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Note

Looking for skin and gill parasites as biological tags for Atlantic bluefin tuna (*Thunnus thynnus*)

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Abstract – Skin and gill parasites found in juvenile Atlantic bluefin tuna (*Thunnus thynnus*) caught in the Bay of Biscay, northeast Atlantic, were examined with the aim of finding biological tags. The degree of infection of different microhabitats was analyzed and the annual prevalence by species of parasite obtained. Of the specimens examined, 98% had parasites, and the gills were by far the most infected microhabitat, followed by the skin and the pectoral fin. Within the gill cavity, parasites were most commonly found in the afferent margins of the primary lamellae. Three species of Copepoda were identified, two species of Monogenea, and eleven Digenea. Thus, ten species (Digenea: Didymozoidae) and a new host record for *Copiatestes thyrsitae* (Digenea: Syncoeliidae) were recorded in *Thunnus thynnus*. The suitability of the different external parasites found is discussed and gill didymozoids are put forward as useful biological tags. Confusing taxonomy within this digenean family makes species identification of the different morphotypes difficult. However, their habitat specificity in hosts can be a good tool to differentiate morphotypes and characterise host individuals.

Key words: Atlantic bluefin tuna / Skin and gill parasites / Stock structure / Biological tags / Didymozoidae

1 Introduction

Atlantic bluefin tuna (*Thunnus thynnus*) is one of the largest bony fishes in the Atlantic Ocean and its fishery and economic interest date back to ancient times. Presently, it is an important commercial species due to the high Japanese market demand for raw tuna products (sushi and sashimi). Atlantic bluefin tuna (ABFT) generates more than $4 billion a year in revenue at the Tokyo fish market. It is this commercial importance that has led to severe overfishing of ABFT stocks. The management of this fish species is based on two Atlantic units delimited by a 45 °W management boundary. The precipitate biomass decline in spawners of the West Atlantic unit over the past few decades and the overexploitation of the fishery in the East Atlantic and Mediterranean unit have led this fishing resource to an unsustainable situation. The increasing evidence of the high contribution of the east unit population to the west, based on electronic tagging and otolith chemistry, has raised concern over the stock structure and population management of ABFT (Block et al. 2005; Fromentin and Powers 2005; Rooker et al. 2007).

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The use of parasites as biological tags has gained increasing recognition for its applicability to the problems of fishery management (MacKenzie 1983a; Lester 1990; Williams et al. 1992; MacKenzie and Abaunza 1998). Their use as tags is difficult since it does not depend only on finding the right parasite but also on studies of parasites of the different distribution areas of the host. Furthermore, the host length must be available, and the parasites' taxonomic status must be clarified (synonyms and taxonomic revisions). Skin and gill epithelia parasites are easily detected and involve a minimum of dissection. In the past these parasites have been used as biological tags in ABFT (Walters 1980; MacKenzie 1983b; Cort and Rey 1983; Cort 1990). Other externally visible parasites, the didymozoid digeneans, have not normally been used as biomarkers as they are endoparasites within fish tissue, which makes them hard to collect in perfect condition and difficult to identify, although their microhabitat specificity has been used as taxonomic criteria. Nevertheless, they are very common in tuna fishes and other pelagic teleosts throughout the entire world (Yamaguti 1970; Munday et al. 2003).

ABFT follow complex migration pathways between spawning and feeding grounds, with differences in movement

patterns and distribution associated with fish size and presumed natal origin (Rooker et al. 2007). The Bay of Biscay is a summer feeding area for ABFT juveniles, where they are found from June to October in the southeast corner of the Bay, 43–47° N, 2–6° W, near the coast (Rodríguez-Marín et al. 2003). Conventional tagging data indicate a significant exchange of juveniles between the Bay of Biscay, the Ibero-Moroccan Bay and the western Mediterranean Sea, and even some transatlantic migrations between the Bay of Biscay and the western Atlantic (Cort 1990; Mather et al. 1995; Rooker et al. 2007). This host's extensive capacity for migration and its wide distribution in the North Atlantic make it a very valuable species to investigate for parasites as biological tags.

A recent study of tuna parasites around the world has postulated that many parasites of tuna species have wide-ranging geographical distributions, which has been confirmed through molecular approaches (Aiken et al. 2007). According to these authors, the factor responsible for the cosmopolitan distribution of these species may be their tuna host, due to its long migrations. This fact makes it difficult to find appropriate parasite biomarkers for wide-ranging stock discrimination and stresses the need for detailed parasitological studies worldwide.

With the aim of finding an ABFT biomarker that can be effectively identified with the least possible handling or dissection of the fish, a preliminary survey of the external parasites of ABFT from the Bay of Biscay was carried out (Barreiro et al. 2006). The aim of this paper is to improve this survey with a more detailed description of the ABFT parasite fauna that could be used as biological tags of this host.

2 Methods

A total of 228 ABFT were captured between July and September from 2001 and 2007 in the Bay of Biscay by professional live bait boats (Fig. 1). A more detailed and systematic parasite examination of the host was performed in 2005, and since then heads have been deep frozen for later inspection. In 2005 and 2006 specimens were collected on board a commercial vessel during a tagging survey, and the rest were obtained through the fish market. All fish were examined fresh, measured (straight fork length), weighed, sexed and aged using dorsal fin first ray sections (Rodríguez-Marín et al. 2007).

All external surfaces, including the gill chamber, nasal cavity and mouth, fins and fins sulci were examined. In the specimens collected since 2005, heads were defrosted and gills were separated and washed with saline water to clean away the mucus and the liquid was filtered in steel sieves of 1 mm and 0.5 mm mesh size. Parasite samples were extracted from different parts of the body and preserved in 70% ethanol. Monogenea were detached using dissecting needles, counted, stained with borax carmine and mounted on a glass slide with Canada Balsam. Digenea were stained with iron acetocarmine (Georgiev et al. 1986) and immersed in Dimethyl phthalate. Crustacea were cleared with lactophenol and examined under a light microscope.

The taxonomic criteria of Yamaguti (1963, 1968, 1970, 1971), Kabata (1992), Ishii (1935) and Pozdnyakov (1996) were followed for the identification of the metazoan parasites. The parasites specimens are deposited at the parasite collection of the Spanish Oceanographic Institute in Santander, Cantabria, Spain (IEOS-BFT-1 to IEOS-BFT-22). *Prevalence*- the proportion of individuals in a population infected with one or more individuals of a particular parasite species (or taxonomic group- and *mean intensity* - "mean number of a particular parasite species per infected host in a sample" were calculated according to Bush et al. (1997). A chi-squared test for differences between prevalence values was used and box and whisker plots were constructed for most frequent parasites. The selection criteria suggested by MacKenzie and Abaunza (1998) were considered in the assessment of the parasite's potential as a biological tag.

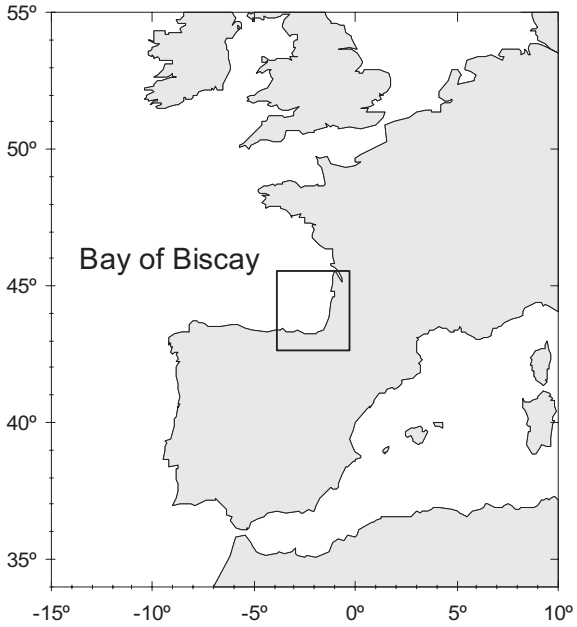

Fig. 1. Map of Bay of Biscay showing Atlantic bluefin tuna sampling area.

3 Results

The total sample size by year, mean size of sampled specimens and prevalence by microhabitat are presented (Table 1). Most of the specimens analyzed were between 1 and 2 years of age with a mean length of 72 cm and mean weight of 7.4 kg. Nearly all fish examined were infected by ectoparasites (98%). The gill chamber was found to be the most infected part of the body, followed by the skin and pectoral fins (Table 1). The level of infection by microhabitat remained relatively constant from year to year except for parasites on the skin where there was considerable annual variation, with samples collected on board a commercial vessel in 2005 and 2006 having significant high values ($p < 0.05$). Within the gill chamber, the outer side of the gill filaments showed a parasite prevalence of 93%, significantly higher than other microhabitats ($p < 0.01$) (Table 2).

The parasite species found in ABFT caught in the Bay of Biscay are summarized and the microhabitat is indicated (Table 3). The locations of parasites in the head and gills of ABFT are shown (Fig. 2). Of the three species of copepods found, *Pseudocycnus appendiculatus* Heller, 1865, was

Table 1. Summary of sample data for Atlantic bluefin tuna and prevalence according to microhabitat for metazoan parasites. Size (SFL) = straight fork length size in cm, SD = standard deviation.

Year	Parasite prevalence according to microhabitat (%)							Total prevalence (%)	No. of bluefin analyzed	Size (SFL)	
	Gills	Nasal cavity	Mouth	Pectoral fin	Dorsal fin	Ventral fin	Skin			Mean	SD
2001	94	0	-	-	-	-	14	94	48	80.2	3.5
2002	93	0	-	27	23	-	7	94	35	72.3	10.0
2003	91	0	0	40	26	3	31	97	36	69.8	9.4
2004	98	0	3	18	18	-	8	98	47	72.2	7.1
2005	100	4	0	48	13	22	65	100	23	64.9	11.1
2006	95	0	0	25	25	5	85	100	20	63.6	1.5
2007	100	5	0	47	37	5	26	100	19	76.4	5.8
Total	96	1	1	25	18	4	27	98	228	72.3	9.1

Table 2. Parasite distribution in gill chamber.

Year	Parasite prevalence by gill area (%)					No. bluefin with parasitized gills
	Gill filaments		Gill arch	Operculum	Branchiostegal membrane	
	Outer side	Inner side				
2005	96	22	9	4	0	23
2006	89	16	5	21	16	19
2007	95	26	32	21	58	19
Total	93	21	15	15	23	61

the most abundant with a prevalence ranging between 15% and 43%. Of the two species of monogeneans recovered, the most prevalent was *Hexostoma thynni* (Delaroche 1811) with an annual prevalence ranging between 53% and 70%. Eleven species of trematode digeneans with ten didymozoid species and one specimen of a syncoeliid species, *Copiatestes thyrsitae* Crowcroft, 1948 (synonym of *C. filiferus* in Shvetzova 2004) were also found. The high diversity of morphotypes and habitats of didymozoid parasites were observed (Table 3). However, some didymozoids could not be identified to species level. Most of these parasite infections were associated with mild lesions that were visible when the parasite was removed. The attachment of the haptor of *H. thynni* was associated with whitish areas that had sucker prints at the two adjacent primary lamellae where the parasite was attached. Didymozoids were located in pseudocysts or cysts within the host tissue causing erosion and malformation. The most evident pathologies were observed in relation to *Didymocystis semiglobularis* Ishii, 1935, located in large periosteum and bone cavities, and to *Didymosulcus* sp. 2 within malformed dental plates. Hypertrophies of the afferent and efferent margins of the primary lamellae were often observed, mostly related to degraded dead didymozoid individuals.

The didymozoid *Didymosulcus katsuwonicola* (Okada, 1926) (synonym *D. wedli*) found in the afferent side of the primary gill lamellae was the most prevalent, with an annual value ranging between 85% and 96%. The didymozoid *Platocystis viviparoides* (Ishii, 1935), found in the laterocaudal skin of the host's flanks, showed significant differences ($p < 0.05$) in prevalence in 2005 and 2006, between 65% and 85%, with respect to that of 2007 (14%). In order to observe annual changes in intensity, a box and whisker plot was obtained for the most frequent gill-chamber didymozoid parasites (Fig. 3)

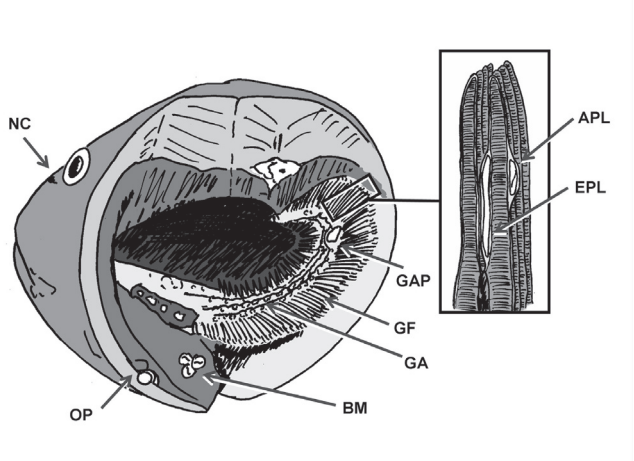

Fig. 2. Schematic drawing of the parasite habitats in head and gills of Atlantic bluefin tuna. APL = afferent margin of primary gill lamellae, BM = branchiostegal membrane, EPL = efferent margin of primary gill lamellae, GA = gill arch, GAP = gill arch periosteum, GF = gill filaments, NC = nasal cavity, OP = operculum inner side.

and some annual variability was found, mainly for *D. katsuwonicola.* No significant differences in prevalence were found for *D. katsuwonicola* and *Didymozoon filicolle* Ishii, 1935 between one and two-year-old ABFT.

4 Discussion

Our findings contribute to increase the description of skin and gill parasites that were acquired by juvenile ABFT captured in the East Atlantic. ABFT stock structure and degree

Table 3. Parasites found on Atlantic bluefin tuna juveniles in the Bay of Biscay. Prev. = prevalence (%), Mi. = mean intensity. Microhabitat: APL = afferent margin of primary gill lamellae, BM = branchiostegal membrane, DF = dorsal fin, EPL = Efferent margin of primary gill lamellae, GA = gill arch, GADP = gill arch dental plates, GAP = gill arch periosteum, GF = gill filaments, NC = nasal cavity, OP = operculum inner side, PF = pectoral fin, SK = skin, VF = ventral fin.

Metazoan parasites	Microhabitat	2005		2006		2007		TOTAL	
		Prev.	Mi.	Prev.	Mi.	Prev.	Mi.	Prev.	Mi.
Copepoda									
Brachiella thynni Cuvier, 1830	PF	4	2	0	0	7	1	3	2
Euryphorus brachypterus (Gerstaecker, 1853)	GF	0	0	5	1	0	0	2	1
Pseudocycnus appendiculatus Heller, 1865	GF	26	2	15	1	43	3	29	2
Monogenea									
Tristomella onchidiocotyle (Setti, 1899)	GA	0	0	0	0	16	6	5	6
Hexostoma thynni (Delaroche, 1811)	GF	61	2	70	2	53	2	61	2
Digenea									
Copiatestes thyrsitae Crowcroft, 1948	GF	0	0	0	0	5	1	2	1
Didymocystis semiglobularis Ishii, 1935	GAP/OP	13	6	20	10	21	2	18	6
Didymocistis sp.2	NC	4	3	0	0	0	0	3	3
Didymocistis sp.3	GAP	9	3	5	3	43	7	19	5
Didymosulcus katsuwonicola (Okada, 1926)	APL	96	20	85	17	95	35	92	24
Didymosulcus sp.2	GADP/BM	0	0	20	3	64	4	23	5
Didymozoidae sp.1	VF	22	2	5	2	7	1	11	2
Didymozoinae sp.1	PF	43	4	30	5	43	7	40	5
Didymozoon filicolle Ishii, 1935	EPL	22	13	15	7	21	17	19	12
Koellikeria sp. Cobbold, 1860	DF	13	1	25	1	64	1	27	1
Platocystis viviparoides (Ishii, 1935)	SK	65	12	85	9	14	2	60	11

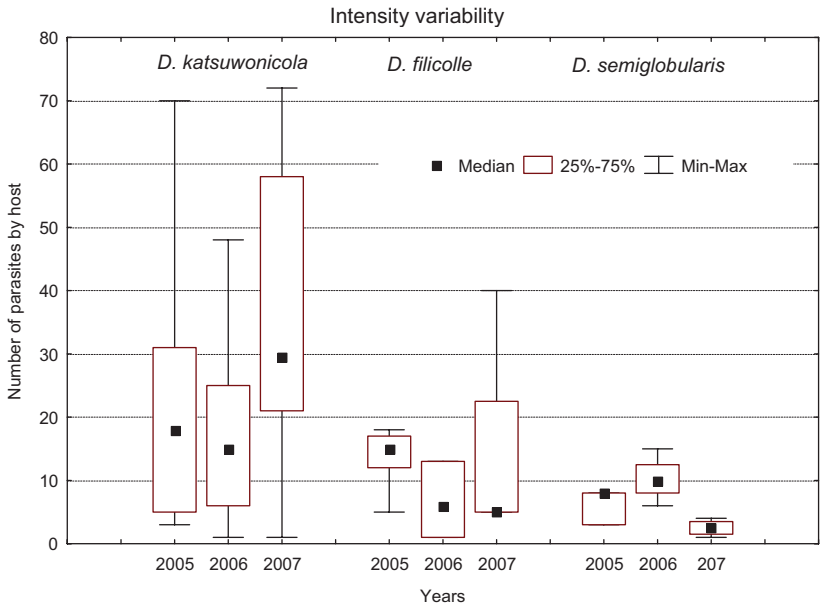

Fig. 3. Box and whisker plot for annual intensity variability of gill didymozoid species found in Atlantic bluefin tuna caught in the Bay of Biscay.

of mixing are currently subjects of study by several methods, including electronic tagging, genetics and otolith chemistry (Block et al. 2005; Carlsson et al. 2007; Rooker et al. 2007). Parasites, in combination with previously cited tools, can contribute towards clarifying exchanges between management units of ABFT. Since the 1980s, parasite studies have been applied to stock discrimination and to the determination of the degree of mixing that has occurred in a certain area (MacKenzie 1985; Lester 1990; Williams et al. 1992; Criscione et al. 2006). This last approach requires knowledge of the parasites of the fish stocks prior to mixing.

Parasite species with single-host life cycles, most of which are ectoparasites, monogeneans, many protozoans and most crustaceans, are the simplest to use (MacKenzie and Abaunza 1998). The gill filaments of ABFT are parasitized by flatworms *Tristomella onchidiocotyle* (Setti, 1899) (synonym *Capsala onchidiocotyle*), and *H. thynni*. Both platyhelminth parasites are considered potential pathogens (Nowak 2004; Deveney et al. 2005) and have been described with a wide-ranging geographic distribution (Aiken et al. 2007). Their pathogenicity and wide distribution decreases their utility as good tags alone. Another known ectoparasitic Monogenea from the nasal cavity of ABFT is the species *Nasicola klawei* (Stunkard, 1962). This species was not detected in our study although it has been used as a biological tag to differentiate east and west populations of ABFT (Walters 1980; Cort 1990).

Three species of copepods were found in ABFT in our survey: *P. appendiculatus*; *Brachiella thynni* Cuvier, 1830 and *Euryphorus brachypterus* (Gerstaecker, 1853) (synonym *Elytrophora brachyptera*). All of these are intrinsically non-pathogenic species although *E. brachypterus* can be pathogenic (Munday et al. 2003; Hayward et al. 2007). The life cycle of these species is direct: eggs are shed into the water, and immatures are free-living. Immatures in the water column usually attach to the skin or gill cavity of the host in response to unknown chemical cues, and they migrate to the preferred site, the gill chamber in the case of *E. brachypterus or P. appendiculatus* or the pectoral fin base in the case of *B. thynni* (Walters 1980). *E. brachypterus* was used as a biological tag for ABFT (Walters 1980; Cort and Rey 1983; Cort 1990), but MacKenzie (1983b) considered this species to be of doubtful value as a biological tag since it occurs in all oceans inhabited by tunas. The values of prevalence and mean intensity found in the present study are much lower than those found by Cort (1990) in the same geographic area of the Bay of Biscay. These differences are very marked despite Cort's (1990) finding that the degree of infection increased with ABFT age, and that this parasite can become dislodged easily before collection (Williams and Bunkley-Williams 1996). Further investigation should be done to determine whether these differences between our findings and those of previous studies are due to changes in infection over time, the different spawning areas from which these ABFT recruits in the Bay of Biscay originate, or the sensitivity of these parasites to changes in environmental conditions.

We found a new host record for *Copiatestes thyrsitae* Crowcroft, 1948 (Trematoda: Syncoelidae). It originally was recorded in the northeast Atlantic in *Trachipterus arcticus*. Subsequent host records include *Nematoscelis megalops*, *Acantholatrix monodactylus*, *Euthynnus alletteratus*, *Katsuwonus pelamis* and *Priacanthus boops* (see Gibson and Bray 1977). It was recently recorded in Brazil in *Thunnus albacares* by Fernandes et al. (2002) and along the Peruvian coasts in *Hemilutjanus macrophthalmos* by Tantaleán and Lefevre, 2004). This parasite was found in the gill filaments of ABFT, but it has also been found in the digestive tract of other hosts.

Among the most prevalent, abundant and diverse parasites acquired during the feeding and migrations of ABFT are Digenea, Didymozoidae (Mladineo 2006). The greatest mean intensity and richness of parasites found in our survey on ABFT corresponded to the didymozoid digeneans, which were located in all microhabitats examined. Four aspects should be borne in mind when considering didymozoids for use as biological tags. First of all, they are very abundant and prevalent with high biodiversity, especially in tuna (Yamaguti 1970; 1971; Munday et al. 2003). Secondly, they are not considered to be severe pathogens for fish, and are normally related to focal hyperplasia and hypertrophy of the epithelia and mild mucus hypersecretion (Kearn 1998). Moreover, infected areas normally become distorted in shape (Kearn 1998; Munday et al. 2003; Deveney et al. 2005; Mladineo 2006), even when parasites have died, so they can be easily detected. Thirdly, their life-cycle is indirect, which implies the need for the presence of intermediate host fauna in each study area (Kearn 1998). Didymozoids have complex life cycles with at least three intermediate hosts before the final one, a large predatory fish, upon which the didymozoids mature (Jones 1991). This heteroxenous cycle could be of advantage to this gregarious species since schools and recent farming practices do not affect its propagation. Lastly, although they have a probable life span of less than one year, their remains are recognizable in the host tissues well after the death of the parasite (Lester 1990; Speare 1995).

The identification of the species within Didymozoidae is extremely difficult and requires great expertise. Moreover, the taxonomy of the group is being revised (Pozdnyakov 1996; Pozdnyakov and Gibson, in press). Didymozoid tissue parasites have particularly narrow microhabitats and this high degree of site-specificity and morphotype have been used for its taxonomy (Ishii 1935; Yamaguti 1970; Pozdnyakov 1996; Rohde 2002). Molecular techniques must be used to carry out detailed studies to discriminate species and confirm their different locations in fish, which can therefore enable them to be used as tags taking into account their morphotype and host location.

The skin didymozoids can easily become detached during fish capture and handling, which limits their use as tags (Barreiro et al. 2006). In contrast, the didymozoids located in the gills are not sensitive to handling, are mainly located in the outer side of the gill filaments and can be easily detected. Thus, these digeneans located in gill filaments are most likely to provide effective biological tags for ABFT. In a similar study carried out in the Mediterranean with 0+ and 5 to 7-year-old ABFT (Culurgioni et al. 2007), five didymozoid species were found in the gills. *D. katsuwonicola* and *D. filicolle* were also highly abundant, the former being the only parasite species found in age-class 0+ specimens. Mariniello et al. (2000) also found *D. katsuwonicola* with significant higher prevalence in

ABFT larger than 50 kg from the Mediterranean Sea, but still with much lower values than in Bay of Biscay specimens, with ours showing wider species diversity. This difference in species composition and the tropical and subtropical distribution of these parasites (Lester 1990; Jones 1991) may suggest that some of them were acquired outside the Mediterranean.

Some didymozoid species have been described in cage-reared ABFT in the Mediterranean Sea (Munday et al. 2003; Mladineo and Tudor 2004; Mladineo 2006; Nowak et al. 2006; Mladineo et al. 2008). Some of these studies of ABFT reared in cages are particularly useful, such as the health surveys carried out in Croatian farms, because the Mediterranean origin of the fish is clear as reared specimens are mainly 0+ or 1-year-old. Comparing our results with an ABFT health survey reported from Croatian cages from 2003 to 2006 (Mladineo et al. 2008), a small number of parasites are shared: two didymozoids and one monogenean, indicating that it seems likely that some other didymozoid species found in our specimens come from the Atlantic. The possibility of infestation from outside the Mediterranean is clear. Large numbers of juvenile ABFT in the eastern Atlantic and in the Bay of Biscay result from the migration of juveniles from nursery areas within the Mediterranean Sea (Cort 1990; Rodríguez-Marín et al. 2005; Rooker et al. 2007).

The present survey provides valuable baseline information about juvenile ABFT skin and gill parasites in the East Atlantic, but more research is needed to know where and when the parasites are acquired. Didymozoids could be helpful in discrimination of ABFT populations and have been used in previous studies (Lester 1990; Jones 1991; Speare 1995; Baker et al. 2007), although the specific identity of the parasites must be studied in detail using morphology, genetics and microhabitat-selection. Analyzing data from several parasites and locations simultaneously should be applied to ABFT to resolve the stock structure of this species and determine which parasites are appropriate tags.

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