitological studies of fish of the genus *Engraulis*. These nematodes have also been detected as parasites of the European anchovy, *E. encrasicolus* (Cuéllar et al., 1991; De la Torre Molina et al., 2000; Osanz, 2001; Pereira Bueno, 1992). Most Spanish cases of anisakidosis caused by *Anisakis* have been ascribed to the consumption of raw anchovies, usually as *boquerones en vinagre*, i.e. anchovies in vinegar (Del Olmo Escribano et al., 1998; Del Olmo Martínez et al., 2000; González Quijada et al., 2005; López Peñas et al., 2000; López-Serrano et al., 2000; Repiso Ortega et al., 2003), a highly popular dish in Spain and other Mediterranean countries. As for *H. aduncum*, it has been described as the etiological agent in at least one case of non-invasive human anisakidosis with digestive symptomatology (Yagi et al., 1996) and may have been involved in some cases of eating allergies (Valero et al., 2003).

Our study hypothesis was that the geographic origin of anchovies may influence the likelihood of their parasitization by anisakids and therefore the risk of anisakidosis from consumption of the raw fish. Although European Union and Spanish norms require food establishments to freeze fish destined for raw consumption, consumption of domestically prepared *boquerones en vinagre* still carries the risk of anisakiasis. However, if it could be demonstrated that fish from some areas carry a low risk of parasitization, these could be preferentially selected by consumers, since point-of-sale information on the origin of fish is mandatory in the European Union. Our hypothesis was tested by examination of anchovies captured in different fishing areas and landed at ports of the Western Mediterranean and Eastern Atlantic (southern Iberian Peninsula, Fig. 1).

We detected third larval stage (L3) *Anisakis* larva type I (*sensu* Berland, 1961) and *H. aduncum* in *E. encrasicolus* from both the Western Mediterranean Sea and the Eastern Atlantic Ocean, and found wide variations in the prevalence and mean abundance of these parasites in anchovies caught in different fishing areas.

2. Materials and methods

Seven hundred and ninety-two anchovies (*E. encrasicolus*) were obtained between October 1998 and September 1999 from the Granada

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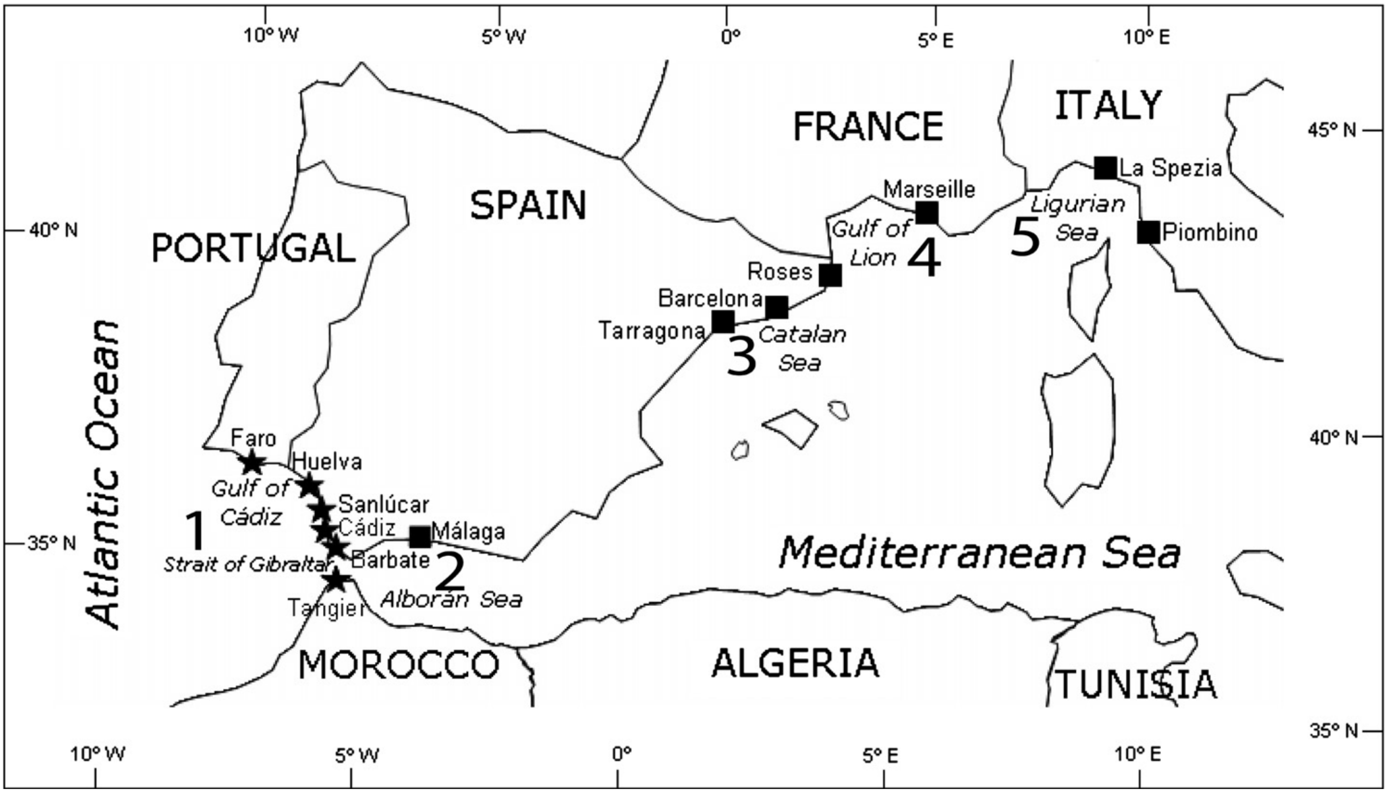

Fig. 1. Area of investigation showing sampling ports from East Atlantic Ocean (*star*) and West Mediterranean Sea (*square*). The prevalence (%) of *Anisakis* larva type I (A) and *Hysterothylacium aduncum* (H) in European anchovies according to fishing area were: A=13.1 and H=4.3 in area 1; A=1.4 and H=2.8 in area 2; A=1.9 and H=37.6 in area 3; A=3.9 and H=68.9 in area 4; A=21.9 and H=70.3 in area 5.

(Spain) wholesale fish market. Among these, 396 were from the Gulf of Cádiz and Strait of Gibraltar in the Eastern Atlantic Ocean (EAtl: Faro, a port in Portugal; Huelva, Sanlúcar de Barrameda, Cádiz and Barbate, ports in Spain; and Tangier, a port in Morocco) and 396 were from the Western Mediterranean Sea (WMed). Fish from the WMed were caught in the NW Sea of Alborán and landed at Málaga (72 fish); in the Catalonia coast and landed at Tarragona, Barcelona and Roses (157 fish); in the Gulf of Lion and landed at Marseille (103 fish); and in the Ligurian Sea and landed at La Spezia and Piombino (64 fish) (Fig. 1).

Specimens measured 9.0 to 18.8 cm. Given that the average lifespan of anchovies is approximately three years and their maximum length is 20 cm (Whitehead et al., 1988), virtually the entire commercial biological cycle of this species was sampled, as catching of specimens under 9 cm is prohibited.

The fish were processed as previously described (Rello et al., 2008a,b). Morphological classification of the nematodes followed Berland (1961) and Petter and Maillard (1988). *Anisakis* spp were not genetically identified.

The association between fish length and anisakid prevalence was analyzed using Fisher's exact test to compare prevalences and a bootstrap 2-sample *t*-test to compare mean intensities and abundances, with 95% confidence intervals being determined when possible. These analyses were performed using free QP 3.0 computer software (Quantitative Parasitology 3.0, Budapest; http://www.behav.org/qp/qp.htm) developed by Reiczigel and Rózsa to address the notoriously left-biased frequency distributions of parasites, based on the theoretical background published by Rózsa et al. (2000).

3. Results

The mean length of the 792 anchovies was 13.1 cm, standard deviation (SD) 1.9 cm. Only larval phases of anisakids identified as L3 of *Anisakis* larva type I (*sensu* Berland, 1961) or L3 of *H. aduncum* were found. The lengths of fish ranged from 9 to 17 cm (mean±SD=12.9±1.6 cm) among those captured in the EAtl area and from 9 to 18.8 cm (mean±SD=13.3±2.2 cm) among those from the WMed.

The findings showed there were a higher prevalence, mean abundance and mean intensity of *H. aduncum* than of *Anisakis* in the anchovies (Table 1). However, analysis by fishing area revealed a higher parasitization by *Anisakis* of fish from the EAtl ($p<0.001$) and by *H. aduncum* of fish from the WMed ($p<0.001$). With regard to the four fishing areas into which the WMed was divided (Table 2), the prevalence and mean abundance of *Anisakis* were highest ($p<0.001$ and $p<0.02$, respectively) in fish caught in the Ligurian Sea, the prevalence of *H. aduncum* infection was higher ($p<0.001$) in fish from the NW Mediterranean (Gulf of Lion and Ligurian Sea) than in those from the Spanish Mediterranean coastal waters (Málaga and Catalonia coasts), and the mean abundance of *H. aduncum* was highest in the Gulf of Lion ($p<0.0001$).

A large majority (75.7%) of *H. aduncum* parasites were detected in viscera and mesenteries, whereas only 42.8% of encapsulated or free *Anisakis* larvae were found in visceral localizations. The remaining parasites were localized in fish muscle (Tables 1 and 2). The highest mean muscle intensities (mmIs) of *H. aduncum* were found in fish from the Gulf of Lion (mmI=4.51) and the Ligurian Sea (mmI=4.90) and the highest mmI of *Anisakis* was in fish from the Ligurian Sea (mmI=3.67). We observed *in situ* the emergence of some *Anisakis* larvae through natural orifices and from muscle through the skin. These observations support the *post-mortem* migration of *Anisakis* larvae.

The prevalence of infection by these parasites increased linearly with increasing length of the host (Fig. 2), with high correlation indexes of $R^2=0.9670$ for *H. aduncum* and 0.9925 for *Anisakis*. Although the intensity of parasitization was also higher with greater fish length, this relationship was not significant ($R^2 < 0.32$ for both parasites).

4. Discussion

Various authors have reported that parasitization by anisakids can vary among fish of the same species but different origins. Thus, our group previously observed a higher prevalence of anisakid larvae in fish from the Atlantic Ocean than in those from the Mediterranean Sea (Adroher et al., 1996; Valero et al., 2000). Several researchers have related high prevalences of *Anisakis* in some fish and fishing areas to the presence of cetaceans (Arthur et al., 1982; Boily and Marcogliese, 1995).

Studies of anisakid parasitization in Spain have found low (≤5.6%) or no infection of European anchovies that were usually purchased in municipal markets (Cuéllar et al., 1991; De la Torre Molina et al., 2000; Osanz, 2001; Pereira Bueno, 1992; Viu et al., 1996). However, with the exception of Osanz, authors did not report the area in which the fish were caught or identify the anisakid species, hampering comparisons with the present results. Osanz (2001) found no infection in anchovies from waters near Tarragona on the Catalonia coast. In the present study, the overall results show a higher parasitization by *H. aduncum* than by *Anisakis*, but the distribution of these parasites differed according to the area in which they were captured.

Differences were also found among different areas of the Mediterranean, with a higher prevalence of *H. aduncum* in anchovies from the northern part of the Western Mediterranean (Gulf of Lion and Ligurian Sea). Anchovies from the Ligurian Sea also showed a higher prevalence and mean abundance of *Anisakis* compared with the other areas studied. Mattiucci et al. (2004) reported an elevated abundance of *A. pegreffii* in hake from the Ligurian Sea. These findings may be explained by the inclusion of this Sea in a Protected Marine Area, the Pelagos Sanctuary for Mediterranean Marine Mammals, which has the largest population of cetaceans in the Western Mediterranean (Notarbartolo di Sciara et al., 2008), including definitive hosts of *A. pegreffii* (Mattiucci and Nascetti, 2008).

Table 1
Prevalence, mean abundance and mean intensity of anisakid larvae in European anchovy (*Engraulis encrasicolus*) as a function of fishing area

Fishing area (number of fish)	Parasite	Prevalence 95% CI[a]	Mean abundance 95% CI	Mean intensity 95% CI (range)	Mean intensity in fish muscle 95% CI (range)
All areas (792)	Anisakids	31.95 28.79–35.28	2.25 1.81–2.73	7.03 5.81–8.45 (1–80)	ND[b]
	Anisakis larva type I	9.35 7.49–11.60	0.18 0.14–0.24	1.92 1.62–2.35 (1–9)	1.74 1.41–2.33 (1–9)
	H. aduncum	24.50* 21.58–27.64	2.07* 1.68–2.58	8.44* 6.87–10.35 (1–78)	4.03* 3.33–5.03 (1–23)
East Atlantic Ocean (396)	*Anisakis* larva type I	13.13 10.07–16.88	0.20 0.15–0.28	1.54 1.29–1.98 (1–8)	1.28 1.09–1.56 (1–4)
	H. aduncum	4.30 2.61–6.78	0.09 0.05–0.16	2.18 1.47–3.06 (1–6)	1.50 1.00–1.75 (1–2)
West Mediterranean Sea (396)	*Anisakis* larva type I	5.56 3.62–8.29	0.16 0.09–0.25	2.82 2.14–3.82 (1–9)	2.79 1.86–4.29 (1–9)
	H. aduncum	44.69 39.77–49.66	4.04 3.18–4.99	9.03 7.41–11.09 (1–78)	4.16 3.37–5.16 (1–23)

Prevalence = 100*N*/*F*, mean intensity = *P*/*N*, mean abundance = *P*/*F*; where *F* is the total number of fish, *N* is the number of infected fish, and *P* is the number of parasites.
[a] CI: confidence interval.
[b] ND: not determined.
* Significant difference ($p < 0.0001$) between the values for *Anisakis* and *H. aduncum*.

Table 2
Parameters of parasitization by anisakids in European anchovy (*Engraulis encrasicolus*) from the West Mediterranean Sea as a function of fishing area

Fishing area (number of fish)	Parasite	Prevalence 95%CI[a]	Mean abundance 95%CI	Mean intensity 95% CI (range)	Mean intensity in fish muscle 95% CI (range)
NW Alborán Sea (72)	*Anisakis* larva type I	1.39 0.08–7.40	0.01 0.00–0.04	1[uc] (1)	0[na] (–)
	H. aduncum	2.78 0.50–9.52	0.11 0.00–0.28	4[uc] (4)	1.50[uc] (1–2)
Catalonia Coast (157)	*Anisakis* larva type I	1.91 0.53–5.60	0.03 0.01–0.10	1.67 1.00–2.33 (1–5)	1.33 1.00–1.67 (1–2)
	H. aduncum	37.58 30.22–45.53	0.80 0.61–1.03	2.12 1.78–2.54 (1–7)	1.62 1.23–2.15 (1–4)
Gulf of Lion (103)	*Anisakis* larva type I	3.88 1.34–9.56	0.06 0.01–0.13	1.50 1.00–1.75 (1–6)	1[uc] (1)
	H. aduncum	68.93 59.26–77.27	11.74 9.03–15.20	17.03 13.61–21.34 (1–78)	4.51 3.47–5.98 (1–23)
Ligurian Sea (64)	*Anisakis* larva type I	21.88 13.06–33.51	0.78 0.41–1.27	3.57 2.64–4.79 (1–9)	3.67 2.44–5.56 (1–9)
	H. aduncum	70.31 57.86–80.66	4.03 2.88–5.95	5.73 4.29–8.18 (1–32)	4.90 3.38–7.21 (1–20)

Prevalence = 100*N*/*F*, mean intensity = *P*/*N*, mean abundance = *P*/*F*; where *F* is the total number of fish, *N* is the number of infected fish, and *P* is the number of parasites.
[a] CI: confidence interval.
[uc] 95% confidence intervals are uncertain.
[na] confidence intervals are not applicable.

The high prevalence and mean abundance of *Anisakis* in the Atlantic waters of the Gulf of Cádiz and the Strait of Gibraltar may be also related to the presence of cetaceans, which are frequently observed in the Strait and adjacent areas, most being members of four dolphin species that are definitive hosts of *A. simplex* s.l. (Mattiucci and Nascetti, 2008), in addition to migratory cetaceans on their way into the Mediterranean or returning to the Atlantic (De Stephanis et al., 2008; Raga and Pantoja, 2004). Within the western Mediterranean Sea, the presence of cetaceans varies according to the

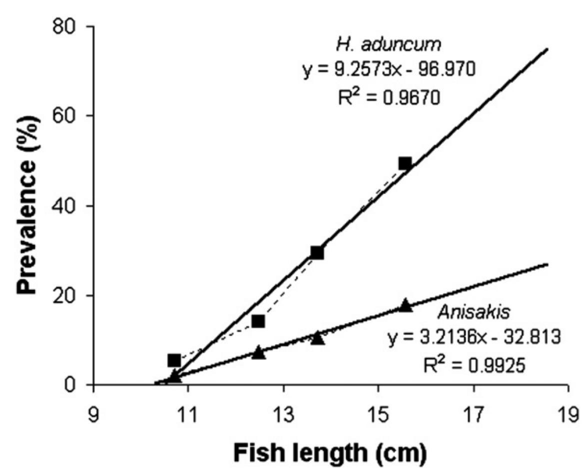

Fig. 2. Prevalences of *Anisakis* larva type I (▲) and *Hysterothylacium aduncum* (■) as functions of fish length. Linear regression analyses were performed. The *X*-axis represents the mean length for each length class of the host, *Engraulis encrasicolus*. The number of examined hosts per length class was as follows: 205 fish measured between 9.0 and 11.9 cm (average 10.71); 193 fish between 12.0 and 13.0 (average 12.49); 191 fish between 13.1 and 14.4 (average 13.71); 203 fish between 14.5 and 18.8 (average 15.57).

topographical, hydrographical and trophic conditions (Gannier, 2005). The areas with highest cetacean density in the present survey appear to be the Sanctuary of Pelagos and the Strait of Gibraltar and adjacent waters (Notarbartolo di Sciara et al., 2008; Raga and Pantoja, 2004).

Among the West Mediterranean fishing areas surveyed, a high prevalence of *H. aduncum* infection was found in the Gulf of Lion and Ligurian Sea, although these two areas significantly differed in mean abundance. While we have found no published data on possible definitive host fish for this parasite in these areas, the high prevalence and abundance of the parasite suggest that the hosts are also very abundant in the northern WMed. In this regard, the Gulf of Lion is host to an important hake fishing industry. The numbers of the first intermediate hosts (small crustaceans) and suitable hydrographic conditions are further key factors (Klimpel and Rückert, 2005).

The present data reveal a marked prevalence of L3 *Anisakis* larva type I (*sensu* Berland, 1961) in both visceral and extra-visceral localizations in anchovies. Various authors have proposed a larval migration from viscera to musculature after the death of the host fish (Smith and Wootten, 1975; Wootten and Waddell, 1977), including fish of the genus *Engraulis*, such as *E. japonica* (Kino et al., 1993). Therefore, larval migration probably produces a higher presence of larvae in fish muscle, increasing the risk of acquiring anisakiasis by the consumption of *boquerones en vinagre.* Moreover, this dish is usually prepared with larger anchovies, which showed higher prevalence and mean intensity values compared with smaller specimens. This relationship between the prevalence of *Anisakis* or *H. aduncum* and greater length, i.e. age of fish, has also been observed in other fish species (Adroher et al., 1996; Hemmingsen et al., 2000; Takao 1990; Valero et al., 2000, 2006a,b). This association suggests an accumulation of parasites over time (Bussmann and Ehrich, 1979), since the diet of anchovies, i.e. copepods and other zooplankton, does not change substantially during their life (Palomera et al., 2007). Hence, the anchovy acts as second intermediate host. Final hosts of *H. aduncum* are larger fish that prey on the smaller, infected fish.

To summarize, these findings indicate that fish captured in areas with an abundant populations of cetaceans have a higher prevalence of *Anisakis* and may carry a greater risk of anisakiasis for the consumer. Further studies are required to identify marine areas with a higher abundance of parasites that may affect human health and to determine whether the abundance in given areas changes over time, allowing measures to be implemented to minimize human exposure to the parasites.

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