Sci Parasitol 13(2):77-81, June 2012
ISSN 1582-1366

SHORT RESEARCH NOTE

Morphometrics for Anisakiasis diagnosis in the North Atlantic horse mackerel *(Trachurus trachurus)*

Ramona Elena Soric[1,2]✉, Luz Calia Miramontes Sequeiros[2], Liviu Miron[1], Antonio Palanca Soler[2]

1 – "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Department of Clinics, Aleea Mihail Sadoveanu nr. 8, 700489 Iași, Romania.

2 – University of Vigo, Faculty of Biology, Department of Ecology and Animal Biology, Laboratorio de Anatomía Animal, Spain.

Correspondence: Tel. +40 232 407315, Fax +40 232 260650, E-mail ramonas@uaiasi.ro

Abstract. Consumption of raw fish or improperly cooked fish can cause large and serious diseases in humans as an intermediate accidental host. Since 1960 when was described Anisakiasis in humans, the number of Anisakiasis cases has been increased worldwide reaching over 34,000, almost half of them recorded in Japan. *Anisakis* spp. is a very common parasite among free-living fishes. Our goal is to find morphological differences in the shape of *Trachurus trachurus* with a high and a low rate of Anisakiasis. We compared the shape of those extreme groups belonging to mentioned Anisikiasis rate, using 10 landmarks. We have analyzed the geometric data in the Morpho J software. We have obtained a T-square: 649.0912 (p-value: 0.14799). The first principal component (PC1) accounts the 83.87% of the total variance. The larvae L3 of *A. simplex* was observed encysted in the fish body cavity, gonades, liver, and the stomach muscle. We have found that in seven of the fishes the number of *Anisakis* spp. was higher than 60. The infection rate was 80%, which means that the infection intensity was 64.6 larvae per fish examined and the abundance of parasites/ infected fish was of 51.73. Our preliminary results state that there are significant differences in the body shape of our studied Horse Mackerel stock between highly parasited and low parasited fishes.

Keywords: Anisakiasis; Diagnosis; Geometric morphometrics; *Trachurus trachurus.*

Received 22/05/2012. Accepted 15/06/2012.

Introduction

In its life cycle, *Anisakis spp.* needs three hosts: two intermediate hosts, one represented by invertebrates, one by marine zooplanktonic fishes (Herring, Hake, Horse Mackerel, Saithe, Anglerfish, Garfish, Cod, Haddock, Whiting, Turbot and Flounder), and a definitive host represented by a marine mammal. Several studies demonstrated that the host specificity of *Anisakis spp*. is low since it was identified in many different fish families (Klimpel et al., 2010; Skov et al., 2009). The most common species of Anisakis found in Horse Mackerel populations from Atlantic Ocean and Mediterranean Sea are *A. simplex* and *A. peregrefii* (Mattiucci and Nascetti, 2008), and they were also identified in fishes from different families as *Macrouridae, Ternoptychidae, Carangidae, Scombridae*, and *Stomidae* (Kellermanns et al., 2009).

Anisakis spp. is very common parasite among free-living fish with a prevalence of 49-100% (Klimpel et al., 2004). Abollo et al. (2001) recorded a prevalence of 100% in five fish species from Galician waters. Due to both *Anisakis spp*. low specificity for fish host and permanent contact between fish and invertebrate host, this parasite can evolve in marine cultured fish, but the incidence of this disease is very low (Skov et al., 2009).

A. simplex and *A. pegreffii* found in *Trachurus trachurus* are usually localized in the muscles, liver, body cavity, and gonades (Santoroa et al., 2010). Anisakis prevalence accounts up to 88%, with an incidence of 15 %, and one of the principal pathogenic effects on fish and paratenic host, are compression on organs and granulomatous inflammation of serous membranes. The post-mortem migration of larvae of *Anisakis spp.* observed in several fish species such as *Scomber scombrus*, *Trachurus trachurus* and *Micromesistius poutassou* could be interpreted as a way of parasite defense (Santoroa, 2010).

Controlling disease-causing nematodes in naturally infected populations of fishes is difficult and presents numerous logistic problems. Pascual et al. (2010) evaluated the survival of larvae of *Anisakis spp*. to CO_2 modified-atmosphere packaging, the most used method of packing in food industry. In anisakiasis, it has been suggested the culling of birds and seal definitive host, as means of controlling the parasite abundance in the fish host. Disease control in wild intermediate host is very difficult (Woo and Bruno, 2006), therefore, the possibility of identifying fish anisakiasis, based on external morphometric changes, can play an important role in preventing disease in humans.

Under this view, our main goal is to identify external morphological differences among Horse Mackerel specimens that could highlight the presence of parasitic larvae *Anisakis* spp.

Material and methods

Study area

We study the mackerel *(Trachurus trachurus)* stock from the Northeast Atlantic Ocean collected in April of 2011 by commercial fisheries in Galicia (Spain). Coastal fishing method used was trawling, usually about 60 miles. Total number of specimens studied was 15, six females and nine males. All samples were weighted in a balance to the nearest 0.1 g. We‘ve used a high-resolution digital camera (PANASONIC LUMIX 18X) in a Kaiser RS1 camera copy stand with a counter-balanced height adjustment and a non-reflecting matt black base plate with grid and height-adjustable feet to take pictures of the left lateral view of each specimen.

Parasite diagnosis

The whole body cavity, and the viscera of each sample, were carefully dissected and thoroughly examined for anisakids. Most larvae were examined directly under a light microscope, but some of anisakids were fixed in 70% ethanol and cleared in glycerin for identification of the species. *Anisakis simplex L3* was identified based on the following morphological characters: the shape and the presence of the boring tooth, the shape of the tail and the presence of the mucron, and the shape of the ventriculus (Choi et al., 2011).

We calculated the infection rate (IR = number of fish positive/number of fish examined × 100), abundance (A = total number of larvae detected/number of fish examined), and intensity of infection (I = total number of larvae detected/number of fish infected) were calculated according to Bush et al. (1997).

2D geometric morphometric analyses

In order to develop this research a two dimensional geometric morphometric approach was used to investigate the morphology variability in the studied species in order to assess any morphological variation and possible relationship with parasite infection. We plotted the fishes into three different classes in function of the *Anisakis* number found during the parasite diagnosis: low, intermediate, and high presence. After we select the extreme classes that is to say low and high parasited fishes to conduct the geometric morphometric analyses. We select four groups of landmarks along the body of the fish (figure 1). Two landmarks compose the first group of landmarks: anterior tip of the premaxilla (1) and the middle point of the eye (2). Three landmarks in the perpendicular cross line to the pectoral fin (4, 3 and 5) as well as to the middle point of the lateral line (6, 7 and 8) that compose the second and third group of landmarks. Finally, the caudal peduncle (9) and the fork of the caudal fin compose the fourth group of landmarks (10).

Figure 1. Groups of landmarks along the body of the fish selected to conduct the shape differences analyses in *Trachurus trachurus* from Northeast Atlantic Ocean

Data was stored in a tpsDIG file and imported to MorphoJ program (version 1.02d, freely available at: http://www.flywings.org.uk/MorphoJ_page.htm. We have performed a subsequent morphometric analysis, such as Generalized Procrustes Analysis, aligned by principal axes. We have conducted a multivariate ordination, in this case a Principal Component Analysis (PCA) classifying our data by pairs of groups in order to compare the shape of parasited and non-parasited fishes.

Results

Discriminant function analysis performed in order to compare the shape between the most and the lowest group of parasited fishes was a T test (T-square: 649.0912, p-value (parametric): 0.14799). In the PCA the first principal component (PC1) accounts the 83.87% of the total variance (figure 2).

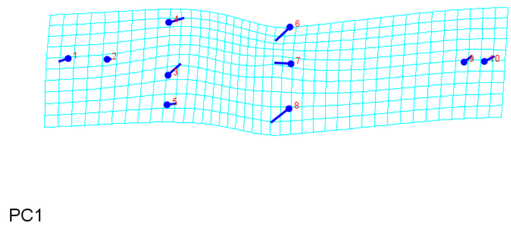

Figure 2. Grid showing the shape differences found after performing the Principal Component analyses (PCA) explained by the first Principal Component (PC1). Comparison was made between fishes with a high and a low number of parasites

The larvae L3 of *A. simplex* were observed encysted in the fish body cavity, gonades, liver, and the stomach muscle. We have found that in seven of the fishes the number of *Anisakis spp*. was higher than 60. The infection rate was 80%, which means that the infection intensity was 64.6 larvae per fish examined and abundance of the parasites/ infected fish was of 51.73 (table 1). These results were calculated for fish harvested in April.

Table 1. Summary of the parasites identified in the specimens of *Trachurus trachurus* from Northeast Atlantic Ocean

Sex	Number of fish:				
	examinated	with 0 parasites	with 1-10 parasites	with 11-60 parasites	with more than 60 parasites
Female	6	0	1	1	4
Male	9	3	2	1	3

Discussion

Our preliminary results state that there are significant differences in the body shape of our studied Horse Mackerel stock between highly parasited and low parasited fishes.

Costa et al. (2003) notes that in *T. picturatus* the nematode load ranged from 1 to 7, he only examined, in one year period, 40 fish ranging in length from 17 to 28 cm, with a total number of nematodes of 66 with a mean intensity of 2.64±0.329, and a mean abundance of 1.65±0.329. This is probably a consequence of their smaller size and feeding on small planktonic crustaceans. In our investigation total length of the fish was more than 28 cm and that can be a reason for the large number of parasites.

A relatively low intensity of infection with *A. simplex* (4.8) in the epipelagic fish *T. trachurus* was previously reported from southern Spanish waters (Adroher et al., 1996) for fish with a total length above 23 up to 30 cm long.

Seasonal variation in infection levels was observed in some fish infected with *Anisakis spp*. (Choi et al., 2011) but in *Trachurus spp*., anisakiasis prevalence, intensity and abundance was reported to be very low (Costa et al., 2003). In contrast, that was not observed in this research. It has to be noted that in our research the fish was analyzed only in one-month period, unlike the anterior authors who analyzed for a period of one year, but on the same number of fish per month. This research must continue for a period of one year to see if anisakiasis diseases in *Trachurus trachurus* present a seasonal variation in our studied area.

The presence of the lipid of host origin in the larvae stage L3 of *A. simplex* (Schaufler et al., 2008) leads to the conclusion that *Anisakis* larval stage uses the fish lipid deposit, especially triacylglycerols (TAG) from fish muscle. The loss of energy, depletion of TAG, and loss of lipids deposits from the muscle, can affect the swimming, speed and swimming depth of the fish (Mika et al., 2010). The nematode larvae can induce this behavior of the infected fish and the purpose could be to weaken the fish organism so it can become an easy prey. The loss of the lipid deposit from the muscle (Schaufler et al., 2008) promotes visceral adhesions that lead to the compression of organs and granulomatous inflammation of serous membranes (Santoroa et al., 2010) with the modification of fish body shape.

High infection rates found in fishes with features of flabby flesh and high fat contents (Choi et al., 2011), might be a result of the loss of the muscle lipids produced by the parasite hydrolysis of host lipids by lipases, and phospholipases. This could be the reason of the accumulation of free fatty acids in the body cavity (Onusiriuka, 2002), as well as, deformation of the fish body shape.

Because anisakiasis is a zoonotic disease, and of the postmortem migration of larvae into the fish muscle (Santoroa et al., 2010), the identification of an external method of diagnostic (before necropsy exam) will facilitates the control of the diseases in humans. Further studies are being undertaken to address more in depth study of such morphological differentiation.

References

Abollo E., Gestal C., Pascual S. 2001. *Anisakis* infestation in marine fish and cephalopods from Galician waters: an updated perspective. Parasitol. Res. 87(6):492-499.

Adroher F.J., Valero A., Ruiz-Valero J., Iglesias L. 1996. Larval anisakids (Nematoda: Ascaridoidea) in horse mackerel *(Trachurus trachurus)* from the fish market in Granada, Spain. Parasitol. Res. 82:319-322.

Bush A.O., Lafferty K.D., Lotz J.M., Shostak A.W. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J. Parasitol. 83:575-583.

Choi S.H., Kim J., Jin O.J., Cho M.K., Yu H.S., Cha H.J., Ock M.S. 2011. *Anisakis simplex* larvae: infection status in marine fish and cephalopods purchased from the Cooperative Fish Market in Busan, Korea. Korean J. Parasitol. 49(1):39-44.

Costa G., Pontes T., Mattiucci S., D'Amelio S. 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.

Kellermanns E., Klimpel S., Palm H.W. 2009. Parasite fauna of the Mediterranean grenadier *Coryphaenoides mediterraneus* (Giglioli, 1893) from the Mid-Atlantic Ridge (MAR). Acta Parasitol. 54(2):158-164.

Klimpel S., Palm H.W., Ruckert S., Piatkowski U., 2004. The lifecycle of *Anisakis simplex* in the Norwegian Deep (northern North Sea). Parasitol. Res. 94:1-9.

Klimpel S., Busch M.W., Sutton T., Palm H.W. 2010. Meso- and bathy-pelagic fish parasites at the Mid-Atlantic Ridge (MAR): Low host specificity and restricted parasite diversity. Deep-Sea Res. 57:596-603.

Mattiucci S., 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.

Mika A., Gołebiowski M., Szafranek J., Rokicki J., Stepnowski P. 2010. Identification of lipids in the cuticle of the parasitic nematode *Anisakis simplex* and the somatic tissues of the Atlantic cod *Gadus morhua.* Exp. Parasitol. 124:334-340.

Onusiriuka B. 2002. The effect of parasitism on the protein and fatty acid content of the Nile tilapia, *Oreochromis niloticus* and African catfish, *Clarias gariepinus* (Teugals) in River Kaduna, Nigeria. J. Aquat. Sci. 17:128-130.

Pascual S., Antonioa J., Caboa M.L, Piñeiroa C. 2010. *Anisakis* survival in refrigerated fish products under CO_2 modified-atmosphere. Food control. 21:1254-1256.

Santoroa M., Mattiuccia S., Paoletti M., Liotta A., Uberti B.D., Galiero G., Nascetti G. 2010. Molecular identification and pathology of *Anisakis pegreffii* (Nematoda: Anisakidae) infection in the Mediterranean loggerhead sea turtle *(Caretta caretta).* Vet. Parasitol. 174:65-71.

Schaufler L.E., Vollenweider J.J., Moles A. 2008. Changes in the lipid class and proximate compositions of coho salmon *(Oncorhynchus kisutch)* smolts infected with the nematode parasite *Philonema agubernaculum.* Com. Biochem. Physiol. Part B 149:148-152.

Skov J., Kania P.W., Olsen M.M. 2009. Nematode infections of maricultured and wild fishes in Danish waters: A comparative study. Aquaculture 298:24-28.

Woo P.T.K., Bruno D.W. 2006. Fish diseases and disorders. Vol. 1, 2nd Ed. Protozoan and Metazoan Infections.