

Morphometric analysis of European clam *Ruditapes decussatus* in Morocco

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Abstract. A morphometric analysis was performed in the European clam *Ruditapes decussatus* from six localities on the Moroccan coast. 240 individuals (40 individuals per site) were collected from Nador (2°86'W 35°17'N), Sidi Moussa (8°74'W 32°98'N), Moulay Bouselham (6°27'W 34°85'N) and Oualidia (9°03'W 32°73'N) lagoon, Bouregreg (6°50'W 34°N) estuarine and Boutalha (15°48'W 23°50'N) (Dakhela bay). The comparison of variance of four morphometric index (elongation index (height/length), compactness index (width/length), convexity index (width/height) and weight linear ratio (weight/length) using Fisher test revealed a significant morphometric difference between the six populations of *R. decussatus* ($p < 0.05$). The principal component analysis and the hierarchical clustering revealed three population groups, suggesting that each group has a defined morphometric characteristic. The generalized additive models (GAMs) showed a significant influence of SST (sea surface temperature) and Chl-a (Chlorophyll a) on the four-morphometric index. The observed morphological variations are likely the consequences of the environmental and ecological conditions.

Key Words: shellfish, bivalve, morphometry, multivariate analysis, Moroccan coast.

Introduction. European clam *Ruditapes decussatus* is one of the most important bivalve in Morocco; it is widely distributed in Mediterranean and along the Atlantic coast from the North Sea to the coast of Senegal (Gharbi et al 2015). For their high filtration capacity, *R. decussatus* is considered as a potential bio indicator species for environmental pollution and contamination (Bebiano et al 2004; Ghazzi et al 2017; Esposito et al 2018). In addition to its ecological importance, *R. decussatus* plays a major socio-economic role in all regions where this species is among the principal target for commercial fisheries. This species constitutes a significant income for local communities in Morocco, especially for women, which are interested in their harvest. Furthermore, for their nutritional and organoleptic values, the European clam is on demand in the European market. For their economic potential, the species is overexploited in Morocco (Kamara et al 2008); intensive fishing pressure can reduce the density of the population and lead to destruction of natural stock.

To ensure suitable exploitation of the species, adequate management measures are required. Identification of morphological variations can be considered in stock discrimination, it can be a useful tool to help identify adequate management measures and strategies to ensure the conservation of species (Caill-Milly et al 2014).

Several authors (Green 1957; Lammens 1967; Innes & Bates 1999; Sousa et al 2007; Caill-Milly et al 2012) have attempted to investigate the morphological variation of bivalves; they recognized that various ecological factors are responsible of bivalve shell morphometry (Cassis et al 2011). This phenotypic plasticity allow bivalves to withstand against environmental condition, it is defined as the ability of a genotype to produce a response according to environmental conditions by more than one alternative form (West-Eberhard 1989; Swain & Foote 1999).

In this work, we focused on morphometric variation analysis of seven *R. decussatus* populations along the Moroccan coast. This study used conventional approach using four morphometric ratios involving metrics and weight. This morphometric analysis can be a useful tool to provide scientific information to help fisheries manager to establish adequate management and conservation measures, for a sustainable exploitation of the resource. This kind of analysis is advisable before exploring more powerful markers such as genetic markers.

The aim of the present study was to report morphometric variation of seven populations of *R. decussatus* using four classic morphometric variables.

Material and Method

Sample collection and study area. Six sites along the Moroccan coast were selected for a morphometric analysis study of *R. decussatus*: Nador (Na), Sidi Moussa (SM), Oualidia (Ou), and Moulay Bouselham (MB) Lagoon, Bouregreg (Bou) Estuary, and Boutalha (Bot) (Dakhela bay) (Figure 1). Fourteen individuals per site were collected by hand during low tide; the clams were stored in a plastic bag with cold accumulator and transferred immediately to laboratory.

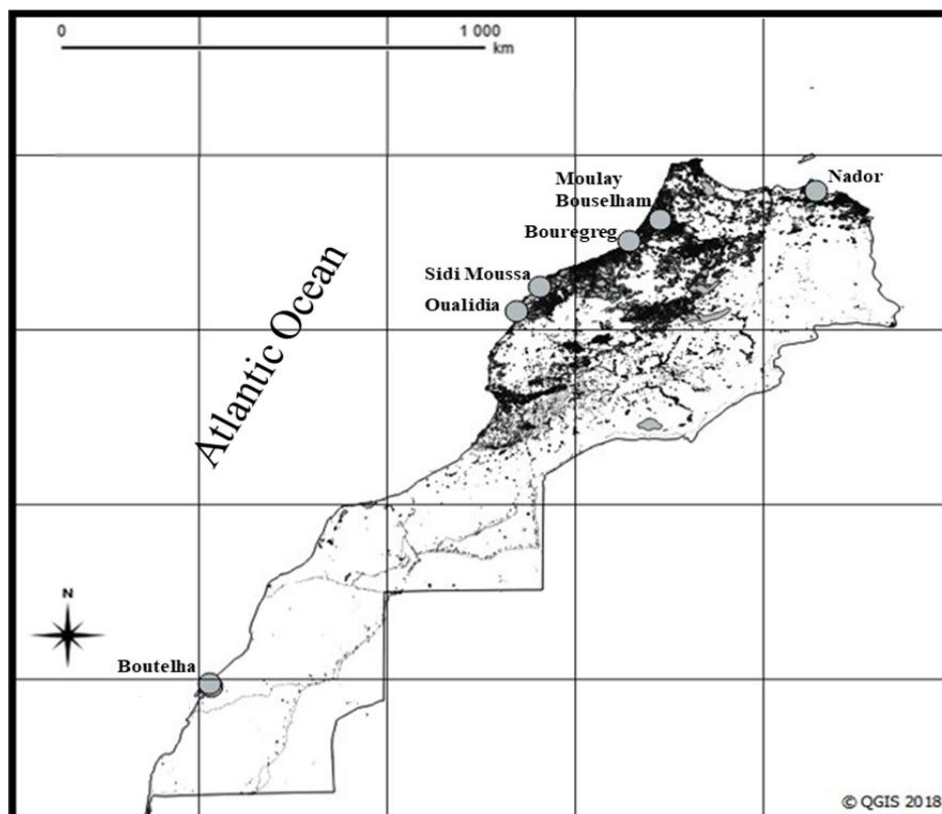


Figure 1. Map of Morocco showing location of the sampling sites (grey dots).

Morphometric characteristics. In the laboratory, all shells were cleaned, and for each individual, three linear measures and one weight measure were obtained. The width (W_i), the height (H_e), the length (L_e) were measured using an electronic digital caliper (0.01 mm accuracy) and the total weight (W_e) was measured by a precision scale (0.01 g accuracy):

The length (L_e): is defined as the longest distance from the front edge to the back. It is the reference length obtained from the lateral view (in millimeters).

The height (H_e): is defined as the distance from the umbo to edge obtained from the lateral view (in millimeters).

The width (W_i): defined as longest distance of the valve in the lateral plane across the valve obtained from the ventral view (in millimeters).

The weight (W_e): is defined as the total mass of shell (in grams).

Four classic variables were used to describe and compare the individuals morphometry of each site (Caill-Milly et al 2014), including three sharpness indices defined as the convexity index (Width/Height), the compactness index (Width/Length), the elongation index (Height/Length) and one linear ratio (Weight/Length).

Univariate analysis. The univariate analysis is a statistical study of a single variable, or of several variables considered independently, in order to describe the sample. In this study, we used an ANOVA to analyze the variance of the four-morphometric indexes for the 6 populations, $p < 0.05$.

Principal component analysis and hierarchical clustering. The multivariate analysis refers to any statistical technique used to analyze data that arise from more than one variable; the technique is used to summarize the continuous information across multiple dimensions while taking into account the effects of all variables on the responses of interest. Two types of statistical investigation were performed.

To summarize and simplify the data carried by the four variables (the convexity index (W_i/H_e) the compactness index (W_i/L_e), the elongation index (H_e/L_e) and the linear weight ratio (W_e/L_e), we constructed a principal component analysis (PCA). In order to identify the homogenous population groups based on the four-morphometric index. A hierarchical cluster analysis was carried out according to Ward's method with Euclidean distance. The simple z standardization was used to rescale the four variables. The statistical methods were carried out by SPSS software version 22.

Environmental conditions. Two parameters, the temperature and the chlorophyll a, were used to assess the influence of environmental conditions on bivalve shell morphometry. The data of sea surface temperature (SST) and chlorophyll a concentration (Chl-a) for each site (Table 1), were imported from the NASA's OceanColor Web by the Moderate Resolution Imaging Spectroradiometer (MODIS) on the Aqua satellite (at a spatial resolution of 4 km) and extracted by the SeaDAS software version 7.4. (Baith et al 2001). The correlation between the four-morphometric index and the environmental conditions were tested by calculate the Pearson's correlation coefficient. We used generalized additive model (GAM) to investigate the influence of the SST and the Chl-a on the four-morphometric index on RStudio, Version 1.1.463.

Table 1
Sampling periods, number of individuals sampled, mean chlorophyll a and mean sea surface temperature ($^{\circ}\text{C}$) at the six study sites

Specification	Boutalha	Nador	Moulay Bouselham	Oualidia	Bouregreg	Sidi Moussa
Sampling date	August 2016	April 2017	October 2016	Mai 2016	September 2016	Mai 2016
Mean chlorophyll a (mg m^{-3})	7.73	0.25	1.69	1.73	0.45	2.27
Mean sea surface temperature ($^{\circ}\text{C}$)	23.83	18.34	18.83	17.95	21.90	18.01

Results

Morphometric characteristics. For the 240 individuals, the length, the height, the width and the weight were, (25.86–49.36) (min-max), (20.29–33.94), (14.18–24.23) and (5.30–22.01) respectively.

The boxplot (Figure 2), showed the differences of length, height, width and weight among the six populations. The median of length, height, width and weight seems to be different amongst all populations.

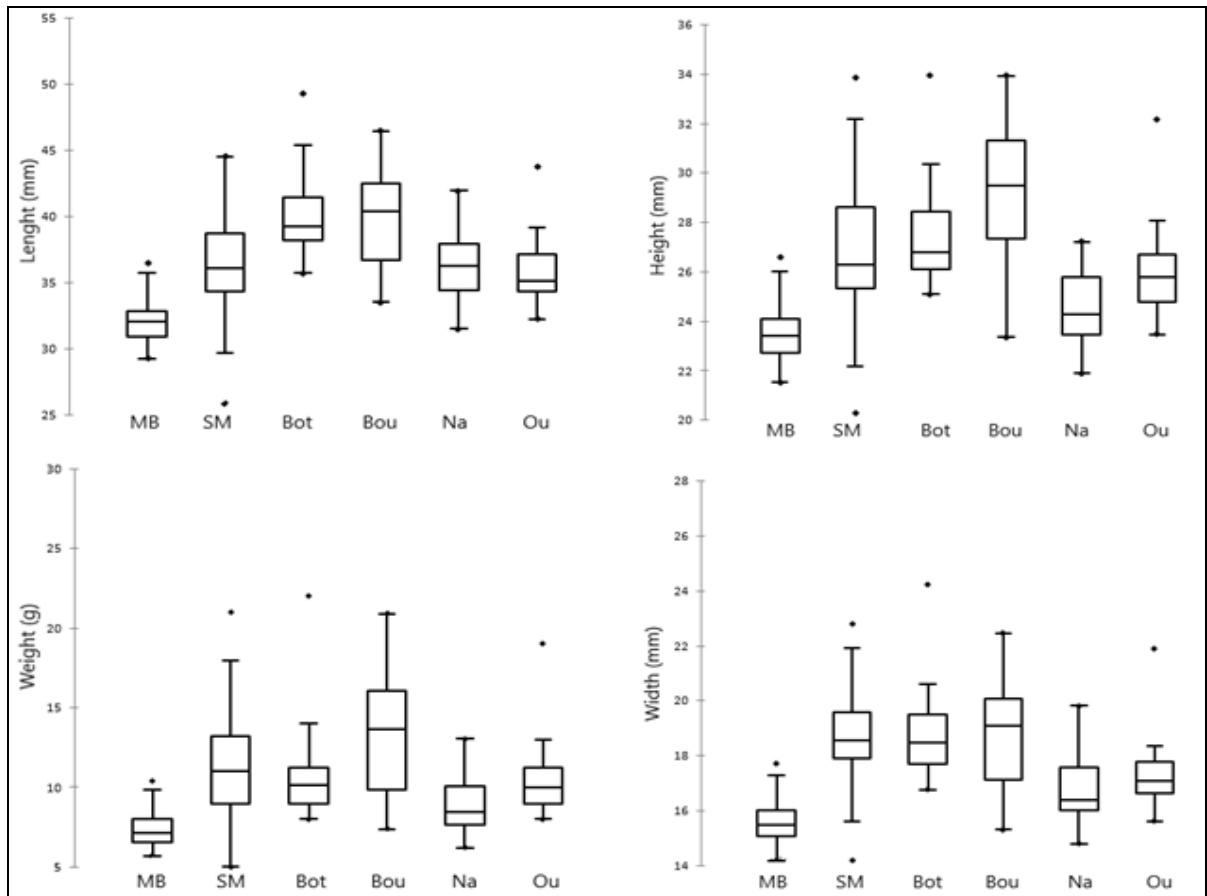


Figure 2. Boxplot displaying the four variables (length, weight, height and width) data for the six populations (Na: Nador, MB: Moulay Bouselham, SM: Sidi Moussa, Bou: Bouregreg, Bot: Boutalha, Ou: Oualidia).

Univariate analysis. The variance analysis of the four morphometric index (Wi/He), (Wi/Le), (He/Le) and (We/le), showed significant differences among sites ($p < 0.05$), see (Table 2). In particular, the shells from Nador had a lower mean (Wi/Le) index, suggesting the dome of the valve are less curved than the valve from Sidi Moussa shell, which had greater mean (Wi/Le) value. Individuals from Sidi Moussa displayed overall greater mean of elongation index (He/Le) and convexity index (Wi/He), suggesting they are dense and more convex. Individuals from Nador had the lower mean value of elongation index (He/Le) suggesting that shells are the more slender. Individuals from Moulay Bouselham had the lower mean (We/Le) ratio, the valve seems more lighter than valve from Sidi Moussa, which had a higher (We/Le) ratio.

Table 2

Variance analysis of four-morphometric index for the six populations of *Ruditapes decussatus*

Parameter	Sites	M	Var	F	P
Wi/Le	Na	0.462	2.70 10 ⁻⁴	36.79	9.55 10 ^{-28*}
	Bot	0.466	3.88 10 ⁻⁴		
	SM	0.514	6.96 10 ⁻⁴		
	MB	0.486	2.60 10 ⁻⁴		
	Ou	0.483	4.33 10 ⁻⁴		
	Bou	0.469	3.91 10 ⁻⁴		
He/Le	Na	0.675	2.56 10 ⁻⁴	78.27	5.39 10 ^{-48*}
	Bot	0.682	4.47 10 ⁻⁴		
	SM	0.739	6.27 10 ⁻⁴		
	MB	0.733	3.28 10 ⁻⁴		
	Ou	0.725	3.61 10 ⁻⁴		
	Bou	0.726	2.79 10 ⁻⁴		
Wi/He	Na	0.684	4.11 10 ⁻⁴	20.34	7.59 10 ^{-17*}
	Bot	0.683	6.29 10 ⁻⁴		
	SM	0.695	6.19 10 ⁻⁴		
	MB	0.663	4.15 10 ⁻⁴		
	Ou	0.667	7.21 10 ⁻⁴		
	Bou	0.647	8.73 10 ⁻⁴		
We/Le	Na	0.242	7.46 10 ⁻⁴	29.79	2.23 10 ^{-23*}
	Bot	0.254	2.07 10 ⁻³		
	SM	0.312	4.53 10 ⁻³		
	MB	0.228	5.65 10 ⁻⁴		
	Ou	0.298	1.53 10 ⁻³		
	Bou	0.334	5.31 10 ⁻³		

M - mean, Var - Variance, F - Fisher test, P - p-value, * - significant, p<0.05.

Principal component analysis. The correlation matrix derived from the principal component analysis, revealed significant correlations between variables, exception for correlation between weight linear ratio and convexity index (Table 3).

Table 3

Correlations matrix for the four-morphometric index

Specification	(Wi/Le)	(Wi/He)	(We/Le)
(He/Le)correlation	0.635*	-0.224*	0.323*
Sig.	0.00	0.00	0.00
N	240	240	240
(Wi/Le) correlation		0.610*	0.291*
Sig.	-	0.00	0.00
N		240	240
(Wi/He)correlation	0.610*		0.036
Sig.	0.00	-	0.291
N	240		240
(We/Le)correlation	0.291*	0.036	
Sig.	0.00	0.291	-
N	240	240	

The eight values and the scree plot allowed to determine only two principal components, accounted for 80.82% of the total variance, (Table 4 & Figure 3). The axe 1 and 2 displayed 48.65% and 32.16%, respectively.

Table 4

Eigenvalues of correlation matrix and related statistics of the PCA

Component	Initial eigenvalues		
	Total	% of variance	Cumulative %
1	1.946	48.656	48.656
2	1.287	32.168	80.825

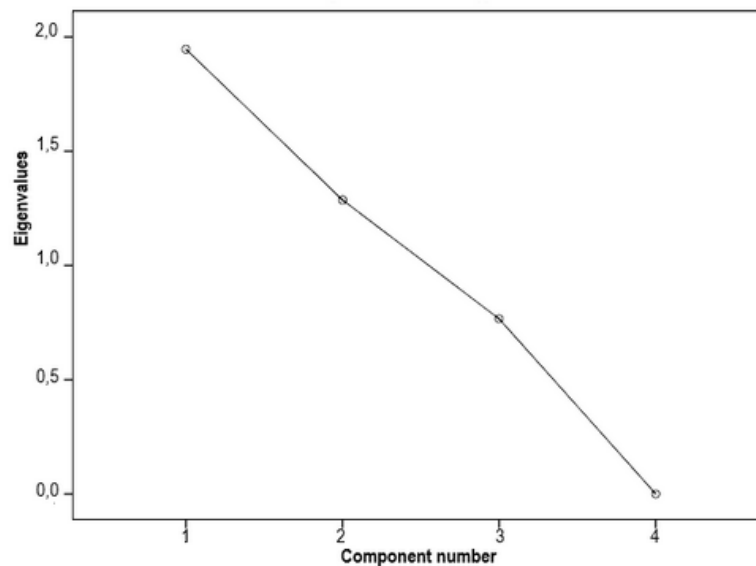


Figure 3. Scree plot indicating the eigenvalues for each of the components derived during the PCA.

Eigenvectors of the two retained components showed that elongation index and weight linear ratio loaded on PC1, The compactness index and convexity index are loaded on PC2, (Table 5 & Figure 4). In this case, the Principal Component 1 is relative to variation in thinness and heaviness, contrariwise the Principal Component 2 account for variation in shape of valve (compactness and convexity).

From the scatter plot, (Figure 5), PCA differentiated Boutalha and Nador from Sidi Moussa and from Bouregreg, Moulay Bouselham and Oualidia. Boutalha was seen to be intercepted within Nador, suggesting that the two populations have almost the similar shape. Same thing was observed for Moulay Bouselham and Oualidia that were intercepted within Bouregreg. This differentiation indicates the morphological variability among all populations.

Table 5

Eigenvectors for PCA on the four-morphometric index

Parameter	Component	
	1	2
Weight linear ratio	0.386	-0.047
Compactness index	0.298	0.422
Elongation index	0.569	-0.157
Convexity index	-0.207	0.694

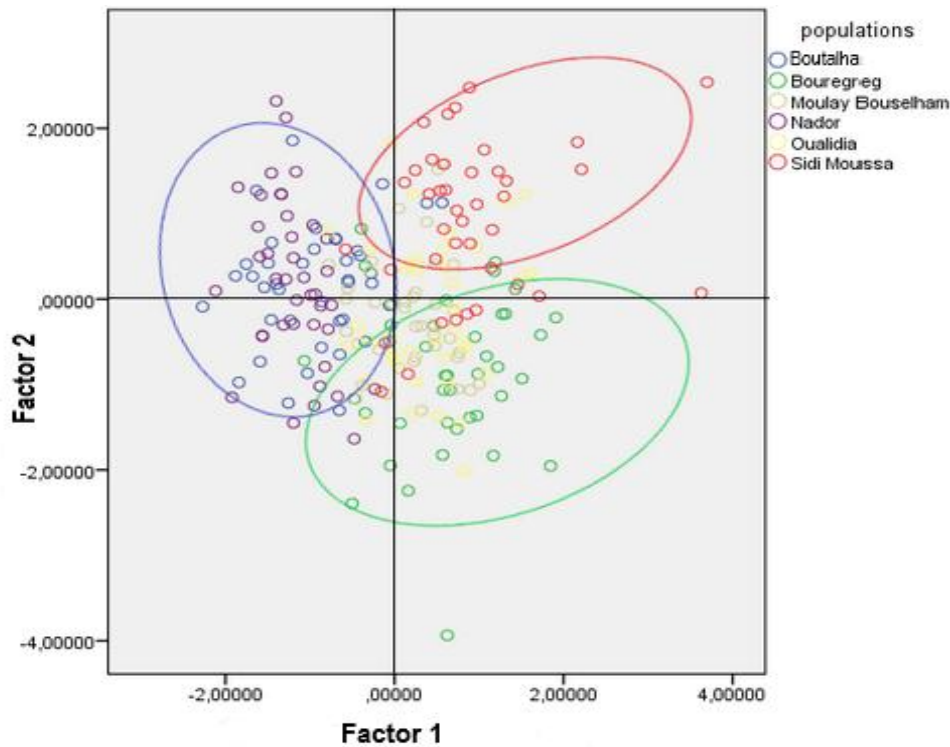


Figure 4. Loading plot derived from the PCA for the four-morphometric index.

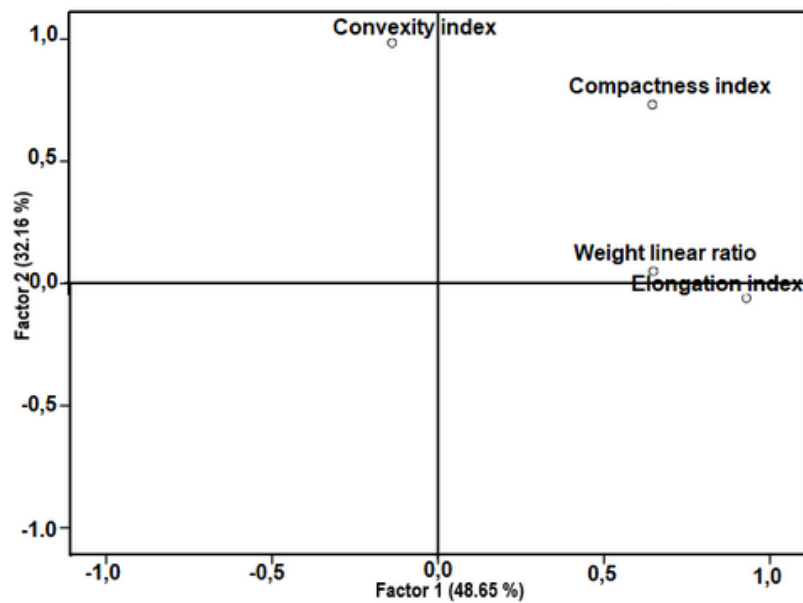


Figure 5. Scatter plot of the PCA of the six populations based on the four-morphometric indexes.

Hierarchical clustering. Based on the dendrogram (Figure 6), we had identified a three clusters, the first cluster associate Boutalha and Nador populations. The second cluster associate Moulay Bouselham, Oualidia and Bouregreg. Sidi Moussa population constitutes the last cluster. This classification confirms the result obtained by the principal component analysis.

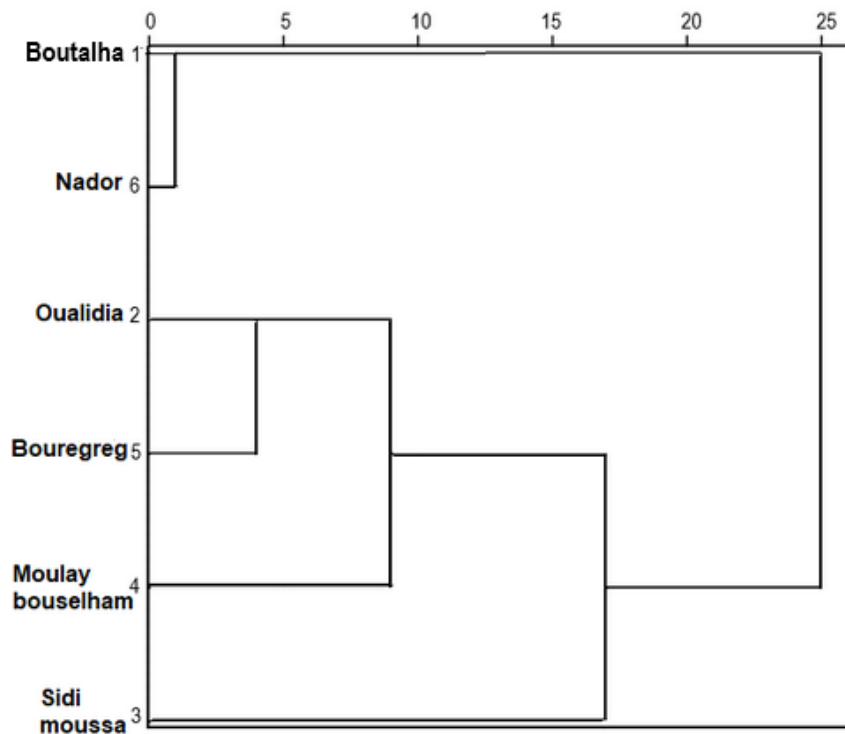


Figure 6. Dendrogram clustering of the 6 populations based on the four morphometric indexes.

Environmental conditions. The morphometric index were significantly related to the environmental conditions (SST and Chl-a), $p < 0.05$, except for the weight linear ratio that was not significantly correlated to SST and the compactness index that was not significantly correlated to Chl-a, (Table 6).

Table 6
Correlation matrix showing relations among the four morphometric index and environmental conditions

Specification		Convexity index	Compactness index	Elongation index	Weight linear ratio
SST	Correlation	-0.131*	-0.351**	-0.305**	0.027
	Sig.	0.043	0.000	0.000	0.673
	N	240	240	240	240
Chl-a	Correlation	0.198**	-0.066	-0.280**	-0.152*
	Sig.	0.002	0.306	0.000	0.019
	N	240	240	240	240

* significant at $p < 0.05$, ** significant at $p < 0.01$.

The generalized additive model showed that the variance explained by the predictors was ranging from 5.41 to 17.2%, (Table 7). The convexity index and the compactness index are the most significant explanatory variables, explaining 16% and 17.2% of the variance, respectively. The sea surface temperature influences negatively the compactness, convexity and the elongation index. The Chl-a influence positively the compactness and the convexity index.

Table 7

Variance and significance values (p levels) for the generalized additive model predictors

Predictors	P	Variance explained (%)
Convexity index	2.10^{-16}	16 %
Elongation index	2.10^{-16}	9.82 %
Weight linear ratio	0.0002	5.41 %
Compactness index	2.10^{-16}	17.2 %

Discussion. A morphometric analysis was carried out for six populations of *R. decussatus* in Morocco, using statistical comparison method and multidimensional analysis methods. It clearly shows a significant morphometric variation among the populations. Comparing variances of the four-morphometric index using Fisher test showed a strong significant heterogeneity between all populations. Derbali et al (2012) who worked on the pearl oyster *Pinctada imbricata radiata* in Tunis found the same result, the comparison of the averages and the variances of the biometric characters using the Fisher test "F" and the Student test "t" revealed an important inter-population morphological variability between El Kraten and El Jorf populations. Sidi Moussa population has a greater value of elongation index that was similar to observations made on Manila clams from Arcachon and Bellevue in France (Caill-Milly et al 2014).

The principal component analysis (PCA), allowed distinguishing three groups of populations, suggesting that each populations group present a defined morphometric characteristics. A group one assembling Boutalha and Nador populations characterized by thin and light shells. A second group constituted by Oualidia, Moulay Bouselham and Bouregreg populations characterized by a heavy and thicker shell. The last group formed by Sidi Moussa population characterized by a dense and thicker shell with convex shape.

Based on the four-morphometric variables, the hierarchical clustering identified also three groups of populations, this confirms the result obtained by the PCA.

A comparative study of the biometric characters in the pearl oyster *P. imbricata radiata* from the Golf of Gabes in Tunis revealed a high divergence between *P. imbricata radiata* populations, using both multidimensional methods (Principal component method and discriminating factor analysis) (Derbali et al 2012).

As many authors have explained, differences found among the shape clams shells could be explained as the outcome of the phenotypic plasticity of the populations subjected to different environmental conditions (Watanabe & Katayama 2010). The salinity, the temperature, the dissolved oxygen, the nature of the substrate and the concentration of food are factors that influence the growth and the morphometry of shell (Sousa et al 2007; Costa et al 2008). In southern African sandy beaches, Laudien (2003), studied morphological variation in four populations of the surf clam *Donax serra*. The morphological comparisons between width vs. height and height vs. length revealed a significant difference in shell shape. Clams from the cold province were significantly rounder, flatter and less wedge-shaped to increased stability in the subtidal habitats and clams from the warm province were more wedge shaped and elongate because they burrow faster and swash-ride more efficiently and thus, are less exposed to predators. From the late Middle Miocene Central Paratethys Sea, a morphometric analysis was performed to *Polititapes tricuspis* species from two localities: the upper Ervilia Zone and the Sarmatimactra Zone using a multimethod approach; the analyses showed significant differentiation between specimens from the two biozones. Neubauer et al (2013) suggest that the phenotypic differentiation to be functional adaptations. The habitats of *P. tricuspis* becomes more exposed to high wave action and tidal activity, for it the species develop a larger and thicker shells with stronger cardinal teeth to offer a higher mechanical stability.

In the six studied populations, Nador and Boutalha clams seems to be light and develop a thin and flat shell, it reflects the post-larval ontogeny of bivalves and the environmental conditions in which they live.

Despite being known as a potential site for aquaculture development, the lagoon of Nador (in the Mediterranean sea) underwent high risks of agricultural, urban and

industrial pollution (Maanan et al 2015) and the high salinity rate during the lagoon closure period (Philippe 1982) which may be at the origin of the morphometric and growth change of shells. The high rate salinity and the bioaccumulation of metallic elements can influence the metabolic process of bivalve (Fuhrmann et al 2016).

The southern Atlantic zone of Morocco is known by a permanent upwelling (Makaoui et al 2017). Despite the trophic richness of the whole zone, Boutalha population possesses a thinner and compact shell. We can suggest that clams do not need to accumulate a lot of nutritive reserve, as long as there is an abundance of nutrients. Watanabe & Katayama (2010) reported also that individuals with better nutritional condition had smaller relative shell thickness.

In addition, The nature thicker rounder and dense of shell from Sidi Moussa, may probably help clams to retain a maximum of water and bury deep because of the presence of predators especially the green crab which is a redoubtable predator next the gastropod *Cymbium* sp. in the lagoon of Sidi Moussa (Maanan 2003). Thicker shells can reveal nutrient storage in different body organs by the species in lower trophic abundance zone.

The General Additive Models revealed significant relationships between the four-morphometric index and the environmental conditions (SST and Chl-a). As an indicator of thickness and density, the elongation index and the weight linear ratio were linked negatively with the Chl-a. Because usually the propitious trophic conditions stimulate the bivalve growth (Steffani & Branch 2003), we hypothesize that the high permanent abundance of nutritional element do not require accumulation of reserve by the bivalve. Same observation were made for the *Venerupis philippinarum* on the French Atlantic coast (Caill-Milly et al 2014), between the chlorophyll a concentration and the elongation index and the weight surface ratio. The SST was negatively correlated to the convexity, compactness and elongation index. Although the species have tolerance for high temperature variation, it appears that it has a negative effect on bivalve growth; high temperatures could alter the productivity and organismal physiology (Talmage & Gobler 2011).

Conclusions. In this work, we revealed the morphometric characteristics of six populations of *R. decussatus* along the Moroccan coast. The identification of morphological variation of shells can constitute a prerequisite to help establish adequate management measures and set up aquaculture trials along the Moroccan coast, because phenotypic variation could reflect about environmental condition. A genetic study on the identification of *R. decussatus* populations is in progress as second steps of this work; it will discriminate between study populations and define the degree of connectivity between them. This information will be used to manage and conserve this species.

Acknowledgements. This study was financially supported by the National Institute of Fisheries Research, Casablanca, Morocco, and the Faculty of Science Rabat, Mohammed V University, Morocco.

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Received: 23 July 2019. Accepted: 23 September 2019. Published online: 30 September 2019.

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How to cite this article:

Amane Z., Tazi L., Idhalla M., Chlaida M., 2019 Morphometric analysis of European clam *Ruditapes decussatus* in Morocco. *AAFL Bioflux* 12(5):1623-1634.