PANAMJAS
Pan-American Journal of Aquatic Sciences

Morphological variation of little tuna *Euthynnus alletteratus* in Tunisian waters and Eastern Atlantic

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Abstract. Meristic counts and morphometric measurements were carried out on little tuna *Euthynnus alletteratus* (Rafinesque 1810) obtained from commercial landings in Tunisian waters (Zarzis, Gabes, Sfax and Teboulbah) and from two samples of the eastern Atlantic Ocean (central-east Atlantic and south-east Atlantic). Nine morphometric and five meristic characters were analysed using hierarchical cluster analysis and multivariate discriminant analysis. In discriminant function analysis, the plotting first discriminant functions explained 76.97% and 60.56% of the between-group variation in the morphometric and the meristic analyses, respectively. All the morphometric analyses indicated the existence of four morphologically differentiated groups of little tuna. The greatest differences were found between the samples from the Atlantic Ocean and all the others sampled, while fish from the Tunisian coast appeared to be more similar among themselves. The meristic characters did not support the morphometric results, showing a clear similarity between the SE Atlantic and Teboulbah Sea specimens, with the Mahalanobis distances among areas being an order of magnitude smaller than those for the morphometric analysis.

Key words: hierarchical cluster analysis, multivariate discriminant analysis, morphometric, meristic

Resumen. Variaciones morfológicas de *Euthynnus alletteratus* en aguas de Túnez y el Atlántico oriental. Se realizaron cálculos merísticos y medidas morfométricas en *Euthynnus alletteratus* (Rafinesque 1810) procedentes de desembarques comerciales en aguas tunecinas (Zarzis, Gabes, Sfax and Teboulbah) y de dos muestras tomadas en el océano Atlántico (Atlántico centro-oriental y sudeste Atlántico). Nueve caracteres morfométricos y cinco merísticos fueron estudiados realizando un análisis cluster jerárquico y un análisis discriminante multivariante. En el análisis multivariante, las funciones obtenidas explican 76.97% y 60.56% de la varianza entre grupos para los rasgos morfométricos y merísticos, respectivamente. Los análisis morfométricos indican la existencia de cuatro grupos de *E. alletteratus* bien diferenciados morfológicamente. Las mayores diferencias se observaron entre las muestras procedentes del Atlántico y todas las demás, mientras que los individuos procedentes de aguas tunecinas se parecían más entre sí. Los caracteres merísticos no respaldan los resultados morfométricos, poniendo de relieve una clara similitud entre los individuos del sudeste Atlántico y los del mar de Teboulbah, cuyas distancias de Mahalanobis resultan de un orden de magnitud menores que las del análisis morfométrico.

Palabras clave: análisis cluster jerárquico, análisis discriminante multivariante, morfométrico, merístico

Introduction

The Little tuna, *Euthynnus alletteratus* (Rafinesque 1810), has shown a wide distribution in the world's oceans. According to Collette & Nauen (1983), this species can be found in the tropical and subtropical waters of the Atlantic Ocean, including the Caribbean Sea and the gulf of Mexico. It also occurs in the Mediterranean Sea and in the Black Sea (Collette & Nauen 1983). Little tuna is among the most important tuna fishing resources in Tunisian waters (Hattour 2000). It is captured by using different fishing gears, such as purse seine, light fishing, gill nets, longlines, pelagic trawl, and beach seine. It is an important commercial fish species across the Tunisian coast. From 1996 to 2005 this species accounted for 35.23% small tuna landed in Tunisian waters (ICCAT 2009).

Studies on the identification of different populations of little tuna are practically nonexistent. From all the stock identification methods available (Rey & Cort 1981, Templeman 1983, Smith & Jamieson 1986) the analysis of morphometric and meristic characters is one of the most commonly used (Taylor & McPhail 1985, Melvin *et al*. 1992). The variation in such characters was assumed to be entirely genetic in early studies (Heincke 1898, McQuinn 1997), but is now known to have both environmental and genetic components (Cabral *et al*. 2003). Studies of morphologic variation among populations continue to have an important role to play in stock identification, despite the advent of biochemical and molecular genetic techniques which accumulate neutral genetic differences between groups (Swain & Foote 1999).

Some data on the biometric characters of tuna in the Atlantic Ocean and in the Mediterranean Sea due to the interest in their taxonomy are available (Godsil 1954, Hattour 2000, Zorica & Sinovčić 2008). However, the number of papers published on this subject, regarding intra- and inter-specific population differences, is still very scarce. It seems that in the Mediterranean Sea an independent tuna population exists, although the tagging results indicated the possibility of little tuna migration into the Atlantic Ocean from the Mediterranean Sea through the Strait of Gibraltar (Rey & Cort 1981). For assessment purposes, the Tunisian little tuna is included in the Mediterranean stock, which is managed together with the other stocks in the eastern Atlantic Ocean.

Nevertheless, the question of whether there is a single stock or multiple stocks of *E. alletteratus* in the Tunisian coast remains unresolved, since Tunisian surveys show a continuous distribution of the resource with a clear pattern of juveniles and adults distribution, as stated above (Hattour 2000, Hajjej *et al*. 2009). Furthermore, no studies have been carried out to verify if there is a possibility of separating the stocks inhabiting the central Mediterranean and eastern Atlantic areas based on biometric analysis. Our objectives were to investigate the morphologic population structure of *E. alletteratus* based on the morphometric and meristic characters of the specimens from the Tunisian coast, and comparing these results with the results of samples taken from eastern Atlantic areas, in order to highlight any possible morphologically differentiated groups of this small tuna species.

Material and methods

Random samples of little tuna were collected between January 2008 and December 2009 with different fishing gears (purse seine, light fishing, gill nets, longlines, pelagic trawl and beach seine) from commercial catches from the four areas of the Tunisian waters indicated in Figure 1: 1) Zarzis coast (76 individuals), 2) Gabes coast (38 individuals), 3) Sfax coast (51 individuals) and 4) Teboulbah coast (62 individuals); and during 1978 (Gaykov & Bokhanov 2008) from area 5, Exclusive Economic Zone (EEZ) of Mauritania (30 individuals), and during 1976 (Gaykov & Bokhanov 2008) from area 6, the Gulf of Guinea and EEZ of Angola (25 individuals). Fishes from Eastern Atlantic were caught in various gears (purse-seine, trawls and rods). The samples from each geographic area are shown in Table I. The fork length was measured to the nearest 0.1 cm. The rest of the morphometric characters were measured to the nearest 0.01 mm. Sexes were pooled for all analyses, since little tuna does not exhibit sexual dimorphism (Gaykov & Bokhanov 2008).

A total of 14 characteristics were used: nine morphometric and five meristic. Figure 2 summarises the morphometric characters:

1. fork length (FL)
2. head length (HL)
3. length of first dorsal fin base (LD1)
4. length of second dorsal fin base (LD2)
5. length of pectoral fin (LP)
6. maximum body height (H)
7. distance of the first dorsal fin (DD1)
8. distance of the second dorsal fin (DD2)
9. distance of anal fin (DA)

The five meristic characters counted were:

1. number of spines in the first dorsal fin (FDR)
2. number of second dorsal rays (SDR)
3. number of anal rays (AR)
4. number of dorsal finlets (DF)
5. number of anal finlets (AF)

In order to ensure that the meristic characters were completely defined, only adults with a fork length larger than 36 cm were used. The morphometric characteristics were calculated as a percent proportion of the fork length. Correlation coefficients between each pair of characters were calculated to check if the data transformation was effective in reducing the influence of size in the measurements.

A hierarchical cluster analysis was

performed using Mahalanobis distance (Dryden & Mardia 1998). This distance was chosen because it is invariant to differences in scale between variables, therefore giving an equal weight to all variables in the calculation of distances (Legendre & Legendre 1979).

Table I. Sampling details of *Euthynnus alletteratus* used in this study.

Code	Area	N	Years	Source	Range (cm)
1	Zarzis	76	2008-2009	Present study	36.6-95.6
2	Gabes	38	2008-2009		
3	Sfax	51	2008-2009		
4	Teboulbah	62	2008-2009		
5	CE Atlantic	30	1978	Gaykov & Bokhanov (2008)	38-63
6	SE Atlantic	25	1976		

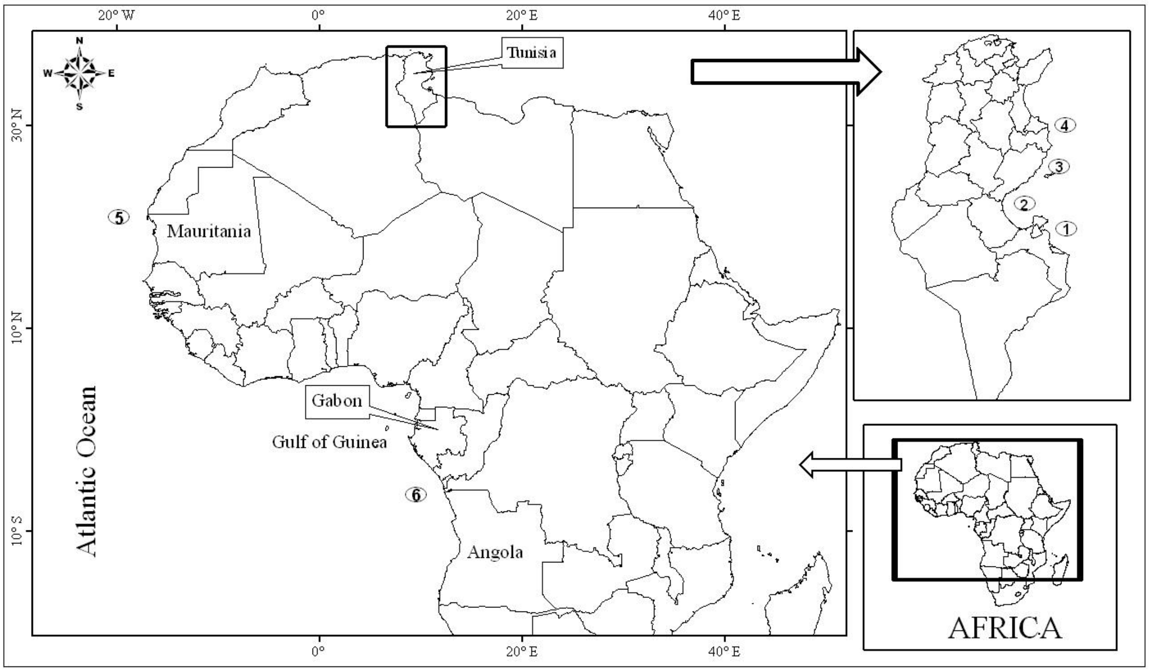

Figure 1. Map of the sampling locations. (1) Zarzis Sea, (2) Gabes Sea, (3) Sfax Sea, (4) Teboulbah Sea, (5) CE Atlantic, (6) SE Atlantic.

A Correspondence Analysis (CA) was used separately for morphometric and meristic data, in order to identify the combination of variables that best separate *Euthynnus alletteratus* samples, since the predictive ability of morphometric and meristic characters differs statistically (Ihssen *et al*. 1981, Hair *et al*. 1996). A Correspondence Analysis was performed with the centroids of the six groups of individuals (corresponding to the six sampling areas), using the Euclidean distance to investigate the geographic effect in the morphometric measurements and meristic characteristics. To estimate the significance level of the differences between groups, a multivariate randomization test was performed between each pair of groups (Sokal & Rohlf 1995). This procedure was preferred to parametric tests, such as the Bonferroni test, to relax the multinormality assumption (Morrison 1990), since q-q plots (Cleveland 1994) showed strong deviations from normality for several characteristics.

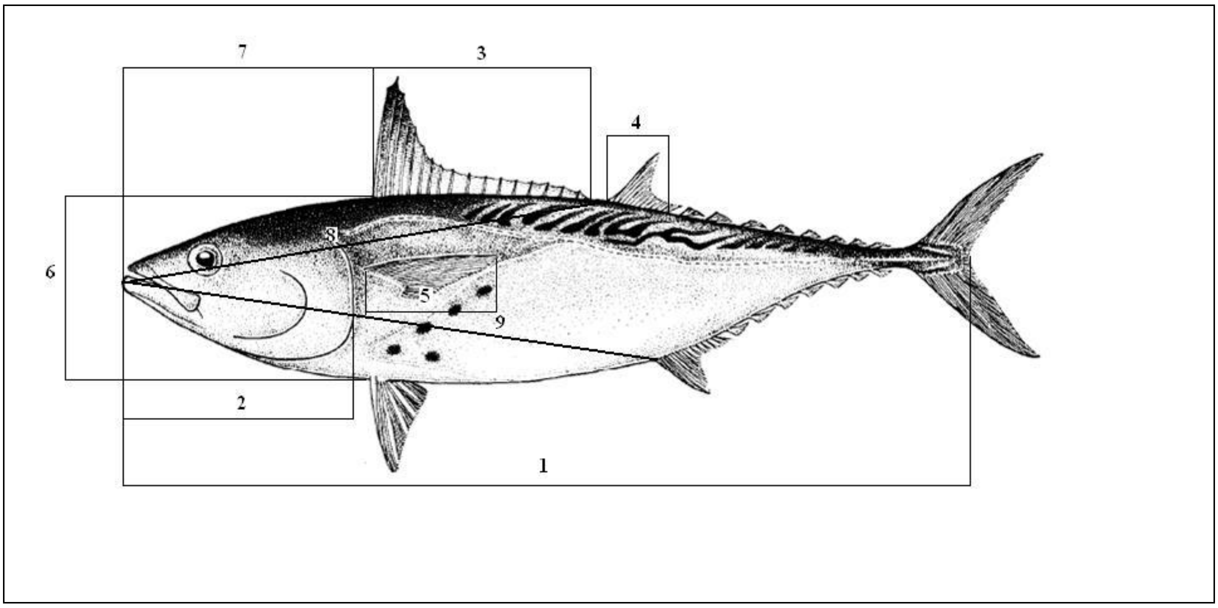

Figure 2. Morphometric characters measured on each fish: 1, FL; 2, HL; 3, LD1; 4, LD2; 5, LP; 6, H; 7, DD1; 8, DD2; 9, DA.

Results

The correlation coefficients between characters are presented in Table II. All the coefficients between morphometric characters were close to 1, except for LD2 and LP measurements, which showed no significant correlation. Regarding meristics, the highest coefficient was 0.31, with most coefficients being lower than 0.2. The correlation results revealed that all of the meristic variables studied were free from the influence of size.

Table II. Correlation coefficients between each pair of characters.

	FL	H	Hl	DD1	DD2	DA	LD1	LD2	LP	FDR	SDR	AR	DF	AF
FL	1.00													
H	0.81	1.00												
Hl	0.74	0.93	1.00											
DD1	0.83	0.98	0.89	1.00										
DD2	0.94	0.92	0.88	0.943	1.00									
DA	0.94	0.77	0.75	0.79	0.94	1.00								
LD1	0.97	0.88	0.84	0.91	0.98	0.933	1.00							
LD2	-0.09	-0.06	-0.17	0.10	0.01	-0.05	-0.00	1.00						
LP	-0.24	0.19	0.37	0.12	-0.07	0.58	0.74	0.02	1.00					
FDR	0.11	0.05	-0.21	-0.14	-0.04	-0.09	-0.12	-0.10	-0.13	1.00				
SDR	0.06	-0.04	-0.04	-0.03	-0.09	-0.10	-0.03	-0.13	-0.04	-0.06	1.00			
AR	0.17	0.21	0.07	-0.05	-0.05	-0.24	-0.12	-0.08	-0.11	-0.05	-0.06	1.00		
DF	0.21	0.15	0.09	0.11	0.27	0.16	0.31	0.23	0.11	007	0.06	0.11	1.00	
AF	0.24	0.11	0.07	0.19	0.14	0.16	0.21	0.12	0.07	012	0.09	0.13	0.08	1.00

The dendrograms for the morphometric and meristic characters (Figs. 3 and 4) present clearly different results. However, the range of Mahalanobis distances for the meristics is an order of magnitude less than for the morphometrics (0 to 1.8 in the former, 1.5 to 4.5 in the latter). This means that if put on the same scale, the morphometric characters reveal much greater differences between groups than the meristic characters.

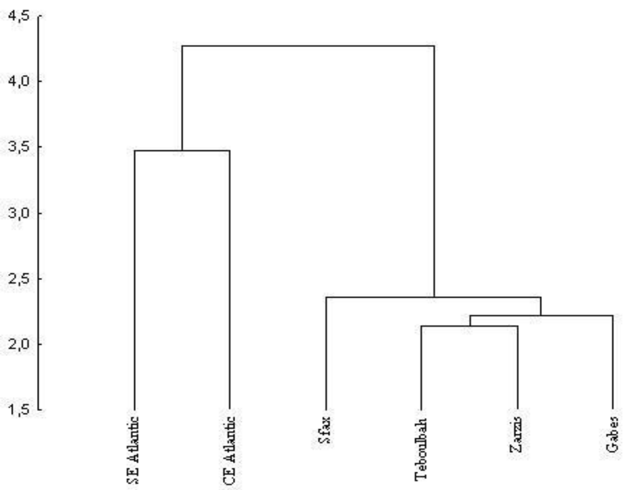

Figure 3. Dendrogram resulting from cluster analysis of Mahalanobis distance based on the morphometric data.

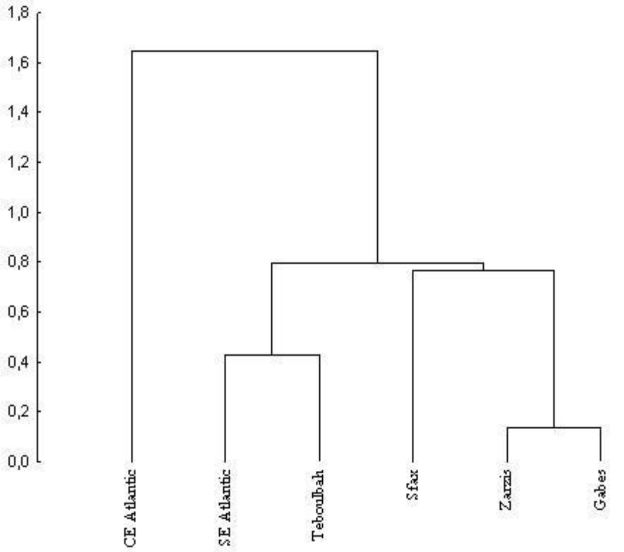

Figure 4. Dendrogram resulting from cluster analysis of Mahalanobis distance, based on the meristic data.

In the morphometric dendrogram all the groups from the Tunisian coast are close to each other, the groups from the Atlantic Ocean appear the furthest from all others. A different picture stems from the meristic dendrogram, where the samples from the CE Atlantic is detached from all the other areas, while the samples from Teboulbah Sea was similar to the SE Atlantic, and Gabes overlapped Zarzis.

According to the results of Multivariate analysis, each of the samples should be classified as a distinct new group. Cluster analysis of Squared Euclidean distances between the different groups centroids calculated produced a correspondence analysis of four groups. Regarding the Correspondence Analysis for morphometrics, the first CA accounted for 76.97% of the between-group variability, showing a clear between-sample differentiation (Fig. 5). For the samples of the Tunisian coast two groups were identified, the first grouped Sfax and Gabes and the second grouped Teboulbah and Zarzis. However, CE Atlantic and SE Atlantic were both highly isolated from all the other samples in discriminant space.

Regarding meristics, the first CA accounted for 60.56% and the second accounted for 28.72% (Fig. 6). Still, the pattern from the dendrogram is also reflected here, with the CE Atlantic and Sfax samples being both isolated from all the other areas. The SE Atlantic sample was grouped together with one sample from the North Tunisian coast (Teboulbah). Taking into account the similarity in the meristic characteristics found in the present study between the individuals from these two areas, the result can hardly be interpreted as evidence of a stock separation because of the presence of CE Atlantic between them, and hence the exclusion of the migration hypothesis. The other Tunisian coast samples (Gabes and Zarzis) overlapped each other and were different from all the other samples. The correlation coefficients between groups for the morphometric and meristic characters presented in Table III support the results of the cluster and discriminant analyses.

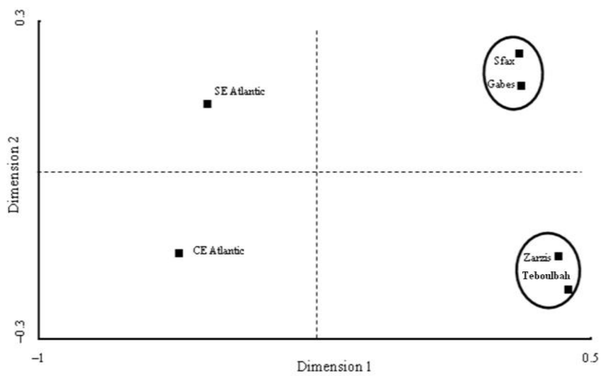

Figure 5. Correspondence Analysis showing a global view of the relationship for the sample groups in three dimensions (bi-dimentional representation) defined by the discriminant variables, based on the morphometric data.

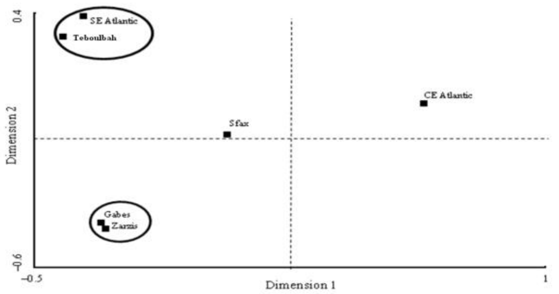

Figure 6 . Correspondence Analysis showing a global view of the relationship for the sample groups in three dimensions (bi-dimentional representation) defined by the discriminant variables, based on the meristic data.

Randomization tests for the morphometric data resulted in significant differences at 5% level between CE Atlantic and SE Atlantic samples and all the other groups, as well as between Sfax and Zarzis, and Gabes and Teboulbah (Table IV), hence reflecting the results from the cluster and discriminant analyses. For the meristic data, significant differences were found between the CE Atlantic sample and all the other groups, as well as between Sfax and Zarzis, and Teboulbah and all the other Tunisian coast groups (Table IV).

Table III. Correlation coefficients between each pair of groups. The upper triangle corresponds to the meristics data, and the lower one corresponds to the morphometric data.

	Gabes	Sfax	Zarzis	Teboulbah	CE Atlantic	SE Atlantic
Gabes		0.59	0.99	0.63	-0.70	0.52
Sfax	0.76		0.60	0.52	-0.43	0.33
Zarzis	0.77	0.71		0.62	-0.69	0.52
Teboulbah	0.69	0.69	0.85		-0.95	0.93
CE Atlantic	-0.80	-0.85	-0.86	-0.95		-0.97
SE Atlantic	-0.40	-0.43	-0.64	-0.74	0.577	

Discussion

The presence of physical barriers among marine fish populations is not always clear (Joyeux *et al*. 2001). Nevertheless, geographic subdivisions allow the establishment of population's traits representing suitable models for biogeographic, ecological and genetic studies. In such cases, biometric analyses can produce valuable information about the phenotypic plasticity of the species and any possible effects of genetic changes on their morphological variation (Hauser *et al*. 1995).

Table IV. Results (p-values) of the multiple comparison randomization tests. The upper triangle corresponds to the meristics data, and the lower one corresponds to the morphometric data.

	Gabes	Sfax	Zarzis	Teboulbah	CE Atlantic	SE Atlantic
Gabes		0.008	0.85	0	0	0.002
Sfax	0.001		0.01	0.02	0	0.009
Zarzis	0.0	0.01		0.003	0	0.003
Teboulbah	0.03	0.001	0.76		0.003	0.53
CE Atlantic	0.003	0.02	0.008	0.01		0.01
SE Atlantic	0	0.007	0.003	0.004	0.73	

Morphometric and meristic characters showed significant phenotypic heterogeneity among the six groups of little tuna samples. The results of this study indicate the likely existence of morphometric variations between four groups at least of little tuna in the areas observed in this study.

On the eastern Atlantic coast it appears to be two stocks, a central-eastern and a southern-eastern stock. Morphometric measurements carried out in the Tunisian coast little tuna appears to demonstrate the existence of clear variations between two samples of the southern coast (Gabes and Sfax) and the other areas (Teboulbah and Zarzis). In the natural environment, many parameters delaying ontogenetic development, such as hypoxia, low temperatures, high salinity and reduced food sources, favour the development of a greater number of body segments (Barlow 1961, Shaklee & Tamaru 1981) and consequently influence the biometric characters of fish. Indeed, this study showed that the samples from Tunisian waters presented greater similarities between themselves than to the Atlantic Ocean samples for both morphometric and meristic characters. There may be enough mixing between these locations to prevent differentiation. The Tunisian coast sample was morphometrically isolated from the Atlantic Ocean samples and can be divided into two subgroups: Gabes-Sfax (located in the south of Tunisia and distant from each other of 136 Km), and Zarzis-Teboulbah, with similar specimens although they correspond to the more distant samples (415 km) in the Tunisian coast. In the case of the Zarzis-Teboulbah group, which represent the important little tuna fishing area in the south and the north of Tunisia respectively, it should be taken into account that most of the samples from these two localities were taken in spring and summer, during the spawning season migration of *E. alletteratus* and, accordingly, an inadvertent sampling between these two areas may have taken place. In fact, in this period, and because of this spawning movement, individuals of each of these two areas can be collected in the other, which may have implications for the results drawn about the structure of the stock. Thus, significant morphological differences do not necessarily demonstrate gene flow restrictions between populations, although they do suggest that fish in each group may not mix extensively. As morphology is especially dependent on environmental conditions during early life history stages (Ryman *et al*. 1984, Cheverud 1988), a morphological differentiation may indicate that the majority of fish spend their entire lives in separate regions.

Nevertheless, the meristic data did not support the homogeneity between Tunisian *E. alletteratus* samples. Data indicate that for meristic analyses, the individuals from one of the Tunisian samples (Teboulbah) are more similar to the SE Atlantic sample than to those from the CE Atlantic specimens. It is clear that from a stock delimitation point of view, these results must be viewed with caution, since CE Atlantic is closest to the Tunisian coast than to the SE Atlantic. Therefore, in order to make inferences on stock identity, the results from this work should be compared with data obtained following a different methodology.

On the other hand it is interesting to observe that in the meristic analysis, the specimens from Zarzis Sea showed a similarity with the specimens from Gabes Sea. The latter sample was detached from the Sfax sample (in different with the morphometric analyses) which in turn, was isolated from all other samples. The disparity between morphometric and meristic data suggests that meristic characters may be more influenced by local environmental conditions (Barlow 1961, Fahy 1980, Lindsey 1988), which increases their differentiation at small geographic scales. Alternatively, there may be some migration between these areas and the meristic data are more sensitive when it comes to detect a low number of migrants between the areas (Fahy 1983, Lindsey 1988, Hulme 1995). It is therefore possible that temporal (intra-stock) variation is higher than geographical (inter-stock) variation, thus masking the differences between stocks (Tremblay *et al*. 1984, Blouw *et al*. 1988). On the other hand, the strong morphometric and meristic differentiation of the CE Atlantic sample from all the other groups suggests that there may be a self-recruiting population of little tuna in this part of the Atlantic Ocean. This result agrees with those given by Gaykov & Bokhanov (2008).

Euthynnus alletteratus population in Tunisian waters, because of its distance from the Atlantic Ocean, seems to form an isolated and selfmaintaining population. It is Interesting to note that the little tuna migration hypothesis between the Mediterranean and the Atlantic Ocean is not confirmed up to now, and the only confirmation has been cited by Rey and Cort (1981) was based on 2 individuals marked in Gibraltar and recaptured in Cadiz's Gulf. Indeed, the similarity of the SE Atlantic samples with little tuna from Teboulbah coast was only depicted by the multivariate analysis, so the result should be viewed with caution. The results from this study on morphometric characters and meristic counts suggest that each of the areas of Tunisian waters included in this study is inhabited by one stock of *Euthynnus alletteratus*. The observed differences are probably influenced by intra-specific factors and environment. It would be interesting to perform a study of the population structure of little tuna along the whole distribution area. Morphometric and meristic data should be collected in conjunction with tissue samples to be genetically analysed.

Biometric analysis is a useful tool not only for stock discrimination, but also for identifying possible temporal changes in the aggregation of fishes from different areas, and in the migration routes. Our study provided the first investigation using multivariate biometry methods between the central Mediterranean and the eastern Atlantic Ocean little tuna populations. If we assume that there are different *E. alletteratus* groups in this study area, then these results should be repeatable in further management analyses. A single stock discrimination method may not be sufficient to split a stock into a number of management units. These results, complemented by future genetic marker analyses and the analysis of other biometric characters such as the base of the caudal fin, could support the effective conservation of this species in the Mediterranean Sea and in the Atlantic Ocean.

Acknowledgements

Financial support was given by the Marine Living Resources Laboratory of the National Institute of Marine Sciences and Technologies (I.N.S.T.M). We are grateful to Susana Artieda for the English review.

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Received September 2010
Accepted March 2011
Published online March 2013