

Morphological variation of horse mackerel (*Trachurus trachurus*) in the Iberian and North African Atlantic: implications for stock identification

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The current delimitation of the Atlanto–Iberian stock of horse mackerel, *Trachurus trachurus* (Linnaeus, 1758), is based on scant biological evidence. Here, the location of the southern boundary of this stock is investigated through the analysis of several morphometric and meristic characteristics. A total of 384 horse mackerel were sampled from three areas off the Portuguese, one off the Spanish (Gulf of Cadiz) and another off the Moroccan coast, and compared using multivariate techniques. Some 14 morphometric and five meristic characters were analysed using hierarchical cluster analysis, multivariate discriminant analysis and randomization tests. Morphometric characteristics showed considerably greater discriminatory power to distinguish individuals from different areas than did the meristic characters. All morphometrical analyses showed similar results: the greatest differences were found between the Gulf of Cadiz and all other groups, while fish from the Portuguese coast appeared more similar to each other than to those from Moroccan waters. Meristic characters did not allow a clear distinction between geographical areas, with the Mahalanobis distances among areas being an order of magnitude smaller than those for the analysis of the morphometrics. If these results are corroborated by further biological evidence (such as genetical or parasitological data), there is a strong argument for the southern limit of the Atlanto–Iberian stock of horse mackerel to be revised.

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Introduction

Horse mackerel, *Trachurus trachurus* (Linnaeus, 1758), is among the most important fishing resources in Iberian waters. For assessment purposes the Iberian horse mackerel is included in the Atlanto–Iberian stock, which is managed independently from the other two stocks in the Northeast Atlantic: the North Sea and Western stocks (Fig. 1). This separation of stocks was based on circumstantial evidence, such as egg distribution and the temporal and spatial distribution of the fishery, and the need for stronger biological justification has been recognized (ICES, 1999). Studies on the northern boundary of the Atlanto–Iberian stock suggest that its current location may be misplaced. Borges (1996) and Nazarov (1976 in Borges, 1996) reported significant

differences in morphometric characters between Iberian horse mackerel and fish from the north of Biscay, Celtic Sea and English Channel. However, Abaunza *et al.* (1995) found differences in the infestation of horse mackerel by *Anisakis simplex* between the east and the west parts of the north Iberian coast.

Although the southern limit of the Atlanto–Iberian stock is currently set at the Gulf of Cadiz, the distribution range of horse mackerel in the Northeast Atlantic includes the Mediterranean Sea and part of the African coast (Whitehead *et al.*, 1986). In the Mediterranean Sea the catches of *Trachurus trachurus* are much smaller than in Atlantic waters, particularly in north African Atlantic waters where an important horse mackerel fishery appears to take place (FAO, 1995). Off the northwest African coast two horse mackerel stocks were

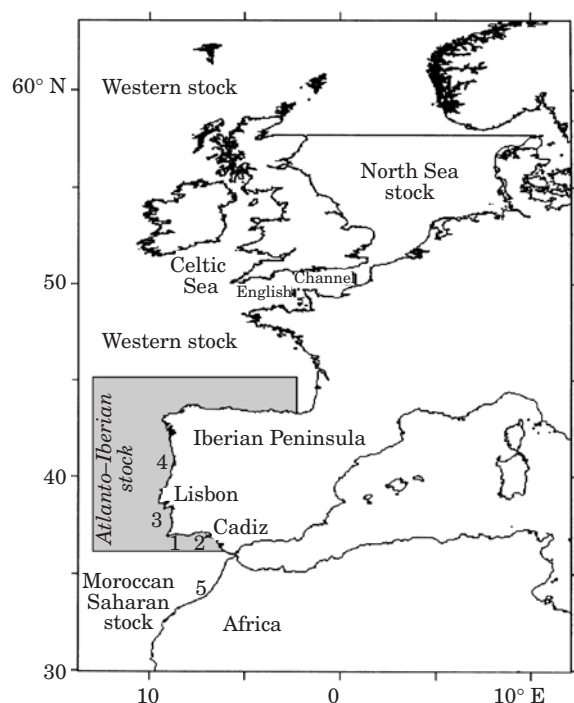


Figure 1. Current stock delimitations and location of the five sampling areas: 1, Algarve (southern Portuguese coast); 2, Gulf of Cadiz; 3, southwest Portuguese coast; 4, northwest Portuguese coast; 5, Moroccan coast.

distinguished based on morphometric characters, weight of the otoliths and population characteristics (Galaktionova and Domanevskiy, 1989): the Senegalese-Mauritanian stock (south of 23°N), and the Moroccan-Saharan stock (from 23°N to the Strait of Gibraltar) (see Fig. 1). Although this latter stock is considered distinct from the Atlanto-Iberian one for assessment purposes, this separation has never been investigated. Hence, it is important to determine whether or not the Moroccan horse mackerel belongs to the Atlanto-Iberian stock.

From all stock identification methods available (Ihssen *et al.*, 1981a; Smith and Jamieson, 1986; Templeman, 1983) the analysis of morphometric and meristic characters is one of the most commonly used (e.g. Hurlbut and Clay, 1998; Melvin *et al.*, 1992; Taylor and McPhail, 1985). Although these characters may be influenced by environmental conditions, they can be as valuable in indicating stock discreteness as other, more genetically related, features (Casselman *et al.*, 1981; Kinsey *et al.*, 1994; Lear and Wells, 1984). Multivariate techniques are used here to study morphometric and meristic characters of horse mackerel from Iberian and north African waters to investigate the validity of the southern boundary of the Atlanto-Iberian stock of horse mackerel.

Table 1. Area, time of sampling and number of fish in each sample.

Area	Year	Month	Sample size
Algarve	1993	February	68
Algarve	1996	March	23
Cadiz	1995	February	31
SW coast	1993	April	7
SW coast	1993	February	5
SW coast	1994	February	26
NW coast	1993	February	92
NW coast	1994	October	13
NW coast	1996	March	61
Morocco	1995	May	18
Morocco	1997	April	40
Total			384

Material and Methods

Horse mackerel were sampled during the spawning season (January–March) (Borges and Gordo, 1991) from the first four areas indicated in Figure 1: 1, Algarve coast (91 individuals); 2, Gulf of Cadiz (31 individuals); 3, southwest Portuguese coast (38 individuals); 4, northwest Portuguese coast (166 individuals); and at the end of the spawning season (April–May) (Berenbeim, 1974) from the fifth area: 5, Moroccan coast (58 individuals). All areas except Cadiz were sampled in different years and at more than one location within years (Table 1). Samples were collected by bottom trawl, during surveys or from commercial trawlers. All fish were deep-frozen (–20°C) and defrosted just before being measured in the laboratory. Measurements were taken to the nearest millimetre, always by the same person. Sexes were pooled for all analyses, since horse mackerel does not exhibit sexual dimorphism (Borges, 1996; Shabonev and Kotlyar 1979).

A total of 19 characteristics were used: 14 morphometric and five meristic. Figure 2 summarizes the morphometric characters:

- total length (TOTLENGTH),
- fork length (FORKLENGTH),
- standard length (STDLENGTH),
- head length (HEADLENGTH),
- left accessory lateral line length (ALLLENGTH),
- first dorsal fin length (DORSAL1),
- second dorsal fin length (DORSAL2),
- length from the snout to the beginning of the anal fin (PREANAL),
- height of the body (HBODY),
- left eye diameter (EYEDIAM),
- inter-orbital distance (IODIST),
- length of the left pectoral fin (LPECT),
- minimum height of the tail peduncle (HTAIL),
- length of the left side of the maxilla (MAXILLA).

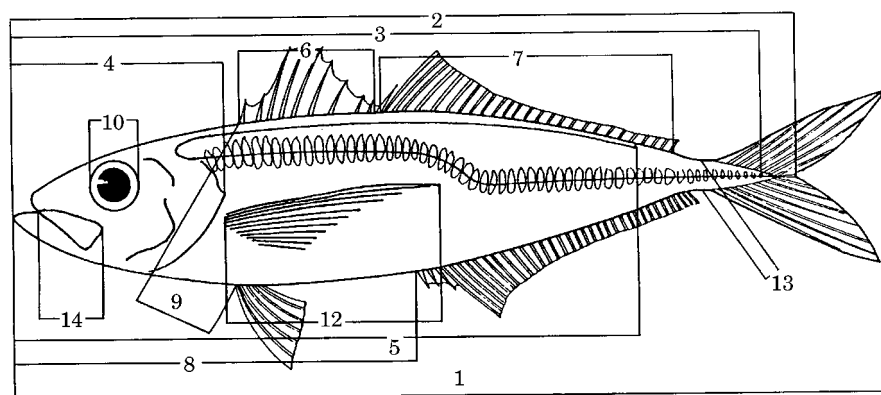


Figure 2. Morphometric characters measured on the left side of each fish: 1, TOTLENGTH; 2, FORKLNGTH; 3, STDLENGTH; 4, HEADLENGTH; 5, ALLLENGTH; 6, DORSAL1; 7, DORSAL2; 8, PREANAL; 9, HBODY; 10, EYEDIAM; 12, LPECT; 13, HTAIL; 14, MAXILLA. The 11th measurement (IODIST) is not shown in the Figure.

The five meristic characters analysed were:

- number of rays in the left pectoral fin (PECTRAYS),
- number of rays in the second dorsal fin (D2RAYS),
- number of soft rays in the anal fin (ANALRAYS),
- number of scales in the left lateral line (SCALESLL),
- number of rays in the second dorsal fin to the end of the left accessory lateral line (R2DEALL).

Body measurements strongly correlate with body size, while in most fish meristic characters do not change during growth beyond some threshold body size (Strauss, 1985). In order to ensure that the meristic characters were completely defined, only adults larger than 20 cm total length were used. Morphometric characters were standardized to the overall mean total length, the standardized measure being given by

$$M_c = M_x \left(\frac{\overline{TL}}{TL} \right)^b$$

(Hurlbut and Clay, 1998; Ihssen *et al.*, 1981b) where TL is the total length, M is the original measurement, \overline{TL} is the overall mean total length and b is the slope, within areas, of the geometric mean regression (Ricker, 1973) on the logarithms of M and TL. This regression model was chosen because none of these variables could be considered either independent or explanatory.

Analyses were carried out separately for morphometric and meristic characters, since these variables are different both statistically (the former are continuous while the latter are discrete) and biologically (the latter are fixed early in development, while the former are more susceptible to the environment) (Allendorf *et al.*, 1987). Morphological variation was analysed considering each fish as a multivariate observation, because a univariate approach would ignore the joint effect of

variables (Bowering, 1988; Misra and Carscadden, 1987; Winans, 1985).

Correlation coefficients between each pair of characters were calculated to check if the data transformation was effective in reducing the influence of size in the measurements. In such a case it is expected that the absolute value of correlation coefficients would decrease after size correction. Also, variables highly correlated after the size effect removal would be considered redundant, and the data set could be reduced.

A hierarchical cluster analysis was performed with the centroids of the five groups of individuals (corresponding to the five sampling areas), using the arithmetic average clustering algorithm (Legendre and Legendre, 1979) and the Mahalanobis distance (Dryden and Mardia, 1998) among centroids. This distance was chosen because it is invariant to differences in scale among variables, therefore giving equal weight to all variables in the calculation of distances (Legendre and Legendre, 1979).

A multivariate discriminant analysis (Johnson and Wichern, 1992) was performed to investigate the integrity of pre-defined groups. To validate the results of the discriminant analysis, the procedure described by Soriano *et al.* (1988) was followed. Each individual was allocated to the group with the nearest centroid, and the proportion of individuals allocated to each group was calculated. The proportion of individuals correctly re-allocated was taken as a measure of the integrity of that group.

To estimate the significance level of the differences among groups, a multivariate randomization test was performed between each pair of groups (Manly, 1997; Sokal and Rohlf, 1995). This procedure was preferred to parametric tests, such as the Bonferroni test or the Wilk's Λ criterion, to relax the multinormality

Table 2. Correlation coefficients between characters, before and after the removal of the size effect, are respectively shown below and above the diagonal. Values higher or equal to 0.95 are in bold.

	totlength	forklength	stdlength	headlength	alllength	dorsal1	dorsal2	preanal	hbody	eyediam	iodist	lpect	htail	maxilla	pectrays	d2rays	analrays	scalesll	r2deall
forklength	1																		
stdlength	1	0.98																	
headlength	0.99	0.99	1.00																
alllength	0.97	0.97	0.97	0.96															
dorsal1	0.99	0.99	0.99	0.96	0.97														
dorsal2	0.99	0.99	0.99	0.98	0.99	0.98													
preanal	0.99	0.99	0.99	0.98	0.99	0.97	0.98												
hbody	0.99	0.99	0.99	0.97	0.98	0.98	0.98												
eyediam	0.99	0.99	0.99	0.97	0.98	0.98	0.98	0.97											
iodist	0.97	0.97	0.97	0.96	0.96	0.96	0.96	0.96	0.97										
lpect	0.97	0.97	0.97	0.96	0.96	0.96	0.96	0.96	0.97	0.98									
htail	0.97	0.97	0.97	0.96	0.96	0.96	0.96	0.96	0.97	0.98	0.96								
maxilla	0.97	0.97	0.97	0.96	0.96	0.96	0.96	0.96	0.97	0.98	0.96	0.97							
pectrays	0.31	0.31	0.31	0.33	0.31	0.33	0.30	0.32	0.32	0.30	0.32	0.34	0.32	0.35	0.03	0.04	0.00	0.00	0.07
d2rays	-0.18	-0.18	-0.18	-0.18	-0.18	-0.20	-0.16	-0.18	-0.20	-0.21	-0.18	-0.20	-0.20	-0.18	-0.02	-0.02	0.05	0.13	0.07
analrays	-0.03	-0.03	-0.03	-0.04	-0.04	-0.04	-0.02	-0.04	-0.04	-0.07	-0.03	-0.04	-0.03	-0.03	0.05	0.60	0.17	0.17	0.19
scalesll	0.34	0.34	0.34	0.25	0.33	0.29	0.38	0.28	0.30	0.14	0.23	0.27	0.34	0.21	0.13	0.06	0.17	0.17	0.11
r2deall	0.10	0.10	0.11	0.10	0.16	0.09	0.11	0.10	0.10	0.06	0.11	0.08	0.09	0.10	0.07	0.37	0.19	0.11	0.11

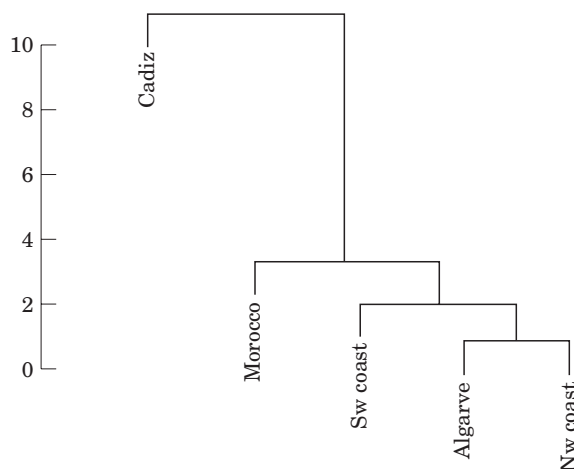


Figure 3. Dendrogram of the cluster analysis for the centroids of the groups, based on the morphometric data. The algorithm used was the arithmetic average clustering, with the Mahalanobis distance as a similarity measure.

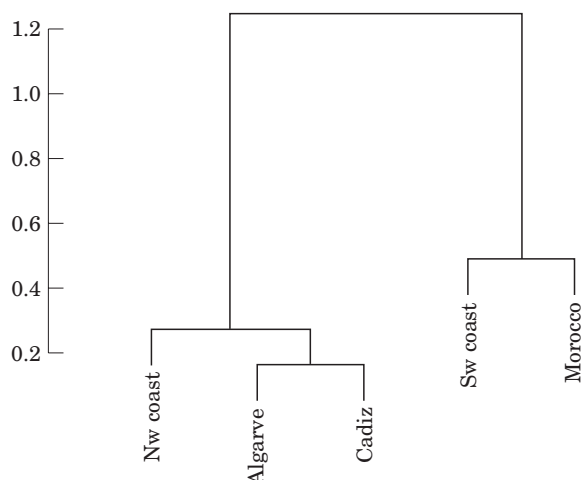


Figure 4. Dendrogram of the cluster analysis for the centroids of the groups, based on the meristic data. The algorithm used was the arithmetic average clustering, with the Mahalanobis distance as a similarity measure.

assumption (Morrison, 1990), since q-q plots (Cleveland, 1994) showed strong deviations from normality for several characteristics. In these tests Euclidean distances were used instead of Mahalanobis distances, since the former are computationally less demanding. However, Euclidean distances are sensitive to differences of scale, thus all characters were re-scaled by dividing every value by the overall standard deviation of the corresponding character.

All calculations were carried out with the software S-Plus 3.3 for Windows (Statistical Sciences, Inc.).

Results

Correlation coefficients between characters before and after the size effect removal are presented in Table 2. All coefficients between morphometric characters were very close to 1 before size correction and were considerably reduced after (values higher than 0.95 are highlighted in Table 2). The lowest correlation before the size-effect removal was 0.86 whereas after removal the highest was 0.61. Most coefficients changed to values close to zero after the removal of the size factor. Within meristics, the highest coefficient was 0.6, with most being less than 0.35, thus confirming the assumption that these characters are independent of size.

The dendrograms for the morphometric and meristic characters (Figs 3 and 4) present clearly different results. However, the range of Mahalanobis distances for the meristics are an order of magnitude less than for the morphometrics (0.2 to 1.2 in the former, 0 to 10 in the latter). This means that if we put them on the same scale, morphometric characters reveal much greater differences among groups than the meristics. In the morphometrics

dendrogram all groups from the Portuguese coast are close to each other, the group from the Moroccan coast is detached from those three, and the group from Cadiz appears furthest from all others. A different picture stems from the meristics dendrogram, where the southwest Portuguese coast and Morocco are separated from the other three areas.

In the discriminant analysis, most of the discriminant power is in the first three discriminant variables (discriminant correlation coefficients 0.89, 0.54, 0.26, 0.16 and 0 respectively for the five morphometric discriminant variables; 0.42, 0.25, 0.13, 0.03 and 0 for the meristics). The plots of the centroids of each group in the space defined by the three first discriminant variables are shown in Figure 5. The radius of the circle around each centroid is one standard deviation of the euclidean distances of the elements of each group to the centroid of that group. As with the cluster analysis for morphometrics, the groups in the Portuguese coast are placed close to each other, while Morocco and Cadiz appear more distinct. Regarding within area variation, the Algarve and the southwest coast are the groups with highest dispersion levels. For the meristics, all groups have similar dispersion and differences between areas are less marked. Still, the pattern from the dendrogram is also reflected here, with Morocco and the southwest Portuguese coast being isolated from the other three areas.

In the validation of the morphometrics discriminant analysis (Table 3) the most well-defined group is Cadiz, with only one misclassified individual. The second most well defined group is Morocco with 22% of misclassifications, most of them to the Algarve. Most misclassified individuals from the groups from the Portuguese coast

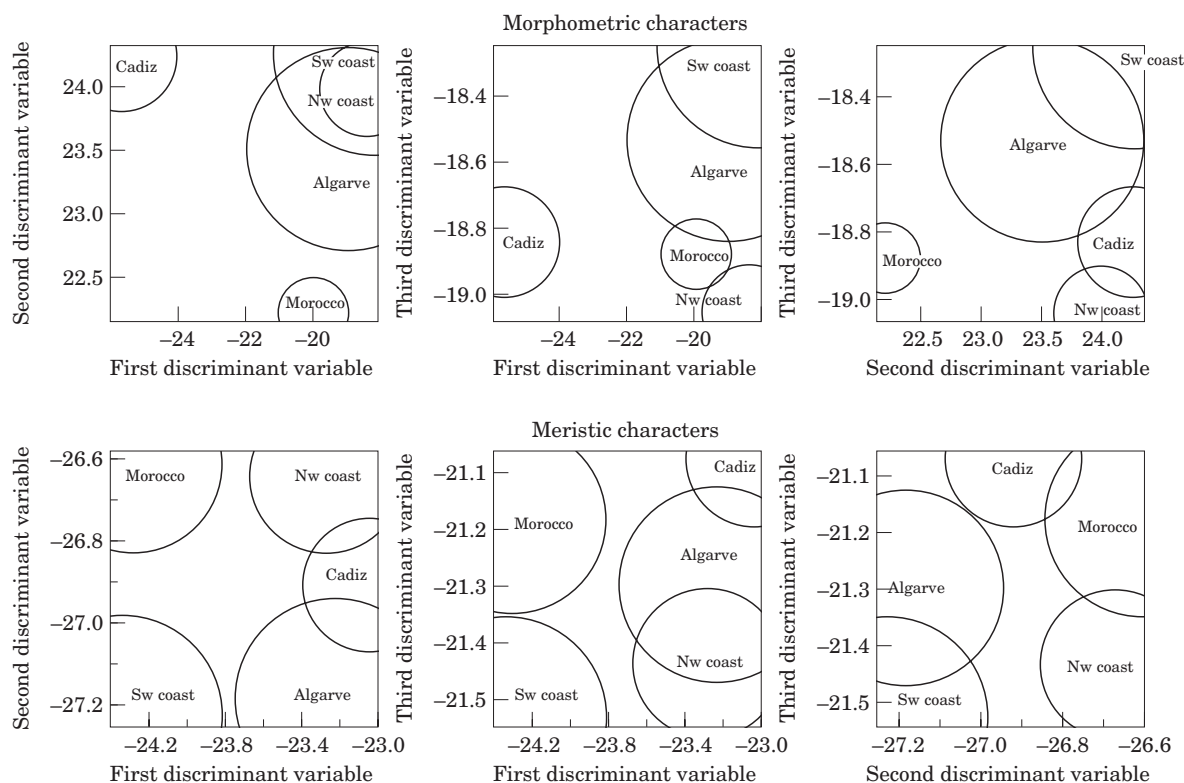


Figure 5. Plots of the centroids of the sample groups in the space defined by the discriminant variables. The radius of the circle around each centroid corresponds to one standard deviation of the Euclidean distances from each individual to its group centroid.

Table 3. Number (and percentage) of individuals reallocated in each group, in the validation of the discriminant analysis for the morphometric data. Rows are the original sample group and columns the reallocation group.

	Algarve	Cadiz	SW coast	NW coast	Morocco	Total
Algarve	41 (45%)	1 (1%)	12 (13%)	20 (22%)	17 (19%)	91
Cadiz	0 (0%)	30 (97%)	0 (0%)	0 (0%)	1 (3%)	31
SW coast	9 (24%)	0 (0%)	19 (50%)	7 (18%)	3 (8%)	38
NW coast	35 (21%)	0 (0%)	37 (22%)	83 (50%)	11 (7%)	166
Morocco	8 (14%)	0 (0%)	1 (2%)	4 (7%)	45 (78%)	58

went to other groups in the Portuguese coast. The worst defined group is the Algarve (55% of misclassifications), most of them to the northwest coast and to Morocco. In the validation of the morphometrics analysis a total of 218 individuals (57%) were well classified. Table 4 shows the validation results for the meristic characters. It is clear that the discriminant variables formed with these data do not allow an effective classification of the individuals (65% of misclassified overall). The lowest percentage of misclassification was obtained for the southwest coast (53%) and the highest for the Algarve (71%).

Randomization tests for the morphometric data resulted in significant differences at 5% level between

Cadiz and all the other groups, and between the northwest coast and Morocco (Table 5), hence reflecting the results from the cluster and discriminant analyses. For the meristic data, significant differences were found between the southwest coast and Algarve and Cadiz, and between Morocco and Algarve, Cadiz and the northwest coast (Table 5).

Discussion

All methods used in this study showed, for the morphometric data, a clear distinction between Cadiz and all other groups. A discontinuity between Morocco and the

Table 4. Number (and percentage) of individuals reallocated in each group, in the validation of the discriminant analysis for the meristic data. Rows are the original sample group and columns the reallocation group.

	Algarve	Cadiz	SW coast	NW coast	Morocco	Total
Algarve	26 (29%)	17 (19%)	14 (15%)	24 (26%)	10 (11%)	91
Cadiz	7 (23%)	13 (42%)	2 (6%)	6 (19%)	3 (10%)	31
SW coast	6 (16%)	1 (3%)	18 (47%)	5 (13%)	8 (21%)	38
NW coast	30 (18%)	25 (15%)	22 (13%)	58 (35%)	31 (19%)	166
Morocco	7 (12%)	7 (12%)	16 (28%)	7 (12%)	21 (36%)	58

groups from Portuguese waters was also evident in the results from the cluster and discriminant analysis, but was not confirmed by the randomization tests. The groups from the Portuguese coast presented greater similarities to each other than to Cadiz or Morocco. It is clear that from a stock delimitation point of view, Moroccan individuals seem closer to the Atlanto-Iberian stock than those from the Gulf of Cadiz. However, these results must be viewed with caution, since Cadiz was the least sampled area. Within Portuguese waters, Algarve and the southwest coast are less well defined than the northwest coast group. This can be seen by the high dispersion rate in the discriminant analysis and high rates of misclassification in its validation. Thus, it seems that the Algarve and southwest coast fish exhibit morphometric characteristics intermediate between the Moroccan and the northwest coast fish, or there is a mixing of individuals with different morphometric types.

Although some agreement was found between the discriminant analysis and the dendrogram for the meristic characters, the randomization tests did not fully support these results. The importance of the differences indicated by the cluster analysis and by the randomization tests must be put on the right scale when compared with the differences indicated by the morphometric data analyses. The Mahalanobis distances among the centroids of the groups in the analysis of the meristics are about one-tenth of the distances calculated with the morphometrics data. This reduced importance of the meristic differences is stressed by the lack of validation of the discriminant analysis. Meristic characters have

been successfully used for stock identification, especially when analysed together with other methods (Fournier *et al.*, 1984; Melvin *et al.*, 1992; Misra and Bowering, 1984; Tibbo, 1956), and evidence of genetically determined morphometric and meristic characters has been reported by several authors (Carscadden and Leggett, 1975; Ihssen *et al.*, 1981b). However, in several situations they have been considered worse than morphometrics for these purposes (e.g. Misra and Carscadden, 1987). Several environmental variables have been shown to affect the determination of meristic characters (e.g. Barlow, 1961; Fahy, 1980; Ihssen *et al.*, 1981a; Lindsey, 1988). It is therefore possible that temporal (intra-stock) variation is higher than geographical (inter-stock) variation, thus masking the differences between stocks (Blouw *et al.*, 1988; Tremblay *et al.*, 1984).

The assumption that meristic characters are independent of fish size was confirmed by the absence of correlations between meristic characters and total length. The method used in this work to correct the measurements for size proved effective, since all correlation coefficients which were close to 1 decreased to low values after the transformation of the data. Morphometric and meristic characters are usually considered non-normal (e.g. Misra and Bowering, 1984; Misra and Carscadden, 1984, 1987), and a usual way to deal with this is to log-transform the data to approximate multivariate normality (Misra and Carscadden, 1987; Pimentel, 1979). However, this does not seem an adequate procedure for this kind of analysis, when the goal is to search for differences in the morphological characteristics and not in their logarithms.

For a reliable identification of a stock more than one method should be used, since different methods may produce different results (Bowering, 1988). Therefore, in order to make inferences on stock identity, the results from this work should be compared with data obtained following a different methodology. In previous assessments, the catches from the area around Cadiz have been considered as belonging to the Atlanto-Iberian stock, but the catches from north African waters have not (ICES, 1999). However, morphometric data indicate that the individuals from Morocco are more similar to the ones from the Portuguese coast than those from the

Table 5. Results (p-values) of the multiple comparison randomization tests. The upper triangle corresponds to the morphometric data, and the lower one corresponds to the meristics.

	Algarve	Cadiz	SW coast	NW coast	Morocco
Algarve		0	0.53	1	0.92
Cadiz	0.85		0	0	0
SW coast	0.02	0		0.99	0.34
NW coast	0.7	0.13	0.78		0
Morocco	0.05	0	0.69	0.01	

Cadiz area. In this latter area, fish may have more Mediterranean characteristics, or there may occur a mixing between Atlantic and Mediterranean populations. Further data are needed to investigate these possibilities. If the results from this morphometric analysis are confirmed by other means, the current delimitation of the Atlanto-Iberian stock of horse mackerel may have to be revised.

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