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The use of parasites as biological tags for stock identification of blue jack mackerel, *Trachurus picturatus,* in the North-eastern Atlantic

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ABSTRACT

The use of parasites as biological tags for discriminating stocks of blue jack mackerel, *Trachurus picturatus* (Osteichthyes, Carangidae), in the northeast Atlantic Ocean is assessed herein. In this study the following parasites have been selected as possible biological tags: *Anisakis* spp. (Nematoda: Anisakidae), *Rhadinorhynchus* sp. (Acanthocephala: Rhadinorhynchidae), *Nybelinia* sp. (Cestoda: Tentaculariidae) and *Bolbosoma* sp. (Acanthocephala: Polymorphidae). *Anisakis* spp. was the most prevalent parasite taxon found in all localities, attaining higher values in fish from Peniche, mainland Portugal. The occurrence of *Rhadinorhynchus* sp. in fish from all studied areas was rare (prevalence <10%) but significantly different between localities, with higher values in both archipelagos. *Nybelinia* sp. specimens were only detected in fish from Madeira and cystacanths of the genus *Bolbosoma* were never detected. The distinctive pattern of infection of these parasite species points to the existence of three stocks of blue jack mackerel in the northeast Atlantic: one in Portuguese mainland waters, one in Madeira archipelago and another in the Canary archipelago. These results support the management strategy which treats the three populations studied as separate stocks.

1. Introduction

The blue jack mackerel, *Trachurus picturatus* (Bowdich, 1825) (Osteichthyes, Carangidae) (Fig. 1), is an oceanic pelagic species that occurs in the Eastern Atlantic (Cárdenas et al., 2005) reaching from the Bay of Biscay (France) southward to Morocco and eastward into the Mediterranean Sea (Eschmeyer, 2003; Karaiskou et al., 2003), between 100 m and 575 m depth (Menezes et al., 2006; Menezes and Giacomello, 2013). In the Northeast Atlantic, it is quite common in the Macaronesian archipelagos, constituting an important fishery resource in Madeira archipelago, although their annual landings have decreased from 2006 t in 1986 to 439 t in 2015 (data from the Fisheries Department of Funchal). Despite this wide distribution, no assessment has ever been made on the species population structure. The knowledge of the stock structure and degree of mixing among populations is important for the rational management of marine resources (Moles et al., 1998), especially when dealing with important commercial species.

Many techniques have been used to identify and discriminate stocks, including the application of artificial and natural tags (Catalano et al., 2014). Parasites have been widely used as indicators of various aspects of fish biology (Williams et al., 1992). One of their most important applications is in stock identification (Williams et al., 1992; MacKenzie and Abaunza, 1998; MacKenzie, 2002; Abaunza et al., 2008). The advantages and limitations of using

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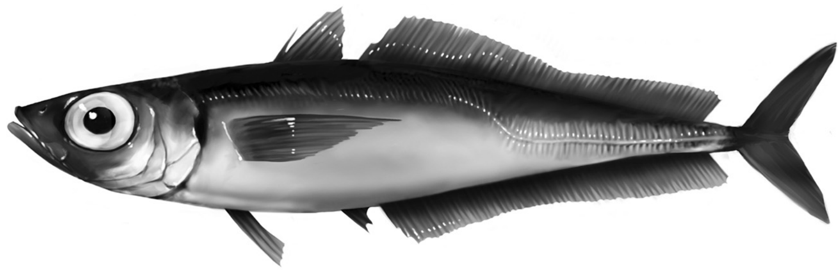

Fig. 1. Illustration of a blue jack mackerel, *Trachurus picturatus* (Bowdich, 1825), caught off Madeira Island (Source: DSI).

parasites as biological tags have been recognized and reported by many works (Sindermann, 1961; MacKenzie, 1987; Williams et al., 1992; MacKenzie and Abaunza, 2014; MacKenzie, 2002; Mosquera et al., 2003).

The study of blue jack mackerel parasite fauna is limited to a few papers from seamounts south of the Azores and off Western Sahara (Gaevskaya and Kovaleva, 1980, 1985), Madeira (Costa et al., 2012) and mainland Portugal (Hermida et al., 2016), highlighting regional differences in the prevalence and intensity of some helminth parasites, which may indicate the possible existence of different populations of this species. In order to evaluate the use of parasites that could be useful as biological tags of *T. picturatus*, Costa et al. (2013) examined samples from two geographic regions, Madeira and Canary Islands, for the presence of anisakids, trypanorhynchs, acanthocephalans and liver coccidians, and discuss the feasibility of using two specific parasites (a protozoan apicomplexan, *Goussia cruciata*, and an acanthocephalan, *Rhadinorhynchus cadenati*), as biological tags for the identification of populations of *T. picturatus*. These authors considered only *G. cruciata* as a useful biological tag.

A deeper insight or more robust conclusions may be gained from using a multidisciplinary approach to determine stock structure. In the present study, the use of parasites as biological tags for discriminating stocks of *T. picturatus* in three geographic regions of the northeast Atlantic Ocean (Peniche, mainland Portugal, and Madeira and Canary archipelagos) was assessed, as part of a multidisciplinary effort.

2. Materials and methods

A total of 607 specimens of *T. picturatus* (180 from Peniche, mainland Portugal, 207 from Madeira archipelago and 220 from Canary archipelago) (Fig. 2) were obtained quarterly from commercial catches in 2015, between January and December (Table 1). Samples from Peniche and Canary Islands were immediately frozen until parasitological examination. After thawing, basic biological information was collected from each specimen in the samples (total length, TL, in cm; total weight, TW, in g; sex and maturity stage) and subsequently they were examined for the presence of parasites. Fish from Madeira were analysed fresh.

For the selection of parasites as biological tags the criteria of MacKenzie (2002) and MacKenzie and Abaunza (2014) were followed. Suitable biological markers should show clear differences in prevalence, intensity and abundance levels among the sampled regions, should be easily detected and, ideally, should not cause disease in the host. Given this information and because small pelagic fishes have lower parasite species richness (Sindermann, 1957; MacKenzie, 1990; Abaunza et al., 1995), the use of a small number of parasites species selected according to the guidelines by MacKenzie (1983) and Sindermann (1983) was followed. As information on the parasitic fauna in this host species is already available, the following parasites were selected as possible biological tags: *Anisakis* spp. (Nematoda: Anisakidae), *Rhadinorhynchus* sp. (Acanthocephala: Rhadinorhynchidae), *Bolbosoma* sp. (Acanthocephala: Polymorphidae) and *Nybelinia* sp. (Cestoda: Tentaculariidae).

MacKenzie and Abaunza (1998, 2014) recommend that parasites used as biological tags should be easily detected and identified, otherwise time becomes a limiting factor. Although *Goussia cruciata* was previously referred as a good biological tag in *T. picturatus* (Costa et al., 2013), this parasite was not used in this study because its exact quantification is not possible and prevalence cannot be determined with certainty without observing the whole liver tissue.

For the collection of parasites in the host, the body cavity was exposed and observed and the viscera removed, placed in Petri dishes and examined for the presence of target parasites. All parasites collected were isolated, counted and preserved in 70% ethanol. The live parasites were fixed with hot 70% ethanol before storage. They were subsequently examined with the help of a binocular microscope after clearing with a suitable agent.

Prevalence (*P*), mean intensity (*I*), and mean abundance (*A*) of infection were determined according to Bush et al. (1997). Prevalence was compared among locations using the Pearsonís chi-square test. Abundances of the parasites species were compared between locations using the Kruskal-Wallis test (three samples).

Correlations between fish size and parasite mean abundance were analysed by Spearman rank correlation (Zar, 1996).

All statistical analyses were performed using R software (R Core Team, 2015). For all tests, statistical significance was accepted when $p < 0.05$.

3. Results

3.1. Length and weight data of blue jack mackerels examined

The fish length and weight varied significantly among locations (Kruskal-Wallis test: $x^2 = 421.19$, $p < 2.2 \times 10^{-16}$ and K-W: $x^2 = 426.39$, $p < 2.2 \times 10^{-16}$, respectively), with fish from Madeira and Canary Islands being the smallest, and those from Peniche the largest.

The number of females, males and fish with undifferentiated sex were significantly different in each region (K-W: $x^2 = 17.43$, $p = 0.0002$). Fish captured off Peniche were predominantly males (61%), those from Madeira females (52%) and the ones from Canaries were undifferentiated (58%).

3.2. Detected parasites and infection levels

Parasite of the genera *Anisakis* and *Rhadinorhynchus* were detected in all studied localities. *Nybelinia* sp. specimens were only detected in fish from Madeira, with a low prevalence (4.8%), and cystacanths of the genus *Bolbosoma* were never detected. Representatives of parasite species selected as possible biological tags and detected in this study are presented in Fig. 3. The infection levels observed in each locality for each parasite species are reported in Table 2.

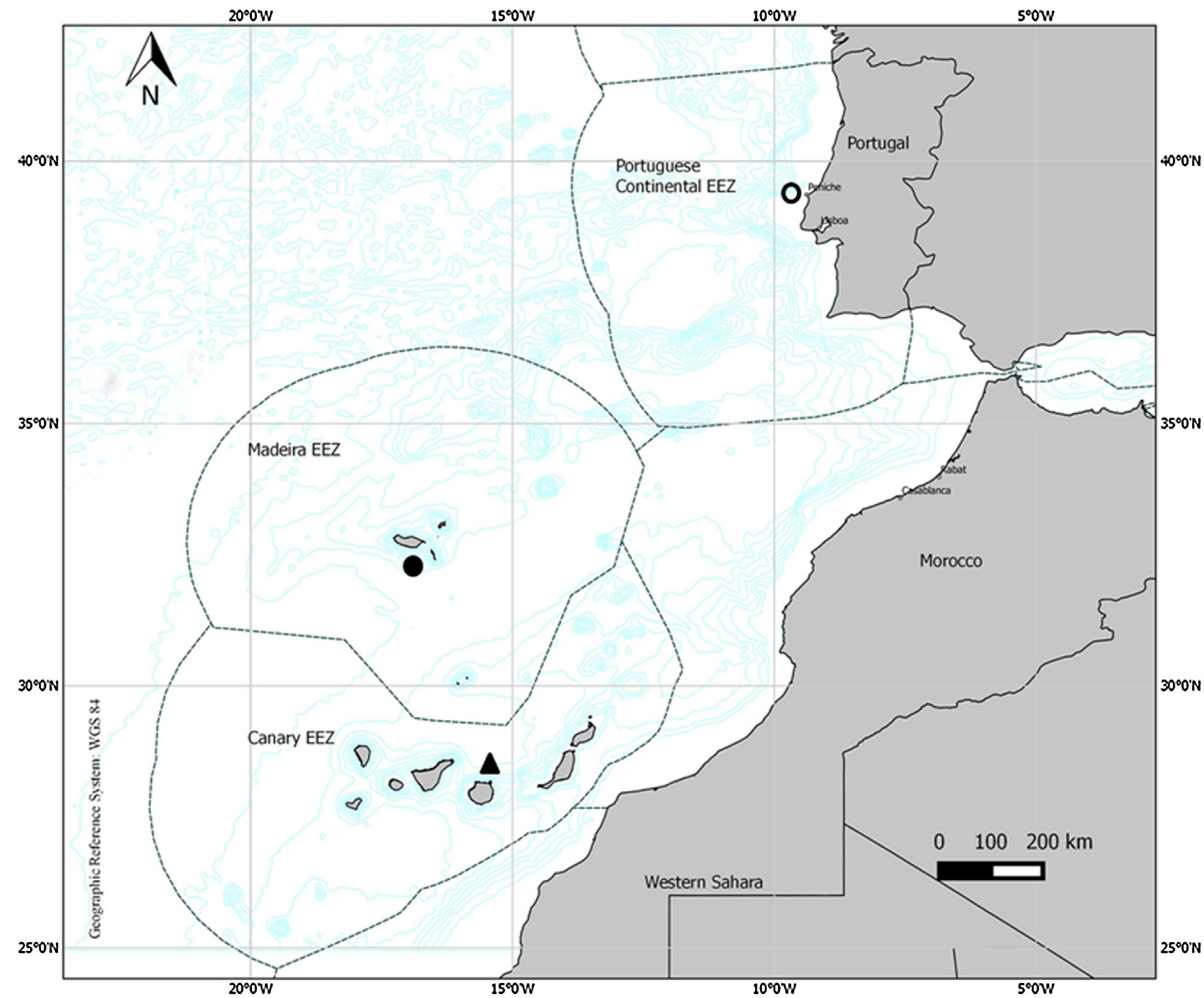

Fig. 2. Map showing the sampling locations of blue jack mackerel: mainland Portugal (open circle), Madeira (black circle) and Canary Islands (black triangle) (Source: DSI).

Table 1
Number of individuals (n) per sampling period (1: January–March; 2: April–June; 3: July–September; 4: October–December) and by sex (F, female; I, indeterminate; M, male) and mean length and weight and respective standard deviation of *T. picturatus* sampled in Madeira, Peniche (mainland Portugal) and Canaries in 2015. Total length (TL, cm) and weight (TW, g) ranges are given in brackets.

Geographic region	Sampling periods	n	Sex			TL range (cm)	TW range (g)
			F	I	M		
Peniche	1	50	19	–	31	25.98 ± 1.90 (22.30–30.80)	159.37 ± 37.52 (84.85–273.07)
	2	34	19	–	15	32.99 ± 2.65 (28.00–37.10)	309.55 ± 69.59 (182.09–471.53)
	3	50	19	–	31	26.05 ± 1.66 (22.80–31.10)	147.03 ± 25.50 (102.70–225.29)
	4	46	13	–	33	30.10 ± 1.71 (26.50–35.00)	229.44 ± 43.91 (172.26–364.81)
	Total	180	70	–	110	28.38 ± 3.42 (22.30–37.10)	202.22 ± 75.39 (84.85–471.53)
Madeira	1	53	31		22	20.40 ± 2.62 (16.50–27.80)	74.49 ± 33.68 (32.99–190.53)
	2	54	24	3	27	20.64 ± 1.83 (16.30–24.80)	78.49 ± 21.89 (39.06–134.58)
	3	50	27	9	14	18.16 ± 1.90 (15.30–24.00)	50.68 ± 17.74 (28.19–114.26)
	4	50	31		19	22.48 ± 1.79 (19.30–28.30)	91.38 ± 26.72 (56.56–196.23)
	Total	207	113	12	82	20.42 ± 2.55 (15.30–28.30)	73.86 ± 29.41 (28.19–196.23)
Canaries	1	61	5	53	3	17.07 ± 1.41 (14.50–21.40)	39.29 ± 10.07 (21.53–79.91)
	2	59	25	9	25	20.25 ± 1.19 (18.00–23.40)	69.58 ± 14.71 (45.17–108.95)
	4	100	14	66	20	16.64 ± 1.05 (14.30–18.70)	36.79 ± 6.76 (22.89–52.77)
	Total	220	44	128	48	17.73 ± 1.95 (14.30–23.40)	46.28 ± 17.51 (21.53–108.95)

Most fish sampled from Peniche (90.6%) were infected with at least one of the 3 detected parasite species (only 17 fish in 180 were uninfected). Most of the fish collected in Madeira and Canaries were not infected with any of the target parasite species (87.4% and 83.6%, respectively).

The most prevalent parasite taxon in all localities was *Anisakis* spp. However, its prevalence was significantly different among

Table 2
Prevalence (P), mean intensity (I) ± sd and mean abundance (A) ± sd of *Nybelinia* sp., Anisakis spp. and *Rhadinorhynchus* sp. in *T. picturatus* from Madeira, Peniche (mainland Portugal) and Canaries. Ranges are given in brackets.

Parasite	Peniche			Madeira			Canaries			Location in the host
	P (%)	I	A	P (%)	I	A	P (%)	I	A	
Nybelinia sp.	–	–	–	4.8	1.3 ± 0.50 (1–2)	0.06 ± 0.30	–	–	–	Visceral cavity
Anisakis spp.	90.6	32.1 ± 40.40 (1–217)	29.05 ± 39.60	5.3	1.2 ± 0.6 (1–3)	0.06 ± 0.30	9.1	1.9 ± 2.70 (1–13)	0.17 ± 0.95	Visceral cavity
Rhadinorhynchus sp.	0.6	1.0	0.01 ± 0.07	3.4	1.7 ± 1.5 (1–5)	0.06 ± 0.40	7.7	1.8 ± 1.10 (1–4)	0.14 ± 0.56	Intestine

Fig. 3. Parasite species selected as biological tags for the stock discrimination of blue jack mackerel (*Trachurus picturatus*) in the Northeast Atlantic: A) *Nybelinia* sp. (Cestoda); B) *Rhadinorhyncus* sp. (Acanthocephala); C) *Anisakis* spp. (Nematoda).

localities ($x^2 = 404.70$, $p < 2.2 \times 10^{-16}$) and highest in fish from Peniche (90.6%). Anisakid prevalence was not significantly different between Madeira (5.3%) and Canaries (9.1%) ($x^2 = 1.73$, $p = 0.188$) when comparing the total sample; however, significant differences in anisakid prevalence were also detected between Madeira and Canary Islands when comparing similarly sized fish (14–23 cm TL) ($x^2 = 7.87$, $p = 0.005$), as well as between Madeira and Peniche (22–29 cm TL) ($x^2 = 82.94$, $p < 2.2 \times 10^{-16}$).

The occurrence of *Rhadinorhynchus* sp. in fish from all studied areas was rare (prevalence < 10%) but significantly different between Peniche and the archipelago samples ($x^2 = 13.33$, $p = 0.001$).

Significant differences were found between regions in the abundance for *Anisakis* spp. (K-W: $x^2 = 439.72$, $p < 2.2 \times 10^{-16}$), *Nybelinia* sp. (K-W: $x^2 = 10.24$, $p = 5.507 \times 10^{-5}$) and *Rhadinorhynchus* sp. (K-W: $x^2 = 13.39$, $p = 0.001$). There were also significant differences in anisakid abundance when comparing fish of similar size (14–23 cm TL) between Madeira and Canary Islands (K-W: $x^2 = 8.38$, $p = 0.004$) and between Madeira and Peniche (22–29 cm TL) (K-W: $x^2 = 81.02$, $p < 2.2 \times 10^{-16}$).

Abundance of *Anisakis* spp. was positively correlated with fish TL ($r = 0.642$, $p < 2.2 \times 10^{-16}$). A negative correlation was found between abundance of *Rhadinorhynchus* sp. and TL ($r = -0.113$, $p = 0.005$). For *Nybelinia* sp., no correlation was found between abundance and TL ($r = -0.002$, $p = 0.969$) in samples from Madeira (the only region where this species occurred).

4. Discussion

4.1. Infection levels

The most prevalent parasite taxon in the three sampled areas was larval anisakids. They have been previously reported in *T. picturatus* from Madeira (Costa et al., 2003a, 2012, 2013), mainland Portugal (Hermida et al., 2016), Irving and Meteor Seamounts and Western Sahara (Gaevskaya and Kovaleva, 1985), and from the Ligurian Sea (Manfredi et al., 2000). However, the *Anisakis* spp. prevalence obtained in the present study in fish from Madeira archipelago was much lower than those observed previously for *T. picturatus* in the same area by Costa et al. (2003a) (62.5%), Costa et al. (2012) (24.3%) and Costa et al. (2013) (12.0%). It is interesting to notice that the prevalence of these parasites in *T. picturatus* has been declining gradually over the past few years in this geographical region. The decrease in the infection level of *Anisakis* spp. in *T. picturatus* from Madeira can be caused by several factors including *Anisakis* spp. host availability.

The geographical distribution of anisakids is affected not only by the definitive host distribution but also by the availability of intermediate hosts (Hermida et al., 2013). *Anisakis* spp. use krill (mainly euphausiids) as first intermediate hosts, teleost fishes and squids as paratenic or transport hosts and marine mammals as definitive hosts (Moravec, 1994; Klimpel and Rückert, 2005). The feeding habits of *T. picturatus* are still poorly known. Studies on blue jack mackerel stomach contents will be helpful to clarify if the decrease in *Anisakis* spp. infection levels is related with food habits or any other factor.

The prevalence observed in this study for *Anisakis* spp. from Peniche (central mainland Portugal) was similar to the one reported by Hermida et al. (2016) in fish from Matosinhos (northern area of the Portuguese mainland coast) whereas the prevalence found in the Canary Archipelago (9.1%) was higher than previously detected by Costa et al. (2013.

Anisakis spp. abundance was positively correlated with fish size. This result corroborates those obtained by Costa et al. (2012, 2013) and by Hermida et al. (2016) in *T. picturatus* from Madeira and mainland Portugal waters, respectively. According to Hermida et al. (2016), this positive correlation would be expected since larger fish consume more, thus increasing potential exposure to trophically-transmitted parasites. In addition, for long-lived parasites such as anisakid nematodes, which are capable of remaining encysted in their paratenic hosts for long periods of time, their accumulation in the host is a function of the total amount of prey consumed by the fish (Gibson and Jones, 1993). Thus, the differences found in the infection levels of *Anisakis* spp. between mainland and islands could be related to the larger fish size in mainland samples. However, the comparison between similarly sized fish from Madeira and Peniche showed significant differences in both prevalence and abundance. Additionally, the comparison between similarly sized fish from both archipelagos also revealed significant differences in anisakid infection levels.

Rhadinorhynchus sp. is a parasite known to occur frequently in *Trachurus* species in the Northeast Atlantic but rarely with prevalence higher than 10% (Gaevskaya and Kovaleva, 1985; MacKenzie et al., 2008; Costa et al., 2013). Contrary to Hermida et al. (2016) a negative correlation was detected between the abundance of *Rhadinorhynchus* sp. and host TL. Blue jack mackerel may become infected with *Rhadinorhynchus* sp. by feeding on isopod crustaceans, which are known to be intermediate hosts of acanthocephalans (Costa et al., 2013). Costa et al. (2013) suggested that the life span of this parasite does not exceed 6 months, diminishing their value as a biological tag for identifying populations; however it remains an important species for assessing migrations between populations.

The prevalence of *Nybelinia* sp. in blue jack mackerel from Madeira has decreased since 2012 (Costa et al., 2012, 2013) until the present study. This decrease may be due, as with *Anisakis* spp., to a decrease in intermediate or definitive hosts around Madeira archipelago. The typical life cycle of Trypanorhyncha includes a copepod as first, euphausiid or schooling fish as second, and an elasmobranch as final hosts (Palm, 2004). Klimpel et al. (2006) referred the Cephalopoda and Myctophidae as possible main second intermediate hosts for *N. lingualis*. As in the previous studies conducted in the Canaries (Costa et al., 2013) and mainland Portugal (Hermida et al., 2016), no cestode *Nybelinia* sp. was found at that archipelago nor in Peniche.

No cystacanths of the genus *Bolbosoma* were found at the archipelagos or in Peniche. However, they have been reported in previous studies conducted in Madeira (Costa et al., 2000, 2012). In samples from 2009, no *Bolbosoma* was recorded in blue jack mackerel from Madeira (Costa et al., 2013). *Bolbosoma* sp. has not been recovered from *T. picturatus* from mainland Portugal (Hermida et al., 2016).

4.2. Parasites as biological tags

To qualify as an ideal tag, a parasite should present different levels of infection in the host captured in different areas (MacKenzie, 1983; Sindermann, 1983). Lester (1990) and MacKenzie and Abaunza (2014) also pointed out the life span as an important criterion for selection of a particular biological tag.

Anisakids have been referred to in the literature as good biological tags because of their long life span (MacKenzie and Abaunza, 2014), and allowed the separation between Atlantic and Mediterranean populations of *Merluccius merluccius* (Mattiucci et al., 2004), *Xiphias gladius* (Mattiucci and Nascetti, 2008) and *Trachurus trachurus* (Mattiucci et al., 2008).

The present study confirms the value of the parasite *Anisakis* spp. as a possible biological tag in *T. picturatus*, given the observed differences in infection levels of fish captured in Peniche when compared to fish caught in Madeira or Canaries waters (total sample) and between Madeira and Canary Islands (similarly sized fish only). However, caution must be taken and a subsequent study performed, including molecular identification of anisakids found in these regions.

The parasite *Nybelinia* sp. seems to be very useful as a biological tag, as it was found only in Madeira archipelago. This parasite species was previously identified in *Trachurus* specimens from Madeira (Costa et al., 2003b, 2012) and Meteor and Irving Seamounts (Gaevskaya and Kovaleva, 1980) but not from the Azores (Gaevskaya and Kovaleva, 1980), Canaries (Costa et al., 2013) or from the northwest coast of mainland Portugal (Hermida et al., 2016).

The use of parasites as biological tags has also been applied for stock discrimination of another species of the genus *Trachurus* in the Northeast Atlantic and Mediterranean, the horse mackerel (*Trachurus trachurus*) (MacKenzie et al., 2008). The distinctive pattern of infection of the larval nematodes *Anisakis* spp. and *Hysterothylacium aduncum* supports the current management strategy which treats the North Sea population as a separate stock.

The definition of the stock limits of blue jack mackerel in the Northeast Atlantic to be considered in the management and assessment of fisheries should be based on more than one methodology, through a holistic approach (e.g., life history parameters, otolith elemental composition, fatty acid profiles, parasites as biological tags, morphometric landmarks, morphometric outlines and genetic analysis). In this study, the distinctive pattern of infection points to a possible existence of three stocks of blue jack mackerel in the southern Northeast Atlantic: one off Portuguese mainland waters, one off Madeira archipelago and another off the Canary Islands. Although there appears to be some amount of mixing between Madeira and Canaries populations, it is possible to distinguish these two regions due to the presence of *Nybelinia* sp. in the former, its absence in the latter, and higher anisakid infection levels in *T. picturatus* of similar size from the Canary Islands. This supports the management strategy of treating the Madeira population as a separate stock.

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