

Morphological variation of bullet tuna *Auxis rochei* (Risso, 1810) from Tunisian Waters

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Abstract: Information about differentiation is essential for evaluating the stock and behaviour of fish species. In Tunisian and Mediterranean waters, differentiations of bullet tuna *Auxis rochei* based on morphological characters were never studied. Thus, morphological variations among 179 specimens of *A. rochei* from three different sampling localities (Ghar El Melh, Sidi Daoud and Mahdia) along the coast of Tunisia were examined. Univariate and multivariate analyses, such as Principal Component Analysis and Canonical Variate Analysis of 13 morphometric and four meristic characters were carried out. Morphological results for morphometric and meristic characters showed a single group for *A. rochei* populations in Tunisian coastal waters. The three sampled populations showed the absence of morphological differentiation as based on the 13 morphometric characters. The only exception was the number of Bronchial spines Right (BrR) and Left (BrL) meristic characters, which showed a significant difference using Scheffé's post-hoc test between Mahdia, Sidi Daoud and Ghar El Melh localities. Our results confirm the existence of a single stock in morphology of *A. rochei* among these Tunisian localities.

Keywords: *Auxis rochei*, morphometric characters, meristic characters, Tunisian waters

Introduction

The bullet tuna *Auxis rochei* (Risso, 1810) is a commercially important scombrid. It is the smallest among all tuna species with a maximum fork length of about 50 cm (RELINI *et al.* 2008). In Tunisian waters, the size of this fish varies from about 31 cm to 40 cm. In 2007, the catches were 841.8 tons (27.90% from total small tunas; ALLAYA *et al.* 2013). This fish has a worldwide distribution in tropical and subtropical waters (SABATÉS, RECASENS, 2001). The bullet tuna is an epi or meso-pelagic fish with a seasonal coastal distribution in the moderate and tropical coastal zones including the Mediterranean waters (COLLETTE 1986). Also, *A. rochei* is abundant in the Strait of Gibraltar, along the north coast of

Africa and along the Spanish Mediterranean coast (KAHRAMAN *et al.* 2011).

So far, numerous studies have been carried out on *A. rochei* studying some biological (TORRES *et al.* 2011), ecological (MOROTE *et al.* 2008), abundance (BIKRAM *et al.* 2014), and reproductive data (MACÍAS *et al.* 2005, KAHRAMAN *et al.* 2010) in order to better understand the biological traits of the bullet tuna. The biology of this species was studied by many authors for the Mediterranean Sea. Indeed, the spawning period is reported to occur from June to September (SABATÉS, RECASENS 2001, MACÍAS *et al.* 2005, KAHRAMAN *et al.* 2010). In the reproductive season, spawning occurs in several bursts as

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oocyte development is asynchronous (KAHRAMAN *et al.* 2010, TORRES *et al.* 2011).

In Tunisian waters, ALLAYA *et al.* (2013) claimed that the main spawning period of bullet tuna is between May and September, but often it only spans from June to August. Recently, KOCHED *et al.* (2013) analysed the spatial distribution and ecology of *A. rochei* in the Gulf of Gabes (Tunisia) and showed that bullet tuna larvae were the most abundant and widespread in comparison with the larvae of other tuna species. The information concerning the migration patterns of this species is scarce and fragmented (SABATÉS, RECASENS 2001). Several authors have suggested that there is migration from the Atlantic Ocean to the spawning areas in the Mediterranean Sea through the Gibraltar Strait (SABATÉS, RECASENS 2001).

In a recent study, RELINI *et al.* (2008) examined the differentiation of *A. rochei* in the Mediterranean Sea and the Atlantic coast of Morocco and concluded that meristic characters and genetic data (nuclear and mitochondrial sequences) of four geographical samples appeared to be very similar. In their recent survey, ALLAYA *et al.* (2015) sequenced an extensive sample of *A. rochei* for mitochondrial DNA control region in Tunisian waters, and showed no genetic heterogeneity between the sampled populations.

Meristic and morphometric characters are powerful tools to analyse the morphological variability of fish species of the family (SIMON *et al.* 2010, NAKAE *et al.* 2014). Currently, the morphological variation of *A. rochei* in Tunisian waters is poorly understood. The aim of this study is to assess and describe the intraspecific variation in morphometric and meristic characters of *A. rochei* from different locations along the Tunisian coast, in order to analyse the morphological variation using univariate and multivariate analyses.

Materials and Methods

Sampling

A total of 179 specimens of *A. rochei* were collected between May 2009 and July 2010 from three localities along the coast of Tunisia (Fig. 1). These were Ghar El Melh, Sidi Daoud and Mahdia (see Table 1 for details). All samples were collected from commercial catches with different fishing gear (purse seine, light fishing, gill nets, longlines, pelagic trawl, and beach seine).

Morphometric analyses

Thirteen morphometric measurements were taken for each of the 179 adult specimens using digital calipers (VERNIER) and values were rounded to the nearest 0.01 mm. These were: (1) Snout length (SnL), (2)

Eye diameter (ED), (3) Body depth (BD), (4) Head depth (HD), (5) Head length (HL), (6) Distance of pectoral fin (DP), (7) Distance of the first dorsal fin (DD1), (8) Distance of the second dorsal fin (DD2), (9) Distance of ventral fin (DV), (10) Distance of anal fin (DA), (11) Standard length (SL), (12) Fork length (FL) and (13) Total length (TL) (Fig. 2). Based on the obtained results, we calculated the report between all morphometric characters and the percentage of fork length (FL), except snout length (SnL), eye diameter (ED) and head depth (HD) which were expressed as a percentage of head length (HL); fork length was expressed as a percentage of total length (TL) (Fig. 2).

Meristic analysis

The four meristic characters considered here were: dorsal finlets (DF), ventral finlets (VF), the number of branchial spines right (BrR) and left (BrL).

Statistical analysis

For each morphological character analysed, the minimal and maximal value were determined, as well as the arithmetic average, the standard error and the coefficient of variation.

One-way analysis of variance (ANOVA) was applied to test whether the studied morphological characters were influenced by localities. Also, ANOVA with Scheffé's post-hoc test (SCHEFFE' 1959) was used to determine which of the morphometric or meristic variables differed significantly between the three populations. We used (Multivariate analysis of variance) MANOVA in order to test the significant effect between the whole morphometric variables in the three localities.

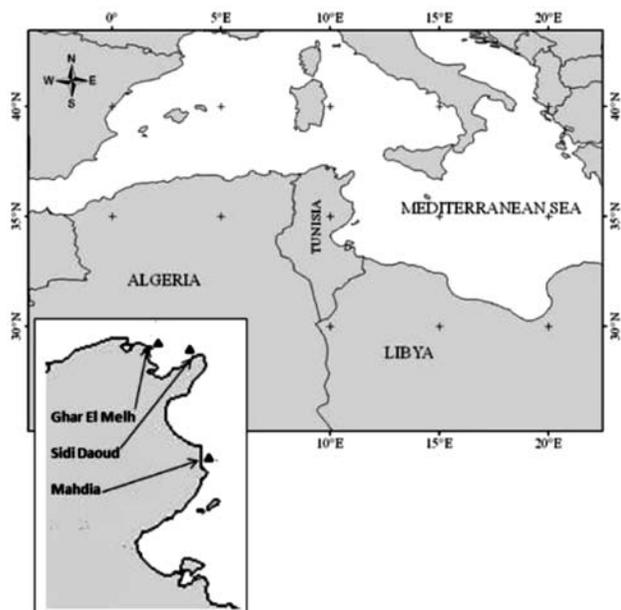


Fig. 1. Sampling localities of *A. rochei* along the coast of Tunisia

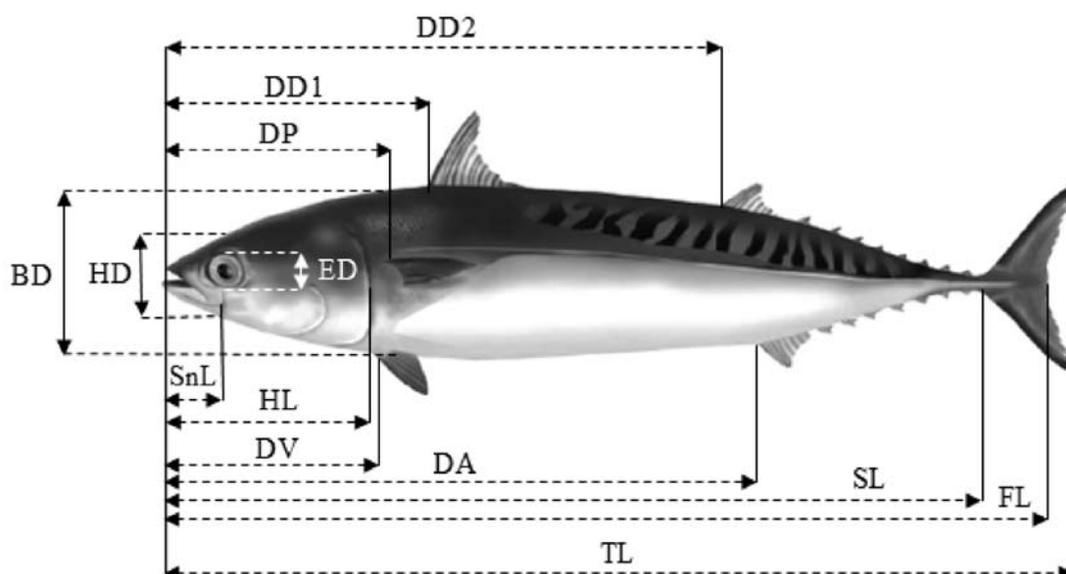


Fig.2. Schematic drawing of *A. rochei* body with measured dimensions [Drawing of an adult *Auxis rochei* (by A. López ‘Tokio’) modified by H. ALLAYA]: (1) snout length (SnL), (2) eye diameter (ED), (3) body depth (BD), (4) head depth (HD), (5) head length (HL), (6) distance of pectoral fin (DP), (7) distance of the first dorsal fin (DD1), (8) distance of the second dorsal fin (DD2), (9) distance of ventral fin (DV), (10) distance of anal fin (DA), (11) standard length (SL), (12) fork length (FL) and (13) total length (TL)

Table 1. Localities and samples sizes from *A. rochei* populations collected along the coast of Tunisia (N is the sample size; Min. and Max are minimum and maximum fork lengths in cm)

Local-ity	Coordinate	N	FL			
			Min.	Max.	X	SD
Ghar El Melh	37°10'07" N 10°10'56" E	9	32.9	39.7	37	2.01
Mahdia	35°29'57" N 11°05'11" E	122	30.7	43.5	36.71	3.12
Sidi Daoud	33°30'08" N 11°07'27" E	48	34	38.9	36.19	1.23
All		179	30.7	43.5	36.58	2.69

The associations of morphometric, meristic characters and populations were assessed using Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA). All variables were transformed into logarithms (\log_{10}) to eliminate the biased effect of large measurements in multivariate analysis (VATANDOUST *et al.* 2014). Principal Components Analysis is basically based upon the variance-covariance matrix of the log-transformed variables. Discriminant function analysis was used to portray relationships based on morphometric variables and to determine to which of the three populations a given individual should be assigned. Morphological character variation was assessed using univariate ANOVA, multivariate analysis (MANOVA). A morphometric variables-based dendrogram was created based on the Euclidean dis-

tance as a measure of dissimilarity and “between three populations” as the clustering algorithm (SNEATH, SOKAL 1973).

All statistical analyses of morphometric parameters were performed using Past v.1.81 (HAMMER *et al.* 2001) and Statistica v.10 (StatSoft, Inc., www.statsoft.com).

Correlation analysis

The relationships between each pair of characters were tested using Pearson’s correlation for data from all three sampling sites and for the whole period. For this statistical analysis, we used Statistica v.10 software and the significance level was at $P < 0.05$.

Results

Morphometric analysis

Results of the morphometric characteristic ratios, length-length equations and relevant parameters are given in Tables 2 & 3. Linear regressions showed the best accuracy for all length-length relationships (Table 2). For Ghar El Melh samples, the best fit was recorded also between distance of pectoral fin (DP) and fork length (FL) ($R^2 = 0.96$), while the lowest value of coefficient of determination was established between head depth (HD) and head length (HL) ($R^2 = 0.352$). For Mahdia samples, the best fit was recorded between standard length (SL) and fork length (FL) ($R^2 = 0.999$), while the lowest value of coefficient of determination was established between HD

Table 2. Length- length relationship of *A. rochei* from Ghar El Melh, Mahdia and SidiDaoud populations

Ghar El Melh		Mahdia		Sidi Daoud	
Equation	R ²	Equation	R ²	Equation	R ²
SnL = 0.328HL+0.451	0.861	SnL = 0.3296HL + 0.4112	0.971	SnL = 0.3151HL + 0.5093	0.696
ED = 0.134HL + 0.402	0.713	ED = 0.1488HL + 0.3128	0.906	ED = 0.0649HL + 0.9634	0.050
BD= 1.127HL – 2.597	0.935	BD = 1.0234HL – 2.0984	0.963	BD = 0.8469HL – 0.9115	0.729
HD = 0.217HL+ 1.609	0.352	HD = 0.3486HL + 0.4161	0.875	HD = 0.1327HL + 2.4381	0.050
HL = 0.272FL- 0.916	0.923	HL= 0.2549FL – 0.1631	0.992	HL = 0.2782FL – 0.9448	0.852
DP = 0.299FL – 1.277	0.960	DP = 0.2815FL – 0.5265	0.988	DP = 0.3217FL – 2.0827	0.827
DD1 = 0.268 FL+ 0.596	0.812	DD1 = 0.2938FL – 0.3426	0.972	DD1 = 0.3532FL – 2.4519	0.745
DD2 = 0.618FL+0.647	0.954	DD2 = 0.6604FL – 1.2251	0.993	DD2 = 0.608FL + 0.6776	0.844
DV = 0.231 FL+ 1.034	0.840	DV = 0.2779FL – 0.567	0.968	DV = 0.2636FL – 0.0224	0.579
DA = 0.727FL – 1.312	0.962	DA = 0.7415FL – 1.9934	0.992	DA = 0.7218FL – 1.4919	0.860
SL = 0.924 FL + 0.511	0.993	SL = 0.9475FL – 0.3734	0.999	SL = 0.9394FL – 0.1132	0.987
FL = 0.272 TL – 0.916	0.923	FL = 0.9675TL – 0.5342	0.996	FL = 0.8592TL + 3.7381	0.917

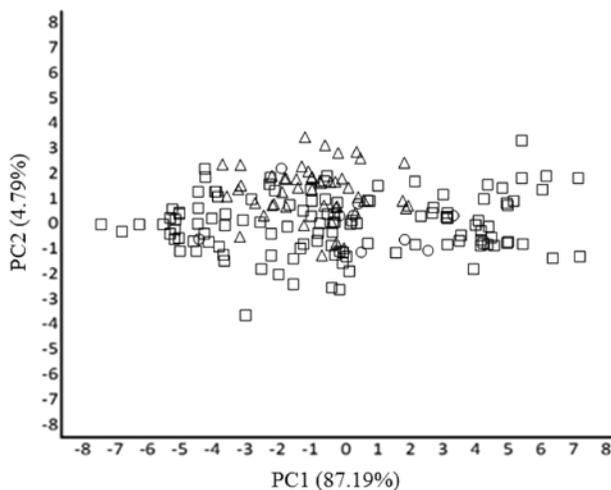


Fig.3. Results of the first (PC1) and second (PC2) principal component analysis for 13 morphometric characters in 179 individuals of *A. rochei* from Tunisia (□,△,○: individuals belonging to Ghar El Melh, Sidi Daoud and Mahdia localities, respectively)

and HL ($R^2 = 0.875$). For Sidi Daoud samples, the best fit was recorded also between SL and FL ($R^2 = 0.987$), while the lowest value of coefficient of determination was established between eye Diameter (ED) and HL ($R^2 = 0.05$; Table 2).

The maximum ratio range of all bullet tuna samples morphometric relationships was noted for HD/HL ($\Delta HD/HL = 14.23\%$). However, the minimum ratio range was noted for HL/FL ($\Delta HL/FL = 2.73\%$). The ratio ranged from 1.13% (DP/ FL) to 5.74% (HD/HL) for Ghar El Melh samples, from 2.74% (HL/FL) to 13.25% (HD/HL) for Mahdia samples and from 2.47% (DP/FL) to 11.36% (HD/HL) for Sidi Daoud samples (Table 3).

General trends of morphometric characters variation among samples of *A. rochei* were surveyed using principal component analysis (PCA) of the 13

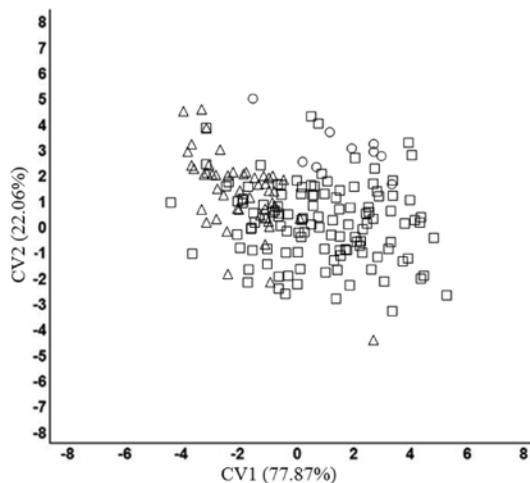


Fig.4. Results of canonical variate analysis (CVA) of the 13 morphometric measurements examined between individuals of *A. rochei*. (□,△,○: individuals belonging to Ghar El Melh, Sidi Daoud and Mahdia localities, respectively)

measurements of 179 individuals clearly showing a single morphometric group for the three populations (Fig. 3). Axis 1 and 2 explained 91.98 % of the total variability. In this analysis, 87.19 % of the total variation was explained by the first component axis while 4.79 % was explained by the second axis.

The Canonical Variate Analysis (CVA) clearly showed the same results as the PCA and demonstrated a single morphometric group for the three sampling sites (Fig. 4). The first canonical axis (CV1) accounted for 77.87% of the total variance while CV2 explained 22.06% of the variance. On the other hand, MANOVA showed no statistically significant difference between the localities for the 13 morphometric characters (Wilk's lambda = 0.394, $F = 0.42$, $P = 0.654$). Moreover, ANOVA did not show any significant difference between the three localities (p

Table 3. Relative relationships of measured body proportion of *Auxis rochei* from Mahdia, Sidi Daoud and Ghar El Melh populations

Variable	Locality	N	Range (%)	X	SD	Variance
SnL/HL	Ghar El Melh	9	35.99-38.85	37.85	0.816	0.666
	Mahdia	122	34.68-40.46	37.63	1.176	1.382
	Sidi Daoud	48	35.07-39.81	37.10	0.865	0.748
	All	179	34.67-40.45	37.50	1.108	1.228
ED/HL	Ghar El Melh	9	16.94-19.49	17.81	0.650	0.423
	Mahdia	122	15.88-21.42	18.42	1.042	1.087
	Sidi Daoud	48	14.05-21.80	17.07	1.248	1.559
	All	179	14.05-21.79	18.03	1.229	1.512
HD/HL	Ghar El Melh	9	36.60-43.34	39.41	2.111	4.459
	Mahdia	122	34.25-47.50	39.37	2.424	5.876
	Sidi Daoud	48	33.27-44.63	40.03	2.580	6.658
	All	179	33.27-47.50	39.54	2.454	6.025
HL/FL	Ghar El Melh	9	24.09-25.42	24.69	0.441	0.195
	Mahdia	122	23.49-26.23	25.02	0.414	0.171
	Sidi Daoud	48	24.49-26.06	25.21	0.409	0.167
	All	179	23.49-26.22	25.05	0.429	0.184
BD/FL	Ghar El Melh	9	19.38-21.91	20.76	0.825	0.681
	Mahdia	122	16.72-22.62	19.71	1.272	1.618
	Sidi Daoud	48	17.58-20.17	18.83	0.616	0.380
	All	179	16.71-22.61	19.54	1.213	1.472
DP/FL	Ghar El Melh	9	25.73-26.86	26.45	0.375	0.141
	Mahdia	122	23.99-28.06	26.72	0.582	0.339
	Sidi Daoud	48	24.84-27.31	26.41	0.544	0.296
	All	179	23.98-28.06	26.62	0.578	0.334
DD1/FL	Ghar El Melh	9	27.03-29.32	28.43	0.703	0.494
	Mahdia	122	25.37-31.01	28.40	0.955	0.913
	Sidi Daoud	48	26.63-29.84	28.54	0.736	0.542
	All	179	25.37-31.01	28.43	0.889	0.791
DD2/FL	Ghar El Melh	9	62.43-64.66	63.59	0.729	0.531
	Mahdia	122	59.72-64.80	62.67	0.993	0.987
	Sidi Daoud	48	60.62-64.27	62.68	0.893	0.797
	All	179	59.72-64.79	62.72	0.974	0.949
DV/FL	Ghar El Melh	9	25.13-26.78	25.91	0.566	0.320
	Mahdia	122	24.32-30.31	26.19	0.893	0.798
	Sidi Daoud	48	24.57-28.86	26.30	0.772	0.596
	All	179	24.32-30.31	26.20	0.849	0.721
DA/FL	Ghar El Melh	9	67.99-70.76	69.17	0.780	0.608
	Mahdia	122	65.30-74.35	68.60	1.389	1.931
	Sidi Daoud	48	66.07-70.80	68.06	0.994	0.988
	All	179	65.29-74.35	68.48	1.298	1.685
SL/FL	Ghar El Melh	9	93.20-94.41	93.79	0.409	0.167
	Mahdia	122	91.26-95.88	93.75	0.464	0.216
	Sidi Daoud	48	92.86-94.28	93.62	0.367	0.135
	All	179	91.25-95.87	93.71	0.440	0.193
FL/TL	Ghar El Melh	9	93.87-96.19	94.82	0.711	0.506
	Mahdia	122	92.66-98.27	95.47	1.063	1.130
	Sidi Daoud	48	93.56-97.79	95.83	1.000	1.000
	All	179	92.66-98.26	95.52	1.052	1.107

> 0.05). However, a significant difference was recorded between the collection date and the 13 morphometric characters (Wilk's lambda = 0.769, F = 0.914, P = 0.023). This result was also confirmed by Scheffé's post hoc test (P < 0.001).

A dendrogram using the Euclidian distance as a measure of dissimilarity between the three populations did not suggest a clear phenotypic differentiation between the three populations, but some relative dissimilarity between both samples from Mahdia and Sidi Daoud on the one hand, and samples from Ghar El Melh on the other hand (Fig. 5). This result showed specimens from Mahdia and Sidi Daoud clustered in the same clade.

Meristic analysis

The meristic characters of *A. rochei*, their range, mode, mean, standard deviation values are given in Table 4. Our results demonstrated the same variation in the three populations (Ghar El Melh, Mahdia and Sidi Daoud) for the number of dorsal finlets (DF) (from 7 to 9). Ghar El Melh locality presents a range of (8-8) whereas for Mahdia and Sidi Daoud the range is (7-9) with 8 for the mode in the three populations. For the ventral finlets (VF) we found a difference between the three localities, so the number ranged from 7 to 7 for Ghar El Melh, from 6 to 8 for Mahdia but from 7 to 8 for Sidi Daoud with mode equal to 7 for the three populations. However, a significant difference of BrL and BrR was revealed between some populations using ANOVA. For BrL meristic character, Scheffé's post hoc test showed a significant difference between Mahdia and Sidi Daoud (P = 0.006) and Mahdia and Ghar El Melh (P = 0.049).

For BrR meristic character, Scheffé's post-hoc test revealed a statistically significant difference between Mahdia and Sidi Daoud populations (P = 0.007). In contrast, MANOVA showed an absence of

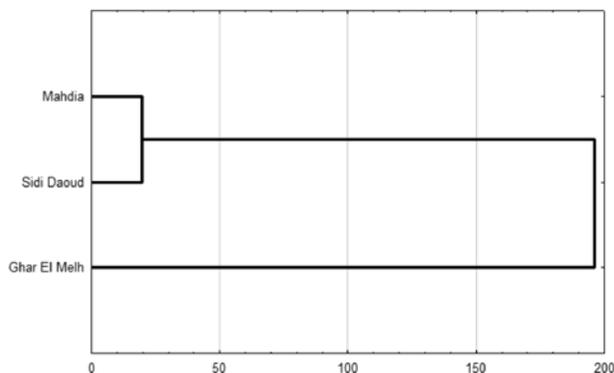


Fig. 5. Relationships between *A. rochei* populations as suggested by cluster analysis based on 13 morphometric variables (Euclidean distances of mean values ± standard deviations)

statistical difference between the four meristic characters and the three localities studied (Wilk's lambda = 0.88, F = 0.06, P = 0.940). However, a significant difference was recorded between the collection date and the four meristic characters (Wilk's lambda = 0.74, F = 1.78, P = 0.002). This result was also confirmed by Scheffé's post-hoc test (P < 0.001).

The scatter plot of the first two principal components for the four meristic characters (PCs) is presented in Fig. 6. Of this total intraspecific variation percentage, component 1 explained 85.69% of this percentage, while component 2 explained 12.80%.

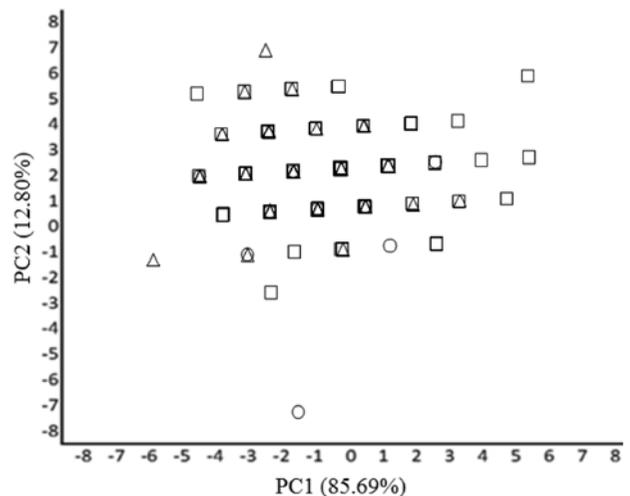


Fig. 6. Results of the first (PC1) and second (PC2) principal component analysis for four meristic characters in 179 individuals of *A. rochei* from Tunisia (□, △, ○: individuals belonging to Ghar El Melh, Sidi Daoud and Mahdia localities, respectively)

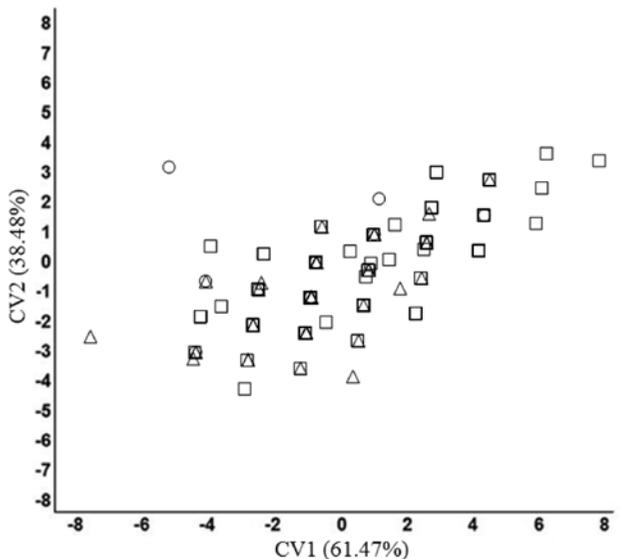


Fig. 7. Results of canonical variate analysis (CVA) of the four meristic characters examined between individuals of *A. rochei* (□, △, ○: individuals belonging to Ghar El Melh, Sidi Daoud and Mahdia localities, respectively)

The distribution of the first two PCs showed a single group for the three populations (Fig. 6). All variables were positively correlated with PC1, which represents an overall size axis. The main loading on this axis was length BrR (0.7300). The second principal component was strongly correlated with BrL (0.7303).

In the CVA, 61.47% of the total variation was expressed by the first canonical variate axis, and 38.48% by the second. The scattergram showed a clear single morphometric group for the three sampling sites (Fig. 7). The first axis was correlated with BrL (0.923) while the second factor was correlated with BrR (0.924).

A dendrogram using the Euclidian distance as a measure of dissimilarity between the three populations clearly revealed a cluster comprising Mahdia and Sidi Daoud samples (Fig. 8).

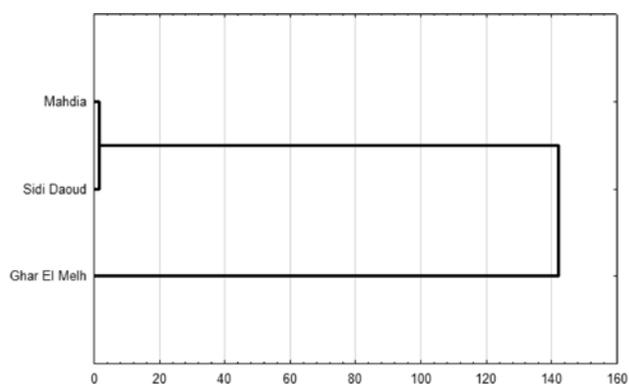


Fig. 8. Relationships between *A. rochei* populations as suggested by cluster analysis based on four meristic variables (Euclidean distances of mean values \pm standard deviations)

Correlation analysis

The correlation coefficients between characters are presented in Tables 5 and 6. Generally, all coefficients between morphometric coefficients were significant for the three populations and close to 1. For the meristic characters, the pairs DF/VF and BrL/BrR showed significant differences. The correlation results revealed that all of the studied meristic variables were not influenced by size.

Discussion

Morphometric analyses using 13 of the size-corrected morphometric characters showed no statistically significant differences among the three populations. Indeed, samples from these three localities had high morphometric similarity between them showing a noticeable overlap of the studied individuals. The similarity revealed here, was likely to have arisen from migrations by adult bullet tuna which could be sufficient to cause global morphological homogeneity. Apart from highly mobile adults, Scombridae species also have buoyant eggs and pelagic larvae (COLLETTE *et al.* 1984). Therefore, dispersal during pre-adult stages may also facilitate this morphometric similarity (ZISCHKE *et al.* 2013). The seasonal movements of several large pelagic species, such as tunas and billfishes, are closely linked with seasonal southward expansion of warm water (Gunn *et al.* 2003, Young *et al.* 2011). Additionally, in the Gulf of Gabes (Southern Tunisian Coasts), the bullet tuna larvae were the most abundant and showed a wide-

Table 4. Meristic characters statistics of *Auxis rochei* from Ghar El Melh, Mahdia and Sidi Daoud populations

Character	Locality	Range	Mode	X	SD
Dorsal finlets (DF)	Ghar El Melh	8-8	8	8	0
	Mahdia	7-9	8	8.007	0.19
	Sidi Daoud	7-9	8	8.02	0.25
	All	7-9	8	8.01	0.20
Ventral finlets (VF)	Ghar El Melh	7-7	7	7	0
	Mahdia	6-8	7	6.97	0.19
	Sidi Daoud	7-8	7	7.02	0.14
	All	6-8	7	6.98	0.18
Number of Branchial spines Right (BrR)	Ghar El Melh	39-44	44	41.8	1.93
	Mahdia	38-46	41	41.98	1.62
	Sidi Daoud	39-45	40	41.14	1.51
	All	38-46	41	41.75	1.64
Number of Branchial spines Right Left (BrL)	Ghar El Melh	38-44	39	40.5	1.84
	Mahdia	39-47	42	41.94	1.52
	Sidi Daoud	37-44	41	41.12	1.31
	All	37-47	42	41.65	1.55

Table 5. Correlation of four meristic characters for the three populations of *A. rochei*

Variable	DF	VF	BrL	BrR
DF	1.00			
VF	0.148045	1.00		
BrL	0.062675	0.085409	1.00	
BrR	0.104603	0.066924	0.739358	1.00

Numbers in bold are significant ($p < 0.05$)

spread distribution over the study areas in comparison with larvae of other tuna species (KOCHED *et al.* 2013). Similar observations have been made in other studies in different parts of the Mediterranean Sea (SABATÉS, RECASENS 2001, ALEMANY *et al.* 2010).

Analyses of the four meristic characters showed that only BrL and BrR had statistically significant differences between the three populations while VF and DF had no such differences. Moreover, the stepwise analysis revealed that these two meristic characters contributed significantly to the multivariate discrimination of the three populations of bullet tuna in Tunisia. Differences in meristic characters between areas could indicate that larval stages have been exposed to different environmental conditions, and this may be interpreted as a probable existence of geographically separated spawning stocks. This hypothesis is supported by RAMLER *et al.* (2014) who claimed that the number of BrR and BrL were determined during their early larval development and influenced by environmental conditions at the sampling sites, especially temperature.

The first two canonical variates showed a very high degree of overlap between individuals from all locations, and they were very close to each other (Fig. 7). According to these findings, two meristic characters (BrL and BrR) showed significant heterogeneity in *A. rochei*. These results are in agreement with results obtained from numerous scombridae species which indicate that differences among samples were associated with the anterior part of the body and coexist within the study area (TURAN 2004; BEKTAS, BELDUZ 2009).

To explore the phenotypic relationships between the examined populations, a dendrogram was constructed based on Euclidean distances, using UPGMA cluster analysis. For both morphometric and meristic characters, the dendrogram showed a relative grouping between samples Mahdia and Sidi Daoud localities indicating that the dissimilarity between specimens from these locations was less important than with specimens from Ghar El Melh. Possible explanation of this phenomenon is that the geographical distance that separate Mahdia from Ghar El Melh is

greater than that between Mahdia and Sidi Daoud. The dissimilarity of Mahdia specimens with those of Ghar El Melh was the highest for both morphometric and meristic characters, thus we could speculate that all studied specimens actually belong to a single population, probably subdivided to many subpopulations, forming schools of fish with high mobility. The morphometric dissimilarity revealed in micro-geographic scales in this paper could be explained by a higher dispersal potential of *A. rochei*, and also was reported in numerous fish species along the coast of Tunisia (ANNABI *et al.* 2013). The meristic characters were much more effective for discriminating between *A. rochei* populations, which expressed lower distances between samples and may be attributable to possible inadvertent sampling of migratory bullet tuna along the Coasts of Tunisia.

The small dissimilarity between morphometric and meristic data showed that unlike meristic characters, which are fixed early in life, morphometric characters typically show ontogenetic changes associated with allometric growth and may be labile to environmental influences throughout life (WAINWRIGHT *et al.* 1991). Besides, there may be some migration between these areas and the meristic data are more sensitive to detect low number of migrants between the areas (HULME 1995). Additionally, the meristic counts are independent of body size or weight (ZAFAR *et al.* 2012).

Here, we found a significant difference between date collection, morphometric and meristic characters for the three localities. The majority of *A. rochei* specimens along Tunisian coast (~ 79%) were collected Between May and December, which was during or immediately after a protracted spawning season (ALLAYA *et al.* 2013). Therefore, if these fish had recently finished spawning and had reached the end of their southward migration, their body condition may be poorer than during other times of the year. In this case, the spawning period in the Mediterranean has been reported to occur from June to September (SABATÉS, RECASENS 2001, KAHRAMAN *et al.* 2010). In Tunisia, ALLAYA *et al.* (2013) suggested that the spawning of *A. rochei* occurred between May and September. We can assume that the missing of differentiation is partly due to sampling which must be done at the same time in all localities and not during the spawning period. This approach will allow the elimination of the possibility of studying samples from the same group of *Auxis rochei* which migrate from one locality to another.

On the other hand, FL of Tunisian specimens of *A. rochei* was comparable to those found in different geographical regions. Indeed, this character

Table 6. Correlation of thirteen morphometric characters for the three populations of *A. rochei*

Variable	SnL	ED	BD	HD	HL	DP	DD1	DD2	DV	DA	SL	FL	TL
SnL	1.00												
ED	0.668670	1.00											
BD	0.887177	0.695805	1.00										
HD	0.658813	0.443658	0.677454	1.00									
HL	0.930979	0.649808	0.910009	0.743210	1.00								
DP	0.908872	0.680653	0.920194	0.693895	0.971589	1.00							
DD1	0.881677	0.609557	0.872033	0.729312	0.939148	0.925203	1.00						
DD2	0.906746	0.667208	0.927780	0.734050	0.967640	0.954133	0.942389	1.00					
DV	0.879035	0.623897	0.880329	0.714057	0.932182	0.909596	0.889917	0.932600	1.00				
DA	0.906762	0.679045	0.924855	0.724626	0.956505	0.952527	0.950301	0.970226	0.924122	1.00			
SL	0.922952	0.676244	0.932061	0.738745	0.977084	0.964544	0.938550	0.982101	0.934103	0.979070	1.00		
FL	0.922551	0.674295	0.930552	0.738274	0.977080	0.964543	0.938259	0.981932	0.930756	0.978425	0.997947	1.00	
TL	0.911550	0.679936	0.932915	0.721919	0.965716	0.954661	0.918397	0.971072	0.919980	0.971156	0.989179	0.9895	1.00

Numbers in bold are significant ($p < 0.05$)

ranged from 34 to 45 cm in the Strait of Gibraltar (RODRIGUEZ-RODA 1966) and from 28.5 to 44.5 cm in Turkish waters (BÖK, ORAY 2001). In addition, FL varied from 33.4 to 47 cm in South-western Spanish Mediterranean (Macias *et al.* 2005) and from 25.9 to 47 cm in Western Mediterranean (MACIAS *et al.* 2006), from 27 to 46.5 cm in Ligurian Sea (PALANDRI *et al.* 2008), and from 34 to 48 cm on the Turkish Mediterranean coasts (KAHRAMAN *et al.* 2010). Consequently, we conclude that our sampling captured a representative size distribution that is similarly reflected in most of the previous studies.

Our morphological results were in agreement with the recent genetic study for *A. rochei* along the coast of Tunisia. ALLAYA *et al.* (2015) sequenced 108 specimens of *A. rochei* sampling from the same populations analysed in this morphological study (Ghar El Melh, Mahdia and Sidi Daoud). They failed to show genetic differentiation between them ($\Phi_{ST} = 0.004$; $P = 0.231$). In addition, this species exhibited high genetic variation yielding a total of 95 different haplotypes with 87% of haplotypes being unique. Also, these three populations had a higher haplotypic and nucleotide diversity ($h = 0.998 \pm 0.002$; $\pi = 0.040 \pm 0.020$, respectively). On the other hand, *A. rochei* demonstrates a history of demographic stability. The timing based on the mismatch value for *Auxis* (between 150 000-170 000), coinciding with the Riss glacial maximum (about 150 000 year ago; ALLAYA *et al.* 2015). The lack of genetic structure is consistent with the expected low degree of genetic differentiation in marine pelagic fishes that have high dispersal capabilities and large effective population sizes (THEISEN *et al.* 2008). In this survey, analyses of morphometric and meristic data provided insight into the phenotypic stock structure of *A. rochei* in Tunisian waters. Our results complement previous research on their global genetic population.

In conclusion, our findings, being the first attempt to study the morphological variation of *A. rochei* in Tunisian waters and the Mediterranean Sea, indicate a morphometric similarity in the bullet tuna among the three sampling localities while two meristic characters have statistically significant differences between populations. We can hypothesize here that *A. rochei* has been evaluated as a single stock in Tunisian coastal waters. Future research should encompass a greater sampling effort with specimens ranging from the extreme north to the extreme south. It should use complementary tools such as other genetic markers, which is needed to compare and validate current research, thereby providing a comprehensive account of the stock structure of the bullet tuna in the Mediterranean Sea.

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References

- ALLAYA H., A. HATTOUR, G. HAJJEJ and M. TRABELSI 2013. Some biological parameters of the bullet tuna *Auxis rochei* (Risso, 1810) in Tunisian waters. – *Cahiers de Biologie Marine*, **54**: 287-292.
- ALLAYA H., A.R. BEN FALEH, A. HATTOUR, M. TRABELSI and J. VIÑAS 2015. Disparate past demographic histories of three small Scombridae species in Tunisian waters. – *Hydrobiologia*, **758**: 19-30.
- ALEMANY F., L. QUINTANILLA, P. VELEZ-BELCHI, A. GARCÍA, D.CORTÉS, J.M. RODRIGUEZ, M.L. FERNÁNDEZ DE PUELLES, C. GONZÁLEZ-POLA, J.L. LÓPEZ-JURADO 2010. Characterization of spawning habitat of Atlantic bluefin tuna and related species in the Balearic Sea (Western Mediterranean). – *Progress in Oceanography*, **86**: 21-38.
- ANNABI A., K. SAID, B. REICHENBACHER 2013. Inter-population differences in otolith morphology are genetically encoded in the killifish *Aphanius fasciatus* (Cyprinodontiformes). – *Scientia Marina*, **77**: 269-279.
- BEKTAS Y., A.O. BELDUZ 2009. Morphological Variation among Atlantic Horse Mackerel, *Trachurus trachurus* Populations from Turkish Coastal Waters. – *Journal of Animal and Veterinary Advances*, **8**: 511-517.
- BIKRAM J.R., K.S. NRIPENDRA, M. GAZIUR RAHMAN, S.M.A. HASAN, M. FOKHRUL ALAM 2014. Abundance of tuna fish species in the Bay of Bengal of Bangladesh region. – *World Journal of Biology and Medical Sciences*, **1**: 26-37.
- BÖK T and I.K. ORAY 2001. Age and growth of bullet tuna *Auxis rochei* (Risso, 1810) in Turkish waters. – *Collective Volume of scientific papers*, **52**: 708-718.
- COLLETTE B.B., T. POTTHOFF, W.J. RICHARDS, S. UEYANAGI, J.L. RUSSO, Y. NISHIKAWA 1984. Scombroidei: development and relationships. In: H. G. Moser *et al.*, eds. Ontogeny and systematics of fishes. – *American Society of Ichthyologists and Herpetologists, Special Publication*, **1**: 591-620.
- COLLETTE B.B 1986. Scombridae. In: White head PJP, Bauchot M-L, Hureau J-C, Nielsen J, and Tortonese E (eds.). Fishes of the North-eastern Atlantic and the Mediterranean. Vol II, 981-997. UNESCO Paris.
- GUNN J.S., T.A. PATTERSON and J.G. PEPPERELL 2003. Short-term movement and behaviour of black marlin *Makaira indica* in the Coral Sea as determined through a pop-up satellite archival tagging experiment. – *Marine and Freshwater Research*, **54**: 515-525.
- HAMMER Ø., D.A.T. HARPER and P.D. RYAN 2001. PAST: paleontological statistics software package for education and data analysis. – *Palaeontology Electronica*, **4**: 1-9.
- HULME T.J 1995. The use of vertebral counts to discriminate between North Sea herring stocks. – *ICES Journal of Marine Science*, **52**: 775-779.
- KAHRAMAN A.E., D. GÖKTÜRK, E.R. BOZKURT, T. AKAYLI and F.S. KARAKULAK 2010. Some reproductive aspects of female bullet tuna, *Auxis rochei* (Risso), from the Turkish Mediterranean coasts. – *African Journal of Biotechnology*, **9**: 6813-6818.
- KAHRAMAN E.A., D. GÖKTÜRK and F.S. KARAKULAK 2011. Age and growth of bullet tuna, *Auxis rochei* (Risso), from the Turkish Mediterranean coasts. – *African Journal of Biotechnology*, **10**: 3009-3013.
- KOCHED W., A. HATTOUR, F. ALEMANY, A. GARCIA and K. SAID 2013. Spatial distribution of tuna larvae in the Gulf of Gabes (Eastern Mediterranean) in relation with environmental parameters. – *Mediterranean Marine Science*, **14**: 5-14.
- MACIAS D., M.J. GÓMEZ-VIVES and J.M. DE LA SERNA 2005. Some reproductive aspects of bullet tuna (*Auxis rochei*) from the South Western Spanish Mediterranean. – *Collective Volume of Scientific papers*, **58**: 484-495.
- MACIAS D., L. LEMA, M.J. GÓMEZ-VIVES and J.M. DE LA SERNA 2006. A preliminary approach to the bullet tuna (*Auxis rochei*) fecundity in the spanish Mediterranean. – *Collective Volume of Scientific papers*, **59**: 571-578.
- MOROTE E.M, P. OLIVAR, P.M. PANKHURST, F. VILLATE and I. URIARTE 2008. Trophic ecology of bullet tuna *Auxis rochei* larvae and ontogeny of feeding-related organs. – *Marine Ecology Progress Series*, **353**: 243-254.
- NAKAE M., K. SASAKI, G. SHINOHARA, T. OKADA, K. MATSUURA 2014. Muscular system in the pacific bluefin tuna *Thunnus orientalis* (Teleostei: Scombridae). – *Journal of Morphology*, **275**: 217-229.
- PALANDRI G., L. LANTERI, F. GARIBALDI, L. R. ORSI 2008. Biological parameters of bullet tuna in the Ligurian Sea. SCRS/2008/057.
- RAMLER D., P. MITTEROECKER, L.N.S. SHAMA, K.M. WEGNER, H. AHNELT 2014. Nonlinear effects of temperature on body form and developmental canalization in the threespine stickleback. – *Journal of Evolutionary Biology*, **27**: 497-507.
- RELINI O.L., G. PALANDRI, F. GARIBALDI, L. LANTERI, F. TINTI 2008. Between lumpers and splitters, which taxonomical approach to mediterranean small tuna of genus *Auxis*? ICCAT/FAO Meeting on small tunas, Malaga May 2008, SCRS/2008/051.
- RODRIGUEZ-RODA J.L 1966. Estudio de la Bacoretta (*Euthynnus alletteratus*) (Raf.) bonito (*Sarda sarda*) (Bloch) y melva, (*Auxis thazard*) (Lac.) capturados por las almadrabas españolas. [in Spanish]. – *Investigaciones Pesqueras Barcelona*, **30**: 120-145.
- SABATÉS A., L. RECASENS 2001. Seasonal distribution and spawning of small tunas, *Auxis rochei* (Risso) and *Sarda sarda* (Bloch) in the northwestern Mediterranean. – *Scientia Marina*, **65**: 95-100.
- SCHIEFFÉ H. 1959. The analysis of variance. John Wiley & Sons, Inc., New York.
- SIMON K.D., Y. BAKAR, S.E. TEMPLE, A. G. MAZLAN 2010. Morphometric and meristic variation in two congeneric archer fishes *Toxotes chatareus* (Hamilton 1822) and *Toxotes jaculatrix* (Pallas,1767) inhabiting Malaysian coastal waters.- *Journal of Zhejiang University Science B*, **11**: 871-879.
- SNEATH P.H.A., R.R. SOKAL 1973. Numerical taxonomy: the principles and practice of numerical classification. W. H. Freeman, San Francisco., 573 p.
- THEISEN T.C., B.W. BOWEN, W. LANIER, J.D. BALDWIN 2008. High connectivity on a global scale in the pelagic wahoo, *Acanthocybium solandri* (tuna family Scombridae). – *Molecular Ecology*, **17**: 4233-47.
- TORRES A.P., P. REGLERO, R. BALBIN, A. URTIZBEREA, F. ALEMANY 2011. Coexistence of larvae of tuna species and other fish in the surface mixed layer in the NW Mediterranean. – *Journal of Plankton Research*, **33**: 1793-1812.
- TURAN C 2004. Stock identification of Mediterranean horse mackerel (*Trachurus mediterraneus*) using morphometric and meristic characters. – *ICES Journal of Marine Science*, **61**: 774-781.
- VATANDOUST S., A. ABDOLI, H. ANVARIFAR, H. MOUSAVI-SABET 2014. Morphometric and meristic characteristics and morphological differentiation among five populations of Brown Trout *Salmo trutta fario* (Pisces: Salmonidae) along the southern Caspian Sea basin. – *European Journal of Zoological Research*, **3**: 56-65.
- WAINWRIGHT P.C., C.W. OSENBERG, G.G. MITTELBACH 1991. Trophic polymorphism in the pumpkinseed sunfish (*Lepomis gibbosus* Linnaeus): effects of environment on ontogeny. – *Functional Ecology*, **5**: 40-55.
- YOUNG J.W., A.J. HOBDDAY, R.A. CAMPBELL, R.J. KLOSER, P.I. BONHAM, L.A. CLEMENTSON, M.J. LANSDELL 2011. The biological oceanography of the East Australian Current and surrounding waters in relation to tuna and billfish catches off eastern Australia. – *Deep Sea Research II*, **58**: 720-733.
- ZAFAR M., A. NAZIR, S.M.H.N. AKHTAR, M. NAQVI, M. ZIAUR-REHMAN 2012. Studies on meristic count and morphometric measurement of Mahseer (Tor putitora) from spawning ground of Himalayan Foot – Hill river Karong Islamabad, Pakistan. – *Pakistan Journal of Biological Sciences*, **5**: 733-735.
- ZISCHKE M.T., S.P. GRIFFITHS, I.R. TIBBETTS and R.J.G. LESTER 2013. Stock identification of wahoo (*Acanthocybium solandri*) in the Pacific and Indian Oceans using morphometrics and parasites. – *ICES Journal of Marine Science*, **70**:164-172.

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