

A morphometric and meristic investigation of Lusitanian toadfish *Halobatrachus didactylus* (Bloch and Schneider, 1801): evidence of population fragmentation on Portuguese coast*

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SUMMARY: A total of 207 specimens of Lusitanian toadfish *Halobatrachus didactylus* from six different localities were analysed in terms of morphometric and meristic characters in order to investigate the hypothesis of population fragmentation on the Portuguese coast. This study confirmed the relative isolation between the Algarve populations and those from the western estuaries, which also showed a high degree of discrimination among them. Morphometric characteristics were much more adequate than meristic characters for a good separation of these populations. The most relevant morphometric characteristics for separation between *H. didactylus* populations were head width, interorbital width and pelvic fin length. These three variables were very useful for separation between western populations and seem to play an important role in species feeding activity. The caudal peduncle development is mainly related to the specimen's swimming ability and was particularly relevant for discrimination between northern and southern populations and between estuarine and adjacent marine populations, apparently due to differences in hydrodynamic conditions. Meristic variation is mostly of a latitudinal nature (mainly nasal tentacles and pores on the lower jaw) but some differences between estuarine and marine populations (principally fin rays counts) related to water temperature were also observed.

Key words: *Halobatrachus didactylus*, Lusitanian toadfish, population fragmentation, Portugal, morphometric and meristic analysis.

RESUMEN: ANÁLISIS MORFOMÉTRICA Y MERÍSTICA EN SAPO LUSITÁNICO *HALOBATRACHUS DIDACTYLUS* (BLOCH AND SCHNEIDER, 1801): EVIDENCIA DE FRAGMENTACIÓN POBLACIONAL EN LA COSTA PORTUGUESA. – Para investigar la hipótesis de fragmentación de las poblaciones portuguesas de sapo lusitánico *Halobatrachus didactylus*, se han analizado un total de 207 ejemplares de esta especie, provenientes de seis localidades distintas, a nivel de sus características morfométricas y merísticas. El estudio ha confirmado el aislamiento relativo de las poblaciones del Algarve y de sus homólogas de los estuarios de la costa occidental, demostrando estas últimas, de igual modo, un elevado grado de aislamiento entre ellas. Las características morfométricas se han revelado mucho más adecuadas que los caracteres merísticos, para la separación de poblaciones de la especie. De las primeras, las más relevantes son la anchura de la cabeza, la distancia interorbital y la longitud de la aleta pélvica. Estas tres características, que parecen desempeñar un papel importante en la alimentación de la especie, son especialmente útiles en la discriminación de las poblaciones de la costa occidental. El desarrollo del pedúnculo caudal está sobretodo relacionado con la capacidad natatoria y es muy relevante para la separación entre poblaciones septentrionales y meridionales, y entre las originarias de medios estuarinos y marinos adyacentes, aparentemente, debido a las diferencias en las condiciones hidrodinámicas que se verifican en estas zonas. Las variaciones en los caracteres merísticos tienen, fundamentalmente, un origen latitudinal (sobretudo los tentáculos nasales y los poros submaxilares), aunque se hayan encontrado también diferencias entre las poblaciones estuarinas y marinas (principalmente en el número de rayos de las aletas) atribuibles a los distintos regímenes térmicos de estos ambientes.

Palabras clave: *Halobatrachus didactylus*, sapo lusitánico, fragmentación poblacional, Portugal, análisis morfométrica y merística.

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INTRODUCTION

The Lusitanian toadfish is a benthic and solitary fish, often buried in soft sediment or concealed in rock crevices (Roux, 1986). It inhabits coastal areas and brackish water environments, but never moves into freshwater (Sobral and Gomes, 1997). Although highly adapted to dominating in habitats with already established populations, this species shows some difficulties in the dispersal and colonisation of new territories due to its ecological characteristics: low fecundity (Almeida *et al.*, 1997), benthic eggs and larvae, parental care with the offspring (Maigret and Ly, 1986) and marked sedentarity of adults (Muzavor *et al.*, 1993).

Its distribution ranges from the southern Bay of Biscay to the Gulf of Guinea, but from Cabo Carvoeiro (centre of Portugal) northwards (Fig. 1) only some digressive specimens can be found (Costa and Costa, in press). Therefore, the south-western Iberian Peninsula is roughly its northern limit of distribution. Because of some abiotic constraints, in Portugal the species seems to be well established only on the Algarve coast, both in marine and brackish water environments, and in three western estuaries, namely Tagus, Sado and Mira (Costa and Costa, in press). Thus, a certain degree of isolation should be expected among western *H. didactylus* populations and between these and Algarve's populations.

Morphometric and meristic studies have provided useful results for identifying marine fish stocks and describing their spatial distributions (Ihssen *et al.*, 1981) and were used here as a first step to analyse the potential fragmentation of Portuguese *H. didactylus* populations.

The objectives of the present study are to (i) assess and describe geographic variation in morphological and meristic characters of *H. didactylus* from different Portuguese coastal locations; (ii) identify the best set of characters for group separation; and (iii) relate the observed variations with the specific ecological constraints of each population.

METHODOLOGY

Samples

To test the hypothesis of population fragmentation along the Portuguese coast, morphometric and meristic characteristics of *H. didactylus* specimens caught during 1995 and 1996 at six different locali-

TABLE 1. – Fishing devices used at each sampling site and sample size. The symbols used in this work to represent each location are also indicated.

Sampling Sites	Symbols	Fishing Devices	Sample Size
Tagus	T	Beam trawl	39
Sado	S	Beam trawl	40
Mira	M	Beam trawl	40
Arade	A	Beam trawl + fishing rod	40
Portimão	P	Beam trawl	29
Monte Gordo	G	Gill nets	19
Total	-	-	207

ties were compared. They were captured with beam trawls, gill nets and fishing rods at Tagus, Sado, Mira and Arade estuaries and in the open sea in front of Portimão and Monte Gordo (Table 1 and Fig. 1). These locations were selected to optimise spatial coverage within *H. didactylus* distribution in Portugal, including marine and brackish water populations. The selected sampling network allowed the comparison among western populations and

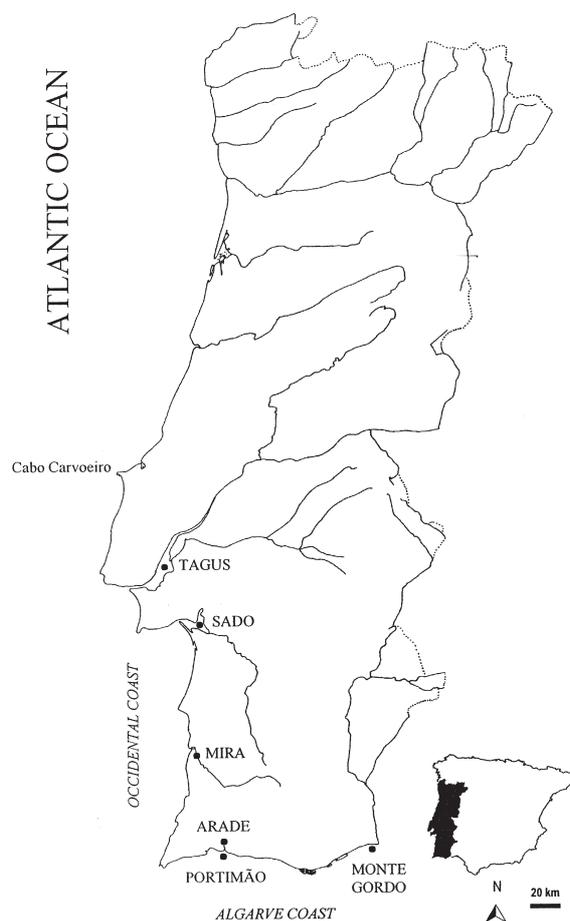


FIG. 1. – Location of the sampling sites.

TABLE 2. – Morphometric and meristic characters used for analysis of *H. didactylus* geographic variation.

Characters	Acronyms
Morphometric Analysis	
Total length	TL
Standard length	SL
Pre-first-dorsal fin length	PDL1
Pre-second-dorsal fin length	PDL2
Pre-anal (anus) length	PAL
Head width	HW
Distance between anterior nostrils	IND1
Distance between posterior nostrils	IND2
Interorbital width	IOW
Caudal peduncle depth	CPD
Pectoral fin length	PTFL
Pelvic fin length	PVFL
Second-dorsal fin length	SDFL
Anal fin length	ANFL
Caudal fin length	CDFL
Meristic Analysis	
Second-dorsal fin rays counts	DFR
Pectoral fin rays counts	PFR
Anal fin rays counts	AFR
Caudal fin rays counts	CFR
Total number of pores on the lower jaw	POR
Total number of barbells on the lower jaw	BAR
Total number of tentacles on the anterior nostril	TNT
Maximum number of rows of teeth on the upper jaw	UJT
Maximum number of rows of teeth on the lower jaw	LJT
Number of rows of teeth on vomer	VMT
Number of rows of teeth on palatines	PLT

between these and the Algarve populations. Furthermore, it permitted the comparison between the specimens from the eastern and western Algarve and even from brackish water environments and adjacent marine areas (Arade and Portimão). Samples ranged from 19 to 40 individuals per location (Table 1) and specimens were frozen before laboratory treatment.

Laboratory work

After complete thawing linear measurements were made with callipers on each specimen and recorded to the nearest 0.5 mm. These operations were performed mainly as described by Roux (1971). However, some additional morphometric

characteristics were considered in this work due to their potential importance for *H. didactylus* population discrimination: second-dorsal, anal and caudal fin lengths and internasal distances. On the other hand, eye diameter was not measured in the present study as a consequence of the orbit distortion induced by the freezing and thawing processes. At the end a total of 15 morphometric characteristics were obtained (Table 2), the length of pectoral and pelvic fins being measured on the left side of the animals.

Meristic counts were also performed mainly as prescribed by Roux (1971). However, in this work the number of caudal fin rays was also recorded, but counts of first-dorsal fin rays, pores of lateral lines and vertebrae were not performed due to their lack of variability (first-dorsal fin rays and vertebrae) or because they were time consuming (pores of lateral lines and vertebrae). As before, counts of pectoral fin rays, tentacles on anterior nostrils and rows of palatine teeth were done on the left side of the animals. Thus, a total of 11 meristic characters were analysed in this study (Table 2).

Sex of 143 specimens was determined by macroscopic examination of the respective gonads, and this subset was used to test the hypothesis of no sexual dimorphism in morphometric and meristic characters of *H. didactylus*.

Data analysis

Because of the variation in size of fish from different areas (Table 3), morphometric and meristic data were statistically adjusted to permit comparative analysis in terms of shape and counts independently of size (Thorpe, 1976). The morphometric measurements were first transformed to common logarithms because linearity and normality are usually more closely approximated by logarithms than by original variables (Hair *et al.*, 1998). Outliers were detected

TABLE 3. – Characteristics of samples obtained for morphometric and meristic analyses.

Groups	Morphometric Analysis		Meristic Analysis		TL Range
	N	Mean TL ± SE	N	Mean TL ± SE	
Tagus	38	186.9 ± 10.5	38	186.9 ± 10.5	103 - 296
Sado	37	182.1 ± 5.7	40	180.2 ± 5.5	103 - 250
Mira	34	185.7 ± 7.1	37	183.0 ± 6.6	100 - 257
Arade	37	164.8 ± 2.9	38	164.7 ± 2.9	136 - 214
Portimão	27	225.9 ± 3.7	26	227.5 ± 3.4	176 - 255
Monte Gordo	19	219.5 ± 6.6	19	219.5 ± 6.6	169 - 263
Total	192	190.2 ± 3.2	198	189.0 ± 3.1	100 - 296

N - sample size; TL - total length in mm; SE - standard error for the mean

by regression analysis of morphometric characters against total length and by scatter plots of residual versus predicted values (Schaefer, 1991a). Any value greater than three standard deviations from the mean was considered an outlier (Anonymous, 1997). When an outlier was found, all the morphometric data (but not the meristic counts) of that fish were withdrawn from further consideration (Schaefer, 1991a). This procedure resulted in the elimination of 15 specimens from the analysis (Tables 1 and 3).

Each of the other 14 morphometric characters showed a linear relationship with total length when analysed by sample (all regressions were significant at $p \leq 0.01$). Analysis of covariance (ANCOVA) was employed to test for differences in allometric relationships among samples, and to estimate the common within-group regression slopes (Sokal and Rohlf, 1995). Within-group regression slopes were significantly different ($df = 5, 180$; $p \leq 0.05$) for six of the morphometric characters and only for IND2 were no differences between adjusted means found at $p \leq 0.05$ ($df = 5, 185$). Coefficients from the common within-group regression are used to allometrically adjust variates when between-group heterogeneity exists (Reist, 1986). Thus, size adjustments were based on the common within-group slopes. The measurements of the morphometric characters were adjusted to those expected for the overall mean total length with a modification of the allometric formula given by Claytor and MacCrimmon (1987):

$$AC_i = \log OC_i - [\beta \times (\log TL_i - \log MTL)];$$

where AC_i is the adjusted logarithmic character measurement of the i th specimen; OC_i is the unadjusted character measurement of the i th specimen; β is the common within-group regression coefficient of that character against total length, after the logarithmic transformation of both variables; TL_i is the total length of the i th specimen; and MTL is the overall mean total length.

Reist (1985) has proved that this allometric adjustment effectively removes size variation and Claytor and MacCrimmon (1987) have shown that it is an appropriate procedure for objective analysis of the data when there is size overlap among the groups examined. Data from male and female Lusitanian toadfish were pooled in subsequent analyses because analysis of variance - ANOVA (Sokal and Rohlf, 1995) for mean values of all adjusted morphometric characters indicated no significant differences ($df=1, 139$; $p > 0.05$) between sexes (Schaefer, 1991a).

Meristics, which are discrete variables, were not normally distributed even after logarithmic, square root or arcsine transformations. However, the analyses applied in this investigation are robust to deviations from normality (Manly, 1989; Sokal and Rohlf, 1995). Hence, we followed Claytor *et al.* (1991) and proceeded as if raw variables were normally distributed but provide this information as a cautionary note. Six of these meristic characters (BAR, TNT, UJT, LJT, VMT and PLT) also showed a linear relationship with total length (regressions were significant at $p \leq 0.001$) and were treated in the same way as morphometric variables for detection and removal of outliers and for size adjustment (within-group regression slopes were significantly different for these six variables at $p \leq 0.001$; $df = 5, 186$). Naturally, for the five meristic characters uncorrelated with total length ($df = 5, 186$; $p > 0.05$) no size adjustment of the data was performed and outliers were detected by the Boxplot procedure (Anonymous, 1997). Following this publication all cases that had values more than 3 hspreads (one hspread corresponds to the interquartile range) below the lower hinge or above the upper hinge were eliminated. Once again, detection of an outlier for any meristic variable caused the removal of that fish from further consideration in meristic analysis (but not for morphometric studies). The application of this procedure resulted in the elimination of 9 specimens from this analysis (Tables 1 and 3).

Conversely to morphometric characteristics, ANOVA for one of the meristic characters (BAR) showed significant differences ($df = 1, 142$; $p \leq 0.01$) between males and females and was not considered in subsequent analyses. Thus, at the end only the 10 meristic characteristics that did not show any difference ($df = 1, 142$; $p > 0.05$) between males and females were studied in this work, the data from both sexes being pooled together.

Meristic characters are represented by discrete or non-continuous data, which means that their predictive ability is likely to be less than that of continuous data (i.e. morphometric data); thus, the two types should clearly be treated separately, especially when multivariate procedures are employed (Ihssen *et al.*, 1981). Discriminant analysis is particularly suitable for this kind of study (Claytor and MacCrimmon, 1987), being the most appropriate statistical technique when separation of more than two groups defined a priori is desired (Schaefer, 1991b). In this study it was used to determine the similarity between Portuguese populations of *H. didactylus*

and the ability of meristic and morphometric characters to correctly identify the origin of the Lusitanian toadfish specimens.

Morphometric and meristic data from the six locations were compared separately by means of multiple discriminant analysis, basically according to the methodological steps and statistical tests indicated in Anonymous (1997). Employing the selection rule that maximises minimum Mahalanobis distance (D^2) between groups, stepwise procedures were used to choose the combinations of variables that best separate those groups (Hair *et al.*, 1998). Canonical variates (scores from the individual discriminate functions, which are linear combinations of the original variables) were used to examine the effectiveness of the size adjustment procedures. Thus, canonical variates 1 and 2 were regressed against total length and size was considered to be effectively removed if regressions were not significant at $p \leq 0.001$ (Clayton and MacCrimmon, 1987). A chi-square transformation (χ^2) of Wilks' lambda (Λ) was used to test equality among the group centroids and whether all canonical variables reflected population differences or only random variation (Anonymous, 1997). When one or more of these canonical variables were deemed not statistically significant, the discriminant models were reestimated with the number of functions to be derived limited to the number of significant functions (Hair *et al.*, 1998). The F statistic proportional to Hotelling's T^2 statistic and to D^2 was used to test the equality of means (centroids) for each pair of groups (Afifi and Clark, 1990). The results of these pairwise group comparisons were corrected by the application of the Dunn-Sidak method using Holm's procedure (Sokal and Rohlf, 1995). The graphical display of canonical variates 1 and 2 was also useful for demonstrating differences between groups because fish that belong to the same group appear closer together on the plot than fish from different groups (Schaefer, 1991a). The resultant discriminant functions were used to classify individual fish into groups. The expected actual error rates of the classification functions were estimated using cross-validation by the leaving-one-out procedure (Anonymous, 1997). The classification accuracy for the analyses was tested by Press's Q procedure (Hair *et al.*, 1998). Discriminant loadings and potency index were used to assess the relative importance of each independent variable in discriminating between the groups (Hair *et al.*, 1998). Discriminant loadings measure the

simple linear correlation between each independent variable and the canonical variables. The potency index is a relative measure among all variables that is indicative of each independent variable's discriminating power.

As a complement to discriminant analysis an unweighted pair group with arithmetic averages (UPGMA) cluster analysis was applied separately to matrices of D^2 for morphometric measurements and meristics counts to illustrate the global relationship between samples (Hair *et al.*, 1998). Mahalanobis distances were obtained from F statistics values computed for each pairwise comparison between groups' centroids by the formula given by Afifi and Clark (1990).

The Mantel test (Sokal and Rohlf, 1995) was used to estimate the degree of association between morphometric or meristic distances (D^2) and geographic separation (in kilometres) for the studied populations. According to those authors, a total of 249 permutations were performed for each Mantel test.

As a complement to multivariate analysis, significant differences in adjusted morphometric characters and in adjusted or unadjusted meristic characters (according to their correlation with total length) were determined using the ANOVA and the a-posteriori SS-STP procedures (Sokal and Rohlf, 1995).

All mathematical procedures were performed using SPSS statistical package (Anonymous, 1997) with the exception of cluster analysis and the Mantel test, which were respectively executed using NT-SYS (Rohlf, 1993) and BIOMstat programs (Rohlf and Slice, 1995).

RESULTS

Morphometric analysis

The regressions for canonical variates 1 and 2 of discriminant analysis against total length were not significant at $p \leq 0.001$ ($r^2 = 0.00$, $df = 190, 1$; and $r^2 = 0.02$, $df = 190, 1$), indicating that size effects had been removed from the morphometric variates.

The Wilks' lambda tests indicated differences between the six groups when their morphometric characters were compared by means of discriminant analysis, all estimated canonical variables being significant (Table 4). Thus, it was worth keeping all five functions for further analysis. The stepwise analysis revealed that 10 of the 14 adjusted morphometric characters contributed significantly to the

TABLE 4. – Results of the Wilks' lambda (Λ) tests (functions 1 through 5) to verify the hypothesis that the means (centroids) of all five functions (the five canonical variables) are equal in the six groups when their morphometric and meristic characters were separately compared by stepwise discriminant analyses. The other successive tests were useful for identifying whether or not the additional functions reflect population differences or only random variation.

Function(s)	Morphometric Analysis			Meristic Analysis		
	Λ	χ^2	df	Λ	χ^2	df
1 through 5	0.07	489.44***	50	0.33	209.38***	30
2 through 5	0.20	296.54***	36	0.58	104.90***	20
3 through 5	0.44	150.95***	24	0.78	47.00***	12
4 through 5	0.66	77.10***	14	0.96	8.31 ^{n.s.}	6
5	0.88	22.40**	6	1.00	0.81 ^{n.s.}	2

*** $p \leq 0.001$; ** $p \leq 0.01$; n.s. - $p > 0.05$

multivariate discrimination of the six groups of fish (Table 5). The correct classification rates estimated from the cross-validation procedure for the 10-variable discriminant function ranged from 56.8 to 79.4%, with an overall rate of 68.2% (Table 6), which was significantly better than chance (Press's $Q = 367.54$; $df = 1$; $p \leq 0.001$).

The complete separation of the centroid values for each group was indicated by all pairwise group comparisons ($df = 10, 177$; $p \leq 0.05$) and confirmed by the plot of the first two canonical variates (Fig.

TABLE 5. – Potency index for variables included in the discriminant functions estimated separately for morphometric and meristic characters. Discriminant loadings for all studied characteristics and percentage of variance explained by the first two canonical variables (CV) are also presented for both analyses.

Variables	Potency index ⁽¹⁾	Discriminant loadings	
		CV1	CV2
Morphometric analysis			
SL	x	0.22	-0.18
PDL1	x	-0.19	-0.26
PDL2	0.09	-0.27	-0.34
PAL	0.09	0.11	-0.21
HW	0.11	0.40	0.24
IND1	0.07	-0.10	0.35
IND2	x	0.00	-0.01
IOW	0.14	0.49	-0.30
CPD	0.09	-0.15	0.48
PTFL	0.08	0.32	-0.17
PVFL	0.14	-0.38	-0.43
SDFL	0.08	0.32	0.21
ANFL	x	0.11	-0.02
CDFL	0.06	-0.22	0.18
% of Variance	-	46.0	29.9
Meristic analysis			
DFR	x	0.02	0.28
PFR	0.11	-0.06	0.51
AFR	0.11	0.11	0.59
CFR	0.11	-0.00	-0.28
POR	0.16	0.48	0.24
TNT	0.43	0.86	0.01
UJT	0.08	-0.11	0.43
LJT	x	0.03	0.15
VMT	x	0.10	0.02
PLT	x	0.02	0.00
% of Variance	-	53.9	26.2

⁽¹⁾x - variables not included in the models

2a), which account for 46.0 and 29.9% of total variation (Table 5). Although there is a noticeable overlap of individuals, particularly of fish from the Algarve, the specimens of the western estuaries (Tagus, Sado and Mira) are fairly distinct from those from the south. Different populations from the western coast also showed much higher heterogeneity than the Algarve ones.

The dendrogram (Fig. 3a) derived from the cluster analysis based on morphometric characters also

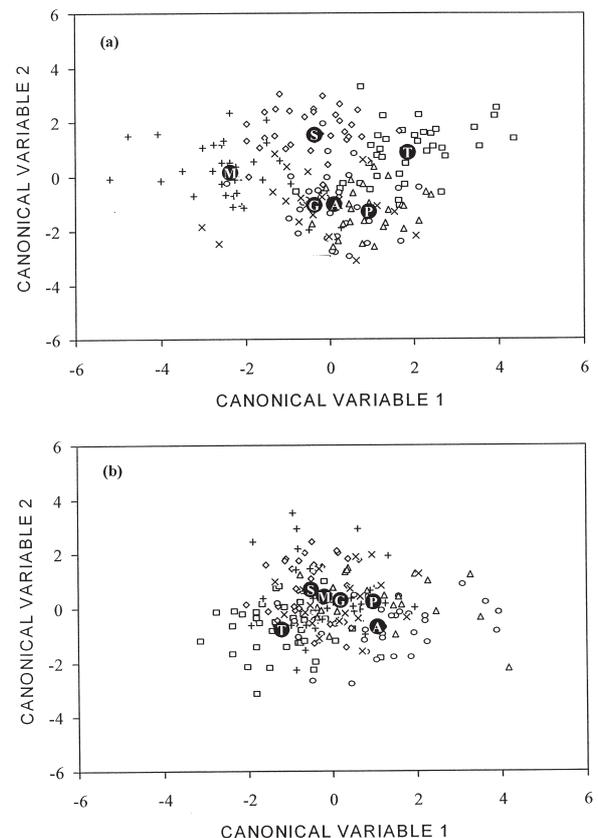


FIG. 2. – Plot of individuals and group centroids on canonical variables 1 and 2 for the six groups of Lusitanian toadfish, based on the morphometric (a) and meristic (b) characters. Symbols for individual fish: (□) Tagus, (◇) Sado, (+) Mira, (○) Arade, (△) Portimão, (x) Monte Gordo. Symbols for population centroids (●) as indicated in Table 1.

TABLE 6. – Classification results of stepwise discriminant analyses for morphometric and meristic characters.

Groups ⁽¹⁾	N	Percent correct	T	Number of Fish Classified into Groups				G
				S	M	A	P	
Morphometric analysis								
T	38	73.7	28	0	0	3	6	1
S	37	73.0	1	27	3	5	1	0
M	34	79.4	0	2	27	0	2	3
A	37	56.8	2	3	2	21	6	3
P	27	63.0	3	0	0	5	17	2
G	19	57.9	0	2	1	2	3	11
Total	192	68.2	-	-	-	-	-	-
Meristic analysis								
T	38	68.4	26	6	3	3	0	0
S	40	57.5	6	23	4	5	0	2
M	37	32.4	8	7	12	1	6	3
A	38	44.7	5	4	2	17	9	1
P	26	23.1	3	6	0	7	6	4
G	19	5.3	3	7	0	3	5	1
Total	198	42.9	-	-	-	-	-	-

⁽¹⁾symbols for the six groups as indicated in table 1; N - sample size

confirms the high degree of heterogeneity among western populations and the relative homogeneity of those from the Algarve. The Mantel test showed that the morphometric distances (D^2) between *H. didactylus* populations were not associated with their geographic distances ($r = 0.26$; $t = 1.01$; $p > 0.05$). Thus, it seems that at least some of the studied populations have been differentiating quite apart from the others, suggesting some degree of isolation from each other.

None of the morphometric variables could be well distinguished from the others with regard to discriminating power (Table 5). However, PVFL, IOW and HW showed slightly superior potency index values and can be considered the most important individual morphometric characters for discriminating *H. didactylus* populations. These three characteristics also showed the highest values of correlation with the first canonical variable of the discriminant analysis (Table 5), which is particularly useful

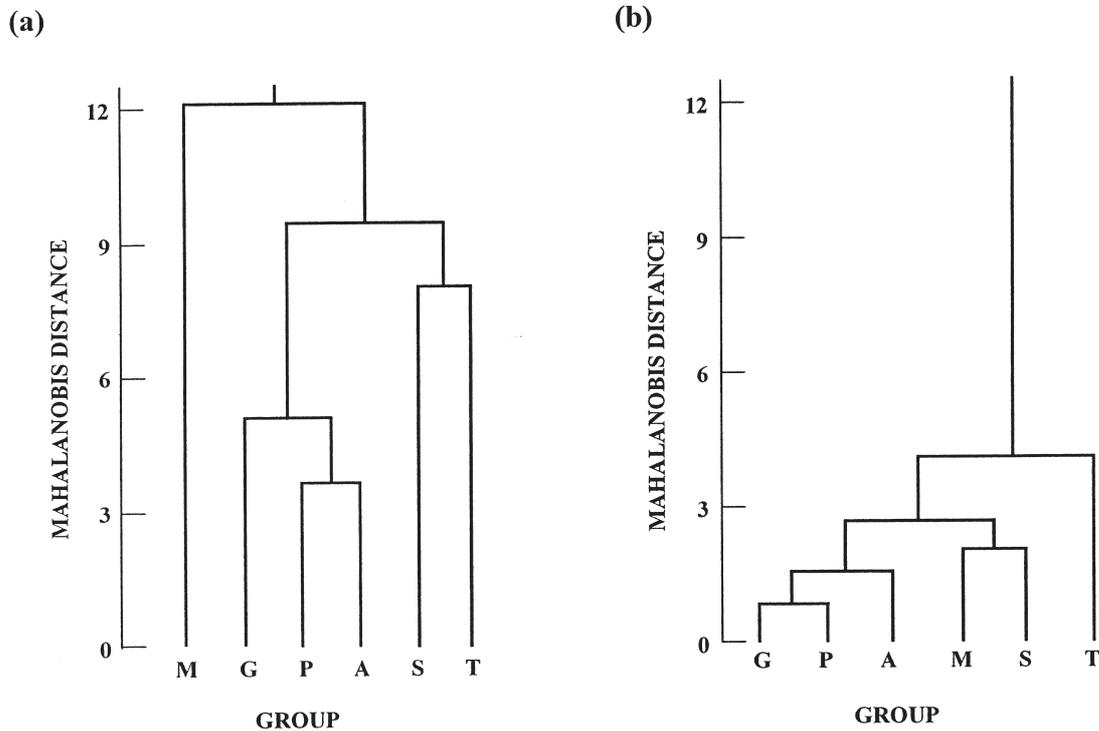


FIG. 3. – Dendrograms derived from cluster analyses of Mahalanobis distances for morphometric measurements (a) and meristic counts (b). Symbols for sampling sites as indicated in Table 1.

TABLE 7. – Univariate comparison of adjusted morphometric characters for the six groups.

Variables	Mean Value for Each Group ⁽¹⁾						ANOVA + SS-STP a-posteriori Test Results ⁽²⁾		
	T	S	M	A	P	G			
SL	2.199	2.194	2.195	2.195	2.200	2.200	5.76***	–	S M A T G P _____
PDL1	1.742	1.746	1.767	1.754	1.749	1.745	13.16***	–	T G S P A M _____
PDL2	1.867	1.862	1.885	1.878	1.874	1.874	12.01***	–	S T G P A M _____
PAL	1.940	1.924	1.936	1.952	1.936	1.921	13.18***	–	G S M P T A _____
HW	1.764	1.735	1.722	1.730	1.742	1.714	15.97***	–	G M A S P T _____
IND1	1.066	1.067	1.077	1.043	1.046	1.020	10.56***	–	G A P T S M _____
IND2	1.282	1.282	1.275	1.272	1.280	1.282	1.21 ^{n.s.}	×	–
IOW	1.443	1.407	1.391	1.438	1.454	1.418	21.30***	–	M S G A T P _____
CPD	1.163	1.191	1.169	1.155	1.152	1.160	14.03***	–	P A G T M S _____
PTFL	1.663	1.663	1.633	1.671	1.675	1.665	11.69***	–	M S T G A P _____
PVFL	1.471	1.498	1.523	1.516	1.520	1.511	21.20***	–	T S G A P M _____
SDFL	1.878	1.861	1.855	1.855	1.868	1.849	12.36***	–	G A M S P T _____
ANFL	1.720	1.712	1.699	1.708	1.718	1.709	4.49***	–	M A G S P T _____
CDFL	1.502	1.530	1.522	1.524	1.500	1.502	9.25***	–	P G T M A S _____

⁽¹⁾symbols for these groups as indicated in table 1; ⁽²⁾df = 5, 186; *** p ≤ 0.001; n.s. - p > 0.05; rules join non-significant subsets

for separation among western populations (Fig. 2a). Specimens from Tagus had large values of HW and IOW and short PVFL, while individuals from Mira showed an inverse variation of these characters and those from Sado an intermediate pattern (Table 7).

The second canonical variable is very important for the discrimination between populations from the Algarve and those from the western coast (Fig. 2a), CPD and PVFL being the most valuable morphometric characters for this separation (Table 5). Southern specimens (including those from Mira) showed large PVFL and western individuals high CPD (Table 7).

For a clear separation among the three populations from Algarve we must make use of the remaining morphometric characteristics. That is the case of PAL, which showed much larger values for the spec-

imens from Arade than for the individuals from Portimão and Monte Gordo (Table 7). Since both marine populations showed also larger SL and shorter CDFL (Table 7), they obviously present great development of the body region included between the anus and the caudal fin. Nevertheless, specimens from Portimão exhibit an intermediate pattern between those from the Arade estuary and Monte Gordo in what concerns to PAL variable (Table 7).

Meristic analysis

As for morphometric data, the regressions for canonical variates 1 and 2 of discriminant analysis against total length were not significant at p ≤ 0.001 (r² = 0.00; df = 196, 1 for both), indicating that size effects had been removed from meristic variates.

Wilk's lambda tests also indicated differences among the six groups when their meristic characters were compared by means of discriminant analysis, but in this case only the first three functions were significant (Table 4). Moreover, the stepwise analysis revealed that only six of the 10 meristic characters contributed significantly to the multivariate discrimination of the six groups of fish (Table 5). Although significantly better than would have occurred only by chance (Press's $Q = 98.33$; $df = 1$; $p \leq 0.001$), the overall correct classification rate for the discriminant function estimated for meristic characters with the three significant canonical variables fell to 42.9% (Table 6).

In this case the plot of the first two canonical variates (Fig. 2b), which account for 53.9 and 26.2% of total variation (Table 5), shows a very high degree of overlap between individuals from all locations and even the centroids for each group are very close to each other. Moreover, the centroid value for the Monte Gordo population did not show a significant difference from those computed for Portimão and the Sado estuary ($df = 6, 187$; $p > 0.05$). The dendrograms derived from both cluster analyses (Figs. 3a and 3b) also confirmed that morphometric characters are much more effective for discriminating

between *H. didactylus* populations than meristic characters as expressed by the lower distances between combined sample means obtained in the last analysis ($0.8 \leq D^2 \leq 4.3$ for meristics against $3.6 \leq D^2 \leq 12.2$ for morphometrics).

Nevertheless, both Figures 2b and 3b reveal a latitudinal trend in meristic characteristics, the pattern exhibited by the dendrogram being much closer to that observed for morphometric analysis: great homogeneity among the Algarve populations and higher heterogeneity among western populations.

As for morphometrics, the Mantel test showed that the meristic distances (D^2) between *H. didactylus* populations were not associated with their geographic distances ($r = 0.27$; $t = 1.00$; $p > 0.05$). This is a complementary evidence of the relative isolation between Portuguese *H. didactylus* populations.

Conversely to the situation observed for morphometric analysis, one of the meristic variables is much more important than the others for the discrimination of the six studied populations. In fact, TNT has a potency index of 0.43, all the other values being much lower (Table 5). Both TNT and POR were best correlated with the first canonical variable and AFR, PFR, UJT and CFR with the second function (Table 5).

TABLE 8. – Univariate comparison of unadjusted (u) and adjusted (a) meristic characters for the six groups.

Variables	T	Mean Value for Each Group ⁽¹⁾			P	G	ANOVA + SS-STP a-posteriori Test Results ⁽²⁾		
		S	M	A					
DFR ^(u)	20.2	20.7	20.4	20.1	20.6	20.6	7.71***	–	A T M P G S
PFR ^(u)	24.2	24.9	24.3	24.1	24.6	24.5	5.75***	–	A T M G P S
AFR ^(u)	16.0	16.4	16.3	16.1	16.5	16.5	5.76***	–	T A M S G P
CFR ^(u)	20.0	19.8	19.4	19.8	20.0	19.8	5.65***	–	M S A G P T
POR ^(u)	13.4	14.3	13.9	14.6	14.5	14.3	8.93***	–	T M G S P A
TNT ^(a)	10.6	12.3	15.7	18.5	18.4	15.3	21.59***	–	T S G M P A
UJT ^(a)	2.8	3.0	3.0	2.7	2.8	2.9	3.94**	–	A T P G S M
LJT ^(a)	2.7	2.9	3.0	3.0	2.9	2.8	2.91*	–	T G P S A M
VMT ^(a)	1.5	1.6	1.5	1.5	1.5	1.4	0.36 ^{n.s.}	×	–
PLT ^(a)	1.3	1.2	1.3	1.2	1.1	1.1	1.45 ^{n.s.}	×	–

⁽¹⁾symbols for these groups as indicated in table 1; ⁽²⁾ $df = 5, 192$; *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; n.s. - $p > 0.05$; rules join non-significant subsets

Naturally, the latitudinal trend observed in Figure 2b results mainly from the effect of TNT and POR variables. However, for some specific meristic variables, namely the number of fin rays (DFR, PFR and AFR but not CFR), specimens from Arade tend to be closer to those from the western coast and the individuals from Sado estuary tend to be more similar to those from the Algarve sea (Table 8). This is why according to the second canonical variable of the discriminant analysis (Fig. 2b) Tagus fish seem to be so close to those from Arade.

DISCUSSION

The hypothesis raised by Costa and Costa (in press) of Lusitanian toadfish population fragmentation along the Portuguese coast was fully confirmed in this work. In fact, both morphometric and meristic analyses revealed a good discrimination between *H. didactylus* populations from Algarve and those from the western coast. Major differences were also observed among the western estuarine populations. This situation seems to result from the low water temperatures and hard hydrodynamic conditions typical of the Portuguese western coast, which hinders species reproduction in the oceanic environment and only allows the development of estuarine populations and small marine nucleuses in more protected areas (Costa and Costa, in press). Thus, communication between the Algarve populations and those from the western coast, and among the latter, is probably restricted to a few numbers of digestive specimens crossing this oceanic region.

However, even in the Algarve, where marine populations are present along all littoral waters (Costa and Costa, in press), a thinner but still clear discrimination between specimens from different localities can be noted. Moreover, differences were observed even between individuals from the Arade estuary and those from adjacent marine waters (Portimão). As stated above, these variations are probably related to species reproductive ecology (low fecundity, benthic eggs and larvae, and parental care with the offspring) and high sedentarity of adults.

Although much of the variation detected for *H. didactylus* morphometric and meristic characters have a latitudinal origin, some of the observed differences are related to specific characteristics of each location. This is particularly true for the shape of the body, since in discriminant analysis for morphometric characters the latitudinal gradient is

mainly related to the second canonical variable, most of the variation detected by the first canonical function being of local nature.

Morphometric discrimination between the three estuarine populations of the western coast is probably a result of the feeding strategy adopted in each of these water bodies. In fact, the diet of *H. didactylus* may include a large fraction of prey associated with the water column, such as fish, shrimps and mysids, or be almost strictly dependent on animals living on the bottom surface or inside the sediments, like crabs, amphipods, isopods and polychaetes (J.L. Costa, unpublished data). Lusitanian toadfish populations from Tagus, Sado and Mira estuaries are quite different from each other mostly regarding HW, IOW and PVFL variables, which seem to be somehow related to the capture and/or processing of prey. A broad head apparently favours the ingestion of hard and massive prey such as most of the crabs and some fish species (Gosline, 1996). Variations in interorbital width may directly derive from variations in head width. However, a close proximity of the eyes could be indicative that these organs are closer to the head dorsal profile, which is a clear advantage for detection of prey associated with the water column, like mysids, shrimps and some fish. Gosline (1996) already documented the importance of pelvic fins in raising batrachoidids front end of the body. Lowering of these fins, which are well forward under the body and have a fibrous pad along the front border of the leading ray, is a crucial contribution for maintaining the elevation of the anterior part of *H. didactylus* specimens when they are resting above the bottom (J.L. Costa, unpublished data). Moreover, aquarium observations have revealed that pelvic fins are also important for a rapid projection of individuals in the water column when they are immobilised at the bottom. In fact, the adoption of this strategy reduces the attrition of the animal with the sediment, minimising the inertia of the fish body while at rest. Thus, a high development of pelvic fins seems to be a very helpful characteristic for the detection and capture of fast prey associated with the water column, like shrimps and fish (Costa *et al.*, 1992).

Specimens from Tagus have large HW and IOW and short PVFL (Table 7), characteristics that apparently favour the consumption of crabs and reduce the capturability of prey associated with the water column. Thus, it is not surprising that *H. didactylus* individuals from the Tagus show the highest rate of ingestion of crabs and the lowest consumption of

TABLE 9. – Selected prey groups occurrence frequency in stomach contents of *H. didactylus* populations from Tagus, Sado and Mira estuaries.

Prey Groups	Tagus ⁽¹⁾	Sado ⁽²⁾	Mira ⁽³⁾
Shrimps and Mysids	22.9	35.0	39.2
Fish	24.6	15.9	23.9
Crabs	49.2	18.5	21.8
Other Prey	3.2	30.6	15.0

⁽¹⁾computed from the original data from Costa *et al.* (2000); ⁽²⁾after Sobral (1981); ⁽³⁾J.L. Costa (unpublished data)

shrimps and mysids (Table 9). Fish from Mira are in the opposite situation with short HW and IOW and large PVFL (Table 7). This is probably the reason why specimens from Mira prey upon a major fraction of shrimps and mysids and reduce their consumption of crabs (Table 9). Individuals from Sado exhibit an intermediate pattern, but HW and IOW values are closer to those observed for Mira (Table 7), and the ingestion of crabs, shrimps and mysids is also very similar in both estuaries (Table 9). On the contrary, the value observed for PVFL in Sado is significantly higher and lower than the ones obtained respectively for Tagus and Mira (Table 7). The relative low values of PVFL and HW presumably reduce the ability of specimens from Sado to capture fish. Naturally, individuals from this estuary eat a greater fraction of less robust benthic prey, like amphipods, isopods and polychaetes, among other minor food items, all designated here as “other prey” (Table 9). The differences detected in these three estuaries may result directly from prey availability in each location or indirectly from any environmental factor, like turbidity, which can influence the ability of *H. didactylus* specimens to detect, pursue and capture organisms in the water column.

Unfortunately, there is no information on Lusitanian toadfish diet in the Arade estuary. On the other hand, the only study concerning the feeding habits of *H. didactylus* marine populations was conducted in the Bay of Cadiz (Cárdenas, 1977), and it would be incorrect to extrapolate those results for Algarve due to the great variability in the species diet from place to place (Costa *et al.*, 2000). Therefore, it is impossible to relate the morphometric characteristics of these southern individuals to their trophic habits in a similar way to that carried out for western estuaries.

Fish from the western coast have higher CPD than those from Algarve (Table 7). In the southern coast specimens that originated in estuarine systems (Arade) also show a reduced development of this

body region, with larger PAL and CDFL (Table 7). According to Le Danois (1961), the caudal region of *H. didactylus* keeps the myological characteristics of pelagic fish while its abdominal portion shows the myological characteristics of a benthic form, which means that its ability for swimming is mainly associated with the posterior region of the body. Thus, the high CPD observed for western coast individuals may result from the characteristics of the ancestors of these estuarine populations, which had to swim in this oceanic region with much harder hydrodynamic conditions than those found in southern areas (Pires, 1989). The influence of undulation on the development of the *H. didactylus* caudal region is confirmed by the fact that fish from the Arade estuary (where waves are only residual) exhibit the lowest length of post-anal region in the Algarve populations. Individuals from Monte Gordo are in the opposite situation since they live more than 6 km from the nearest estuarine nucleus (Guadiana estuary). The intermediate pattern presented by the fish from Portimão obviously results from their proximity to the Arade estuary and consequently from the expected high rate of cross-fertilisation between both populations. These results are in agreement with those obtained for species with diadromous and resident forms, which usually show a higher development of caudal peduncle in the case of migrant populations (Copeman, 1977; O'Maoileidigh *et al.*, 1988; Damsgard, 1991).

It is noteworthy that in spite of inhabiting habitats with only residual undulation, specimens from western estuaries still keep higher values of CPD. Therefore, the characteristics related to movement apparently show less plasticity than those associated with feeding. Thus, it seems that this species presents an important adaptative capacity to cope with variations in trophic conditions but keeps a much more conservative pattern with regard to swimming ability. The conjunction of both factors will allow an effective exploration of the available feeding resources in each habitat and improve the ability for recolonisation or colonisation of new territories, according to its limited capacity of dispersal.

In the Northern Hemisphere the number of meristic elements is progressively greater to the north (Taning, 1952). Meristic characters of *H. didactylus* also show a latitudinal trend but the two most important factors for discriminating between Portuguese populations (TNT and POR) present an inverse pattern (Table 8). On the other hand, fin ray counts (DFR, PFR and AFR) generally associate specimens from the Tagus, Mira and Arade estuaries

and those from the Algarve sea with the ones from the Sado estuary (Table 8). Meristic counts are usually determined during a relatively short span of time during early development and the characteristics that are last to appear during ontogeny are the most labile (Barlow, 1961). According to Taning (1952), the referred cline seems to be related to temperature since there is a good correlation between cooler environmental temperatures and higher meristic numbers. Apparently the number of serial elements is determined by developmental rate, with larger developmental periods usually producing higher counts in meristic structures (Barlow, 1961). This then is the mode of action of lower temperatures since developmental rate varies directly with temperature (Taning, 1952). Since during reproduction and early development of *H. didactylus* (late Spring and early Summer) the water temperature reaches much higher values in brackish water environments than in open sea (Costa and Costa, in press), thermal variations could explain how fish from estuaries show generally lower fin ray counts than those from the sea (Table 8). Similarity of specimens from Sado with those from Portimão and Monte Gordo can be easily explained by the fact that fish from the estuary were captured just near the river mouth, where water temperature is very close to that observed in the sea. This must also be the reason why individuals from Sado show very high values of CPD (table 7): specimens from a western population with a strong marine influence may be favoured in their swimming capacity by well developed caudal peduncles. However, as seen above, temperature can hardly explain the latitudinal variation of TNT and POR. Therefore, both these characteristics must be controlled by other factors, such as photoperiod, which plays an important role in the early development of batrachoidid fish (Ryder, 1887; Campana, 1984). Liability of nasal tentacles to variation is obvious since these meristic characters depend on the length of individuals. Thus, their importance for the separation of *H. didactylus* populations may be due to the fact that they are subject to environmental influence during the whole life of the animals.

As was found before for other fish species (e.g. Sharp *et al.*, 1978), morphometric characteristics are more suitable than meristic characters for a good discrimination between *H. didactylus* populations. According to Barlow (1961), these differences are caused by the fact that variation in shape is usually more related to genetic causes while meristic char-

acters are much more dependent on environmental fluctuations. The discrete nature of meristic data also reduces its suitability for the application of more powerful statistical analysis (Ihssen *et al.*, 1981) and contributes to the lower ability of meristic characters to discriminate between *H. didactylus* populations. However, usefulness of morphometric characteristics depends on the application of multivariate statistical techniques since none of these individual variables promotes a good separation between the studied *H. didactylus* populations. On the other hand, for meristic characters both TNT, and to a lesser extent POR, play a determinant role in discrimination of these populations, being responsible by themselves for a great amount of the variation detected. As a result of these findings future investigations about this subject should focus mainly on the study of morphometric characteristics, and it is acceptable for meristic analysis to become limited to the study of nasal tentacles and lower jaw pores. This option enables an optimisation of the sampling effort, allowing the study of new morphometric variables and the examination of more specimens and populations. As stated above, multivariate statistical techniques should be preferred to univariate procedures, which must be used only as a complement to the formers.

Observed morphometric and meristic differences in this investigation are probably influenced by both genes and environment. Thus, it would be valuable to conduct also some genetic studies in the future. The accumulated information from morphometrics, meristics and genetics, along with other life-history information such as parasitological data, could then be evaluated for a better understanding of the population structure of *H. didactylus*.

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