Ecology of the Atlantic black skipjack *Euthynnus alletteratus* (Osteichthyes: Scombridae) in the western Mediterranean Sea inferred by parasitological analysis

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SUMMARY

Between 2008 and 2011, the head of 150 *Euthynnus alletteratus* (Osteichthyes: Scombridae) caught inshore off the southeastern Iberian coast (western Mediterranean Sea) were examined for parasites. Two monogeneans, four didymozoid trematodes and four copepods were found. Parasite abundance showed a positive relationship with the annual sea surface temperature, except for *Pseudocycnus appendiculatus*, but negative with the sea depth (*Capsala manteri, Neonematobothrium* cf. *kawakawa* and *Caligus bonito*). Prevalences and mean abundances differed significantly among sampling areas, except for *C. manteri, Oesophagocystis* sp. 2 and *Ceratocolax euthynni*, and sampling years (*Melanocystis* cf. *kawakawa, N.*cf. *kawakawa, P. appendiculatus* and *Unicolax collateralis*). Results indicate that the parasite abundances of *E. alletteratus* in the western Mediterranean Sea depend mainly on regional environmental variables, which can show interannual variations. The presence of pelagic parasites, i.e. didymozoids and *P. appendiculatus*, could indicate that *E. alletteratus* migrates between inshore and offshore pelagic domains. The different parasite faunas reported in *E. alletteratus* populations from the western Atlantic Ocean and the Mediterranean Sea appear to point out the geographical host isolation. These results suggest that *E. alletteratus* inhabiting the western Mediterranean Sea performs inshore-offshore small-scale migrations, and not transoceanic migrations between the western Atlantic Ocean and Mediterranean Sea.

Key words: Scombridae, parasite Copepoda, Didymozoidae, Monogenea, migration.

INTRODUCTION

The Atlantic black skipjack *Euthynnus alletteratus* (Rafinesque, 1810) is a pelagic scombrid fish that inhabits the coastal tropical and subtropical waters of both sides of the Atlantic Ocean, including the Mediterranean Sea, Caribbean Sea and Gulf of Mexico (Collette and Nauen, 1983). Three main populations of *E. alletteratus* are known in the Atlantic Ocean, located in the central eastern, in the southeastern and in the western Atlantic Ocean (Yoshida, 1979). Moreover, there is a population in the Mediterranean Sea, which is considered independent from the Atlantic ones (Gaykov and Bokhanov, 2008). The status of the Mediterranean population is scarcely known, although *E. alletteratus* is a species of commercial interest (El-Haweet *et al.* 2013), whose fishery in the region represents up to 25% of the global catches of this species (5 230 t in 2013) (Catarci, 2004; FAO, 2014) and it plays a key role in the Mediterranean pelagic trophic webs (Falautano *et al.* 2007).

The parasite fauna of *E. alletteratus* has been investigated only in the western Atlantic Ocean (Alves and Luque, 2006), remaining neglected in the central and southeastern Atlantic Ocean and in the Mediterranean Sea.

The aim of this study is to investigate the metazoan parasite populations of *E. alletteratus* from the western Mediterranean Sea, evaluating how the geographical, environmental and biological factors can influence their structure and inferring the biological and ecological traits of the species that can be derived from the spatial and temporal variations.

MATERIALS AND METHODS

Host and parasite collection

A total of 150 Atlantic black skipjacks, caught off the Iberian Peninsula in the western Mediterranean Sea

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between 2008 and 2011 were examined for parasites (Table 1). Environmental data, as bottom depth (Depth) and sea surface temperature (SST), from each collection site per sampling date were obtained as monthly means from the SeaWiFS dataset (http:\\oceancolor.gsfc.nasa.gov; Table 1).

Immediately after landing, fish were measured (FL, fork length range 205–731 mm), weighed (110–6214 g) and sexed (54 males, 72 females and 44 unsexed/hybrid specimens). After each sampling, the heads (including gills and heart) of fish were excised, stored individually in plastic bags and frozen at −20 °C. Subsequently, the heads were defrosted and examined under a stereomicroscope for metazoan parasites. All parasites were identified according to their morphological characteristics, counted and stored in 70% ethanol.

Morphological and molecular analysis of didymozoid parasites

Molecular data were used to support the morphological identification of Didymozoidae, family of trematodes of fish with a highly complex and controversial taxonomy (Abe and Okamoto, 2015). Parasites of each didymozoid morphotype were cut into two portions at about 500 μm to the tip of the posterior end: the anterior part was stored in ethanol 70% for morphological control and the rest dehydrated with absolute ethanol and processed according to the following protocol. Total DNA was isolated placing the samples in 200 μL of a 5% suspension of deionised water and Chelex containing 0·1 mg mL^{-1} proteinase K (Georgieva *et al.* 2013). The polymerase chain reaction (PCR) amplification was performed on two loci of interest: D1-D2 domain of the ribosomal large-subunit (partial 28S) and second internal transcribed spacer (ITS-2) of DNA (Anderson and Barker, 1993; Chambers and Cribb, 2006). Three microliters of DNA were used in 25 μL reactions using Ready-To-Go PCR beads (GE Healthcare, UK) and 2 μL of each primer. Partial 28S was amplified using primers ZX-1 (5′-ACCCGCTGAATTTAAGCATAT-3′) and 1500R (5′-GCTATCCTGAGGGAAACTTCG-3′), according to Palm *et al.* (2009). The ITS2 portion was amplified using primers 3S (5′-GGTACCGGTGGATCACTCGGCTCGTG-3′) and BD2 (5′-TATGCTTAAATTCAGCGGGT-3′) (Bowles *et al.* 1993). PCR products were stained with Gel Red (Biotium, Italy) and visualized on 1·5% Tris-acetate-EDTA agarose gel electrophoresis. PCR products were purified with QIAquick PCR purification kit (QIAGEN, Italy) according to the manufacturer's instructions, and sequenced by an external core service (Macrogen Europe Inc, the Netherlands) using the PCR primers.

Newly-generated sequences were aligned using Clustal W as implemented in MEGA v6 (Tamura *et al.* 2013) and then deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/Genbank/GenbankSearch.html). Species boundaries were assessed via maximum likelihood (ML) analyses of Tamura-Nei parameter distances using MEGA v6 with nodal support estimated using 1000 bootstrap resamplings (Tamura *et al.* 2013). The outgroup-rooted trees were drawn using the species *Quadrifoliovarium pritchardae* (GenBank accession number AY897561 for ITS2 and AY897567 for 28S) as outgroup. A six-rates custom model (general time reversible) of sequence evolution was set, allowing the estimation of proportion of invariable sites and the gamma distribution. Nodes with a percentage of bootstrap <70% were considered to be not statistically supported (Hillis and Bull, 1993). Voucher specimens were deposited in the Parasite Collection of the Cavanilles, Institute of Biodiversity and Evolutionary Biology, University of Valencia, Spain (PCICBiBE).

Data analysis

Differences between mean fork lengths of host specimens were evaluated with Student's *t*-test; the mean FL of the specimens from the Gulf of Valencia of 2011 was significantly lower than that of tuna from the southeastern Spain of the same year (Student's $t = -11{\cdot}824$, P-value $< 0{\cdot}0001$), the mean FL of tuna from the southeastern Spain differed among the years 2008, 2009 and 2011 ($t = 5{\cdot}36$–$7{\cdot}55$, $P < 0{\cdot}0001$). Fish samples were grouped according to size as juvenile (FL = 200–400 mm) and adult specimens (401–800 mm), and then according to locality and year. Hosts were divided into five groups (Table 1): juvenile fish from the Gulf of Valencia of 2011, adult fish from the southeastern Spain of 2008, 2009 and 2011, and adult fish from the Strait of Gibraltar of 2008.

Prevalence (P%), abundance and mean abundance (mA) of each parasite species were calculated according to Bush *et al.* (1997). The confidence intervals of prevalence and mean abundance were estimated with the Sterne exact method and the bias-corrected and accelerated Efron-Tibshirani's bootstrap. Differences between the parasite assemblages of the five host groups and between host sexes (including all adult specimens) were evaluated using the Fisher's exact test for prevalence and the bootstrap rank Welch test for mean abundance. Results were compared with previously published quantitative data on the parasites of *E. alletteratus* from the western Atlantic Ocean (Alves and Luque, 2006). Correlations between abundance of parasite species and host size were evaluated with the Spearman rank correlation coefficient. All these analyses were performed using the free software Quantitative Parasitology web 1·0·9 (Reiczigel *et al.* 2013).

Table 1. Sampling data and biological parameters of *Euthynnus alletteratus* from the western Mediterranean Sea

Date of sampling	Locality (latitude, longitude)	Depth (m)	SST (ºC)	N	mean FL (mm)	mean W (g)	Host development	Sex (M/F/U)
22/10/2011	Gulf of Valencia (39°35′52·5″N 0°04′33·5″E)	60	17·9	23 (23/0/0)	306 (205–367)	512 (110–844)	Juvenile	0/0/23
27/05–26/06/2008	Southeastern Spain La Azohía (37°32′58·9″N 1°10′43·5″W)	40	20·1	63 (0/30/33)	605 (540–686)	3612 (2480–4346)	Adult	25/38/0
18/05–25/06/2009		40	17·3	22 (0/3/19)	646 (575–731)	4342 (3030–6214)	Adult	11/11/0
21–29/06/2011		40	20·2	20 (0/15/5)	528 (371–637)	2547 (1488–4248)	Adult	9/10/1
12/06/2008	Strait of Gibraltar – Tarifa (36°00′58·9″N 5°37′44·3″W)	50	23·1	22 (0/8/14)	613 (580–654)	3931 (3330–4600)	Adult	9/13/0

SST, sea surface temperature; FL, fish fork length, W, fish total weight, M/F/U, male, female, unknown sex; N, number of hosts according to mean FL classes (201–400/401–600/601–800 mm).

Bayesian hierarchical spatial models (Muñoz *et al.* 2013) were used to examine whether the abundance of each parasite species was related to independent variables, namely the bottom depth, SST, length and weight of the hosts. Moreover, a random spatial effect was introduced in the analysis in order to verify the spatial variability of the data within each area. Specifically, the number of each parasite in each location sampled, Y_i was considered Poisson distributed with rate $t_i \lambda_{i.}$, where t_i is the observation time at site i and λ_i is proportional to a relative species abundance at the site i and measures the expectation of samplings for a unit observation time, according to the general formulation,

$$\log(\lambda_{ij}) = \alpha_{+} X\beta_{ij} + Z_j + W_i$$

where α is the intercept, X_{ij} is the vector of the explanatory covariates at the year j and the location i, Z_j is the component of the temporal unstructured random effect at the year j, and W_i represents the spatially structured random effect at the location i.

Once the model was determined, the next step was to estimate its parameters. Following Bayesian reasoning, the parameters were treated as random variables, and prior knowledge was incorporated via the corresponding prior distributions of the selected parameters. In particular, for the parameters involved in the fixed effects, non-informative Gaussian distributions N(0, 100) were used. A prior Gaussian distribution with a zero mean and covariance matrix was assumed for the spatial effect. This component depends on two hyperparameters k and τ, which determined, respectively, the range and the total variance of the spatial effect (see Muñoz *et al.* 2013 for more detailed information about spatial effects). In addition, a Log Gamma prior distribution was assumed on the log-precision λy ($a = 1$, $b = 5e{-}05$) for the temporal component.

A model selection with both backwards and forwards approaches was used to select relevant variables. Specifically, the Deviance Information Criterion (DIC), a well-known Bayesian model-choice criterion for comparing complex hierarchical models (Spiegelhalter *et al.* 2002), was used. Lower values of DIC represented the best compromise between fit and parsimony.

The *Integrated Nested Laplace Approximation* (INLA) methodology (Rue *et al.* 2009) and software (http:\\www.r-inla.org), which is implemented in the R software (R Development Core Team, 2015), were used as an alternative to the Markov chain Monte Carlo methods for speed of calculation (Roos *et al.* 2015).

RESULTS

A total of 10 parasite species/taxa were found in the head of *E. alletteratus* from the western

Mediterranean Sea (Table 2). The monogenean *Hexostoma thunninae* was the most prevalent species (P% = 55%) and the didymozoid *Melanocystis* cf. *kawakawa* the most abundant one (mA = 14·4).

Morphological and molecular analysis of didymozoid parasites

The 3 236 didymozoid specimens found in the head tissues of *E. alletteratus* were morphologically classified into four species/morphotypes (Fig. 1): *Melanocystis* cf. *kawakawa* (66% of all specimens), *Neonematobothrium* cf. *kawakawa* (4%), *Oesophagocystis* sp. 1 (28%), and *Oesophagocystis* sp. 2 (2%). All these parasites consisted of a capsule including a couple of similar hermaphroditic individuals, encapsulated in host tissues. *Neonematobothrium* cf. *kawakawa* had a very long, ribbon-like body, not divided into regions. Oral sucker and pharynx were present, whereas prepharynx and ventral sucker were absent. Testes parallel, reaching genital junction level. Ovary and vitellaria simple, thin and long, located in the anterior and posterior parts of the body, respectively. Uterus three looped. *Melanocystis* cf. *kawakawa, Oesophagocystis* sp. 1, and *Oesophagocystis* sp. 2 had the body divided into two regions. The anterior regions were lancelolate, filiform and racket–like, respectively, and attached to the middle of the ventral surface of the posterior regions. The posterior regions, which included ovarium, vitellaria and testes, were disk-like in *M.* cf. *kawakawa*, conical in *Oesophagocystis* sp. 1, and bell-shaped in *Oesophagocystis* sp. 2. Oral sucker and pharynx were present, whereas prepharynx and ventral sucker absent. Two blind caeca with black amorphous contents were visible in *M.* cf. *kawakawa*.

A total of 384 bp of ITS2 and 595 bp of 28S fragments of 18 DNA sequence were generated (GenBank accession number: KU216165-KU216182 and KU290344-KU290361; PCICBiBE voucher numbers: 12126–12143). The comparison of ITS2 fragments showed that there was no genetic variation within *M.* cf. *kawakawa* and *N.* cf. *kawakawa* groups, whereas 0·001 ± SD 0·001 of difference was found within *Oesophagocystis* sp. 1 and *Oesophagocystis* sp. 2 groups. The ML analysis of these fragments revealed four well-supported genetic clusters within the family Didymozoidae (Fig. 1), which were consistent with the morphological types previously identified. ITS2 fragments of *M.* cf. *kawakawa* were more similar to those of *Oesophagocystis* sp. 1 (0·035 ± SD 0·009) than to those of *Oesophagocystis* sp. 2 (0·047 ± SD 0·011) and those of *N.* cf. *kawakawa* (0·255 ± SD 0·030). The ITS2 sequences of *N.* cf. *kawakawa* were more similar to those of *M.* cf. *kawakawa* than to those of *Oesophagocystis* sp. 1 (0·270 ± SD 0·011) and those of *Oesophagocystis* sp. 2 (0·279 ± SD 0·032). Finally, the sequences of *Oesophagocystis* spp. were more similar between them (0·039 ± SD 0·010) than to those of the other species. The phylogenetic tree from 28S region (not shown) had similar topology to that deduced from ITS2. Bootstrap support values were generally lower than the ITS2 ones, with some basal nodes of the tree not resolved.

Parasite population analysis

The abundance of *H. thunninae* and *N.* cf. *kawakawa* was positively correlated with the host length (Spearman rank correlation index, Rs = 0·25, *P* = 0·0043 and Rs = 0·18, *P* = 0·0415, respectively), whereas that of *Unicolax collateralis* was negatively correlated with host length (Rs = –0·35, *P* = 0·0001). Bayesian GLM analyses showed a positive relationship between the abundance of *Capsala manteri* and North eastward latitude and longitude (posterior mean = 0·0021, 95% CI = –0·0439–0·0483; 0·1079, 95% CI = –0·568–0·7758, respectively), whereas a negative one between that of *Ceratocolax euthynni* and the eastward longitude (posterior mean = –0·3646, 95% CI = –1·4649-0·8126). Positive relationships were also found between the abundance of most of parasites and SST (Table 3), except that negative of *Pseudocycnus appendiculatus*. The relationship between the abundance of *C. manteri, N.* cf. *kawakawa* and *Caligus bonito* and the sea bottom depth was also negative. No significant differences in P% and mA of each parasite species were found between host sexes.

Figures 2–3 show the prevalence and mean abundance of parasites according to the locality. *Ceratocolax euthynni* was found only in the tuna from the southeastern Spain; whereas three species were not collected in those of the Strait of Gibraltar: *C. manteri, N.* cf. *kawakawa* and *C. bonito*. This caligid parasite and *U. collateralis* had the highest P% and mA in tuna of the Gulf of Valencia; *H. thunninae* and *P. appendiculatus* had the highest P% and mA in the samples of the southeastern Spain. *Melanocystis* cf. *kawakawa* and *Oesophagocystis* sp. 1 had the highest P% in the Gulf of Valencia and in the southeastern Spain, and had the highest mA in the southeastern Spain and in the Gulf of Valencia, respectively.

Table 4 shows the P% and mA of the parasites found in the southeastern Spain according to the year. The P% of didymozoids and of *U. collateralis* was higher in 2011 than in 2008 and 2009, being higher in 2009 than in 2008, but *Oesophagocystis* sp. 2 had similar prevalences among years. The mA of *M.* cf. *kawakawa* was higher in 2009 than in 2008 and 2011; the mA of *P. appendiculatus* were higher in 2008 than 2011, and that of *N.* cf. *kawakawa* was higher in 2011 than in 2008.

Table 2. Prevalence (P%), mean abundance (mA), 95% confidence intervals (in brackets), Spearman rank correlation index (Rs) between abundance and fish length and microhabitat, of the parasites of the head of *Euthynnus alletteratus* from the western Mediterranean Sea.

Species	*P*%	mA	Rs (*P*-value)	Microhabitat
Monogenea				
Capsala manteri Price, 1951	5 (2–9)	0·8 (0·1–4·0)	0·06 (0·4767)	Gill arch
Hexostoma thunninae Parona *et* Perugia, 1889	55 (47–63)	1·6 (1·2–2·2)	0·25 (0·0043)	Gill filaments
Trematoda				
Melanocystis cf. *kawakawa* Yamaguti, 1970	36 (29–44)	14·4 (6·3–29·5)	−0·03 (0·5949)	Oesophagus and pharynx
Neonematobothrium cf. *kawakawa* Yamaguti 1965	23 (17–30)	0·8 (0·5–1·1)	0·18 (0·0415)	Pharynx and operculum
Oesophagocystis sp. 1	39 (31–47)	6·0 (4·2–8·9)	0·02 (0·7994)	Gill filaments and nasal sinus
Oesophagocystis sp. 2	11 (7–17)	0·4 (0·2–0·7)	0·02 (0·8109)	Gill rakers
Crustacea				
Caligus bonito Wilson, 1905	21 (15–29)	0·3 (0·2–0·4)	−0·02 (0·822)	Gills and operculum
Ceratocolax euthynni Vervoort, 1965	2 (1–6)	0·03 (0–0·1)	−0·07 (0·539)	Olfactory organ
Pseudocycnus appendiculatus Heller, 1865	49 (41–57)	1·0 (0·8–1·2)	0·09 (0·3309)	Gill filaments
Unicolax collateralis Cressey *et* Cressey 1980	11 (7–18)	0·2 (0·1–0·3)	−0·35 (0·0001)	Nasal sinus

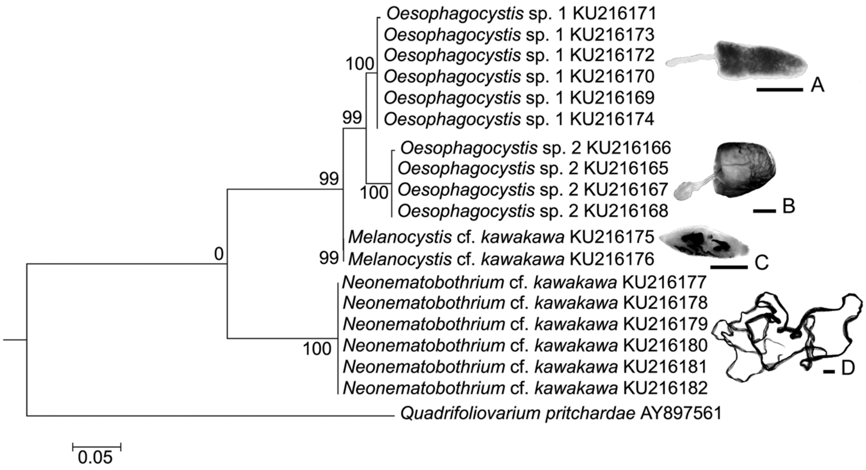

Fig. 1. Micrographs and phylogenetic tree of didymozoids of the head of *Euthynnus alletteratus* from the western Mediterranean Sea inferred by using the maximum likelihood method based on the Tamura-Nei model of internal transcribed spacer 2 (ITS-2) sequences. Bootstrap support (>70% for 1000 replicates) of the percentage of trees in which the associated taxa clustered together is shown next to the branches. Bar in microns: A-B, 500; C, 100; D, 1000.

Table 3. Bayesian analysis of the relationship between environmental factors and the abundance of the parasites of the head of *Euthynnus alletteratus* from the western Mediterranean Sea

	Intercept	SST	Bottom depth
Capsala magronum	0·085 (−1·6511–1·8236)		−0·0019 (−0·0408–0·0372)
Hexostoma thunninae	−6·1269 (−14·2665–1·8037)	0·1312 (−0·0985–0·37)	
Oesophagocystis sp. 1	−6·825 (−13·8141–0·4852)	0·4046 (0·1036–0·7422)	
Melanocystis cf. *kawakawa*	−56·9288 (−89·0855–22·4681)	2·1506 (0·8481–3·3741)	
Neonematobothrium cf. *kawakawa*	4·2626 (−14·9237–22·5256)	0·3448 (−0·1091–0·8686)	−0·2363 (−0·3832–0·108)
Caligus bonito	−5·0908 (−14·4585–5·4462)	0·3217 (0·0118–0·6213)	−0·0754 (−0·2429–0·0551)
Ceratocolax euthynni	−21·336 (−47·576–0·8104)	0·8059 (−0·0836–1·9292)	
Pseudocycnus appendiculatus	6·1964 (1·2137–11·2907)	−0·2385 (−0·4691–0·014)	
Unicolax collateralis	−2·354 (−17·6347–13·8325)	0·3231 (−0·2198–0·869)	

SST, sea surface temperature.
95% confidence intervals in brackets. Only significant data are shown ($P \leqslant 0{\cdot}05$).

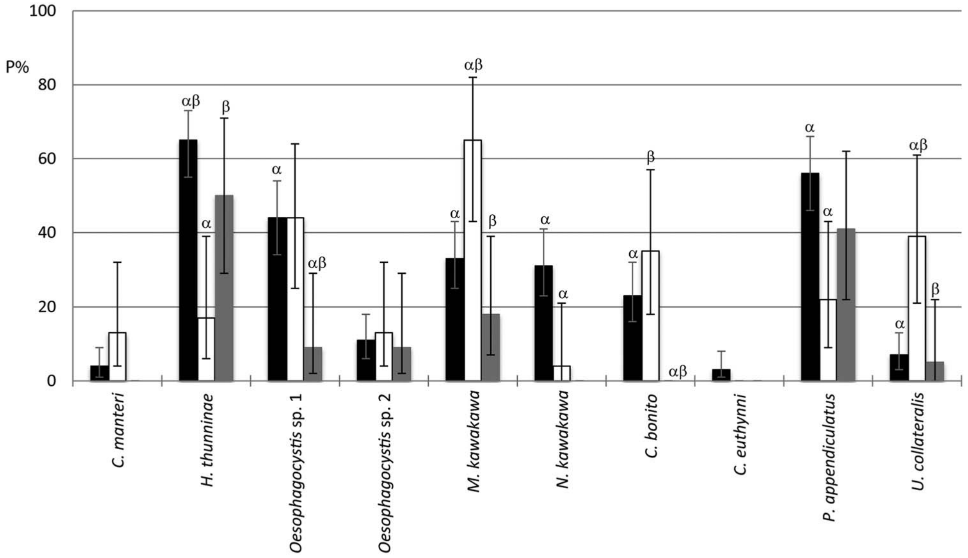

Fig. 2. Prevalence of the parasites of the head of *Euthynnus alletteratus* according to locality. Black squares, southeastern Spain samples; white squares, Gulf of Valencia; and grey squares, Strait of Gibraltar. Lines indicate 95% confidence intervals. Greek letters indicate significant differences ($P \leqslant 0{\cdot}05$).

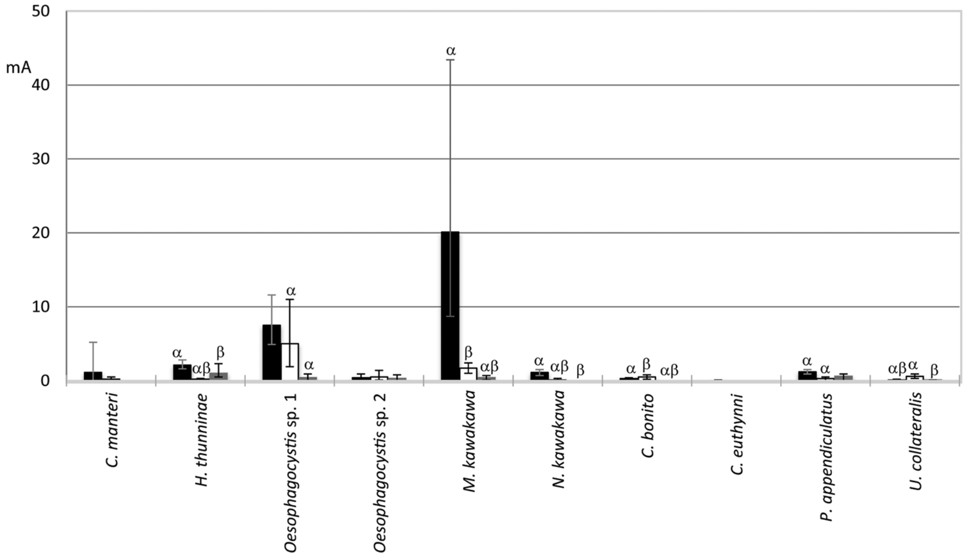

Fig. 3. Mean abundance of the parasites of the head of *Euthynnus alletteratus* according to locality. Black squares, southeastern Spain samples; white squares, Gulf of Valencia; and grey squares, Strait of Gibraltar. Lines indicate 95% confidence intervals. Greek letters indicate significant differences ($P \leqslant 0{\cdot}05$).

DISCUSSION

This study describes for the first time the parasite assemblage of the head of *E. alletteratus* in the western Mediterranean Sea, adding information on the parasite fauna of this tuna, which has been previously investigated only in the western Atlantic Ocean (Alves and Luque, 2006, Table 5). This is also the first contribution to the study of the molecular taxonomy of the didymozoids of this host. Considering the parasites recorded in formerly published studies, the known metazoan parasite fauna of the head of the Atlantic black skipjack includes a total of 24 species/taxa. Prior to this study, only three parasitic species had been reported in *E. alletteratus* from the Mediterranean Sea: the hexostomatid monogenean *H. thunninae* in tuna from the Ligurian Sea and the bomolochid copepods *Unicolax collateralis* and *Unicolax mycterobius* in hosts caught off Lebanon (Palombi, 1949; Lin and Ho, 2006). The finding of the first two species in *E. alletteratus* from the westernmost areas of the Mediterranean Sea enlarges their range of distribution, confirming their presence in the whole Mediterranean Sea. *Unicolax mycterobius* was not observed in this study, but it has been found in *Auxis rochei* (Scombridae) from the southeastern of

Table 4. Percent prevalence, mean abundance and their 95% confidence intervals of the parasites of the head of *Euthynnus alletteratus* from the southeastern Spain according to the sampling year

Species	2008	2009	2011
Capsala magronum	3 (1–11) 0·4 (0–2·1)	9 (2–29) 4·2 (0–16)	0 (0–17) 0
Hexostoma thunninae	64 (51–75) 1·8 (1·3–2·6)	64 (42–81) 3 (1·6–6·5)	70 (47–86) 2 (1·2–4)
Melanocystis cf. *kawakawa*	8 (3–17) *αβ* 0·3 (0·1–1) *αβ*	46 (46–66) *αγ* 92·5 (41·1–176) *αγ*	100 (83–100) *βγ* 2·5 (2·1–2·8) *βγ*
Neonematobothrium cf. *kawakawa*	19 (11–31) *αβ* 0·6 (0·3–1·2) *α*	41 (22–62) *α* 1·5 (0·6–2·5)	60 (37–79) *β* 2·1 (1·2–3) α
Oesophagocystis sp. 1	27 (17–40) *αβ* 4·2 (2·1–7·9)	59 (38–78) *α* 7·3 (3·5–12·5)	80 (58–93) *β* 18 (8–26·4)
Oesophagocystis sp. 2	8 (3–17) 0·2 (0–0·4)	9 (2–29) 0·3 (0–0·7)	20 (7–42) 1·2 (0·2–3·5)
Caligus bonito	19 (11–31) 0·2 (0·1–0·3)	32 (15–55) 0·4 (0·1–0·6)	25 (10–48) 0·5 (0·2–0·9)
Ceratocolax euthynni	2 (0–9) 0·02 (0–0·1)	0 (0–15) 0	10 (2–32) 0·2 (0–0·5)
Pseudocycnus appendiculatus	60 (48–72) 1·5 (1–2) *α*	50 (29–71) 0·6 (0·3–1)	50 (29–71) 0·7 (0·4–1) *α*
Unicolax collateralis	3 (1–11) *α* 0·1 (0–0·2)	0 (0–15) *β* 0	25 (10–48) *αβ* 0·5 (0·2–0·9)

Greek letters indicate significant differences ($P \leqslant 0{\cdot}05$).

Table 5. Summary of the parasite fauna of the head of *Euthynnus alletteratus*

Locality parasite	Mediterranean Sea	Eastern Atlantic Ocean	Western Atlantic Ocean
Monogenea			
*Allopseudaxine macrova**			E
*Capsala magronum**			E, G
*Capsala manteri**	X	B	D, G
Capsala maccallumi			G
*Hexostoma euthynni**			D, E
*Hexostoma keokeo**			E
*Hexostoma lintoni**			E
*Hexostoma thunninae**	X, A		
Udonella caligorum			D
*Sibitrema poonui**			E
Trematoda			
*Lobatozoum multisacculatum**			E
Melanocystis cf. *kawakawa**	X		
Neonematobothrium cf. *kawakawa**	X		
Oesophagocystis sp. 1*	X		
Oesophagocystis sp. 2*	X		
Crustacea			
*Caligus bonito**	X		E
*Caligus pelamydis**			E
Caligus productus			C
Ceratocolax euthynni	X	C	C
Isopoda gen. sp.			E
*Pseudocycnus appendiculatus**	X	C	E
Unicolax anonymous		F	F
*Unicolax collateralis**	X, F		F
Unicolax mycterobius	F		F

Capital letter represent sources: A, Palombi (1949); B, Bussiéras (1972); C, Cressey and Cressey (1980); D, Hendrix (1994); E, Alves and Luque (2006); F, Lin and Ho (2006); G, Chisholm and Wittington (2007); X, present results. Asterisk indicates significant differences between the present results and Alves and Luque (2006) ($P \leqslant 0{\cdot}05$).

Spain and the Strait of Gibraltar during a contemporary parasitological survey (Mele *et al*. 2015). In addition, the capsalid monogenean *C. manteri* and the bomolochid copepod *C. euthynni*, which have been observed in *E. alletteratus* from the Atlantic Ocean (Cressey and Cressey, 1980; Chisholm and Whittington, 2007), were found for the first time in the western Mediterranean Sea, suggesting that these parasites can infect the Atlantic black skipjack throughout its range of distribution. Each single species of the families Bomolochidae and Hexostomidae infects a different tuna species, showing a high degree of host specificity that is an uncommon character for the ectoparasites of the western Mediterranean tunas (Mele *et al.* 2010, 2012; Culurgioni *et al.* 2014). For instance, the other ectoparasites found, *Caligus bonito* and *P. appendiculatus*, have been reported in several tuna species from the Mediterranean Sea, as well as from the Atlantic Ocean (Culurgioni *et al.* 2014; Mele *et al.* 2015). However, the host specificity is not always straightforward, for example three ectoparasites from the western Mediterranean Sea, *Allopseudaxine macrova* of *Auxis rochei* and *Capsala magronum* and *Caligus coryphaenae* of *Thunnus thynnus*, were absent in *E. alletteratus* from the western Mediterranean Sea, whereas they have been found in some tuna species from the Atlantic Ocean, including *E. alletteratus* (Alves and Luque, 2006; Culurgioni *et al.* 2014; Mele *et al.* 2015). This fact suggests that the habitat overlapping of *E. alletteratus* and *T. thynnus* in the western Mediterranean Sea does not allow the sharing of these parasites. Probably for the same reason, several parasites observed in tunas from the western Atlantic Ocean, including *E. alletteratus*, were not found in the Mediterranean Sea: i.e. *Capsala maccallumi, Hexostoma lintoni, Udonella caligorum, Sibitrema poonoui* and *Unicolax anonymus* (Alves and Luque, 2006; Justo and Kohn, 2015; Mele *et al.* 2015).

Morphological and genetic analyses of the didymozoid trematodes revealed the presence of four species that had not been previously described in *E. alletteratus*. Among them, *Melanocystis* cf. *kawakawa* and *Neonematobothrium* cf. *kawakawa* have been also found in *Euthynnus affinis* and *Euthynnus lineatus* (Yamaguti, 1970; Madhavi and Ram, 2000), whereas the other two, *Oesophagocystis* sp. 1 and *Oesophagocystis* sp. 2, can be considered as putative new species. Conversely, *Lobatozoum multisacculatum*, reported in *E. alletteratus* from the Brazilian waters of the Atlantic Ocean (Alves and Luque, 2006) was not found in the present study. However, in a contemporary parasitological survey Mele *et al.* (2012) reported this species in one specimen of *Katsuwonus pelamis*, a migratory species of tuna between the Atlantic Ocean and the western Mediterranean Sea. Didymozoids are food-borne parasites with a partial known life cycle that presumably involves several possible intermediate hosts, including molluscs, crustaceans and fish, and one or more definitive hosts, mainly scombrid fish (Nikolaeva, 1985; Pozdnyakov and Gibson, 2008). The fact that no one of the didymozoids found in *E. alletteratus* have been reported in other scombrids of the western Mediterranean Sea shows that this species plays a specific key role as host reservoir for the maintenance of these infections in this area. On the other side, the absence of *L. multisacculatum* in *E. alletteratus* of western Mediterranean Sea can be attributed to the lack of suitable environmental conditions and/or the absence of the intermediate hosts in this area.

The limited number of parasite species shared between the parasite faunas of the head of *E. alletteratus* from the western Mediterranean Sea and the western Atlantic Ocean indicates that the distance between these areas could represent a barrier to the dispersion of both the host and its species-specific parasites. This is in accordance with other studies on scombrids, e.g. *Scomber colias* that showed differences in the helminth fauna between the opposite sides of the Atlantic Ocean (Mele *et al.* 2014). Moreover, the difference between the Atlantic and Mediterranean domains can influence the dispersion and recruitment of the generalist parasites infecting large migrant fish, e.g. the common dolphinfish *Coryphaena hippurus* (Carbonell *et al.* 1999). Therefore, the presence of several parasites exclusively either in the western Atlantic Ocean (i.e. *Hexostoma keokeo, H. lintoni, Hexostoma euthynni, S. poonoui*) or in the western Mediterranean Sea (all the didymozoids and *Hexostoma thunninae*), as well as the differences in the structure of the parasite assemblages between these areas, could be useful to distinguish the fish populations from the opposite coasts of the Atlantic Ocean.

Concerning the differences between the parasite assemblages of the fish from the Gulf of Valencia and southeastern Spain, it must be considered that the samples from Valencia had the lowest fork length, and this fact could influence the occurrence of some parasites (Madhavi and Ram, 2000). Particularly, the abundance of the gill monogenean *H. thunninae* appears to be positively correlated with the fish length, because of the enlargement of the suitable habitat (gill area). Conversely, the abundance of *U. collateralis*, a copepod that lives embedded in the mucous of the nasal sinus, was negatively correlated with the fish length, suggesting that the parasite find an ideal microhabitat in the nasal cavities of the small hosts, as indicated for other nasal copepods (Cressey *et al.* 1983; Sharples and Evans, 1995). Moreover, the infection of didymozoids is related to the diet of *E. alletteratus*, that changes as fish grow, becoming the large specimens more piscivorous (Manooch *et al.* 1985; Falautano *et al.* 2007).

Differences of didymozoid infections between the fish from the southeastern Spain and the Strait of Gibraltar can be due to differences in environmental conditions, which influence the presence of infected preys in the areas. Although no specific information is available on the metacercariae of the didymozoids of *E. alletteratus*, it is important to note that several prey items of *E. alletteratus* (Falautano *et al*. 2007) have been reported infected by didymozoid metacarcariae also in the areas herein studied (Culurgioni *et al*. 2014, PhD thesis at http://veprints.unica.it/1010/ and author personal comments; Mateu *et al*. 2015). Because most of these intermediate hosts are pelagic species, it is more probable that *E. alletteratus* acquires the didymozoids, as well as other pelagic parasites as *P. appendiculatus* (Mladineo *et al*. 2011; Culurgioni *et al*. 2014), in the offshore pelagic areas close to the sampling points than in the adjacent inshore coastal regions.

The abundance of several parasites, especially of *M.* cf. *kawakawa*, is likely associated to the heating of the western Mediterranean waters in the warm season (Álvarez *et al*. 2015). Analogously, Lester *et al*. (1985) and Jones (1991), studying the parasites of tuna that seasonally migrate from the warm tropical Pacific Ocean to the temperate southern Pacific areas of New Zealand, observed higher abundance of didymozoids in tropical areas than in the temperate ones, suggesting that these trematodes infect tuna only in the warm waters. Conversely, *P. appendiculatus* is found in the western Mediterranean Sea during the winter and early spring seasons, and its abundance seems to be favoured by the low SST (Hayward *et al*. 2008). In fact, this parasite showed significant differences of abundance between sampling years that can be related with the regional oceanography conditions. According to Álvarez-Berastegui *et al*. (2014) the westernmost areas of the Mediterranean Sea are characterized by highly dynamic oceanographic process that promote a seascape shaped by filaments, fronts and eddies, whose location varies between years and this variability can influence the presence of direct life cycle parasites.

In conclusion, molecular and morphological results evidence the findings of two putative new species of *Oesophagocystis* in *E. alletteratus*, which motivate further efforts aimed at their morphological description and systematics analysis. Interannual changes of the environmental factors, such as temperature, are the main factors influencing the abundance of most of the parasite species of *E. alletteratus* in the western Mediterranean Sea, but also the changing of these variables between sampling areas and the host length modify the presence of monogeneans and bomolochid copepods. The parasite fauna of *E. alletteratus* differs between the western Mediterranean Sea and the western Atlantic Ocean, suggesting that the parasite assemblages are isolated because this host does not seem able to cross the Atlantic Ocean. Although *E. alletteratus* is considered a near-shore species, the finding of pelagic parasites, like didymozoids and *P. appendiculatus*, suggests that this tuna can make local migrations between inshore and offshore pelagic areas in the western Mediterranean Sea.

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