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# de Comunicaciones a texto completo

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El libro de capítulos de las III Jornadas Internacionales y V Nacionales de Ciencias de la Salud comprende el compendio de comunicaciones aceptadas a texto completo y enviadas siguiendo las pautas establecidas por el Comité Científico. Éstas debían seguir diferentes premisas de formato y metodología aceptados por la comunidad internacional como requisitos imprescindibles para una trabajo de calidad científica. Se llevó a cabo un proceso ciego de revisión por pares para la aceptación o el rechazo de los trabajos aquí publicados, cuyo resultado recoge el presente libro de capítulos.

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# SURVEY OF PARASITIZATION BY *ANISAKIS SIMPLEX*, ETIOLOGICAL AGENT OF THE HUMAN ANISAKIASIS, IN SARDINES

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## INTRODUCTION

*Anisakis simplex* is a nematode of the family Anisakidae which parasitizes a large number of fishes, crustaceans and cephalopods, as intermediate/paratenic hosts, and cetaceans, as final hosts. This parasite affects the man causing the human anisakiasis. The man is usually infected by eating raw, marinated or undercooked fish and squid with third larval stage (L3) of *A. simplex*. Anisakiasis is a concern of public health in those countries where the fish is consumed raw. For example in Japan, 2000-3000 new cases per year are recorded (Umehara et al., 2007). Also in Spain, new cases every year are recorded and three cases caused by sardines ingestion were described in 1992 (Barros et al., 1992; López-Vélez et al., 1992). The presence of *Anisakis* larvae in commercial fishes is frequent, especially in fishes such as European hake (*Merluccius merluccius*) with a prevalence of 87.97% (Valero et al. 2006), blue whiting (*Micromesistius poutassou*) 81.7% (Martín-Sánchez et al., 2005) and horse mackerel (*Trachurus trachurus*) 43.9% (Sanmartín et al., 1989).

## OBJECTIVES

This epidemiological survey has been carried out in order to know the parasitization by *Anisakis simplex* in sardines (*Sardina pilchardus*) and determine the risk of human anisakiasis by raw, marinated or undercooked sardine consumption.

## MATERIALS AND METHODS

A total of 190 sardines (*Sardina pilchardus* Walbaum, 1792, family Clupeidae) were examined. The samples come from 5 fishing ports, 3 from Southern Spain: Málaga, Cádiz, and Isla Cristina (Huelva); and two from Northern Spain: A Coruña, and Ondarroa (Biscay). The fish were bought in the fish market of Granada (Southern Spain) and transported to laboratory in ice. In the laboratory, the fish were measured and weighted and then, they were dissected to examine the presence of *Anisakis* larvae in the body cavity. The viscera were examined for parasites, extracted, and weighted. The larvae collected were morphologically identified as L3 of *Anisakis simplex* s.l. Rudolphi, 1809 (according to Hartwich, 1974, and Peter and Maillard, 1988), and they were washed with NaCl 0.9% (w/v) solution and immediately placed at -20 ºC. Next, the viscera and musculature were separately digested with a solution of pepsin/HCl at pH 2.0 during 2 h for the former and 6 h for the latter. Then, the digested materials were examined for worms. Again, the larvae identified as *A. simplex* s.l. were collected, washed and frozen, as above.

The epidemiological parameters, prevalence (proportion of fish infected in the sample surveyed), mean intensity (parasites per infected fish in the sample), and mean abundance (parasites per examined fish in the sample), were determined according to methods used by Bush et al. (1997).

## RESULTS

The sardine average total length is similar in all fishing ports surveyed (Fig. 1A), between 19.7 cm (Málaga) and 21.7 cm (A Coruña). The mean weight was between 68 and 76 g, except in A Coruña with a mean of 100 g (Fig. 1B).

All *Anisakis* larvae collected were morphologically identified as L3 of *A. simplex* s.l. The determined epidemiological parameters showed variations among the fishing ports. So, the ports of Northern Spain showed higher prevalence (P) than in the ports of southern (Fig. 2A), being maximum in A Coruña (P = 35%) and minima in Málaga and Isla Cristina (P = 0%). Regard to mean intensity (MI), the fishing port of A Coruña showed the sardines more parasitized with MI = 2, while the infected sardines from Ondarroa and Cádiz only

hosted 1 larva (MI = 1) (Fig. 2B). Consequently, the mean abundance (MA) was much higher in Ondarroa (MA = 0.7) than in the other ports (MA = 0-0.05) (Fig. 2C).

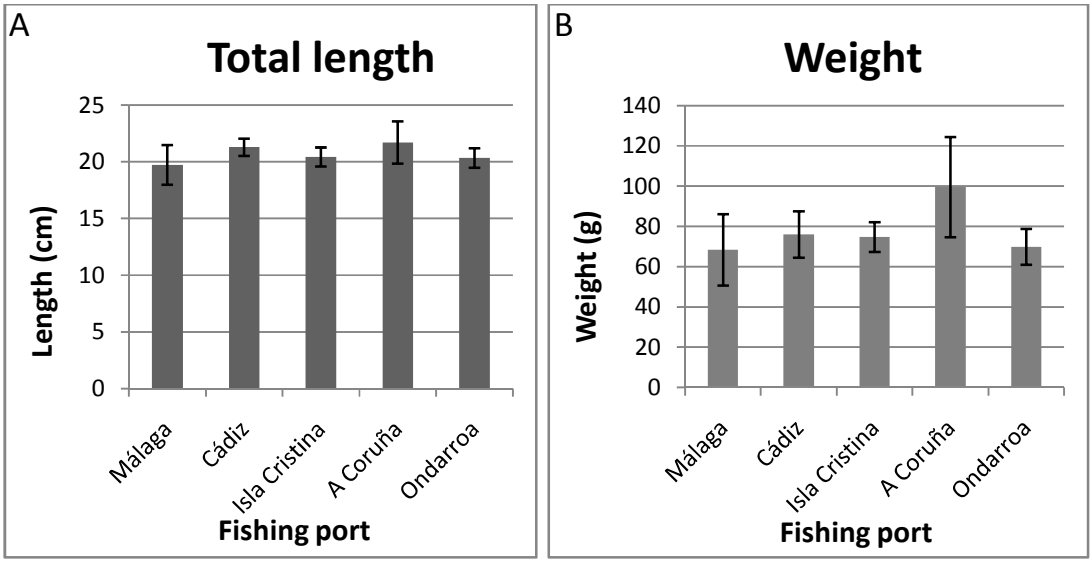


Fig. 1.- Total length ± SD (A) and total weight ± SD (B) of the sardines according to fishing port.

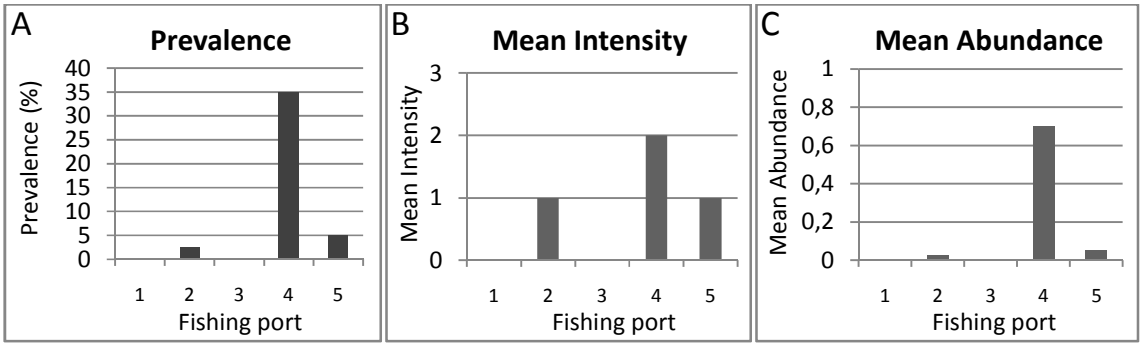


Fig. 2.- Epidemiological parameters of *Anisakis simplex* s.l. in sardines according to fishing port (1, Málaga; 2, Cádiz; 3, Isla Cristina; 4, A Coruña; 5, Ondarroa). A) Prevalence. B) Mean Intensity. C) Mean Abundance.

## DISCUSSION

The genus *Anisakis* comprises two major clades. The first one includes the complex *Anisakis simplex* s.l. (*A. simplex* s.s., *A. pegreffii*, *A. simplex* C), as well as *A. typica*, *A. nascettii* and *A. ziphidarum*; the second one consists of the complex *A. physeteris* (*A. brevispiculata*, *A paggiae*, *A. physeteris*) (Mattiucci and Nascetti, 2008; Mattiucci et al., 2009). The species belonging to complex *A. simplex* s.l. causing 97% of cases of human

anisakiasis, especially *A. simplex* s.s. and *A. pegreffii*, according to genetic studies (Umehara et al., 2007).

In accordance with previous studies related with the presence of parasites of the complex *A. simplex* around Iberian Peninsula, *A. simplex* s.s. is mainly distributed within the Atlantic Ocean coasts and *A. pegreffii* within Mediterranean ones (Mattiucci y Nascetti, 2006). Experiments carried out with rats in our Department (Rello, 2003) showed *A. simplex* s.s. have a higher capacity of penetration through digestive mucosa than *A. pegreffii*. By these reasons, we suggest the hypothesis that *A. simplex* s.s. is able to infect the sardines and *A. pegreffii* is not. This could explain the results of several authors which find *Anisakis simplex* s.l. in sardines from Atlantic waters and no *Anisakis* in sardines of Mediterranean waters (Silva and Eiras, 2003; Rello et al. 2008). In this sense, Umehara et al (2007) have analyzed *Anisakis* larvae from human infections and they have molecularly determined that these larvae are mainly *A. simplex* s.s. This shows that *A. simplex* s.s. and *A. pegreffii* have different pathogenic abilities. Further genetic studies are needed to highlight this question.

Finally, according to epidemiological parameters determined in this survey, the sardines suppose a low risk for human health in southern Spain (P = 0.9%), being higher risk in the northern Spain (P = 27.5%).

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