POPULATION GENETIC OF ATLANTIC BONITO IN THE NORTH EAST ATLANTIC AND MEDITERRANEAN

J. Viñas[1]*, J. Ollé[1], G. Hajjej[2], D. Macias[3], S. Saber[3], P. G. Lino[4], R. Muñoz-Lechuga[4], S. A. Baibbat[5], B. M. Habibe[6], F. Ngom Sow[7], N'G. C. Diaha[8], F. Lucena Frédou[9]

SUMMARY

This study assesses the stock structure of Atlantic bonito (Sarda sarda) using the mitochondrial control region as a genetic marker. About 615 individuals distributed in seven locations were analyzed. Two of the locations were in the Mediterranean Sea (MD/BIL95) (Spain and Tunis), three in the northeast Atlantic (AT-NE/BIL94B) (Portugal, Tunis, Morocco and Mauritania), and one in the southeast Atlantic (AT-SE/BIL97) (Côte d'Ivoire). All these samples were obtained thanks to the participation of all authors in two Small Tuna Research Programs funded by ICCAT. The analysis of the genetic variability of the sequence of mitochondrial control regions depicts a clear heterogeneity among locations. The shared genetic pool that comprises the locations within the Mediterranean (Spain and Tunis), including also a sample from the northeast Atlantic (Portugal), is clearly different from the African locations (Senegal and Côte d'Ivoire). Moreover, these two African locations are also genetically differentiated between them. Morocco and Mauritania locations seems to be located in an intermediate situation between these two groups of locations. These results can be used to infer a management policy by ICCAT on the fisheries of this species.

RÉSUMÉ

*Cette étude évalue la structure du stock de la bonite à dos rayé (*Sarda*) en utilisant la zone mitochondriale de contrôle comme marqueur génétique. Environ 615 spécimens présents dans sept sites ont été analysés. Deux de ces sites se trouvaient en Méditerranée (MD/BIL95) (UE-Espagne et Tunisie), trois dans l'Atlantique Nord-Est (AT-NE/BIL94B) (Portugal, Tunisie, Maroc et Mauritanie) et un dans l'Atlantique Sud-Est (AT-SE/BIL97) (Côte d'Ivoire). Ces échantillons ont été obtenus grâce à la participation de tous les auteurs à deux programmes de recherche sur les thonidés mineurs financés par l'ICCAT. L'analyse de la variabilité génétique de la séquence des régions mitochondriales de contrôle montre une nette hétérogénéité entre les régions. Le patrimoine génétique commun qui comprend les sites de la Méditerranée (UE-Espagne et Tunisie), y compris un échantillon de l'Atlantique Nord-Est (UE-Portugal), est clairement différent des sites africains (Sénégal et Côte d'Ivoire). En outre, ces deux sites africains sont également génétiquement différenciés entre eux. Les sites du Maroc et de la Mauritanie semblent se situer dans une situation intermédiaire entre ces deux groupes de sites. Ces résultats peuvent être utilisés pour sous-tendre une politique de gestion de l'ICCAT sur les pêcheries de cette espèce.*

RESUMEN

*Este estudio evalúa la estructura del stock de bonito (*Sarda sarda*) utilizando la región de control mitocondrial como marcador genético. Se analizaron aproximadamente 615 ejemplares distribuidos en siete localizaciones. Dos de las localizaciones eran en el Mediterráneo*

[1]Laboratori Ictiologia Genètica, Departament de Biologia, Universitat de Girona. 17071, Girona, Spain.
*Corresponding author: jordi.vinas@udg.edu
[2]National Institute of Marine Science and Technology, Tunisie
[3]Instituto Español de Oceanografía, Centro Oceanográfico de Málaga, Spain
[4]Instituto Português do Mar e da Atmosfera, Portugal
[5]Laboratoire des Pêches (Dakhla), Morocco
[6]Laboratoire Evalutaion des Ressources Vivantes Aquatiques, Mauritania
[7]Centre De Recherches Oceanographiques de Dakar, Senegal
[8]Centrer of Oceanology Research,Côte d'Ivoire
[9]Departamento de Pesca e Aquilcutura – Universidad Federal Rural de Pernambuco, Brasil

(MD&BIL95) (España y Túnez), tres en el Atlántico nororiental (AT-NE/BIL94B) (Portugal, Túnez, Marruecos y Mauritania) y una en el Atlántico suroriental (AT-SE/BIL97) (Côte d'Ivoire). Todas estas muestras se obtuvieron gracias a la participación de todos los autores en el Programa de investigación de pequeños túnidos financiado por ICCAT. El análisis de la variabilidad genética de la secuencia de las regiones de control mitocondrial describe una clara heterogeneidad entre las localizaciones. El acervo genético compartido que comprende las localizaciones dentro del Mediterráneo (España y Túnez), incluida también una muestra del Atlántico nororiental (Portugal), es claramente diferente del de las localizaciones africanas (Senegal y Côte d'Ivoire). Además, estas dos localizaciones africanas están también diferenciadas genéticamente entre sí. Las localizaciones de Marruecos y Mauritania parecen estar en una situación intermedia entre estos dos grupos de localizaciones. Estos resultados pueden usarse para inferir una política de ordenación por parte de ICCAT para las pesquerías de esta especie.

KEYWORDS

Small tuna, Stock structure, Atlantic Bonito (BON), Sarda sarda*, Northeast Atlantic, Southeast Atlantic, Mediterranean, Population genetics*

1. Introduction

The Atlantic Bonito (BON) (*Sarda sarda*), along with Little tunny (LTA) (*Euthynnus alletteratus*) and Wahoo (WAH) (*Acanthocybium solandri*), has been identified as key species for implementing working program to gather information for of growth, maturity and stock structure (Anon. 2019). Knowledge of these biological parameters are key to implement correct management strategies for the fisheries of these species.

One of the most useful and traditionally applied methods for inferring stock structure is based on population genetics (Ward 2000). This parameter is essential to infer the fishery stocks and finally to determine the correct measure of fishery management. For all the possible methodologies for inferring the population structure of the species, the use of mtDNA sequence variation is one of the most traditionally used in marine pelagic species. Although, nowadays other methods for inferring population structure such as microsatellites or Single Nucleotide Polymorphism (SNPs) are also widely used. The use of sequence variation of the Control Region (D-loop region) of mtDNA (mtDNA CR) is still extremely useful for inferring a preliminary assessment of the population's structure and a first step for subsequent application other methodologies with high power of resolution. One of the clear advantages of the mtDNA CR from other methods is that this methodology is already optimized for the majority of Scombridae species in (Allaya *et al*. 2015; Ollé *et al*. 2019; Viñas *et al*. 2010; Viñas *et al*. 2011). That implies that it could be applied to large number of samples with a relative low cost.

In this study, we have analyzed 615 individuals of Atlantic bonito from seven locations distributed between the Mediterranean, northeast and southeast Atlantic (**Table 1**). The samples have been acquired due to the participation of two short terms ICCAT contracts under the Small tuna year program. We can consider that this is probably the most exhaustive sampling of this species realized to date.

2. Material and methods

Up to 615 Atlantic bonito distributed in seven different locations from three different ICCAT areas were analyzed. Of these, three of the locations two temporarily differentiated samples were included in the analysis (see **Table 1** and **Figure 1** for a detailed description of the sampling).

Genetical variability of all individuals was assessed following the methodological procedure in Viñas *et al.* (2004). Briefly, once the samples arrived at the LIG-UdG total genomic DNA was isolated. Following extraction, DNA was resuspended in 100 µl of deionized water. We amplified approximately 450 base pairs (bp) of the first (left) domain of the mitochondrial control region (mtDNA CR) with the L-strand primer L15998 (5'-TAC CCC AAA CTC CCA AAg CTA-3'), in combination with the H-strand primer CSBDH (5'-TgA ATT Agg AAC CAg ATg CCA g-3'). Amplification was carried out in 12.5 µl reaction volumes using approximately 50 ng (0.5 µl) of the isolated DNA as the template. Each PCR reaction contained 1X Taq DNA polymerase buffer, 1.5–2 mM $MgCl_2$,

200 mM of each dNTP, 10 pmol of each primer, and 0.5 U Taq DNA polymerase. Thermal cycles involved an initial denaturing step of 5 min at 94°C, followed by 35 cycles of denaturing at 94°C for 45 s, annealing at 50°C for 45 s, and extension at 72°C for 1 min. Negative controls were included in all PCR runs to ascertain that no cross-contamination took place. Double-stranded DNA products were purified and subsequently were sequenced unidirectionally using the BigDye Kit v3.1 (Applied Biosystems) on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems). When sequencing results were ambiguous, the amplicon was sequenced in both directions. Sequence alignments were inspected using the Geneious v.R7. Sequence Phylogenetic tree was constructed using the Neighbor joining (Saitou and Nei 1987) procedure with the kimura 2-distance (Kimura 1980) with a resampling of 1000 bootstrap pseudoreplicates to assess the robustness of the branches in the tree. Haplotype (*h*) (Nei and Tajima 1981) and nucleotide diversity (π) (Nei 1987) were estimated from haplotype frequencies and haplotype divergence based on a pairwise distance matrix in ARLEQUIN v. 3.5 (Excoffier and Lischer 2010). The geographical structure for each species was estimated using analysis of molecular variance (AMOVA) (Excoffier et al. 1992) based on the pairwise matrix of distances between haplotypes. The haplotypic correlation measure (Φ_{ST}) was estimated for all possible permutations among regions for each species. The significance level of each haplotypic correlation was tested by conducting a non-parametric permutation procedure 10,000 times in ARLEQUIN.

3. Results

Genetic variation in Atlantic bonito was relatively high with 132 variable sites out of 392bp of the alignment. We identified up to 340 distinct haplotypes from 615 sequences (**Table 1**). Accordingly, in all locations the haplotypic diversity was close to one, ranging from 0.974 to 0.996. Nucleotide diversity was also high compared to the ones observed in other species of *Sarda* (Viñas *et al.* 2010), but within the range observed in the Mediterranean Sea for the Atlantic bonito (Viñas *et al.* 2004). This high sequence variation diversity is probably consequence of the presence of two highly divergent groups of sequences (**Figure 2**), previously described in Viñas *et al.* 2004 as Clade I and Clade II. Clear genetic differentiation was observed, with a highly significant overall value of Φ_{ST}= 0.146 (*P-value* = 0.000). Pairwise comparisons of genetic differentiation among locations (**Table 2**) revealed a pattern of differentiation between the locations from Northeast Atlantic-Mediterranean locations (Spain, Portugal, Tunis) and the rest of locations (Morocco, Mauritania, Senegal and Cote D'Ivoire). In addition, there is a clear differentiation between Mauritania, Senegal and Cotê d'Ivoire. These locations are also genetically differentiated among them. The location of Morocco seems to be situated in intermediate situation between the group of Atlantic-Mediterranean locations (Tunis, Spain, and Portugal) and the group of Northeast and southeast Atlantic locations (Senegal and Cotê d'Ivoire). In addition, the clade distribution among locations (**Table 2**) seem to confirm these results. The two clades were highly heterogenous distributed (χ^2 = 41.25; df = 4; *P-value* < 0.0001), in a shift of distribution with Clade I individuals clearly increasing up to more than 80% in the south east Atlantic. Genetical temporal stability was detected in the locations of Spain and Portugal, but the samples of 2018 versus 2019 in Côte d'Ivoire were genetically differentiated (Senegal).

4. Discussion

Atlantic bonito presents a pattern of genetic heterogeneity along the locations analyzed. To our knowledge this is the most extensive study including samples from the largest area studied to date (see **Figure 1** for a detailed location of samples analyzed). This is the first study to confirm that in the northeast Atlantic, the Atlantic bonito also presents genetic heterogeneity. Pairwise Φ_{STs} identified at least four differentiated genetic pools (see **Figure 1**). Lack of genetic heterogeneity is detected within the Mediterranean (Tunis and Spain), this genetic pool extends to the Atlantic side of the Strait of Gibraltar (Portugal). On the other end, in the east Atlantic and south Atlantic: Senegal and Côte d'Ivoire are also two genetically differentiated locations. Morocco and Mauritania locations seems to be located in an intermediate situation between these two groups of locations. The capacity of Atlantic bonito in establishing genetically isolated populations has been already observed between relatively close locations. Genetic differentiation was previously observed within the Mediterranean (Viñas *et al.* 2004) and between the east Mediterranean and Black Sea (Turan 2015) and also between both sides of the Atlantic Ocean (Viñas *et al.* 2010).

All this geographic structure seems to be related to the distribution of the two mtDNA clades. There is a clear shift of clade distribution between the north and south locations (**Figure 1**). Remarkably, the European anchovy presents an extremely similar pattern of clade distribution in a similar geographical region (Silva *et al.* 2014). The authors postulated that this distribution can be correlated to a genetic selection of water temperature, with one clade being more abundant in warmers waters.

The lack of genetic heterogeneity between the two Mediterranean locations (Tunis and Spain) and the location in the Atlantic out of the strait of Gibraltar (Portugal) is unexpected. In other studies of Atlantic bonito, locations within the Mediterranean and with a relative less geographical distance and have being observed as genetically differentiated (Viñas *et al*., 2004). It should be noted, however, that in swordfish the genetical pool of the Mediterranean extends out the Strait of Gibraltar towards north east Atlantic (Smith *et al*. 2015). A situation similar was observed here for the Atlantic bonito. Other surprising result if the genetic different ion between the two temporal samples of Côte d'Ivoire. This is surprising since the other locations where there are samples from two consecutive years denoted a temporal stability. Further inspection is needed to confirm this last result.

Finally, this study can be a starting point for stablishing correct fishery management measures by ICCAT for Atlantic bonito. However, the results presented here disagree with the management areas adopted by ICCAT. The locations of the ICCAT area MD/BIL95 (Spain and Tunis) shares the genetic pool with one location in the NE/BIL94B (Portugal). This genetic unit is clearly separated from Senegal (NE/BIL94B), and from Côte d'Ivoire (AT-SE/BIL97). There is also a clear differentiation between Senegal and Côte d'Ivoire. The locations of Morocco and Mauritania (NE/BIL94B) seem to be placed in an intermediate situation. However, it has to be kept in mind the presence of type II statistical error in the analysis of population structure (false negatives): actual presence of local structures, but not detected by the analysis (Waples *et al*. 2008). Thus, a management in a broad area, where actually exists small and local stock could produce the irrevocable loss of the small stocks. Thus, considering the results and the impact of having undetected small structures, it can be proposed at least four clear differentiated stocks: (i) one in the west Mediterranean; (ii) the location of Portugal, out of the Strait of Portugal; (iii) initially Morocco and (iv) Mauritania can be managed separately. Clear separation of (v) Senegal and (vi) Côte d'Ivoire.

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Table 1. Results of Atlantic bonito sampling and molecular diversity indices. Year, year of sampling. Samples with asterisks are the ones analyzed in the 2019 contract. N, number of individuals; M, number of haplotypes; *h*, haplotypic diversity; π, nucleotide diversity. Distribution of clades along locations according the phylogenetic tree in **Figure 2**.

Location/	ICCAT area	Code	Year	N	*M*	*h*±*SD*	π±*SD*	Clade distribution		
								I	***II***	*%Clade I*
Spain	MD/BIL95	ESP2018	2018	108	76	0.985 ± 0.005	0.070 ± 0.034	49	59	45.4
Spain	MD/BIL95	ESP2019	2019	96	54	0.960 ± 0.013	0.066 ± 0.032	45	51	46.9
Portugal	AT-NE/BIL94B	PRT2018	2018	65	46	0.975 ± 0.010	0.069 ± 0.034	31	34	47.7
Portugal	AT-NE/BIL94B	PRT2019	2019	38	28	0.979 ± 0.012	0.067 ± 0.034	20	18	52.6
Tunisia	MD/BIL95	TUN2018	2018	49	30	0.974 ± 0.010	0.066 ± 0.033	28	21	57.1
Morocco	AT-NE/BIL94B	MAR2018	2018	40	28	0.968 ± 0.016	0.048 ± 0.024	31	9	77.5
Mauritania	AT-NE/BIL94B	MRT2019	2018	48	45	0.996 ± 0.005	0.047 ± 0.024	40	8	83.3
Senegal	AT-NE/BIL94B	SEN2018	2018	49	43	0.990 ± 0.009	0.039 ± 0.020	43	6	87.8
Cotê d'Ivoire	AT-SE/BIL97	CIV2018	2018	50	38	0.975 ± 0.013	0.017 ± 0.009	48	2	83.3
Cotê d'Ivoire	AT-SE/BIL97	CIV2019	2019	72	51	0.975 ± 0.010	0.032 ± 0.016	65	7	90.3
All				615	340	0.988 ± 0.002	0.064 ± 0.031	400	215	65.0

Table 2. Pairwise genetic differentiation among Atlantic bonito samples. Below diagonal, Φ_{ST}s values. Above diagonal, *P*-values. In bold, *P*-values significant after multiple testing. Samples code as **Table 1**.

	ESP2018	ESP2019	PRT2018	PRT2019	TUN2018	MAR2018	MRT2019	SEN2018	CIV2018	CIV2019
ESP2018	--	0.013	0.905	0.060	0.178	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
ESP2019	0.031	--	0.036	0.493	0.012	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
PRT2018	-0.009	0.031	--	0.100	0.341	0.003	**0.000**	**0.000**	**0.000**	**0.000**
PRT2019	0.031	-0.007	0.026	--	0.058	0.004	0.001	**0.000**	**0.000**	**0.000**
TUN2018	0.011	0.057	-0.001	0.037	--	0.040	**0.000**	**0.000**	**0.000**	**0.000**
MAR2018	0.129	0.182	0.115	0.148	0.055	--	0.001	0.025	0.004	**0.000**
MRT2019	0.213	0.174	0.207	0.133	0.171	0.140	--	**0.000**	**0.000**	0.041
SEN2018	0.221	0.265	0.219	0.250	0.167	0.058	0.178	--	**0.000**	**0.000**
CIV2018	0.323	0.379	0.332	0.390	0.274	0.093	0.279	0.113	--	**0.000**
CIV2019	0.286	0.250	0.289	0.219	0.248	0.176	0.034	0.205	0.269	--

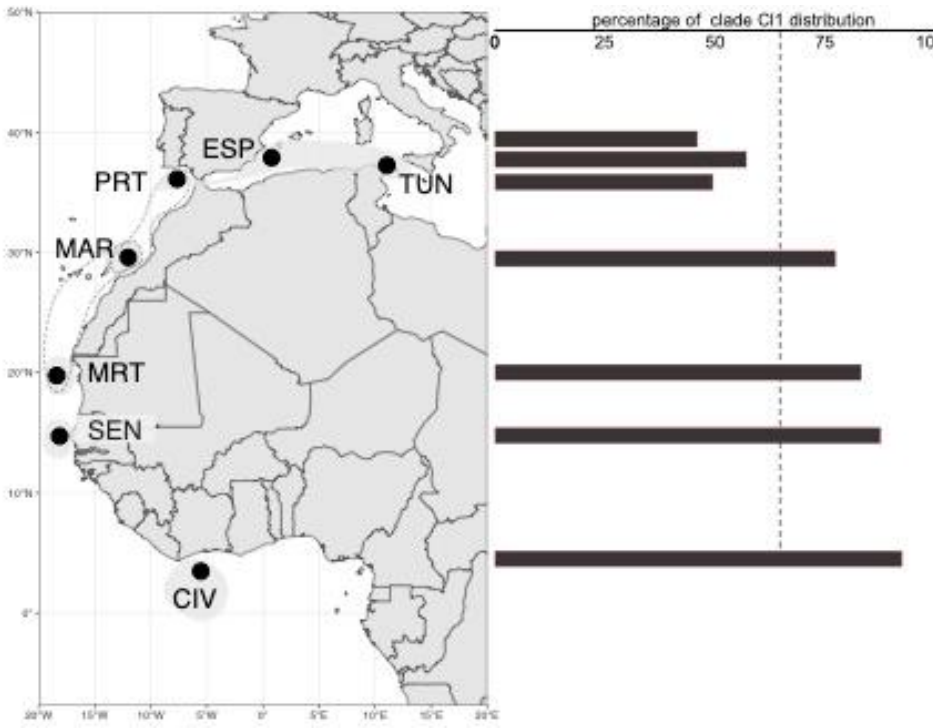

Figure 1. Distribution map of locations analyzed. On the right proportion of Clade I distribution. Grey dashed line, average of Clade I along all localities. In the map, grey shapes denote genetically differentiated locations, and with dashed line possible contact between locations.

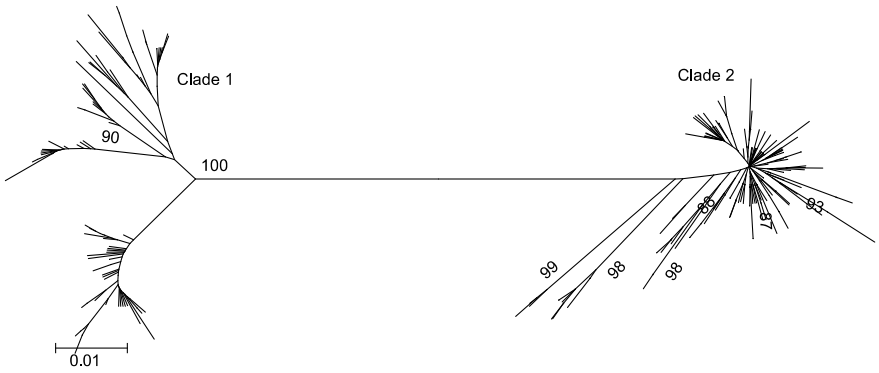

Figure 2. Unrooted phylogenetic tree of the 340 Atlantic bonito mtDNA-CR haplotypes. Values in branches are bootstrap percentages above 80% consistency.