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Metazoan gill parasites of wild albacore *Thunnus alalunga* (Bonaterre, 1788) from the Balearic Sea (western Mediterranean) and their use as biological tags

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ABSTRACT

Metazoan gill parasites of 30 albacore *Thunnus alalunga* caught in the Balearic Sea (western Mediterranean) were examined for parasites with the aim to evaluate their possible use as biological tags. A total of 9 species of parasites were found: 1 capsalid monogenean, 6 didymozoid trematodes and 2 crustaceans. Most of the parasites collected were didymozoids (95.8% of all specimens) and *Didymozoon longicolle* was the dominant species. Albacore is a new host record for *Capsala paucispinosa* and *Didymozoon pretiosus*, while *Didymosulcus aahi*, *Didymosulcus dimidiatus*, *Nematobothrium latum* and *Rocinela* sp. are for the first time reported from the Mediterranean Sea. Significant differences were found grouping data by host size, with lower infection levels in the larger sized fish, whereas no differences were found between host sex. Most of the parasites showed a high site selection: *D. aahi*, *D. dimidiatus* and *D. longicolle* had significant differences of prevalence between internal and external margins of gill filaments, and almost all specimens of *Pseudocycnus appendiculatus* were attached to the gill filaments of the second and third holobranchs. The usefulness of parasites as biological tags is discussed; particularly, *D. longicolle* and *D. pretiosus* could be used to separate Mediterranean and northeast Atlantic stocks of albacore.

1. Introduction

The albacore *Thunnus alalunga* (Bonnaterre, 1788) (Teleostei: Scombridae) is a migratory cosmopolitan tuna distributed throughout tropical and temperate areas of all oceans, including the Mediterranean Sea (Collette and Nauen, 1983). It is a top level predator, and its diet varies according to size and availability of prey, e.g. pilchard, anchovy, mackerel, squid and crustaceans (Consoli et al., 2008). The populations of this fish from different oceans are managed as separate stocks, based on the available evidence of geographical separation, and distinct spawning areas and seasons (Alonso et al., 2005; Joseph, 2003). Therefore, the North and South Atlantic populations (separated by 5°North latitude), and the Mediterranean one are considered as three distinct management units (Anon, 1996). The Mediterranean populations have been separated from the North Atlantic ones by genetics (Nakadate et al., 2005), spawning areas (Dicenta et al., 1975; Duclerc et al., 1973), growth (Megalofonou, 2000), and size and age at first maturity (Arena et al., 1980). Despite this separation for management purposes, the knowledge about the extent of the transzonal migrations of this fish is meagre (Arrizabalaga et al., 2003).

Parasites have been used with success to point out differences between host populations and/or to study migrations of several fish species (Lester, 1990; Lester and MacKenzie, 2009; MacKenzie and Abaunza, 1998). Therefore, the information about the parasite fauna of albacore could be a complementary tool for stock assessment and management, as reported for other tunas, such as the southern bluefin tuna, *Thunnus maccoyi* (Castelnau, 1872), and the Atlantic bluefin tuna, *Thunnus thynnus* (Linnaeus, 1758) (Hayward et al., 2007; Nowak et al., 2006). The parasite fauna of albacore has been investigated by Jones (1991), Pozdnyakov (1990) and Schwartz (1939) in the Pacific Ocean, and by Aloncle and Delaporte (1974), Dollfus (1952), Guiart (1938), Legendre (1940), Postel (1963, 1964) and Priol (1944) in the Atlantic Ocean, while no parasitological data are available for the Mediterranean Sea. Gill parasites of tunas are often used as biological tags because gills are not affected by handling manipulation, can be easily dissected during the evisceration and do not have any commercial value (Lester et al., 1985; Rodríguez-Marín et al., 2008).

The aim of this paper is to describe the metazoan parasites on the gills of *T. alalunga* from the Balearic Sea (western Mediterranean) and to evaluate the possible use of parasites as biological tags.

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2. Materials and methods

On July 2008, 30 specimens of albacore were caught by trolling lines in the Balearic Sea (western Mediterranean) (Fig. 1).

Immediately after landing, fish were measured (range fork length = 50–96 cm), weighed (range of total weight 3.8–16.2 kg), and sexed (sex ratio = 1:1) (Table 1). Gills were extracted, stored individually in plastic bags and frozen at −20 °C. In the laboratory, the gills were unfrozen and carefully examined for parasites. Each holobranch was numbered from 1 to 4, from the anterior-external to the posterior-internal, and their surfaces named A, B, C and D (Fig. 2).

Location and possible macroscopic pathological alterations were recorded for each parasite. All parasites found were counted and stored in 70% ethanol. For microscopical examination and species identification, parasites were processed according to standard protocols (Berland, 1984; Roberts, 1989). Fresh and mounted parasites were micrographed and measured with a digital system. Among the literature used for species identification: Bussieras (1972), Chisholm and Whittington (2007), Lamothe-Argumedo (1997), Palombi (1949) and Price (1939) for monogeneans; Ariola (1902), Guiart (1938), Ishii (1935), Legendre (1940), Pozdnyakov (1990), Pozdnyakov and Gibson (2008) and Yamaguti (1958, 1970) for didymozoids; Brian (1906), Hewitt (1969) and Kabata (1992) for copepods; Bruce (1983), Brusca and Iverson (1985), Haswell (1882) and Richardson (1905) for isopods.

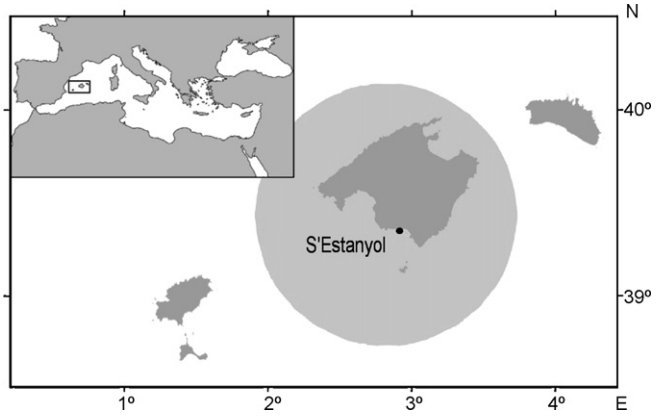

Fig. 1. Site of sampling of albacore (*Thunnus alalunga*) in the Balearic Sea (western Mediterranean).

Table 1
Sampling data of the specimens of albacore (*Thunnus alalunga*) examined, grouped according to fork length. FL = fork length; *W* = total weight.

Group of size	*N*	Mean FL ± s.d. (cm)	Mean W ± s.d. (kg)	Sex ratio F:M
FL 50–66 cm	16	62.2 (4.1)	4.9 (0.8)	1:0.45
FL 67–74 cm	6	69.8 (1.1)	6.3 (0.7)	1:2
FL 75–96 cm	8	81.7 (6.8)	10.4 (2.6)	1:1
All samples	30	68.9 (9.5)	8.5 (2.8)	1:1

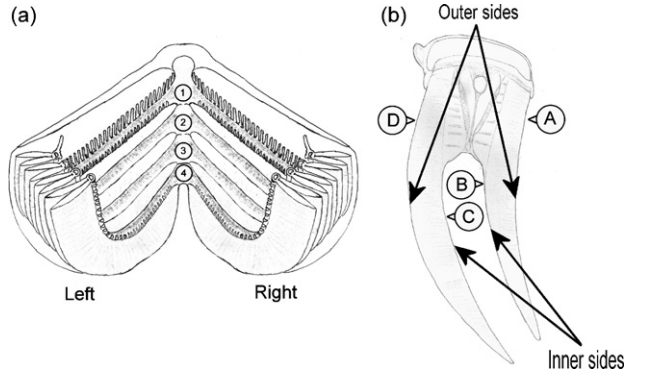

Fig. 2. Diagram of the gills of albacore (*Thunnus alalunga*) illustrating the division in microhabitats. (a) Gill holobranchs; (b) transversal section of the whole holobranch, sides (A, B, C and D); outer sides (A and D) and inner sides (B and C).

Prevalence (P%), range of intensity (IR) and mean intensity (MI) of each parasite species were calculated according to Bush et al. (1997). Confidence intervals of prevalence and of mean intensity were calculated with Sterne's exact method (Reiczigel, 2003) and Efron-Tibshirani bootstrap (Rózsa et al., 2000), respectively. The levels of infection were calculated according to site of location, host size (hosts were divided into three fork length categories: 50–66 cm, 67–74 cm, 75–96 cm), and sex. Fisher's exact test and bootstrap test were used to evaluate differences between prevalences and mean intensities (Rózsa et al., 2000). Calculations were made using the free software Quantitative Parasitology 3.0 (Reiczigel and Rózsa, 2005). To evaluate possible correlations between host size and prevalence (with previous arcsine transformation) and intensity of infection, the Spearman's rank correlation coefficient was tested with Student's *t*-test (Zar, 1996). Only species with prevalence ≥10% were considered for calculations (Bush et al., 1990).

3. Results

On the gills of examined albacores, a total of 9 species of metazoan parasites were found (Table 2): 1 capsalid monogenean (*Capsala paucispinosa* (Mamaev, 1968)); 6 didymozoid trematodes (*Didymosulcus aahi* Pozdnyakov, 1990; *D. dimidiatus* Pozdnyakov, 1990; *Didymozoon longicolle* Ishii, 1935; *D. pretiosus* Ariola, 1902; *Nematobothrium latum* Guiart, 1938; *Wedlia bipartita* (Wedl, 1855)); 2 crustaceans (*Pseudocycnus appendiculatus* Heller, 1868; *Rocinela* sp.).

Most of the parasites collected were didymozoids (95.8% of all specimens), followed by crustaceans (2.9%) and monogeneans (1.3%). *D. longicolle* was the dominant species, with 113 specimens (36.1%), and the copepod *P. appendiculatus* represented almost all crustaceans. Overall, 83.3% of all sampled tunas were infected with at least one parasite (total MI 12.5).

Prevalence and mean intensity of parasites are shown in Table 2. Grouping data according to host size, significant differences were found between total prevalences of the smaller and the larger sized fish (Table 2), but prevalence and intensity of the single parasite species were not correlated with host size. Prevalence and mean intensity according to host size are reported in Fig. 3. In general, the lower values of infection were recorded in the larger sized hosts, and two species (*D. pretiosus* and *P. appendiculatus*) were not recorded in fish of this group. *C. paucispinosa* was only found in 2 specimens of the smaller sized fish. No significant differences were recorded between host sex.

According to location, no significant differences were found between right and left gills, and between holobranchs. The first holobranch showed the higher total mean intensity of infection (4.9), and the third the higher total prevalence (73.3%), in particular: 1st holobranch, P% = 70.0%, MI = 4.9; 2nd, 63.3%, 3.4; 3rd, 73.3%, 3.3; 4th, 66.7%, 3.1. The only 4 *C. paucispinosa* specimens found in situ were located on the first three holobranchs between the gill filaments. Didymozoids had well separated location on gills (Fig. 4): *D. aahi* was found on the outer margin of gill filaments; *D. dimidiatus* on the outer side of gill arches skin; *D. longicolle* and *D. pretiosus* on the inner margin of gill filaments, on the basal and middle third of lamella, respectively; *N. latum* and *W. bipartita* on the connective tissue of the proximal and distal parts of gill arches, respectively.

Table 2
List of the parasites found on the gills of albacore (*Thunnus alalunga*), with indication of prevalence (P%), range of intensity (IR), mean intensity (MI) and 95% confidence intervals (second row).

Parasite	FL 50–66 cm		FL 67–74 cm		FL 75–96 cm		Total		
	P%	MI	P%	MI	P%	MI	P%	MI	IR
Monogenea									
C. paucispinosa	12.5	2.0	–	–	–	–	6.7	2.0	–
	2.3–37.2	–	–	–	–	–	1.2–21.3	–	
Digenea									
D. aahi	43.8	2.0	16.7	2.0	12.5	2.0	30.0	2.0	1–4
	20.8–69.5		0.1–58.9	–	0.6–50.0	–	16.3–48.3	1.4–2.6	
D. dimidiatus	68.8	3.7	50.0	8.7	25.0	5.5	53.3	4.9	1–15
	43.6–86.8	1.3–2.7	15.3–84.7	4.0–12.3	46.4–63.5	–	34.8–70.2	3.3–7.1	
D. longicolle	68.8	7.4	50.0	8.0	62.5	1.6	63.3	6.0	1–53
	43.6–86.8	2.5–5.4	15.3–84.7	3.0–11.3	28.9–88.9	1.0–1.8	45.0–78.7	2.8–16.0	
D. pretiosus	12.5	8.0	16.7	1.0	–	–	10.0	5.7	1–11
	2.3–37.2	2.3–25.6	0.9–58.9	–	–	–	2.8–26.3	1.0–9.0	
N. latum	50.0	4.3	33.3	6.5	12.5	2.0	36.7	4.5	1–12
	27.2–72.8	5.0–8.0	6.3–72.9	3.0–6.5	0.6–50.0	–	21.4–55.1	2.5–7.2	
W. bipartita	31.3	3.0	16.7	8.0	25.0	1.0	26.7	3.1	1–8
	13.2–56.4	1.8–7.6	0.9–58.9	–	46.4–63.5	–	13.1–44.9	1.5–5.4	
Crustacea									
P. appendiculatus	25.0	1.5	33.3	1.0	–	–	20.0	1.3	1–3
	9.0–50.0	1.2–5.2	6.3–72.9	–	–	–	9.1–38.2	1.0–1.7	
Rocinela sp.	–	–	–	–	12.5	1.0	3.3	1.0	–
	–	–	–	–	1.0–50.0	–	0.2–17.7	–	
Total	100.0[a]	13.2	66.7	19.0	61.5[a]	8.1	83.3	12.5	2–57
	79.2–100.0	1.0–2.0	27.1–93.7	4.0–31.8	34.2–83.4	4.4–16.6	65.3–93.2	8.6–19.1	

[a]Significant difference.

D. aahi, *D. dimidiatus* and *D. longicolle* showed significant difference of prevalence between internal and external margins of gill filaments. The pooled didymozoid species occupied with similar total prevalence and mean intensity the inner (P% = 63.3%, MI = 7.5) and outer margins of gill filaments (P% = 60.0%, MI = 6.5) (Fig. 4). Almost all specimens of *P. appendiculatus* were attached to the second and third holobranchs, on the distal part of gill filaments.

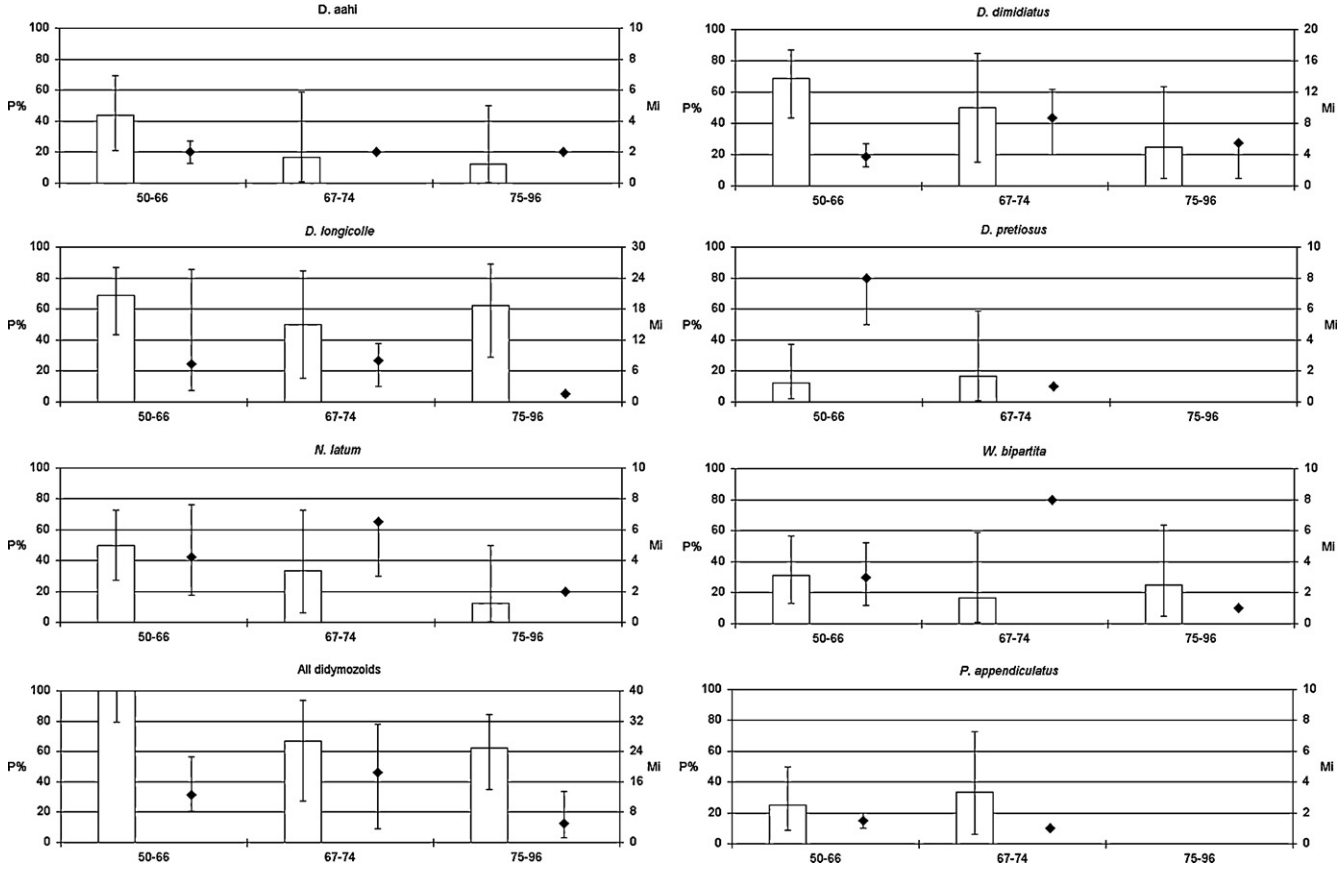

Fig. 3. Prevalence (□), mean intensity (♦) and 95% confidence intervals (—) of the gill parasites of albacore (*Thunnus alalunga*) per host size class.

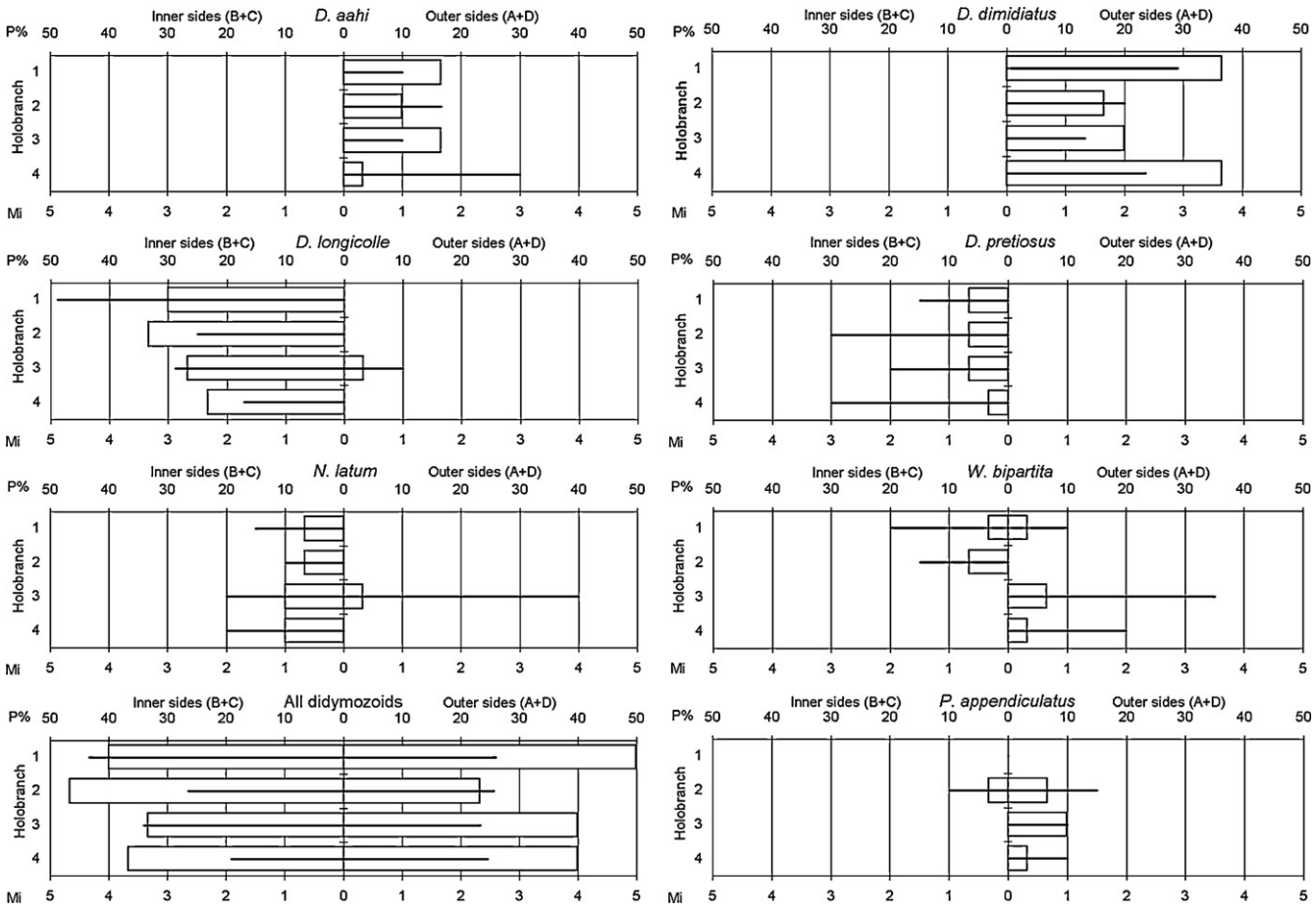

Fig. 4. Location of parasites on gills of albacore (*Thunnus alalunga*). Prevalence (□) and mean intensity (—).

No evident pathological alterations caused by parasites were found, except a light gill swelling on the site of infection caused by some dead didymozoids and *P. appendiculatus*.

4. Discussion

This study, although based on a relatively small number of samples, is the first contribution to the knowledge of the parasites of *T. alalunga* from the Mediterranean Sea. Among the 9 species of metazoan gill parasites found, albacore is a new host record for *Capsala paucispinosa* and *Didymozoon pretiosus*, while *Didymosulcus aahi*, *D. dimidiatus*, *Nematobothrium latum* and *Rocinela* sp. are for the first time reported from the Mediterranean Sea (Chisholm and Whittington, 2007; Junoy and Castelló, 2003; Munday et al., 2003). *D. dimidiatus* and *N. latum* were previously reported only on *T. alalunga* (Guiart, 1938; Pozdnyakov, 1990), while the other species were already recorded on several hosts: *C. paucispinosa* on *Euthynnus affinis* (Cantor, 1849), *Thunnus albacares* (Bonnaterre, 1788), *T. obesus* (Lowe, 1839) and *T. orientalis* (Temminck and Schlegel, 1844) (Chisholm and Whittington, 2007); *D. aahi* on *T. alalunga* and *T. albacares* (Pozdnyakov, 1990); *D. longicolle* on several scombrids from the Pacific and Atlantic Oceans (Ishii, 1935; Munday et al., 2003); *D. pretiosus* on *T. thynnus* (Ariola, 1902; Culurgioni et al., 2007, 2008); *W. bipartita* on *T. alalunga*, *T. thynnus* and *Seriola dumerili* (Risso, 1810) (Culurgioni et al., 2007, 2008; Grau et al., 1999; Guiart, 1938); and *P. appendiculatus* on *Euthynnus* spp., *Katsuwonus pelamis* (Linnaeus, 1758) and *Thunnus* spp. from all oceans (Cressey et al., 1983).

The available literature on the gill parasites of albacore is relatively scarce. Guiart (1938), Legendre (1940) and Priol (1944) reported first qualitative data on the parasites of this species from the northeastern Atlantic Ocean. Among the species listed by these authors (overall 16 species, 9 of which on the gills) only *N. latum*, *W. bipartita* and *P. appendiculatus* overlap with the parasites found, but on the basis of the descriptions and drawings given by Guiart (1938), it is likely that the new species there described as *Didymocystis macrorchis* Guiart, 1938 and *Didymocystis lanceolata* Guiart, 1938 could be synonyms of *D. aahi* and *D. dimidiatus*, respectively. On the other hand, Jones (1991) reported quantitative data on the parasites of albacore from the southern Pacific Ocean, but only crustaceans were identified at species level, while didymozoid types where simply listed with labels. The great species richness and high infection levels of didymozoids reported on the gills of albacore, and confirmed in the present study, indicate that these parasites are the principal component of the gill parasites of this host species. This pattern was also observed in *K. pelamis* (Lester et al., 1985), *T. albacares* (Lardeaux, 1982) and *T. thynnus* (Culurgioni et al., 2008; Mladineo et al., 2008; Rodríguez-Marín et al., 2008), suggesting that tunas are among the preferred hosts for didymozoids (Nikolaeva, 1985).

Concerning the site of infection, each species showed a specific location on the 4 holobranchs, as well as on the outer and the inner margins of gill filaments. The dimension and surface area of holobranchs, decreasing from the 1st to the 4th, did not affect the parasite distribution. In fact, no significant differences were found between holobranchs.

According to MacKenzie and Abaunza (1998), the ideal tag parasite should have significantly different levels of infection in different localities of the study area and it should persist in the host for a long period of time, the minimum time depending on

the nature of the study. Moreover, the parasite should be easily detected and identified, and examination of the host should involve the minimum of dissection. Aloncle and Delaporte (1973) proposed *Hirudinella fusca* Mantez (1926) as a possible biological tag to characterize the northeastern Atlantic population of albacore migrating from the Azores to the Gulf of Biscay, and Jones (1991) used the differences in parasite assemblages to describe the genetic migrations of this species between temperate waters of New Zealand and the spawning areas in tropical waters of the Coral Sea and the Tonga Sea.

Although prevalence and mean intensity of gill parasites in Mediterranean albacore were not correlated with host size, the total prevalence of the smallest and largest sized fish showed significant differences, suggesting a reduction of the infection levels in the larger sized hosts. This inverse relation was also reported by Jones (1991) for the Pacific albacore, and could be related to the change of habitat and feeding habits of the host with age (Laurs and Lynn, 1991). The lack of *C. paucispinosa* and *P. appendiculatus* in the larger hosts could be related to the change of life style and habitat of adults, which become less gregarious and migrate toward deeper oceanic waters, reducing the chance of transmission of monoxenous parasites, as observed for other big pelagic fish (Garcia et al., 2008; Hayward et al., 1998; Merella et al., 2003). Jones (1991) and Lardeaux (1982) suggested *P. appendiculatus* and *C. paucispinosa* for stock discrimination of *T. alalunga* and *T. albacares*, respectively, but the easy dislodging, short life span and seasonality of these parasites make them unreliable indicators. On the other hand, the lower infection of didymozoids reported on adult fish could be related to the change of feeding habits, indicating that these heteroxenous parasites would be more present in organisms preyed on by juveniles.

Didymozoids are considered as semi-permanent parasites, because their cysts are easily detectable in their specific location, and their remains are recognizable in the host tissues well after their death (Rodríguez-Marín et al., 2008; Speare, 1995). Moreover, the significant site selection showed by these parasites facilitates their detection and could be useful for species identification (Culurgioni et al., 2007; Rodríguez-Marín et al., 2008). Among didymozoids, *D. pretiosus* and *D. longicolle* could be used to discriminate Mediterranean and northeastern Atlantic albacore, since they have been found only on tunas from the Mediterranean Sea, although *D. longicolle* has also been described on tunas from the Gulf of Mexico (Nikolaeva and Parukhin, 1968) and the Pacific Ocean (Ishii, 1935). *W. bipartita* could be useful to study the migrations of tunas from the Mediterranean Sea or the northeastern Atlantic to other zones of this Ocean, since this species has only been recorded on albacore and Atlantic bluefin tuna from the Mediterranean Sea and the Gulf of Biscay. Therefore, didymozoids seems to be the most reliable group to be used as biological tags to investigate the albacore ecology and migrations. Nevertheless, taking into account the lack of biological data on their life cycle, the record of many species in separate geographical areas, and of similar species on sympatric hosts, their use would require some caution. In this sense, molecular techniques should be applied to assess the genetic differences among and within the species of this group (Mele et al., 2008; Rodríguez-Marín et al., 2008; Žilic et al., 2007). Moreover, considering that some of them may also infect the opercula and buccal cavity, the analysis of the whole head would probably give more complete information on the parasite fauna of the host.

Comparing the gill parasitic infections between albacore and bluefin tuna from the Mediterranean Sea, the only Mediterranean tuna studied for parasites (Culurgioni et al., 2008; Mladineo et al., 2008), although the total prevalences and mean intensities were similar, the higher values of infection of shared didymozoid species were always found in albacore: Culurgioni et al. (2008) reported lower values of *D. pretiosus* (P% = 5.9%, MI = 3.0) and *W. bipartita* (P% = 3.1%, MI = 1.0); Mladineo et al. (2008) described *D. longicolle* as rare (P% = 1.1%, MI = 1.9), while it was the dominant species in the present study. Therefore, in spite of the analogous feeding habits of both species (Consoli et al., 2008), considering that movements of Mediterranean albacore are limited to the Mediterranean Sea (Alonso et al., 2005; Arrizabalaga et al., 2003) while adult bluefin tuna resides in the Mediterranean only during the summer spawning season (Rooker et al., 2007), albacore could have a primary role in the life cycle of these Mediterranean food borne parasites (e.g. *D. longicolle* and *D. pretiosus*), while bluefin tuna should be of secondary importance.

Finally, no macroscopic pathological alterations were observed on the gills of the examined hosts, except a light gill swelling caused by *P. appendiculatus* and by the remains of some dead didymozoids. It is important to stress that monogeneans and copepods can be harmful at high intensities of infection, causing histopathological lesions, such as hyperplasia of the gill epithelium, fusion of gill lamellae, haemorrhages, etc. (Deveney et al., 2001; Hayward et al., 2007). Conversely, gill didymozoids cause few pathological alterations (Al-Bassel and Ohida, 2006; Deveney et al., 2005; Mladineo, 2006), and they are not considered as harmful. However, in rearing systems where fish is daily exposed to stress, the effects of any infection should be investigated over long time periods, in order to gain accurate insight into the pathology and its effect on the host (Mladineo and Tudor, 2004).

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