

IMPLICATIONS FOR FISHERY MANAGEMENT IN SMALL TUNAS THE CASE OF GENETIC POPULATION STRUCTURE OF BULLET TUNA IN THE WEST MEDITERRANEAN

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SUMMARY

*Knowledge of population structure of species is needed to establish appropriate management regulations. Of particular concern are those commercial species; these exploited fish populations can undergo loss of genetic variability that ultimately may lead the loss of regional small populations. This situation could occur in the small tuna species, which in some cases are heavily targeted by artisanal fisheries. Here, we focused on the Bullet tuna (*Auxis rochei*) with an extensive sampling ($n = 431$) along the north and south coast of the west Mediterranean and one location on the east Atlantic. The analysis of the mtDNA control region revealed that seven (about 1.6%) individuals were not identified as Bullet tuna suggesting a species misidentification with possible implications in stock assessment. Population genetics results showed clear genetic differentiation between the Iberian Peninsula and North African locations. These results have a clear impact on the conservation and management strategies, and if it is confirmed in other small pelagic species, the pattern of population structure in the Mediterranean is more complex than initially expected.*

RÉSUMÉ

*Des connaissances sur la structure de la population de l'espèce sont nécessaires pour établir des réglementations de gestion adéquates. Les espèces commerciales sont particulièrement préoccupantes. Ces populations exploitées de poissons peuvent souffrir une perte de variabilité génétique qui peut se traduire au bout du compte par la perte de petites populations régionales. Cette situation peut se produire dans le cas des thonidés mineurs qui sont intensément ciblées par les pêcheries artisanales à certains endroits. La présente étude se concentre sur le bonitou (*Auxis rochei*) dont un échantillonnage exhaustif ($n = 431$) a été réalisé le long des côtes sud et nord de la Méditerranée occidentale et à un endroit dans l'Atlantique Est. L'analyse de la région de contrôle d'ADNmt a fait apparaître que sept spécimens (environ 1,6 %) n'ont pas été identifiés comme bonitou, ce qui suggère une identification erronée ayant des implications potentielles pour l'évaluation des stocks. Les résultats de la génétique des populations ont montré une différenciation génétique claire entre la péninsule ibérique et l'Afrique du Nord. Ces résultats ont un impact clair sur les stratégies de gestion et de conservation et, s'il se confirme pour d'autres espèces de petits pélagiques, le schéma de la structure de population en Méditerranée serait plus complexe que ce qui avait initialement été escompté.*

RESUMEN

*Se requiere un conocimiento de la estructura de la población de las especies para establecer reglamentos de ordenación apropiados. Las especies comerciales generan inquietud; estas poblaciones explotadas pueden experimentar una pérdida de variabilidad genética que, en última instancia, puede dar lugar a una pérdida de pequeñas poblaciones regionales. Esto puede producirse en especies de pequeños túnidos a las que, en algunos casos, se dirigen en gran medida las pesquerías artesanales. Este estudio se centra en la melvera (*Auxis rochei*) ($n = 431$) en la costa norte y sur del Mediterráneo occidental y en el Atlántico del este. Los análisis de la región de control de ADNmt revelaron que siete (aproximadamente 1,6%) ejemplares no se*

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habían identificado como melvera y sugerían una identificación errónea de las especies con posibles implicaciones para la evaluación de stock. Los resultados de la genética de la población mostraban una clara diferenciación genética entre localizaciones de la península Ibérica y del norte de África. Estos resultados afectan a las estrategias de ordenación y conservación y, de confirmarse en otras especies de pequeños pelágicos, el patrón de la estructura de la población en el Mediterráneo sería más complejo de lo que se consideró inicialmente.

KEYWORDS

Auxis rochei, Bullet tuna, Small tuna, Population genetics, Stock identification

Introduction

Previous to establish correct management regulations of any species it is crucial to know the population structure (Punt, 2006; Waples *et al.*, 2008). A particular concern is established in commercial fish species that in most of the cases are overfished (Worm *et al.*, 2006). Under these circumstances exploited fish populations can undergo a loss of genetic variability (Hauser *et al.*, 2002; Hauser and Carvalho, 2008) that ultimately may lead to a decline in their capacity to adapt to new circumstances.

This situation could occur in the small tuna species. Taking in consideration the ICCAT nominal catches data of the five most small tuna species captured (Atlantic bonito, *Sarda sarda*; Atlantic black skipjack, *Euthynnus alletteratus*; frigate tuna, *Auxis thazard*; king mackerel, *Scomberomorus cavalla*; bullet tuna, *Auxis rochei*) with a total combined landing of about 10,500 t in 2013 (Anon., 2016). Thus, although these species are not the main target species captured, in some locations the small tuna have a high commercial value and they are targeted by artisanal fisheries (Anon., 2016). For instance, there is a general trend of an increase of landings of these species in the last ten years (2004 to 2013) (ICCAT, 2016) (**Figure 1**) with some of the species doubling the number of catches (i.e., frigate tuna).

These five species belongs to the family Scombridae, and all have a relative migratory behavior (Collette and Nauen, 1983). To our knowledge very few studies are reported about the population structure of this species in the Atlantic - Mediterranean area. For Atlantic bonito, a series of studies reported genetic differentiation among populations within the Mediterranean and between West Atlantic and Mediterranean (Viñas *et al.*, 2004; Viñas *et al.*, 2010a). More recently, in a preliminary study the bullet tuna also showed signs of genetic differentiation within the Mediterranean (Pérez-Bielsa *et al.*, 2017), but not differentiation is detected at small scale (Kumar *et al.*, 2013; Allaya *et al.*, 2015).

Thus, it is crucial a more detailed study about the population structure of these species previous to the correct management fisheries policies. In this study, we focused on the populations structure of bullet tuna, with a large sampling effort encompassing samples along the Iberian Peninsula and African Mediterranean coasts and a sample form the East Atlantic close to the Strait of Gibraltar using the sequence variability of the mitochondrial control regional (mtDNA CR) as genetic marker.

Material and Methods

We analyzed a large data set of 431 bullet tuna individuals collected from commercial catches using different fishing gear (purse seine, light fishing, gill nets, longline, pelagic trawl, and beach seine). Sampling is a combination of various resources. Samples from Tunisia (n = 109) proceed from our previous published study of Allaya *et al.* (2015), were combined with samples from Algeria (n = 45) already included in other study (Pérez-Bielsa *et al.*, 2017) and newly obtained individuals (n = 277) from Atlantic Iberian Peninsula, Portuguese coast (n = 52), and Mediterranean Iberian Peninsula (n = 225), distributed in 5 locations (Strait of Gibraltar, n = 20; Cartagena, n = 97; Malaga, n = 38; Valencia, n = 21; and Tarragona, n = 49). See **Table 1** and **Figure 2** for sampling description.

Sequences of new individuals, from DNA extraction to DNA sequence read, were obtained following the same procedures described in (Allaya *et al.*, 2015). Once the new sequences were obtained, all sequences were pooled together using the software platform GENEIOUS v7.0.6 (Kearse *et al.*, 2012). All new sequences were checked by a BLAST (Altschul *et al.*, 1990) analysis to confirm the species identification. After these deparating procedures haplotype collection data set was set up and aligned together with a previous data collection (Allaya *et al.*, 2015). Neighbor-joining haplotype tree (Saitou and Nei, 1987) was performed using Kimura 2 parameters distance (Kimura, 1980) and branch tree robustness was tested with a 1000 pseudoreplicates bootstrap (Felsenstein, 1985).

Haplotype diversity (h) (Nei and Tajima, 1981) and nucleotide diversity (π) (Nei, 1987) was obtained using ARLEQUINv3.5 (Excoffier and Lischer, 2010) pairwise population differentiation and analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992) based on the pairwise distance matrix was also tested by ARLEQUIN.

Results

The blast analysis of the new sequences resulted in seven sequences (1.6%) not identified as Bullet tuna. All seven were identified as Frigate Tuna. Once these sequences were discarded, one hundred sixty-eight variable sites were detected, yielding a total of 401 different haplotypes occurring at a maximum frequency of three individuals in a single haplotype and with 93.3 % (374 of 401) of haplotypes being unique. These polymorphism yields an overall haplotype diversity of $h = 1.000 \pm 0.000$ and overall nucleotide diversity of $\pi = 0.044 \pm 0.022$. Northern African locations, Tunisia and Algeria, showed the lowest genetic diversity $h = 0.998 \pm 0.002$, $\pi = 0.041 \pm 0.020$ and $h = 0.996 \pm 0.006$, $\pi = 0.043 \pm 0.021$, respectively (See **Table 1** for detailed information of genetic variability). Clear genetic differentiation was detected among samples $\phi_{ST} = 0.036$; $P = 0.000 \pm 0.000$. However, when the samples of Mediterranean Iberian Peninsula were compared, they failed to show genetic heterogeneity $\phi_{ST} = 0.036$; $P = 0.472 \pm 0.017$. Accordingly these samples were pooled together a considered a single group (named Mediterranean Iberian Peninsula). In contrast, the two samples of northern African showed genetic differentiation were genetic differentiated $\phi_{ST} = 0.098$; $P = 0.000 \pm 0.000$, and thus were considered different. A global pairwise comparison of the four remaining location: Atlantic Iberian Peninsula ($n = 52$); Mediterranean Iberian Peninsula (a combination of five locations, $n = 225$), Algeria ($n = 45$) and Tunisia ($n = 109$) (**Table 1**). All comparisons were significantly different, except the comparison between Atlantic versus Mediterranean Iberian Peninsula with a P value higher than significance when the Bonferroni correction is applied (**Table 2**).

Discussion

Bullet tuna and Frigate tuna are small tuna species closely related, with similar worldwide distribution, behavior pattern and similar morphology (Collette and Nauen, 1983; Collette, 1999). According to the result of this study, there is about 2% of misidentification between these species. Laboratory error can be discarded since we used all the positive and negative controls to ascertain that there is no contamination in the sample analysis. Alternatively, confusion during the sample processing can also occur between the bullet and frigate tuna at small sizes. However the individuals with this mtDNA variability were large with an average FL of 41.2 cm. Otherwise, mtDNA introgression between species can also account for this result. For instance about 3-6% of Atlantic bluefin tuna individuals present mtDNA closely related to albacore and Pacific Bluefin tuna (Alvarado Bremer *et al.*, 2005). Further analysis using nuclear genetics markers would definitively disentangle the origin of this result.

To our knowledge, the analysis of more than 400 individuals of Bullet tuna is the most the ambitious study about any small tuna species in the Mediterranean – Atlantic region. The genetic variability of the mitochondrial control region obtained in analysis is the range of previous studies of Bullet tuna (Allaya *et al.*, 2015) and other small pelagic species, such as Atlantic bonito (*Sarda Sarda*) (Viñas *et al.*, 2004; Viñas *et al.*, 2010a). The population structure analysis clearly depicts a scenario of genetic heterogeneity among north and south Mediterranean locations with a large area of lack of genetic heterogeneity encompassing the Atlantic – Mediterranean Iberian coast. Even in the south Mediterranean (north African coast), the locations included in the analysis (Algeria and Tunisia) were significantly different. These two locations are closer than the locations in the Iberian Peninsula. One plausible scenario can be accounted for this result is the migratory pattern of this species. It is suspected that the bullet tuna enters to the Mediterranean from the Atlantic following the coast of Peninsula Iberia through the Strait of Gibraltar up to the North Mediterranean (Sabatés and Recasens, 2001; Mele *et al.*, 2015). Thus, all the samples from the Iberian Peninsula included in this study could belong to the same population. However, no genetic flow occurs across Mediterranean, between North Atlantic and Iberian Peninsula locations. Similar to the present results, there are an increase number of studies reporting genetic differentiation among locations within the Mediterranean as observed for other small Scombridae species (i.e, Atlantic bonito, *Sarda sarda*) (Viñas *et al.*, 2004), small pelagic fish species, including European anchovy (*Engraulis encrasicolus*) (Magoulas *et al.*, 1996; Grant, 2005; Magoulas *et al.*, 2006; Sanz *et al.*, 2008; Zarronaindia *et al.*, 2009; Montes *et al.*, 2013; Vinas *et al.*, 2014), and even large pelagic fish species, such as the swordfish, *Xiphias gladius* (Viñas *et al.*, 2010b). Thus, it can be concluded from this study and taking into account the studies of similar species, that the initial hypothesis genetic homogeneity of migratory species among low distant locations is likely challenged in most species. For most pelagic species the pattern of population structure in the Mediterranean is more complex than initially expected. This result has a clear impact in the conservation and management strategies, since the worst-case scenario is the management directives aimed at a broad geographic area when actually there structure does exist (Waples and Punt, 2008). This management strategy could lead to the irrevocable loss of regional populations. Thus, it is imperative to realize deep and focused studies in population structure not only in bullet tuna but also in all related and commercial important species to establish correct and proper management policies.

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Table 1. Sampling description. Code, number in Figure 2. n , number of individuals. M , number of haplytpes. h , haplotypic diversity. π , nucleotide diversity. SD , standard error.

<i>Location</i>	<i>Code</i>	<i>n</i>	<i>M</i>	<i>h</i>		<i>SD</i>	π		<i>SD</i>
Atlantic Iberian Peninsula	1	52	51	0.999	\pm	0.004	0.049	\pm	0.024
Strait of Gibraltar (Ceuta)	2	20	20	1.000	\pm	0.016	0.041	\pm	0.021
Mediterranean Iberian Peninsula		225	219	1.000	\pm	0.001	0.044	\pm	0.022
Málaga	3	38	37	0.999	\pm	0.007	0.040	\pm	0.020
Cartagena	4	97	97	1.000	\pm	0.002	0.046	\pm	0.023
Valencia	5	21	21	1.000	\pm	0.015	0.044	\pm	0.023
Tarragona	6	49	48	0.999	\pm	0.004	0.046	\pm	0.023
Northern Africa									
Algeria	7	45	41	0.996	\pm	0.006	0.043	\pm	0.021
Tunisia	8	109	96	0.998	\pm	0.002	0.041	\pm	0.020
All samples		431	401	1.000	\pm	0.000	0.044	\pm	0.022

Table 2. Pairwise comparison of genetic differentiation between the four locations described in the text. IP, Iberian Peninsula. Below diagonal ϕ_{ST} values. Above diagonal, P values.

	<i>Med IP</i>	<i>Algeria</i>	<i>Tunisia</i>	<i>Atlantic IP</i>
Med. IP		0.000 \pm 0.000	0.000 \pm 0.000	0.022 \pm 0.005
Algeria	0.032		0.000 \pm 0.000	0.000 \pm 0.000
Tunisia	0.035	0.098		0.000 \pm 0.000
Atlantic IP	0.012	0.063	0.032	

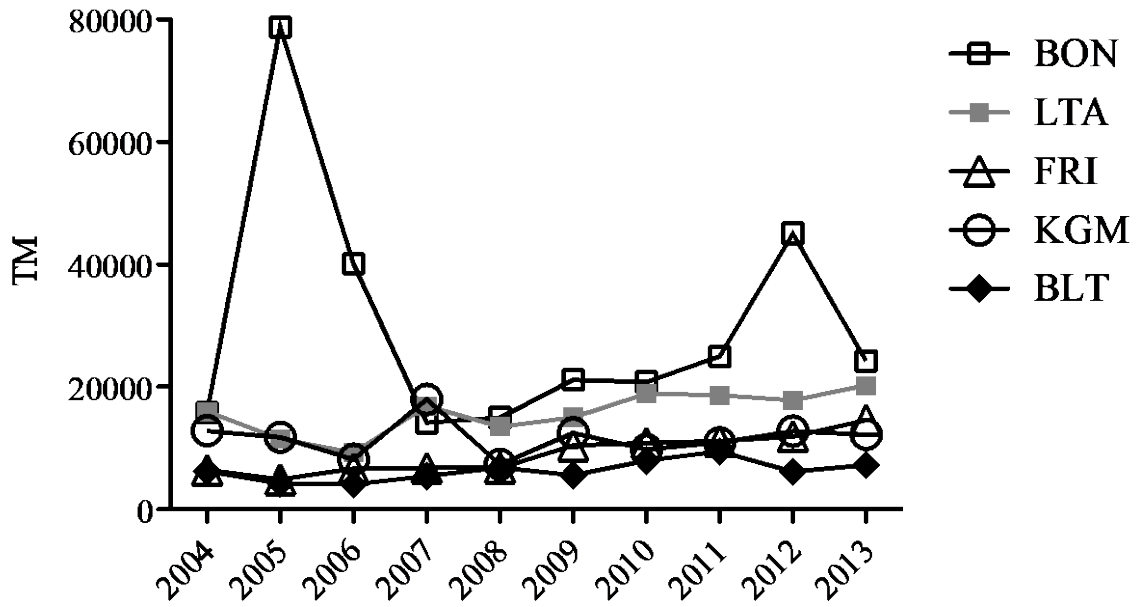


Figure 1. Landings during the last ten years of the five most exploited Small tuna species. BON, Atlantic bonito; LTA, Atlantic Black Skipjack; FRI, Frigate tuna; KGM, King Mackerel; BLT, Bullet tuna. Data from Anon. (2016).

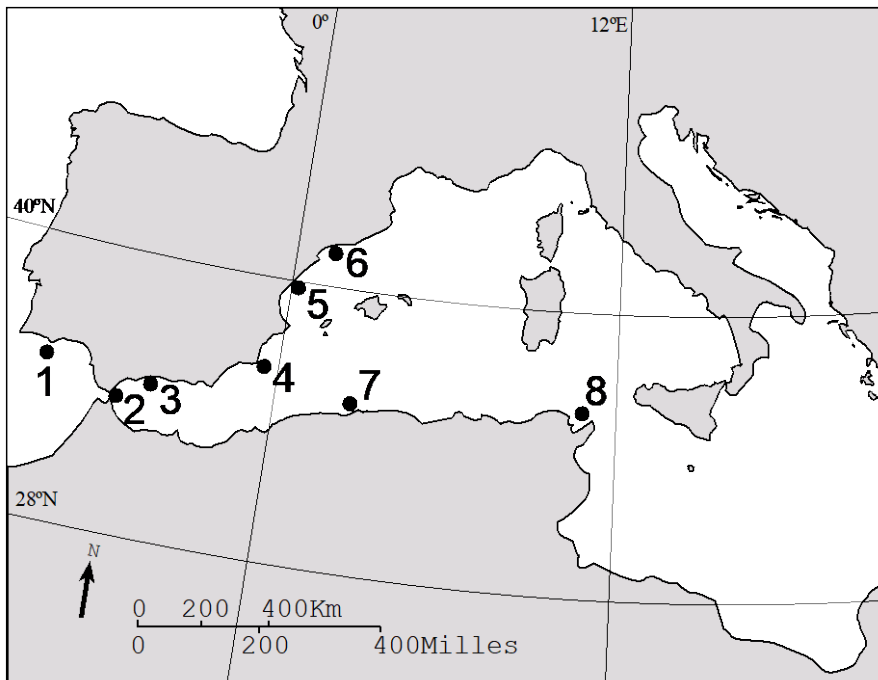


Figure 2. Map of sampling localities. Number codes are detailed in **Table 2**.