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Metazoan parasites in the head region of the bullet tuna *Auxis rochei* (Osteichthyes:
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Introduction

The bullet tuna *Auxis rochei* (Risso, 1810) (Osteichthyes: Scombridae) is a pelagic fish distributed in tropical and subtropical areas, including the Mediterranean Sea (Uchida, 1981). The limits of its distribution are not well known, mainly because this species is often confused with its congener, the frigate tuna *Auxis thazard* (Lacepède, 1800), another cosmopolitan fish (Di Natale *et al.*, 2009). In fact, the systematics of the genus *Auxis* is still controversial: some authors consider the species synonymous (Collignon, 1961; Nair *et al.*, 1970), while others recognise the existence of two distinct species (Yesaki & Arce, 1991; Collette & Aadland, 1996). Although at present the latter hypothesis is the one considered valid (Catanese *et al.*, 2008), recent genetic and morphometric studies showed that *A. rochei* is the only species distributed in the Mediterranean Sea and in the adjacent areas of the Atlantic Ocean (Orsi Relini *et al.*, 2008), suggesting that some occurrences of this species from this area could have been misidentified as *A. thazard*, even in scientific papers (Orsi Relini *et al.*, 2009).

Auxis rochei, which is the most abundant tuna in the Mediterranean Sea, represents an important component of the food web (Mostarda *et al.*, 2007) and is exploited by artisanal fisheries, representing 39% of the total tuna landings in the Mediterranean area (9829 t in 2010; FAO, 2011). The migrations of *A. rochei* in the Atlantic Ocean have been rarely studied; Grudtsev (1992) suggested that it may undertake a trophic migration along the north west coast of Africa, and Richards & Simmons (1971) suggested that spawning took place south of the Islands of Cape Verde. In addition, little is known about its life cycle and migrations in the Mediterranean Sea. Tortonese (1963) and Reglero *et al.* (2012) reported that *A. rochei* performs local migrations around the spawning areas in the neritic habitat of the mainland and islands, whereas Sabatés & Recasens (2001) proposed a spawning migration from the

Atlantic Ocean to the western Mediterranean Sea.

Parasites are useful tags to investigate the biology, ecology, migration and population structure of marine organisms (MacKenzie and Abaunza, 2014), and they have also been successfully used to clarify taxonomic relationships between hosts (Whittington, 2005). Although the parasites of the head region of representatives of the genus *Auxis* have been studied in several areas of the world, the confusion in the systematics of *A. rochei* and *A. thazard* makes specific information on their parasite fauna perplexing, e.g. in the Atlantic Ocean and Mediterranean Sea records of several parasites assigned to unidentified *Auxis* sp. (Dollfus, 1926; Palombi, 1949; Cressey & Cressey, 1980) could refer to *A. rochei*. The aim of this study is to describe the metazoan parasites of the head of *A. rochei* from the western Mediterranean Sea.

Materials and methods

Collection and examination of fish

A total of 63 *A. rochei* (32 males and 31 females), caught in the traditional trap fishery of La Azohía (37°32'59"N, 1°10'44"W, south east Spain, western Mediterranean Sea), were examined for parasites: 21 specimens were collected in May 2008 and 42 in May 2011. An additional group of nine specimens of bullet tuna (six males and three females), caught in the traditional trap fishery of Tarifa (36°00'59"N, 5°37'44"W, Strait of Gibraltar) in May 2008, were examined for comparative purposes. For each individual fish, the fork length to the nearest centimetre was recorded (FL = 34-44 cm).

The heads of fish were excised, stored individually in plastic bags and frozen at -20° C. Subsequently the samples were defrosted and examined for parasites according to Mele *et al.* (2012). The following literature was used for species identification: for monogeneans,

Palombi (1949), Fuentes Zambrano (1997), Mogrovejo & Santos (2002) and Mogrovejo *et al.* (2004); for didymozoids, Skrjabin (1955), Yamaguti (1970) and Pozdnyakov & Gibson (2008); for copepods, Vervoort (1962, 1965), Cressey & Cressey (1980), Boxshall & Halsey (2004) and Lin & Ho (2006).

Data analysis

The prevalence of infection and mean abundance of each parasite species was calculated according to Bush *et al.* (1997). Confidence intervals of prevalence and mean abundance were assessed with the Sterne's exact method and the bias-corrected and accelerated Efron-Tibshirani bootstrap, respectively, using the free software Quantitative Parasitology 3.0 (Reiczigel & Rózsa, 2005).

Possible correlations between abundance of infection and host size were evaluated using the Spearman rank correlation coefficient, and its significance tested using the R software ("spearman" method, "cor.test" function, "stats" library of the R-software; R Development Core Team, 2014). Levels of infection of each parasite species were calculated for each of two size groups: (1), FL 33-37 cm; (2), FL 38-44 cm. Samples from south east Spain comprised four groups according to sampling year and host size: group (1) and (2) of 2008 ($n = 11$ and 22 , respectively) and 2011 ($n = 10$ and 20 , respectively). Samples from the Strait of Gibraltar of 2008 belonged only to the host size group (2) ($n = 9$). Differences between the parasite infections of the five host groups and between host sexes were evaluated using the Fisher exact test for prevalence and the Welsh bootstrap t-test for mean abundance (Reiczigel & Rózsa, 2005).

Published and new data on the parasites of the head region of *Auxis* spp. worldwide were used to assess the dissimilarity between the parasite faunas of the head region of the

bullet and frigate tunas from four geographical regions: *A. thazard* from the Atlantic Ocean (data from: Vervoort, 1965; Fuentes Zambrano, 1997; Mogrovejo *et al.*, 2004; Chisholm & Whittington, 2007), *A. thazard* from the Indian Ocean (data from: Silas, 1962; Mogrovejo *et al.*, 2004; Chisholm & Whittington, 2007), *A. thazard* from the Pacific Ocean (data from: Silas, 1962; Yamaguti, 1970; Mogrovejo *et al.* 2004; Chisholm & Whittington, 2007), and *A. rochei* from the Mediterranean Sea (present results). Parasite fauna dissimilarity was evaluated with the Marczewski-Steinhaus distance (“cc” method, “betadiver” function, “vegan” library of the R-software) and the Bray-Curtis index (“-1” method, “betadiver” function, “vegan” library of the R-software) (Culurgioni *et al.*, 2014; Mele *et al.*, 2014).

Results and discussion

This study is the first description of the parasite fauna of the head region of *A. rochei* from the western Mediterranean Sea. A total of seven parasite species were found in and on the hosts from south east Spain, with 72% being adult didymozoids belonging to two species, *Didymozoon auxis* Taschenberg, 1879 and Nematobothriinae gen. sp. (table 1). Up to 21% were monogeneans, including *Allopseudaxine macrova* (Unnithan, 1957), *Churavera triangula* (Mamaev, 1967) and *Hexostoma auxisi* Palombi, 1943. The remaining 7% comprised two copepod species, *Caligus bonito* Wilson, 1905 and *Unicolax mycterobius* (Vervoort, 1965). Unidentified post-larval stages of didymozoids were found in the gill arch tissues. Only three parasite species were detected in *A. rochei* from the Strait of Gibraltar: *D. auxis* (86.7% of all specimens), *A. macrova* (6.7%) and *U. mycterobius* (6.6%). Only *D. auxis* and *H. auxisi* have been previously reported in *Auxis* sp. from the Mediterranean Sea (Dollfus, 1926; Palombi, 1949).

The unidentified Nematobothriinae gen. sp. has never been described before,

therefore it could be a specific parasite of *A. rochei* in the Mediterranean Sea, although the lack of previous records of this parasite could also be due to the difficulty of detection in the retrobulbar fat tissue. The other parasites have been reported in a wide range of hosts and regions: the congener *A. thazard* was found to harbour *C. triangula* in the Atlantic and Pacific Oceans and *H. auxisi* in the Atlantic Ocean only (Mogrovejo *et al.*, 2004); *A. macrova* infects several tunas such as *A. thazard*, *Euthynnus* spp., *Thunnus albacares* and *Katsuwonus pelamis* from the three oceans (Bussi  ras & Baudin-Laurencin, 1973; Rohde *et al.*, 1980; Gibson *et al.*, 2005; Alves & Luque, 2006); *C. bonito* infects pelagic fish from the Mediterranean Sea (*Coryphaena hippurus*, *K. pelamis*; Carbonell *et al.*, 1999; Mele *et al.*, 2012), the north west Atlantic Ocean (*Thunnus thynnus*) and the tropical areas of the Atlantic, Indian and Pacific Oceans (*Euthynnus* spp., *K. pelamis* and *Sarda* spp.; Cressey & Cressey, 1980;   ktener & Trilles, 2009). In the Mediterranean Sea *A. macrova* seems to be restricted to *A. rochei*, since this monogenean was not found in 156 *E. alletteratus* and 35 *K. pelamis* caught within the same sampling program (Mele, 2013). The cross-infection of *A. macrova* among the Mediterranean tuna could be limited by the different life history and habitat of hosts (Reglero *et al.*, 2012; Rodr  guez *et al.*, 2013).

No significant differences in prevalence and mean abundance were found ($p > 0.05$) between host sexes and years of sampling; therefore the south east Spain data were pooled across sex and years, and the south east Spain hosts were only divided according to host size (table 1). The highest prevalence was that of *D. auxis* in the host size group (2) (71% in the south east Spain and 78% in the Strait of Gibraltar samples), being twice than in the host size group (1) (31% of the south east Spain samples). Significant differences between the mean abundance of three parasites were found: *A. macrova* had higher mean abundance in the host size group (2) from the south east Spain than in host size group (1); Nematobothriinae

gen. sp. had higher mean abundance in both host groups from the south east Spain than in that from the Strait of Gibraltar; and *C. bonito* had higher mean abundance in the host size group (2) from the south east Spain than in that from the Strait of Gibraltar (table 1). The higher prevalence of *D. auxis* and the higher mean abundance of *A. macrova* in the larger *A. rochei* than in the smaller ones could be due to the different origin of the fish. However, considering that *D. auxis* infects the definitive host through the food web, these differences could also indicate a change of the diet with size. In fact large fish (> 35 cm) can predate on fast-swimming prey including juvenile and adult fish and cephalopods (Mostarda *et al.*, 2007), which are among the intermediate hosts of didymozoids (Felizardo *et al.*, 2011). The difference of the mean abundance of Nematobothriinae gen. sp. and *C. bonito* according to geographical region could also be influenced by the small number of hosts sampled from the Strait of Gibraltar. The level of infection of Nematobothriinae gen. sp. did not change with host size (table 1). Considering that its geographical range is limited to the Mediterranean Sea and that the host cannot lose this tissue parasite with migration, the occurrence of Nematobothriinae gen. sp. only in the Mediterranean *A. rochei* could indicate that this host population is separated from that of *Auxis* spp. from other areas. However, the presence of this parasite could go unnoticed in routine parasitological analysis.

A negative relationship between the abundance of *U. mycterobius* and the host size (-0.27 , $p = 0.003$) was found. This fact could be due to the increase of the distance between the narine walls in the larger hosts that will not offer an ideal microhabitat for the parasite to live wedged in the nasal cavities (Cressey & Cressey, 1980).

The parasite fauna of *A. thazard* from the Atlantic and Pacific Oceans has the highest richness, with nine and eight species, respectively. The poorest is the parasite fauna of *A. thazard* from the Indian Ocean, with only six species, although this could be due to the

scarcity of parasitological studies on this fish from this area. The parasite fauna of *A. rochei* from the Mediterranean Sea showed the closest similarity with that of *A. thazard* from the Atlantic Ocean, while the other groups were largely dissimilar (fig. 1). Considering the current debate on the host taxonomy and identification, the affinity between the parasite assemblages of the Mediterranean *A. rochei* and the Atlantic *A. thazard* could be compatible with the existence of a unique *Auxis* species throughout the Atlantic Ocean and the Mediterranean Sea (Orsi Relini *et al.*, 2009).

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Table 1. Prevalence (%), mean abundance (MA), 95% confidence intervals (CI) and microhabitat of the metazoan parasites in the head region of *Auxis rochei* from south east Spain and the Strait of Gibraltar; FL – fork length, na – no data given, * and ^- level of significant differences between pairs of samples given as $p \leq 0.05$.

Geographical region	South east Spain				Strait of Gibraltar		Microhabitat
Host size class	FL 33 - 37 cm		FL 38 - 44 cm		FL 38 - 44 cm		
Parasite	% (CI)	MA (CI)	% (CI)	MA (CI)	% (CI)	MA (CI)	
Monogenea							
<i>Allopseudaxine macrova</i>	5(0-23)	0.1 (0-0.1)*	24 (13-39)	0.3 (0.1-0.4)*	11 (1-44)	0.1 (0.0-0.3)	Gill filaments
<i>Churavera triangula</i>	5(0-23)	0.1 (0-0.1)	7 (2-20)	0.1 (0.0-0.3)	0 (0-32)	0.0 (na)	Gill filaments
<i>Hexostoma auxisi</i>	14 (4-35)	0.9 (0.1-3.9)	2 (0-13)	0.1 (0.0-0.1)	0 (0-32)	0.0 (na)	Gill filaments
Didymozoidae							
<i>Didymozoon auxis</i>	33 (16-55)*^	1.4 (0.4-4.1)	71 (56-84)*	2.2 (1.4-3.3)	78 (44-96)^	1.4 (0.7-2.3)	Gill filaments
Nematobothriinae gen. sp.	24 (10-46)	0.3 (0.1-0.4)*	24 (13-39)	0.3 (0.2-0.6)^	0 (0-32)	0.0 (na)*^	Retrobulbar fat tissue
Copepoda							
<i>Caligus bonito</i>	5(0-23)	0.1 (0-0.1)	17 (8-31)	0.2 (0.1-0.3)*	0 (0-32)	0.0 (na)*	Gill chamber and mouth
<i>Unicolax mycterobius</i>	20 (7-47)	0.2 (0.0-0.4)	5 (0-16)	0.1 (0.0-0.1)	11 (1-44)	0.1 (0.0-0.3)	Nasal cavities

Legend of the figure

Fig. 1. Cluster dendrograms (group-average linkage) of the parasites of the head of *Auxis rochei* from the western Mediterranean Sea (Ar-Med), and of *A. thazard* from the Atlantic Ocean (At-Atl), Indian Ocean (At-Ind) and Pacific Ocean (At-Pac), using Marczewski-Steinhaus (A) and Bray-Curtis (B) dissimilarity measures based on the presence/absence data with the cophenetic correlation coefficients having a similar value of 0.89. Source: Silas (1962); Vervoort (1965); Yamaguti (1970); Cressey & Cressey (1980); Muruges & Madhavi (1995); Fuentes Zambrano (1997); Mogrovejo *et al.* (2004); Chisholm & Whittington (2007); and present results.

