

## Endoparasites of *Trachurus picturatus* (Pisces: Carangidae) from the Madeira and Canary Islands: Selecting parasites for use as tags

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**SUMMARY:** In order to evaluate the use of parasites as biological tags to identify populations of the oceanic horse mackerel, *Trachurus picturatus*, two samples of 100 fish each, caught off the Madeira and Canary Islands from January to June 2009, were examined for the presence of anisakids, trypanorhynch, acanthocephalans and liver coccidians. In *T. picturatus* from Madeira, prevalence of the coccidian *Goussia cruciata* reached 82%, whereas prevalence of the nematode *Anisakis* sp., the acanthocephalan *Rhadinorhynchus cadenati* and the trypanorhynch *Nybelinia lingualis* were 12.0%, 8.9% and 7.0%, respectively. In samples from the Canary Islands, prevalence of *G. cruciata* was 8.0%, *Anisakis* sp. and *R. cadenati* reached 5.0% and 42.0% respectively, and *N. lingualis* was not recovered. The absence of *N. lingualis* and the lower prevalence of *Anisakis* sp. appear to be related to the smaller size of the oceanic horse mackerels examined from the Canary Islands. Although significant differences in prevalence of two parasites, *G. cruciata* and *R. cadenati*, were found between the two regions investigated ( $\chi^2=125.13$ ,  $df=1$ ,  $p=0.000$  and  $\chi^2=40.77$ ,  $df=1$ ,  $p=0.000$ ), only *G. cruciata* was considered useful as a biological tag for the identification of populations of *T. picturatus*. In order to reach sound conclusions, an expansion of the temporal and spatial sampling strategy is recommended.

**Keywords:** parasites, biological tags, *Trachurus picturatus*, Madeira Islands, Canary Islands.

**RESUMEN:** ENDOPARÁSITOS DE *TRACHURUS PICTURATUS* (PISCES: CARANGIDAE) DE MADEIRA E ISLAS CANARIAS: SELECCIÓN PARA SU USO COMO MARCADORES BIOLÓGICOS. – Con objeto de evaluar el uso de parásitos como marcadores biológicos para identificar las poblaciones del chicharro, *Trachurus picturatus*, se analizaron dos muestras de 100 ejemplares capturados en Madeira y en las Islas Canarias, desde enero hasta junio de 2009, para determinar la presencia de anisákidos, trypanorhyncha, acantocéfalos y coccidios en el hígado. En *T. picturatus* de Madeira, el coccidio *Goussia cruciata* alcanzó un 82% de prevalencia, mientras que el nemátodo *Anisakis* sp., el acantocéfalo *Rhadinorhynchus cadenati* y el trypanorhyncha *Nybelinia lingualis* el 12.0%, 8.9% y 7.0%, respectivamente. La ausencia de *N. lingualis* y la escasa presencia de *Anisakis* sp en los ejemplares procedentes de las Islas Canarias podrían estar relacionadas con el menor tamaño de los ejemplares analizados. Aunque se hallaron diferencias significativas en la presencia de *G. cruciata* y *R. cadenati* entre las dos regiones estudiadas ( $\chi^2=125.13$ ,  $df=1$ ,  $p=0.000$  y  $\chi^2=40.77$ ,  $df=1$ ,  $p=0.000$ ), sólo *G. cruciata* fue considerado como marcador biológico útil para la identificación de poblaciones de *T. picturatus*. Se recomienda una ampliación espacio-temporal del muestreo con el fin de obtener conclusiones definitivas.

**Palabras clave:** parásitos, marcadores biológicos, *Trachurus picturatus*, Madeira, Islas Canarias.

## INTRODUCTION

Several studies have examined the parasite composition of commercially exploited fish species in distinct geographical regions with the aim of identifying different fish populations or stocks (MacKenzie 1990, George-Nascimento and Arancibia 1992, Williams *et al.* 1992, MacKenzie *et al.* 2008, Santos *et al.* 2009). Two different approaches have been recognized by a number of researchers for the use of parasites as indicators of fish population structure: one using a small number of parasite species (Sindermann 1957, MacKenzie 1985, Lang *et al.* 1990, Abaunza *et al.* 1995) and one using entire parasite assemblages (George-Nascimento 2000, Timi 2003, Brickle and MacKenzie 2007, MacKenzie *et al.* 2008, Henriquez *et al.* 2011). Because small pelagic fishes have lower parasite species richness, the first approach has been favoured in the study of population structuring in small pelagic fishes (Sindermann 1957, Reimer 1978, MacKenzie 1990, Abaunza *et al.* 1995).

The oceanic horse mackerel, *Trachurus picturatus* (Bowdich, 1825) (Pisces: Carangidae) is a small benthopelagic fish species that is commercially exploited in the Macaronesian region of the Azores, Madeira and Canary Islands. Its geographical range extends from the Mediterranean to the eastern central North Atlantic in neritic zones and island shelves, banks and seamounts (Smith-Vaniz 1986). According to Shabonayev and Ryazantseva (1977), there appeared to be differences in growth parameters and morphological characteristics between *T. picturatus* from the Azores and Canary Islands. Data obtained by Gouveia (1993) on the biology of this fish species suggested differences between populations of the Azores and Madeira Islands based on morphological characteristics.

Parasite species richness of this fish species was studied in the Azores Archipelago and Western Sahara Atlantic region (Gaevskaya and Kovaleva 1980, 1985) and in the Madeira Archipelago (Gonçalves 1996, Costa *et al.* 2012), evidencing regional differences in prevalence and intensity of some helminth parasites, which could indicate the existence of different populations of this fish species. Here we report the occurrence of some internal parasites of the oceanic horse mackerel from two geographic regions, the Madeira Islands and the Canary Islands, and discuss the feasibility of using two horse mackerel specific parasites, a protozoan apicomplexan and an acanthocephalan, as biological tags for the identification of populations of *T. picturatus*.

## MATERIALS AND METHODS

Samples of oceanic horse mackerel, *T. picturatus*, were obtained from commercial fish landings at the Madeira Islands (33°7'30''N, 16°16'30''W) and Canary Islands (28°16'7''N, 16°36'20''W) from January to March 2009 (first sampling period) and April to June 2009 (second sampling period) (Fig. 1). Additionally,



FIG. 1. – Map of the central North Atlantic (AO) showing sampling locations (black circles) at the Madeira Islands (MA) and Canary Islands (CA). (ME) Mediterranean Sea.

samples of oceanic horse mackerel from the Canary Islands were obtained in September and November 2009 (third sampling period) with the purpose of determining the life span of acanthocephalans.

After total fish length had been recorded in centimetres (cm), fish were kept deep frozen at  $-17^{\circ}\text{C}$ , until parasitological examination was possible. Initially the intention was to analyse all parasites (ecto and endoparasites). However, since fish were kept frozen for several months prior to examination, monogeneans and digeneans would be adversely affected. Therefore, it was decided to concentrate the analysis on coccidians, acanthocephalans, larval trypanorhynch and anisakid nematodes of the digestive tract and body cavity, which can still be studied after prolonged freezing. Fresh squash preparations of liver were examined unstained for the presence of coccidians, using a Zeiss Axioplan Photomicroscope equipped with DIC optics and an MC-Camera. Stomach and intestine were dissected and examined for the presence of helminths, using a Stemi 2000 binocular stereomicroscope. The body cavity was examined for the presence of larvae of helminth parasites.

Liver coccidians were identified according to Lom and Dyková (1992). Recovered helminths were preserved in either 70% or 95% ethanol. Those in 70% ethanol were cleared in lactophenol or in glycerine, mounted in glycerine jelly and identified according to Golvan (1969) for acanthocephalans, Palm (2004) for larval trypanorhynch, and Berland (1961) for nematodes. Prevalence (P, number of fish infected divided by the number of examined fish), mean intensity (total number of parasites of a particular taxon found divided by the number of infected fish), abundance (A, number of individuals of a particular parasite in a single infected or uninfected host) and mean abundance (total number of parasites of a particular taxon found divided by the total number of fish examined) were calculated according to Bush *et al.* (1997). Intensity (I, number of individuals of a particular parasite species in a single infected host) of infection with coccidians was evalu-

ated using a semi-quantitative method, on the basis of number of oocysts seen in a single squash from individual livers, rated as 1 (low) when fewer than 10 oocysts were seen, 2 when fewer than 50 oocysts were seen, 3 when 50-100 oocysts were seen and 4 (heavy) when more than 100 oocysts were seen in the liver smear.

Relationships between fish length and mean abundance of parasites were analysed with the Spearman's correlation coefficient. Differences in the abundance of parasites between sampling periods were examined with the Mann-Whitney U test (Fowler *et al.* 1998). Significance of prevalence differences between regions was studied with the chi-square test. Differences in fish length between sampling periods and regions were evaluated with the Mann-Whitney U test because the data were non-normal (Kolmogorov-Smirnov and Shapiro Wilk tests). Statistical analysis was performed with SPSS 11.0. The aggregation indices, variance/mean ratio ( $s^2/x$ ) and index of dispersion ( $D$ ) of Poulin (1993) were calculated using the program QP 3.0 (Rozsá *et al.* 2000).

## RESULTS

### Length and diet data of examined *Trachurus picturatus*

Two hundred *T. picturatus* were examined, 100 from the Madeira Islands, ranging in length from 16.20 to 30.40 (20.95±3.58; mean±sd), and 100 from the Canary Islands, with lengths from 13.50 to 22.50 cm (18.52±1.94), from January to June 2009. Significant differences in fish length were found between the two sampled regions, the smallest fish being caught in the Canary Islands (Mann-Whitney U=2900.5,  $z=-5.13$ ,  $p=0.000$ ). In each region, significant differences in fish length were also found between sampling periods,

TABLE 1. – Total body length (cm) of the oceanic horse mackerel, *Trachurus picturatus*, examined from two geographical regions, the Madeira Islands and the Canary Islands, in the first (January-March), second (April-June) and third (September-November) sampling periods.

Geographical region	Mean length ± SD (min.-max.)		
	Jan-March	April-June	Sept-Nov
Madeira Islands	19.21±1.95 (16.20-23.60)	22.70±3.99 (17.10-30.40)	–
Canary Islands	20.24±0.73 (19.10-22.30)	16.81±1.04 (13.70-18.60)	17.28±1.39 (14.40-20.0)

with bigger fish found in the second sampling period (April to June) in the Madeira Islands, whereas in the Canary Islands smaller fish were found in the second sampling period (Mann-Whitney,  $z=-2.20$ ,  $p=0.03$  and  $z=-8.62$ ,  $p=0.000$ , respectively) (Table 1). Stomach contents of fish sampled from the Canary Islands in the second sampling period contained mostly isopods and some remains of small fishes, whereas in the first and third sampling periods the stomachs were filled with remains of small fishes. Stomach contents of fish sampled from the Madeira Islands consisted of remains of fishes and euphausiids.

### *Goussia cruciata*

Oocysts of *Goussia cruciata* (Thélohan, 1894) Labbé, 1896), with sporocysts characteristically arranged forming a cross, were seen in livers of oceanic horse mackerel. Numbers of oocysts in the livers of *T. picturatus* from the Madeira Islands were higher than those found in fish from the Canary Islands. On a scale of 1 to 4 (low to heavy intensity of infection), 26.0% of livers of fish from the Madeira Islands reached intensity level 4, followed by 30.0% with intensity level 3 and 31.0% with intensity levels 1 and 2. Overall prevalence of *G. cruciata* in fish from the Madeira

TABLE 2. – Prevalence (P), mean intensity (MI) and mean abundance (MA) of parasites, with range in brackets, found infecting *Trachurus picturatus* from the Madeira Islands and the Canary Islands in the first (January-March) and second (April-June) sampling periods. (A) Adult stages; (L) larval stages.

Parasite species	P (%)	Madeira Islands		P	Canary Islands	
		MI	MA		MI	MA
<i>Goussia cruciata</i>						
January-March	100	–	–	16.0	–	–
April-June	71.10			0.0		
Total prevalence	82.0			8.0		
<i>Rhadinorhynchus cadenati</i> (A)						
January-March	0	0	0	0	0	0
April-June	8.90	3.25 (1-5)	0.29 (0-5)	84.0	18.74 (1-148)	15.74 (0-148)
Total prevalence	4.0			42.0		
<i>Anisakis</i> sp. (L)						
January-March	3.6	1	0.04	6.0	1.67	0.10
April-June	22.2	5.9 (1-15)	1.31 (0-15)	4.0	1.0	0.04
Total prevalence	12.0			5.0		
<i>Nybelinia lingualis</i> (L)						
January-March	10.90	1.67 (1-3)	0.18 (0-3)	0	–	–
April-June	2.20	1.0	0.02	0	–	–
Total prevalence	7.0			0		

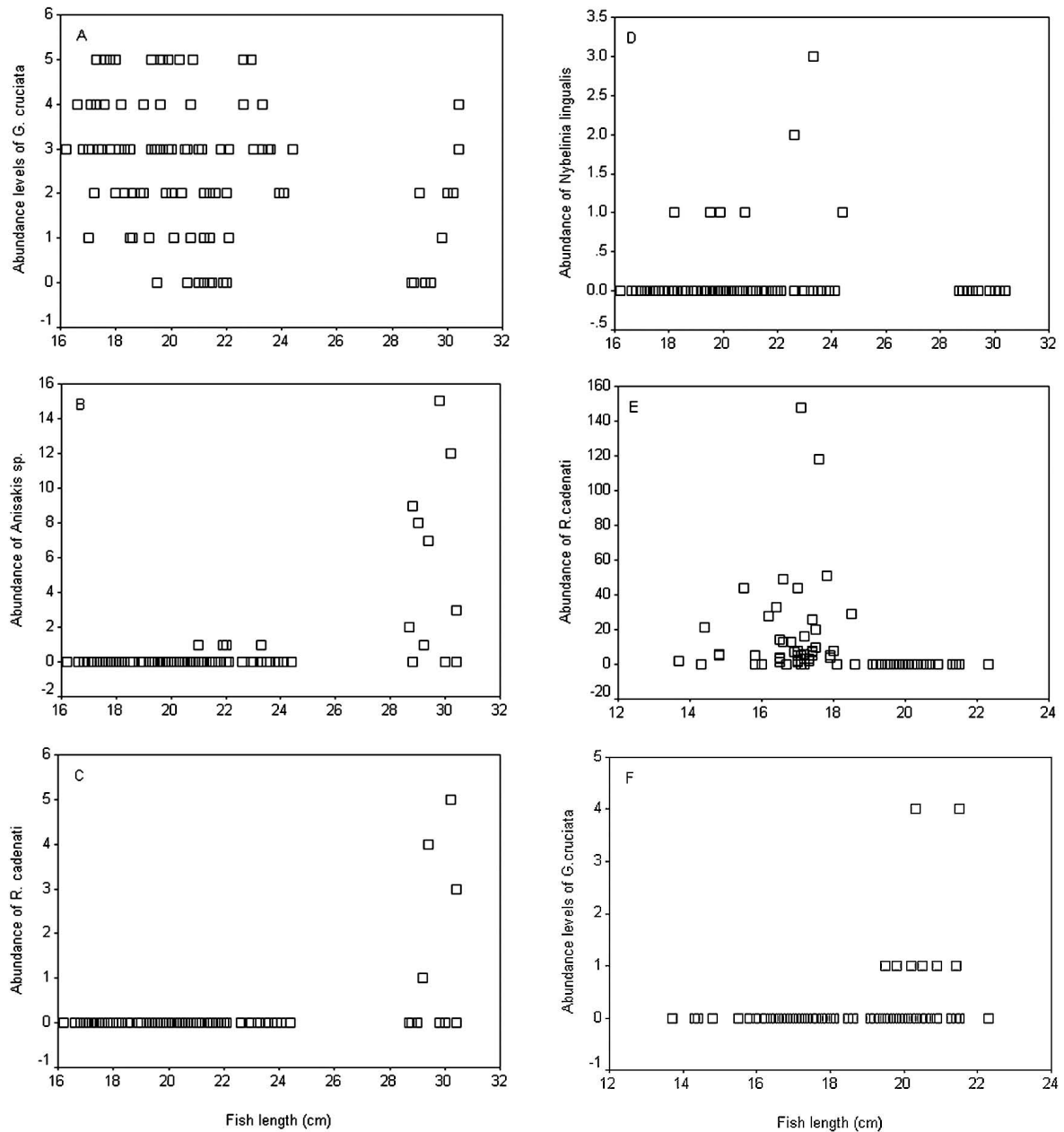


FIG. 2. – Abundance of endoparasites of *T. picturatus*, in relation to fish length, at the Madeira Islands: (A) *G. cruciata*, (B) *Anisakis* sp., (C) *R. cadenati*, (D) *N. lingualis* and at the Canary Islands: (E) *R. cadenati*, (F) *G. cruciata*.

Islands was 87.0%, with variation from 100% in the first sampling period (January to March) to 71.10% in the second sampling period (April to June). In fish from the Canary Islands, on the other hand, prevalence was 16.0% in the first sampling period, whereas no infected fish were found in the second sampling period. Intensity level 1 represented 6.0% and intensity level 4 represented 2.0% (Table 2). A significant negative correlation was found between fish length and mean abundance of *G. cruciata* in oceanic horse mackerel from the Madeira Islands ( $r=-0.33$ ,  $p=0.001$ ). Significant differences in prevalence of this coccidian were found

between the Madeira and Canary Islands ( $\chi^2=125.13$ ,  $df=1$ ,  $p=0.000$ ).

#### *Rhadinorhynchus cadenati*

A total of 787 individuals of *Rhadinorhynchus cadenati* (Golvan et Houin, 1964) were found in the intestines of fish from the Canary Islands and 13 individuals only were recovered from the intestines of fish from the Madeira Islands. Aggregation indices, variance/mean ratio ( $s^2/x$ ) and index of discrepancy (D) for *R. cadenati*, recovered from *T. picturatus* from

TABLE 3. – Prevalence (%) of *Goussia cruciata* and *Rhadinorhynchus cadenati* in relation to fish length at the Madeira and Canary Islands in two seasons of the year. (R) *R. cadenati*; (G) *Goussia cruciata*.

Length class (cm)	Madeira Islands		Canary Islands	
	Spring	Summer	Spring	Summer
13-18	62.9 (n=35)* (G) 0.0 (R)	46.1 (n=115)* (G) 0.0 (R)	no data no data	0.0 (G) 84.0 (R)
19-22 and ≥22 (only Madeira)	81.4 (G) 0.0 (R)	77.3 (G) 18.2 (R)	16.0 (G) 0.0 (R)	no data no data

\* Data from 1996: Spring (March-April); Summer (May-June); Winter (January-February). At the Madeira Islands 43 fish in spring, 22 in summer and 35 in winter; at the Canary Islands 50 in spring and 50 in June. Winter values are not shown because there were no fish samples from the Canary Islands in winter.

the Canary Islands, suggested that the distribution of this acanthocephalan is overdispersed ( $\chi^2=18.05$ ,  $df=12$ ,  $p=0.05$ ;  $s^2/x=56.77$ ;  $D=0.84$ ). While overall prevalence reached only 4.0% at the Madeira Islands, 42.0% of fish were infected at the Canary Islands. Infections with this parasite were mostly found in the second sampling period ( $P=84.0\%$ ,  $n=50$ ; see Table 2). Significant differences were found in the prevalence of *R. cadenati* between the Madeira and Canary Islands ( $\chi^2=40.77$ ,  $df=1$ ,  $p=0.000$ ). A negative significant correlation was observed between mean abundance and length for *R. cadenati* in the Canary Islands ( $r=-0.69$ ,  $p=0.000$ ). All infected fish were found in the second sampling period, so the factor most influencing the mean abundance of this parasite could be sampling period rather than length ( $U=200.00$ ,  $z=-8.07$ ,  $p=0.000$ ,  $r=-0.81$ ). At the Madeira Islands, on the other hand, the sampling period effect on the mean abundance of *R. cadenati* was weaker ( $U=1150.00$ ,  $z=-2.03$ ,  $p=0.04$ ,  $r=-0.20$ ). However, only 4 fish were infected with a maximum of 5 parasites, so the significance of this conclusion is not statistically strong. At the Canary Islands, 42 fish were infected, so the significance of the statistical results is more robust. An additional sample of 50 fish, from September ( $n=30$ ) and November 2009 ( $n=20$ ) composed of fish ranging in length from 14.40 to 20.0 cm ( $17.28\pm 1.39$ ) aimed to establish the timing of infection with *R. cadenati* and its life span, in order to further evaluate the validity of this parasite as a tag. Although fish were still found infected with *R. cadenati* in September ( $p=33.30\%$ ), mean intensity (1.30) and mean abundance values (0.43) were much lower than those found in June, and no infections were found in November 2009.

#### Larval stages of *Anisakis* sp. and *Nybelinia lingualis*

Sixty-one larval L3 stages of *Anisakis* sp. (Dujardin, 1845) were found in the visceral cavity of *T. picturatus* from the Madeira Islands and 7 in oceanic horse mackerel from the Canary Islands. Prevalence reached 12.0% in the Madeira Islands and 5.0% in the Canary Islands (Table 2). Ten larval stages of *Nybelinia lingualis* (Cuvier, 1817) were found in 7 oceanic horse mackerel from the Madeira Islands only ( $P=7.0\%$ ). Aggregation indices, variance/mean ratio ( $s^2/x$ ) and

index of discrepancy ( $D$ ) for *Anisakis* sp. suggested an overdispersed distribution ( $\chi^2=3.73$ ,  $df=5$ ,  $p=0.05$ ;  $s^2/x=8.70$ ;  $D=0.93$ ). Fish length was positively correlated with the mean abundance of *Anisakis* sp. in the Madeira Islands only ( $r=0.48$ ;  $p=0.000$ ).

Table 2 shows mean intensity and mean abundance values of the helminth taxa *Anisakis* sp., *N. lingualis* and *R. cadenati*, as well as the prevalence of both helminths and the coccidian parasite. The abundance of the parasites in relation to fish length for *T. picturatus* caught at the Madeira and Canary Islands are represented in Figure 2.

#### DISCUSSION

Previous surveys of parasites of *T. picturatus* (and *T. trachurus*) suggested that some of their endohelminth parasites, such as digeneans, were unsuitable to be used as biological tags for stock identification due to their short life span and low prevalence of infection (Gaevskaia and Kovaleva 1985, MacKenzie *et al.* 2008, Costa *et al.* 2012). Other parasites with promising roles in elucidating aspects of the population biology and migrations of *T. picturatus* included larval cestodes, acanthocephalans and *G. cruciata* (see Gaevskaia and Kovaleva 1985, Costa *et al.* 2012).

#### *Goussia cruciata*

*Goussia cruciata* is known to infect the livers of members of the genus *Trachurus*, namely *T. trachurus*, *T. mediterraneus* and *T. picturatus*, from the Mediterranean and the Atlantic Ocean (Kalfa-Papaioannou and Athanassopoulou-Raptopoulou 1984, Daoudi 1987, Gonçalves 1996, Diouf *et al.* 2000, Abollo *et al.* 2001, MacKenzie *et al.* 2008). Our results showed that *G. cruciata* is very common at the Madeira Islands, as previously described by Gonçalves (1996), who also reported the presence of this parasite all year round, and even infecting small fishes of 12 cm length. On the other hand *G. cruciata* is less common in the Canary Islands (Table 2). It was not found on the banks of the Azores Archipelago (Irving and Meteor banks) or in the Western Sahara, specifically in *T. picturatus*, but it was found in other *Trachurus* species from off West Africa (see Table 3) (Gaevskaia and Kovaleva 1980, 1985, Diouf *et al.* 2000, MacKenzie *et al.* 2008).

Differences in prevalence of this coccidian have been reported previously, with higher prevalence around the Iberian Peninsula and in the central Mediterranean than in the western Mediterranean and the Canary Islands (Kalfa-Papaioannou and Athanassopoulou-Raptopoulou 1984, Daoudi 1987, MacKenzie *et al.* 2008). As in the present results, variations in the prevalence of this coccidian suggested a relationship with sampling period rather than with fish length, with higher prevalence in the winter-spring period in comparison with summer (Gonçalves 1996, MacKenzie *et al.* 2008). When the values of the prevalence and intensity of infection data of this coccidian at the Madeira and Canary Islands in the present survey are compared, the differences found cannot be attributed to sampling period, as fish from these two regions were caught at the same time of the year.

Although there were significant differences in the fish length between the two regions, previous results have concluded that prevalence was not related to host length (Gonçalves 1996, MacKenzie *et al.* 2008). It appears that the southern boundary for this parasite is located at the Madeira Islands, with some infected fish in the Canary Islands due to migration of fish from the Madeira Islands to Canary Islands, or from the Mediterranean, where *G. cruciata* is common in *Trachurus* species.

#### *Rhadinorhynchus cadenati*

Differences were also found in the prevalence and mean abundance of *R. cadenati*, a parasite known to be common in *Trachurus* species off West Africa and the Azores Islands (Golvan 1969, Gaevskaya and Kovaleva 1985, MacKenzie *et al.* 2008). Our results suggested that prevalence and mean abundance of *R. cadenati* is not entirely length-related as was previously thought (MacKenzie *et al.* 2008), but rather is more dependent on the sampling period, with fish of different lengths being infected in the second sampling period but not in the first (see Table 3).

Previous studies of the parasites infecting *T. picturatus* conducted at the Madeira Islands concluded that *R. cadenati* was absent in fish of less than 20 cm length, in three seasons of the year (winter, spring and summer, see Gonçalves 1996). By contrast, at the Canary Islands *R. cadenati* infected fish of less than 20 cm. The infection with this parasite is possibly due to isopods, which are known to be intermediate hosts of acanthocephalans and filled the stomachs of the fish caught in June 2009, thus explaining the higher infections with *R. cadenati* in this region. However, in September, and in particular in November, feeding shifted mainly to fishes, as was demonstrated by the presence of fish scales in the stomachs leading to a lower infection and complete absence of *R. cadenati* in September and November 2009, respectively.

It appears that infection takes place in late spring and that the life span of this parasite is not more than

6 months, decreasing its value as a biological tag for population identification. Nevertheless, it could still be used as an indicator of migratory behaviour of *T. picturatus*. Since infection with this parasite probably occurs in late spring, the observed decrease in the infection levels in September and November 2009 could be due to either immigration of fish from the Madeira Islands to the Canary Islands or emigration of infected fish from the Canary Islands to the Madeira Islands during the autumn period. These results indicate some degree of mixing of *T. picturatus* between the Madeira and Canary Islands, with the area of infection with *R. cadenati* located in the Canary Islands, within the known endemic area of this parasite (Gaevskaya and Kovaleva 1985, MacKenzie *et al.* 2008).

#### Larval stages of *Anisakis sp.* and *N. lingualis*

Larval *Anisakis sp.* and *N. lingualis* could not be considered effective biological tags, as present and previous data on the infection of *T. picturatus* and other *Trachurus* species infected with these parasites determined that their prevalence and mean abundance were length-dependent, with fish of less than 21 cm showing low prevalence of infection (MacKenzie *et al.* 2008, Costa *et al.* 2012).

#### Importance of diet for parasite transmission

An earlier study of the feeding habits of *T. picturatus* along the shelf region of the northwestern Atlantic African coast concluded that mysids and euphausiids were not common in the zooplankton samples, and that the diet of fish of less than 25 cm consisted mostly of copepods and malacostracan larvae (Isopoda) (Kompowski 1976). This finding is in agreement with the higher prevalence of infection with *R. cadenati* found in our Canary Islands samples, which consisted of fish less than 25 cm in length.

In contrast, fish of the 25- to 30-cm length class feed predominantly on euphausiids and fishes, and to a lesser extent on malacostracan larvae, which may explain the higher prevalence of anisakids and the very low prevalence of *R. cadenati* at the Madeira Islands in the second sampling period, when some fish of that length class (n=11) were concurrently more infected with *Anisakis sp.*

Nevertheless, fish from different regions may have different parasites as a result of the availability or lack of suitable intermediate hosts in their diet (Marcogliese 1995, 2002), so one should be careful in the choice of the parasites as biological tags and in the conclusions drawn from parasitological surveys (Lester and MacKenzie 2009).

#### CONCLUSIONS

Although two parasites, *R. cadenati* and *G. cruciata*, showed significantly different prevalence in the

two study areas, only *G. cruciata* could fulfil most of the criteria for the first approach of the usefulness of parasites as biological tags, namely a long life span in the host, significant levels of infection (prevalence) between the study areas in different sampling periods, and a level of infection that was fairly constant from year to year and was easily detected and identified (MacKenzie and Abaunza 1998, MacKenzie *et al.* 2005).

Expanding the temporal and spatial sampling strategy to include other geographical areas such as the Mediterranean and the Iberian Peninsula, and examining more samples in all seasons of the year, would be appropriate in order to add further information on the biology of this species throughout its complete distributional range, and to determine the relationships between the different geographic areas in terms of occurrence of parasites.

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