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CAN THE PARASITES OF THE HEAD OF JUVENILE *THUNNUS THYNNUS* L. HELP TO IDENTIFY ITS NURSERY AREAS IN THE MEDITERRANEAN SEA?

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SUMMARY

*Between 2009 and 2013, the head region of 102 juveniles of Atlantic bluefin tuna (*Thunnus thynnus L.*) caught in four nursery areas of the Mediterranean Sea (Balearic Sea, Ionian Sea, Ligurian Sea and Tyrrhenian Sea) were analysed for parasites. Eleven parasite species were found:* Capsala magronum, C. onchidiocotyle, C. paucispinosa, Nasicola klawei, Hexostoma thynni, Didymocystis sp. 2 *(sensu Rodríguez-Marín* et al*., 2008),* Didymosulcus sp. 2 *(sensu Rodríguez-Marín* et al. *2008),* Didymosulcus wedli, Didymozoon pretiosus, Nematobothriinae gen. sp. *and* Wedlia sp. *The prevalence of some food-borne parasites (*Didymocystis sp. 2, D. pretiosus, Nematobothriinae gen. sp. *and* Wedlia sp.*) had significant differences between localities ($p \leq 0.05$). The results showed that the parasite fauna of juvenile tunas is not homogenously distributed in the Mediterranean Sea: parasite assemblages differed between hosts from the Balearic, Ionian, Ligurian, and Tyrrhenian seas, suggesting parasites as possible tags to identify the different tuna populations from the corresponding nursery areas.*

RÉSUMÉ

*Entre 2009 et 2013, la région céphalique de 102 juvéniles de thon rouge de l'Atlantique (*Thunnus thynnus L.*), capturés dans quatre zones de nourricerie de la Méditerranée (mer des Baléares, mer Ionienne, mer de Ligure et mer Tyrrhénienne), a été analysée afin de détecter des parasites. Onze espèces de parasite ont été observées :* Capsala magronum, C. onchidiocotyle, C. paucispinosa, Nasicola klawei, Hexostoma thynni, Didymocystis sp. 2 *(Rodríguez-Marín et al., 2008),* Didymosulcus sp. 2 *(Rodríguez-Marín et al. 2008),* Didymosulcus wedli, Didymozoon pretiosus, Nematobothriinae gen. sp. *et* Wedlia sp. *La prévalence de certains parasites d'origine alimentaire (*Didymocystis sp. 2, D. pretiosus, Nematobothriinae gen. sp. *et* Wedlia sp.*) présentait des différences significative d'un lieu à l'autre ($p \leq 0,05$). Les résultats indiquaient que la faune parasitaire des juvéniles de thons n'est pas distribuée de manière homogène dans la mer Méditerranée. Les assemblages parasitaires différaient entre les hôtes des mers des Baléares, Ionienne, de Ligure et Tyrrhénienne, ce qui donne à penser que les parasites pourraient servir de marques permettant d'identifier les différentes populations de thons provenant des zones de nourricerie correspondantes.*

RESUMEN

Entre 2009 y 2013, se analizó la región craneal de 102 juveniles de atún rojo del Atlántico (Thunnus thynnus L.) *capturados en cuatro zonas de cría del Mediterráneo (mar Balear, mar Jónico, mar de Liguria y mar Tirreno) en busca de parásitos. Se hallaron once especies de parásitos:* Capsala magronum, C. onchidiocotyle, C. paucispinosa, Nasicola klawei, Hexostoma thynni, Didymocystis *sp. 2 (sensu Rodríguez-Marín* et al*., 2008),* Didymosulcus sp. *2 (sensu Rodríguez-Marín* et al. *2008),* Didymosulcus wedli, Didymozoon pretiosus, Nematobothriinae gen. sp. *y* Wedlia sp. *La prevalencia de algunos parásitos transmitidos por los alimentos (*Didymocystis sp. 2, D. pretiosus, Nematobothriinae gen. sp. *and* Wedlia sp.*) presentaba diferencias significativas entre localidades ($p \leq 0.05$). Los resultados demostraron que la fauna de parásitos de los túnidos juveniles no se distribuye homogéneamente en el Mediterráneo: los*

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conjuntos de parásitos diferían entre los anfitriones de los mares Balear, Jónico, de Liguria y Tirreno, lo que sugiere que los parásitos podrían ser posibles marcas para identificar las diferentes poblaciones de túnidos de las correspondientes zonas de cría.

KEYWORDS

Parasites assemblages, Juvenile, Biological tags, Atlantic bluefin tuna, Nursery areas

Introduction

The Atlantic bluefin tuna (BFT), *Thunnus thynnus*, is a pelagic fish inhabiting the Atlantic Ocean and the Mediterranean Sea. It represents one of the most economically important fish in Atlantic Ocean and Mediterranean Sea, which sustains important recreational and commercial fisheries as well as the capture-based tuna aquaculture industry (Milatou and Megalofonou 2014). At present, the Atlantic bluefin tuna is still considered overexploited and it is included in a multiannual recovery plan by International Commission for the Conservation of Atlantic Tuna (ICCAT), which is the responsible for the management of this fisheries.

Parasites have been used with success to point out differences between host populations and/or to study migrations of several fish species (Mackenzie and Abaunza 2014). The analysis of parasite assemblages throughout the whole area of the host distribution has been demonstrated to be useful to follow the migrations of tunas in the Atlantic (Lardeaux 1982, MacKenzie 1983) and Pacific Ocean (Lester *et al*., 1985, Jones 1991). However, no extensive studies have done on the parasite fauna of *T. thynnus* (MacKenzie 1983). Walters (1980) analysed the parasite data of *T. thynnus* from different areas of Atlantic Ocean suggesting the usefulness of *Nasicola klawei* and *Euryphorus brachypterus* to discriminate tunas from western and eastern Atlantic Ocean. Rodríguez-Marín *et al*. (2008) studied the parasite populations of juvenile bluefin tuna from the Gulf of Biscay suggesting that some direct life cycle parasites (monogeneans, protozoans and crustaceans) and most the food-borne parasites found in the head region would be useful to characterize tuna from this area. Culurgioni *et al.* (2014) reported differences in the parasite populations of smaller and larger sized tunas from the Mediterranean Sea, suggesting the school of large size bluefin tuna include specimens resident in the Mediterranean Sea and belonging from the Atlantic Ocean. Currently, the parasite fauna of young-of-the-year (YOY) tuna is unknown. The current study describes the metazoan parasite assemblages of the head of young-of-the-year *T. thynnus* from the Mediterranean Sea.

1. Materials and methods

Between 2009 and 2013, a total of 102 *T. thynnus*, caught in four locality of the Mediterranean Sea were examined for parasites: 41 specimens were collected from the Balearic Sea (length fork, LF = 22-40 cm), 21 from the Ionian Sea (LF=21-41 cm), 20 from the Ligurian Sea (LF =21-40 cm), and 20 from the Tyrrhenian Sea (LF=21-41 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) and Chisholm and Whittington (2007); and for didymozoids Yamaguti (1970) and Pozdnyakov and Gibson (2008).

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 and Rózsa 2005). Levels of infection of each parasite species were calculated according to locality. Differences between the parasite infections of the host groups were evaluated using the Fisher exact test for prevalence and the Welsh bootstrap t-test for mean abundance (Reiczigel and Rózsa 2005). Multivariate analyses were carried out on the data of the parasites that showed significant difference of prevalence and mean abundance between at least one pair of localities, using R-software (see Mele *et al.*, 2012). Hierarchical cluster analysis of prevalence and mean abundance were performed on a Bray-Curtis similarity matrix to identify possible differences between the examined localities.

2. Results

Eleven parasite species/taxa were found in the head region of juvenile bluefin tuna from the Mediterranean Sea (**Table 1**). Most of the parasites were didymozoid trematodes belonging to six species: *Didymocystis* sp. 2 (*sensu* Rodríguez–Marín *et al*., 2008), *Didymosulcus* sp. 2 (*sensu* Rodríguez–Marín *et al*., 2008), *D. wedli* (Ariola, 1902), *Didymozoon pretiosus* (Ariola, 1902), Nematobothriinae gen. sp. and *Wedlia* sp. Five monogenean species were found: *Capsala magronum* (Ishii, 1936), *C. onchidiocotyle* (Setti, 1899), *C. paucispinosa* (Mamaev, 1968), *Hexostoma thynni* (Delaroche, 1811), and *Nasicola klawei* (Stunkard, 1962). The parasite assemblage of bluefin tuna from the Ionian Sea was the highest parasite richness (9 species), followed by bluefin tuna from the Tyrrhenian (8), Balearic (7) and Ligurian seas (4). *H. thynni* was the dominant species in the Balearic Sea, *Didymosulcus* sp. 2 in the Ionian and Ligurian seas, and *Wedlia* sp. in the Tyrrhenian Sea.

The prevalence of four parasites (*Didymosulcus* sp. 2, *D. pretiosus*, Nematobothriinae gen. sp. and *Wedlia* sp.) showed differences between localities (**Table 1**). The prevalence of *Didymosulcus* sp. 2 and *D. pretiosus* was higher in bluefin tuna from the Ionian Sea than bluefin tuna from the remaining areas ($p < 0.05$). The prevalence of Nematobothriinae gen. sp. and *Wedlia* sp. was higher in BFT from the Ionian and Tyrrhenian seas than bluefin tuna from the Balearic and Ligurian seas. The mean abundance of *Didymosulcus* sp. 2 and *D. pretiosus* was higher in bluefin tuna from the Ionian Sea than bluefin tuna from the other localities. The mean abundance of *Wedlia* sp. was higher in fish from Tyrrhenian Sea than bluefin tuna from the Ligurian Sea. The cluster analysis of prevalence and mean abundance (**Figure 1**) of the parasite assemblages showed that bluefin tuna from Ligurian Sea and Balearic Sea had the lowest level of dissimilarity, whereas bluefin tuna from the Ionian Sea were the most dissimilar.

3. Discussion

In this study eleven parasites were found in the head region of young-of-the-year *T. thynnus* from the Mediterranean Sea. Only *Didymosulcus* sp. 2 has been previously described in the YOY from the Mediterranean Sea (Culurgioni *et al.*, 2014). Most of the parasites found, have been previously described in bluefin tuna from the Mediterranean Sea and the Atlantic Ocean. Interestingly, *N. klawei*, previously reported only in the Atlantic, is also present in the juvenile Mediterranean tunas (Walters 1980, Rodríguez-Marín *et al.* 2008, Mladineo *et al.*, 2011, Culurgioni *et al.*, 2014). This is the first report of *Didymocystis* sp. 2, Nematobothriinae gen. sp. and *Wedlia* sp. in bluefin tuna from the Mediterranean Sea.

The parasite fauna of YOY bluefin tuna from the Mediterranean Sea had a lower richness than larger bluefin tuna from the Mediterranean Sea (Adriatic and Sardinia seas) and the North East Atlantic Ocean (Gulf of Biscay) (Walters 1980, Rodríguez-Marín *et al.*, 2008, Mladineo *et al.*, 2011, Culurgioni *et al*,. 2014). Surprisingly, *D. wedli* was only found in one YOY bluefin tuna, whereas it was the dominant species everywhere in the larger tunas. Other two food-borne parasites (*Copiatestes thyrsitae* Crowcroft, 1948 and *Didymocystis* sp. 3(*sensu* Rodríguez-Marín *et al.*, 2008) reported in bluefin tuna from the Atlantic Ocean did not infect YOY bluefin tuna from the four areas herein analysed. Moreover, copepod parasites, such as *Caligus coryphaenae*, *Euryphorus brachypterus* (Gerstaecker, 1853) and *Pseudocycnus appendiculatus*, reported in larger tuna from Sardinia Sea and the Atlantic Ocean, were not present in YOY bluefin tuna.

The comparison of the parasite populations indicates that some species (*Didymosulcus* sp. 2, *D. pretiosus*, and *Wedlia* sp.) had differences in prevalence and mean abundance between areas. According to the general principle to select the parasites as biological tags illustrated by MacKenzie and Abaunza (2014), these didymozoids can be considered reliable tags to follow its migrations, because: (i) they showed differences of infection levels between localities; (ii) they are permanent parasites (*sensu* Lester *et al.* 1985) and their remains are recognizable in the host tissues well after their death; and (iii) the host cannot lose them when it migrates outside the endemic area of these parasites (Lester *et al.* 1985). In fact, the absence of *D. pretiosus*, Nematobothriinae gen sp. and *Wedlia* sp. in fish from Ligurian Sea suggest that (i) these parasites did not infect the praise of *T. thynnus* in this area, (ii) and that no infected fish migrated from Tyrrhenian and Ionian seas to the Ligurian one.

The analysis of the parasite assemblages clustered tuna (**Figure 1**) in three groups belonging to the Ionian, Tyrrhenian and a third group that includes Balearic and Ligurian samples. This result seems to agree the general oceanographic division of the Mediterranean Sea, suggesting that tuna from Ionian Sea and Tyrrhenian Sea stay around of their breeding areas (Ionian and Tyrrhenian Sea, respectively; Alemany *et al.* 2010), while the Balearic one, probably migrates throughout the northernmost areas of the western Mediterranean Sea (Balearic and Ligurian seas).

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Table 1. Prevalence and mean abundance of parasites of juvenile *Thunnus thynnus* from the Mediterranean Sea, 95% confident interval given in parenthesis. Bal., Balearic Sea; Ion., Ionian Sea; Lig., Ligurian Sea; Tyr., Tyrrhenian Sea. Uppercase letters, significant P-value<0.05.

Parasite	**Prevalence (%)**				**Mean abundance**			
	Bal	**Ion**	**Lig**	**Tyr**	**Bal**	**Ion**	**Lig**	**Tyr**
Capsala magronum	25 (12-40)	34 (15-57)	10 (2-32)	30 (12-55)	0.5 (0.2-0.9)	0.3 (0.1-0.5)	0.25 (0-0.9)	0.5 (0.2-1.0)
Capsala onchidyocotile	-	5 (1-24)	-	-	-	0.1 (0.0-0.1)	-	-
Capsala paucispinosa	-	5 (1-24)	-	10 (2-32)	-	0.1 (0.0-0.1)	-	0.1 (0.0-0.2)
Nasicola klawei	-	-	-	5 (1-25)	-	-	-	0.1 (0-0.2)
Hexostoma thynni	42 (26-58)	33 (15-57)	30 (12-55)	35 (16-60)	0.8 (0.5-1.3)	0.4 (0.2-0.7)	0.4 (0.1-0.6)	0.5 (0.2-0.8)
Didymocystis sp.2	17 (8-32)	18 (8-33)	-	5 (1-25)	0.6 (0.2-1.5)	0.6 (0.2-1.4)	-	0.1 (0.0-0.3)
Didymosulcus sp.2	8 (2-20)^{a}	72 (48-89)^{abc}	25 (9-50)^{bd}	-^{cd}	0.5 (0.1-1.4)^{a}	2.1 (1.3-3.8)^{ab}	0.5 (0.2-1.0)^{b}	-
Didymosulcus wedli	-	-	5 (1-25)	-	-	-	0.1 (0.0-0.2)	-
Didymozoon pretiosus	12 (5-27)^{a}	77 (53-92)^{abc}	-^{b}	20 (6 -44)^{c}	0.4 (0.1-0.9)^{a}	1.6 (1.0-2.4)^{abc}	-^{b}	0.4 (0.1-1.1)^{c}
Nematobothriinae gen. sp.	3 (1-13)^{ab}	19 (6-42)^{a}	-^{c}	25 (9-50)^{bc}	0.02 (0.0-0.1)	0.2 (0.1-0.3)	-	0.3 (0.1-0.4)
Wedlia sp.	18 (8-33)^{ab}	34 (15-57)^{c}	-^{acd}	45 (24-69)^{bd}	0.4 (0.2-0.9)	1.1 (0.4-2.4)	-^{a}	0.8 (0.4-1.3)^{a}

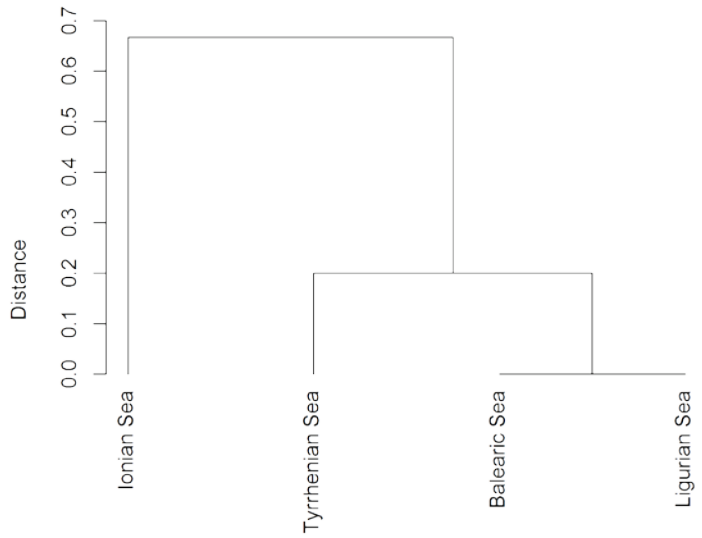

Figure 1. Cluster dendrogram (group-average linkage method) of the parasites of the head of the young-of-the-year *Thunnus thynnus* from the Mediterranean Sea using a Bray-Curtis (B) dissimilarity indices based on prevalence.