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# MORPHOLOGICAL VARIATION IN EIGHT SPARIDAE SPECIES OF THE EASTERN ATLANTIC AND MEDITERRANEAN SEA

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Dissertação apresentada à Universidade do Algarve para a obtenção do grau de Doutor no ramo de Ciências do Mar especialidade de Zoologia Marinha

JORGE AFONSO MARTINS DA PALMA

FARO

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## Resumo

A presente dissertação teve como objectivo principal o estudo das características morfológicas de oito das espécies mais importantes da família Sparidae, nomeadamente, a dourada, *Sparus aurata* Linnaeus 1758, o pargo, *Pagrus pagrus* (Linnaeus, 1758), o sargo, *Diplodus sargus* (Linnaeus, 1758), o sargo bicudo, *Diplodus puntazzo* (Cetti, 1777), a ferreira, *Lithognathus mormyrus* (Linnaeus, 1758), o goraz, *Pagellus bogaraveo* (Brünnich, 1768), o pargo capatão, *Dentex dentex* (Linnaeus, 1758), e a choupa, *Spondyliosoma cantharus* (Linnaeus, 1758), provenientes da costa Europeia Sul Atlântica e do Mar Mediterrâneo. Como casos particulares da especiação morfológica das espécies em estudo foram analisados, o efeito exercido por condições artificiais de cultivo intensivo sobre uma das espécies estudadas (dourada), e o resultado da hibridação entre duas espécies simpátricas da mesma família, o híbrido *Sparus auratax Pagrus pagrus* (*Sa*×*Pp*).

A tese foi dividida em três capítulos principais, versando o primeiro capítulo a análise merística das espécies referidas, o segundo a análise morfométrica dessas mesmas espécies, e o terceiro a análise do híbrido *Sa*×*Pp*. Neste capítulo, para além da aplicação da metodologia utilizada nos capítulos 1 e 2 (análise merística e morfométrica), analisou-se ainda a morfologia craniana dos exemplares, nomeadamente, a estrutura dentária e a morfologia óssea. Sob um ponto de vista comparativo com os progenitores, foram ainda efectuadas experiências de crescimento com os referidos híbridos.

Do ponto de vista merístico, as barbatanas anal, caudal, dorsal revelaram uma menor variabilidade nas características merísticas estudadas. Pelo contrário, as estruturas pares, como sejam as barbatanas peitorais e os arcos branquiais (superiores e inferiores) mostraram ser significativamente diferentes para a maioria dos casos ($p$<0,05). Estas características variaram de uma forma independente. Nas espécies em estudo não foi possível, no entanto, estabelecer um padrão claro de variação geográfica entre as amostras. Apenas foram encontradas diferenças quando a comparação foi feita entre duas amostras ou entre uma amostra e todas as restantes. No universo analisado ocorreu apenas uma excepção com *P. pagrus*. Apesar deste facto, foi encontrada uma proximidade entre as amostras portuguesas e italianas de dourada (quer selvagens quer cultivadas) e de ferreira. Esta semelhança parece estar relacionada com o isolamento das respectivas populações.

A comparação do conjunto total das contagens das características merísticas entre todas as amostras, para cada uma das espécies analisadas, e os valores extraídos da bibliografia revelaram que, em 33,3% dos casos, os valores calculados neste estudo se incluem dentro dos intervalos propostos por outros autores. Quando, em 63,3% dos casos, os valores encontrados ultrapassaram estes intervalos, tal ficou a dever-se à presença de um a três indivíduos que excederam os intervalos propostos pelos diferentes autores. Nos restantes casos, o número de indivíduos que excederam os intervalos propostos variou entre 4 e 50, verificando-se que, em alguns casos, tal aconteceu em todas as amostras.

De forma complementar à análise merística, foi analisada a relação entre a estabilidade de desenvolvimento e a variabilidade genética (heterozigotia) entre as amostras selvagens e cultivadas de dourada. A estabilidade de desenvolvimento foi avaliada através da análise da assimetria flutuante. Apesar dos valores obtidos (0,305±0,147), esta ocorreu na maioria das amostras, os valores mais elevados foram calculados nas amostras cultivadas. Os valores de heterozigotia resultantes da análise aloenzimática foram sempre elevados. No entanto, verificou-se que os valores das amostras cultivadas foram sempre inferiores aos valores das amostras selvagens. Na análise de DNA mitocondrial os valores de heterozigosidade foram superiores nas amostras de indivíduos cultivados, excepto nas amostras gregas. Estes resultados indicam que, nas amostras cultivadas ocorre uma diiminuição de variabilidade genética, o que se pensa ser devido principalmente, à perda dos alelos mais raros, presentes nas populações selvagens. A deriva genética causada, provavelmente, por técnicas selectivas de cultivo, é a razão mais provável para este decréscimo. Neste estudo, não foi possível, no entanto, distinguir uma variação geográfica entre as amostras.

Relativamente à análise morfométrica, a análise de componentes principais (CP) revelou, através da componente principal 1 (CP1), valores semelhantes, quer em sinal, quer em magnitude, o que indica que esta característica representa uma medida geral do tamanho do peixe. Este facto verificou-se para ambos os métodos morfométricos utilizados (método tradicional e método truss). Esta componente mostrou para todas as espécies uma correlação sistemática e significativa com o comprimento standard (CS) ($p$<0,05). Às componentes principais 2 a 5 corresponderam sempre menores percentagens de variação, não se correlacionando, na maioria dos casos, com o CS. Quando tal aconteceu, foi apenas de uma forma esporádica e com o CP2. Neste caso,

apenas uma (num universo de 12 características para o método tradicional e 31 características para o método truss) se correlacionou com uma das componentes.

As características que apresentaram os valores mais elevados de correlação com a CP2 foram, de uma forma geral, características recolhidas na zona da cabeça (no caso da morfometria tradicional) ou recolhidas da zona da cabeça e do pedúnculo caudal (no caso do método truss). Este resultado aponta para um crescimento diferenciado de cada uma dessas áreas corporais, comparativamente com o resto do corpo.

O número de características que contribuíram para a análise discriminatória das amostras variou entre 4 e 12, para o método tradicional, e entre 5 e 22, para o método truss. Numa perspectiva de alinhamento geográfico Oeste/Este, observou-se um gradiente em duas espécies (*D. puntazzo* e *L. mormyrus*) através do método tradicional, e em três espécies (*D. sargus*, *D. puntazzo* e *D. dentex*) através do método truss.

No caso da dourada, a análise discriminante através do método truss identificou um conjunto de características morfométricas que asseguraram a discriminação morfológica das amostras. A taxa de classificação correcta nas amostras estudadas através da análise discriminante, baseada em 18 e 22 características morfométricas, foi de 97,3% e 98,7% para as amostras cultivadas e selvagens, respectivamente.

Quer as amostras selvagens, quer as amostras cultivadas, evidenciaram uma clara diferença geográfica, bem como um elevado grau de diferenciação entre si. Estes resultados indicam diferenças morfologicamente significativas entre as amostras, o que sugere que cada uma das áreas de estudo é representada por populações com elevado grau de diferenciação mútuo.

No caso do sargo, do sargo bicudo e da ferreira, a análise discriminante assegurou também um elevado grau de diferenciação, com base nas características seleccionadas. A taxa de classificação correcta para as quatro amostras através da análise discriminante, baseada em 7, 9 e 10 características ajustadas (sargo, sargo bicudo e ferreira) foi de, respectivamente, 93,7%, 90,6% e 96,3%. Foram ainda calculadas diferenças significativas entre as amostras de cada uma das espécies. Em cada uma destas três espécies foi também encontrado um claro gradiente geográfico entre a amostra Atlântica e as amostras Mediterrânicas, o que indicia que cada uma destas áreas é habitada por populações separadas.

Tal como nas espécies anteriores, foram calculadas diferenças significativas entre as amostras de pargo, de goraz, e de pargo capatão. A taxa de classificação correcta das amostras, obtida através da análise discriminante, baseou-se em 9

características, para o pargo, e 14 características, para o goraz e para o pargo capatão com valores percentuais de 96,8%, 100% e 97,9%, respectivamente. Para o pargo capatão, foi também possível encontrar um gradiente geográfico entre as amostras. Nas restantes espécies, este gradiente não foi encontrado, apesar do elevado grau de diferenciação entre as amostras.

No caso da choupa, a análise restringiu-se apenas à comparação entre a amostra portuguesa e espanhola. No entanto foram calculadas diferenças significativas entre as amostras, com uma taxa de identificação correcta de 100% (para ambas as amostras) e com os critérios estatísticos $D^2$ e $F$ a apresentarem resultados altamente significativos ($p$<0,0001). Estes resultados revelaram também uma diferença acentuada entre a amostra Atlântica e a amostra Mediterrânica.

No caso da informação obtida através da morfometria tradicional, os resultados foram semelhantes aos obtidos com o método truss, apresentando um grau elevado de discriminação entre as amostras, mas não alcançando, na maioria dos casos, o grau de excelência obtido através do método truss. Só em duas das espécies (*L. mormyrus* e *S. cantharus*) os valores obtidos através das medições efectuadas com o método tradicional foram globalmente superiores aos obtidos com o método truss. No caso de *L. mormyrus*, foi mesmo possível ajustar um gradiente geográfico e, no caso de *S. cantharus*, os valores dos critérios estatístico melhoraram substancialmente.

A análise do híbrido *Sa*×*Pp* mostrou que as características merísticas (barbatanas e arcos branquiais) variaram dentro dos intervalos observados para as espécies parentais. A contagem do número de dentes, embora feita separadamente no lado direito e no lado esquerdo de cada uma das maxilas, foram globalmente simétricos, apresentando o mesmo número de dentes em ambos os lados da boca. A maior diferença foi calculada para o número de filas de dentes molares, com cinco na dourada, quatro no híbrido e somente duas no pargo. Numa perspectiva global, o híbrido evidenciou uma maior grau de semelhança com a dourada.

A comparação das características osteológicas entre o híbrido *Sa*×*Pp* e as espécies parentais mostrou que, na análise do neurocrânio, a maioria das estruturas observadas (supraoccipital, vomer, parasfenóide, basioccipital e basisfenóide) evidenciaram um maior grau de semelhança entre os híbridos e o pargo. Apenas duas características, o etmóide e o exoccipital se assemelharam à dourada. Outras estruturas ósseas, como sejam os ossos lacrimais, o opérculo, o sub-opérculo, o inter-opérculo, o

pré-opérculo, a maxila, a pré-maxila, o dentário e o articular partilharam uma maior semelhança com o pargo.

A análise morfométrica do híbrido, baseada no método truss, produziu resultados ligeiramente inferiores aos obtidos através da morfometria tradicional. Através desta, nove medições asseguraram a diferenciação das amostras. A taxa de classificação correcta foi de 100%, 98,8% e 98,3% para *S. aurata*, *Sa×Pp* e *P. pagrus*, respectivamente, enquanto que através do método truss, 16 características asseguraram a discriminação entre as amostras. A taxa de identificação correcta foi superior para os progenitores, 98,8% e 99,2% para *S. aurata* e *P. pagrus*, respectivamente, e ligeiramente inferior para o híbrido, com 94,7%.

Os valores médios das características morfométricas relacionados com a forma da cabeça do híbrido encontram-se mais próximos dos obtidos para o pargo, enquanto que os relacionados com a forma do corpo, se encontram mais próximos da dourada. Este resultado, denota que as características transmitidas através do património genético dos progenitores não se manifestaram de uma forma aleatória. Através de ambos os métodos foi possível estabelecer uma diferenciação entre as amostras. Apenas uma fracção de híbridos partilhou as suas características com as espécies parentais.

O estudo da relação entre a taxa de crescimento do híbrido e as diferentes dietas administradas mostrou que a temperatura da água (15,9-23,5°C), o oxigénio (5,15-6,65 $mg.l^{-1}$) e uma densidade de acondicionamento baixa (até 2,7 $kg.m^{-3}$) providenciaram condições de crescimento adequadas. Durante o primeiro período de experiências, os melhores resultados foram obtidos com o alimento A (38,43±11,68g), enquanto que no segundo período, o alimento B proporcionou uma taxa de crescimento mais elevada (32,67±12,31g). O alimento C apresentou sempre os piores resultados em ambos os períodos, respectivamente 33,81±13,35g e 18,01±6,41g. As taxas de crescimento mais elevadas foram obtidas durante o primeiro período em que a temperatura da água foi mais elevada. Nestas circunstâncias, a taxa de crescimento do híbrido foi bastante semelhante à da dourada, enquanto que, a temperaturas inferiores, o crescimento foi mais lento. Este ensaio permite concluir que a produção do híbrido *Sa×Pp* deve ser feita dentro de parâmetros ambientais criteriosos, de forma a providenciar taxas de crescimento mais elevadas.

**ABSTRACT**

The objective of this dissertation was to study the morphological characteristics of eight of the most important species of the Sparid family, namely the gilthead seabream, *Sparus aurata* Linnaeus 1758, the red porgy, *Pagrus pagrus* (Linnaeus 1758), the white seabream, *Diplodus sargus* (Linnaeus 1758), the sharpsnout seabream, *Diplodus puntazzo* (Cetti, 1777), the striped seabream, *Lithognathus mormyrus* (Linnaeus 1758), the red seabream, *Pagellus bogaraveo* (Brünnich, 1768), the common dentex, *Dentex dentex* (Linnaeus 1758), and the black seabream, *Spondyliosoma cantharus* (Linnaeus 1758), along the South European Atlantic coast and in the Mediterranean Sea.

The influence of intensive rearing conditions was also tested in *S. aurata*, in order to evaluate its influence on the morphology of the species.

The artificial hybrid *Sparus aurata*×*Pagrus pagrus* was also studied, to analise the result of the cross between two sympatric species of the same family.

From the meristic point of view, the anal, dorsal and caudal fins were the less variant of the studied meristic traits. In contrast, the pectoral fin rays (both right and left side) and the gill rakers (both upper and lower limbs) proved to be significantly different in the majority of the cases ($p<0.05$). These two traits were found to vary independently. A clear geographical variation between samples was not found, with the major differences being only found between paired samples, or between one sample and all the others, except for *Pagrus pagrus*. However, closeness between Portuguese and Italian samples was found for the *S. aurata* (both wild and reared samples) and *L. mormyrus* samples. Such similarity is mainly due to the isolation of those populations.

A specific case study concerning the relationship between the developmental stability and genetic heterozygosity was tested in the wild and cultured samples of gilthead seabream (*S. aurata*). The developmental stability was assessed by the analysis of the fluctuating asymmetry, which was shown to exist in the majority of the samples although their values were consistently low, (0.305 ±0.147), but higher in the cultured samples. The allozyme heterozygosity values were always high, but lower in the cultured samples. The microsatellite DNA analysis produced similar results. Heterozygosity was higher in cultured individuals (except for the Greek samples). These findings seem to be early evidence that the reared samples are losing some genetic variation, especially due to the loss of the rarest alleles (which were present in the wild

populations). Genetic drift, probably caused by propagation practices, is most likely responsible for the decrease of the genetic variation. No distinct pattern of geographic separation was identified.

The comparison between the pooled counts of the meristic traits of all samples for each of the studied species and the values found in the literature revealed that in 33.3% of the cases the values found in this study lie within the range found by other authors. When the counts extended beyond or below is range, in 63.3% of the cases it occurred only due to the presence of one to three fishes in the whole sample. In the remaining cases, the number of individuals that exceeded the ranges varied between 4 and 50, and in some cases in all of the studied samples.

In the morphometric analysis, the principal component 1 (PC1) loadings were similar in size and sign indicating it was a general measure of fish size and occurred in both methods (traditional morphometry and truss networks) and for all the species in study. This component was always correlated significantly with the standard length (SL). PC2 to PC5 accounted for much smaller percentage of variation not correlating with SL. Correlations between the morphological measurements and the remaining principal components occurred sporadically, and usually with PC2. When occurring, only one of them (in a universe of twelve and thirty-one morphological characteristics for the traditional morphometrics and truss networks, respectively) correlated with one of those components. For PC2 the largest loadings were usually head related for the traditional morphometry and head and the caudal peduncle related in truss analysis, which is indicative of differential growth of these body regions compared to rest of the body.

The number of characteristics that contributed for the sample discrimination varied between four and twelve, and between five and twenty-two for the traditional morphometry and truss networks, respectively. From a geographical perspective a geographic gradient in a West/East sequence was observed in *D. puntazzo* and *L. mormyrus* samples through the traditional morphometry and for *D. sargus*, *D. puntazzo*, and *D. dentex* samples through the truss networks.

Based on the truss measurements, the stepwise discriminant analysis yielded a reduced variable set that identified significant differences among all five samples of gilthead seabream, *S. aurata*. The overall percent-correct classification rate for the five samples from the stepwise discriminant analysis, based on 18 and 22 adjusted morphometric characters was 97.3% and 98.7% for the reared and wild samples,

respectively. Both wild and reared fish exhibited a clear geographical gradient. Furthermore, when wild and reared samples were compared, there was a significant degree of morphological dissimilarity between them. These results indicated significant morphological differences of gilthead seabream, suggesting that fish from these areas represent separate groups.

For white seabream, sharpsnout seabream, and striped seabream, stepwise discriminant analysis yielded a reduced variable set that identified significant differences between all samples. The overall percent-correct classification rate for the four samples from the stepwise discriminant analysis, based on 7, 9 and 10 adjusted morphometric characters (white seabream, sharpsnout seabream, and striped seabream samples, respectively) was 93.7%, 90.6% and 96.3%. For each species, there was a significant degree of morphological dissimilarity between samples. A clear geographical gradient occurred between the Atlantic and the Mediterranean samples, suggesting that in some cases the fish from these areas represent separate groups.

Like the previous species, significant differences were found between samples of red porgy *P. pagrus*, red seabream *P. bogaraveo*, and common dentex *D. dentex*. The overall percent-correct classification rate for the four samples from the stepwise discriminant analysis, based on 9, 14 and 14 adjusted morphometric characters (*L. mormyrus P. bogaraveo*, and *D. dentex*) was 96.8%, 100% and 97.9%. For *D. dentex*, a geographic gradient was found between all samples, whereas, for the other two species a good discrimination between samples was found but with no specific gradation between them.

For the black seabream, the analysis was restricted to two samples (Portugal and Spain). Nevertheless, a considerable difference was found between samples with an overall percent-correct classification rate of 100% for both samples. $D^2$ and $F$ statistics also presented highly significant results, which imply a large difference between the Atlantic and the Mediterranean sample.

Based on the traditional morphometric data, results were similar, indicating a good discrimination between samples, but never achieving for the majority of the species the values obtained with the truss networks. The traditional morphometry overlapped the results obtained with the truss networks only in two cases (*L. mormyrus* and *S. cantharus*). For *L. mormyrus*, a geographic gradient was established through traditional morphometry, whereas, for *S. cantharus* the results obtained from each of the statistical methods overlapped those obtained through the traditional morphometry.

The analysis of the hybrids showed that the meristic characters (fins and gill rakers) fall within the range observed for the parental species. Tooth counts, although separately quantified in the left and right side of the jaws, were symmetrical, presenting the same number of teeth in the left and right sides of the mouth. A major difference was the number of molar rows, which was five in the *S. aurata*, four in the *Sa×Pp* and only two in the *P. pagrus*. In an overall perspective, the hybrid evidenced a higher degree of similarity with *S. aurata*.

The osteological characteristics between the hybrids *Sa×Pp* and the parental species were compared. The neurocranium analysis indicated that the majority of the observed substructures (supraoccipital, vomer, parasphenoid, basioccipital, basisphenoid) evidenced a higher degree of similarity between hybrids and *P. pagrus*. Only two, the ethmoid and exoccipital, were similar to *S. aurata*. Other osteological structures such as the lacrimal bones, the opercle, the subopercle, the interopercle, the preopercle, the maxilla, the premaxilla, the dentary and the articular also shared a higher degree of resemblance with *P. pagrus*.

In the morphometric analysis of the hybrids, the truss protocol produced slightly worse results than the traditional morphometry. Through the traditional morphometry, nine measurements assured sample differentiation. The overall percent-correct classification success (PCS) was 100%, 98.7% and 98.3% for *S. aurata*, *Sa×Pp* and *P. pagrus*, respectively, whereas, through the truss networks sixteen truss elements were selected for sample differentiation. PCS was higher for the progenitors, 98.83 and 99.19% for *S. aurata* and *P. pagrus*, respectively, and slightly worse for the hybrids with 94.74 %.

The mean values calculated for the hybrids using body shape related characters are much closer to those calculated for the gilthead seabream, with the head being more closely related to the red porgy. Such findings show that characters did not manifest a random influence of the parental gene pool.

The morphological analysis based on both methods was able to differentiate the three samples. Only a small hybrid sample fraction shared similar characteristics with the parental species.

A comparison of the growth performance of the hybrid *Sa×Pp* fed with different diets was carried out under intensive farming conditions. Water temperature (15.9-23.5°C) and oxygen (5.15-6.65 $mg.l^{-1}$) as well as low stock density (max. 2.7 $kg.m^{-3}$)

provided favourable rearing conditions. Food A presented the best weight increase in the first period (38.43±11.68g), while food B was best in the second (32.67±12.31g). Food C presented the worst results in both periods, with 33.81±13.35g and 18.01±6.41g, respectively. The best growth rates were obtained in the first period of experiments with higher temperatures. Under these circumstances, the hybrid general growth was quite similar to the gilthead sea bream. In contrast, under low temperatures, growth was very poor. The present work showed that rearing of the hybrid *Sa*×*Pp* must be undertaken within a specific temperature range to provide the maximum growth capacity.

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## INTRODUCTION

Fishes have been ecologically dominant in aquatic habitats through much of the history of the complex life, inhabiting all aquatic environments regardless of the local conditions. They occupy essentially all habitats that have liquid water throughout the year, including thermal and alkaline springs, hypersaline lakes, sunless caves, anoxic swamps, temporary ponds, torrential rivers, wave-swept coasts, high-altitude, high-latitude environments (Helfman *et al.*, 1997) and sub-zero water temperatures (Eastman & McCune, 2000). To colonize and thrive in such a variety of environments, fishes have evolved obvious and striking anatomic, physiological, behavioural, and ecological adaptations. A living animal is the result of past evolutionary events and those animals will be adapted to current environmental forces only if those forces are similar to what has happened to the individual's ancestors. Such phylogenetic constraints arise from the long-term history of a species (Helfman *et al.*, 1997).

This dispersion of fishes, tightly related with their great capability of adaptation to the environment, is relevantly reflected by the number of species. Valid scientific descriptions exist for approximately 24,600 living species of fishes (Nelson, 1994), which represent 48.1% of the vertebrate diversity (Lagler *et al.*, 1977). Marine fishes comprise about 58% of this number, whereas freshwater fishes make up about 41% and 1% move between freshwater and the sea during their life cycles (Cohen, 1970). The majority of marine fish species (78%), which represent 44% of all fishes inhabit the narrow water layer less than 200 m deep along the land masses (Moyle & Cech, 1996).

Colonisation of new habitats is driven by several factors that influence the survival of species, and can be resumed to three main aspects: environmental conditions, food availability and niche availability. Fishes like other animals live preferentially in habitats with environmental conditions that benefit their life style. If the conditions are not attained, the habitat adaptability can, in a long term, induce a new life style in a new environment, when available (niche availability). However, such adaptation is only viable if a reliable food source is present.

Although many fish radiations display phyletic diversity but little morphological and ecological diversity (Brooks & McLennan, 1991; Mayden, 1992) others can exhibit substantial morphological and ecological diversification. The adaptation to a new habitat induce several changes in the body morphology of the fish, implying alterations that on a long term scale can transform two different populations of a single species in

two different species. Such body changes are mainly determined by feeding abilities (Ruiz & Lorencio, 1987, 1988; Hyndes *et al.*, 1997), local orographic conditions (Riddell & Leggett, 1981; Tortonese, 1983), depth dependency (Uiblein *et al.*, 1994), or even locomotion (swimming ability) (Webb, 1984; Gozlan, 1998).

Morphological studies consist in one of the many available ways to study fish adaptability to the environment. They are closely related to other areas such as ecology, systematics or even paleogeography, as well as other disciplines such as geology and biogeography. Several groups of fishes exhibit a remarkable range of morphological adaptive radiation (Fermon & Cibert, 1998). Particular cases, that are well documented such as the high abundance of African Cichlids species that live in the Lakes Tanganyika, Victoria and Malawi compared with other geographical locations (North and South America) (Brooks, 1950; Fryer & Iles, 1972; Meyer *et al.*, 1990) illustrate how the geological movements of the earth crust represent a major and determinant factor in the fluctuations of the levels of the African Great Lakes, and so act as a leading factor for the diversification and speciation of this fish family.

Throughout the years, numerous publications have focused on morphological related subjects, but only recently with the development of computer techniques have more detailed and insightful analyses been carried out. Until recently, morphological analysis was mainly based on recordable features, such as meristic characteristics, with small attempts in the allometric relationships of the animals.

Nowadays the objects of the morphometric studies are not the forms themselves, but rather their associations, causes and effects (Bookstein, 1997). Characters derived from the external phenotype of organisms provide useful information on the similarity or dissimilarity of taxa (Reist, 1985), conspecifics populations or ontogenetic stages (Strauss & Bookstein, 1982). These orientations of objectives lead the morphometric studies from the so called traditional morphometrics to the new morphometrics. The approach now referred as traditional morphometrics is only a few decades old and it is characterized by the application of multivariate statistical methods to sets of variables. Such variables usually correspond to various measured distances on the organisms. The measurements are usually length and width of structures or distances between certain landmarks (Rohlf & Marcus, 1993).

New morphometrics is characterized by a new approach where the data is recorded to capture the structure being studied. This is in the form of two-dimensional (2-D) or three-dimensional (3-D) coordinates of morphological landmark points (Rohlf

& Marcus, 1993). The analysis of the form of whole biological organs or organisms is done by the acquisition of geometric locations of the landmark points. These points not only have their own locations but also have the "same" locations in every other form of the study. These measurements of the shapes of configurations of landmark locations reduce to multiple vectors of shape coordinates. These come in pairs that represent the shape of one triangle of landmarks in a manner completely independent of size (Bookstein, 1997). These landmark points are usually analysed by truss morphometrics protocol (Strauss & Bookstein, 1982; Bookstein *et al.*, 1985).

Geographic variation in species characteristics is a function of the genetic variability of individuals comprising local populations and of environmental differences they experience in time and space (Ridell & Leggett, 1981). In such a context a significant number of authors have tested the relationship between genetics and morphological variability (e.g. Beacham & Withler, 1985a, 1985b; Strauss, 1989; Beacham, 1990; Pepin & Carr, 1993; Bembo *et al.*, 1996; Hänfling & Brandl, 1998; Zawadzki *et al.*, 1999). Nevertheless, in fish populations, genetic sources of phenotypic variation have generally been deemed less important than environmental sources in determining interpopulation differences. Beside the morphological characters, genetics is frequently compared with meristic characteristics (e.g. Epperly, 1989; Beacham & Withler, 1985a, 1985b; Bembo *et al.*, 1996) due to the same reasons stated above.

Meristic traits are often considered to be the most reliable taxonomic characteristics because most are easy to determine (Moyle & Cech, 1996). Meristic traits include anything on a fish that can be counted, such as vertebrae, fin rays and spines, scale rows, pyloric ceca, lateral line pores and gill rakers. Within species variability usually occurs, so the interpretation of meristic data must be conscientious and not be unequivocally attributed to differences between species. Another factor that can lead to erroneous results is human error, especially in small fish (Moyle & Cech, 1996). Standardization of methodology is always important in studies where more than one reader is involved in data analysis.

Published morphological studies have focused on a vast number of species, with a special emphasis on salmonids (e.g. Winans, 1984; Winans & Nishioka, 1987; Bailey & Irvine, 1991; Wilkins *et al.*, 1994; Kacem *et al.*, 1998) and cyprinids (Child & Solomon, 1977; Corti *et al.*, 1988; Claytor & Verspoor, 1991, Claytor *et al.*, 1991; Bogutskaya & Collares-Pereira, 1997; Hanfling & Brandl, 1998; Cibert *et al.*, 1999). Published information on the morphology of Sparid species is scarce, especially if

carried out over distinct geographical locations. During the preparation of the present dissertation only two publications on the morphology of Sparid species morphology were found in the literature. Due to this fact, this dissertation tries to discriminate and characterize the most important species of the Sparid species in the Eastern Atlantic Ocean and Mediterranean Sea. Due to the high commercial value of these species there is a constant and some times extreme pressure on their stocks, so an in depth understanding deep knowledge of these fish species is extremely important to their management and conservation.

In recent years, a huge effort has been made to increase the research on new species for aquaculture in order to supplement natural production. Presently, among Sparids, only gilthead seabream is produced in a reliable commercial perspective. However, species like sharpsnout seabream are in a short term, promising marine fish species for aquaculture (Sará *et al.*, 1999), whereas others, like red porgy, white seabream or common dentex remain in a preliminary research (Divanach *et al.*, 1993; Kentouri *et al.*, 1994a; Kentouri *et al.*, 1995a). Furthermore, the production of hybrids has been concidered as another possibility for increasing the supply of high value fish.

Several authors have described natural hybridisation (e.g. Child & Solomon, 1977; Crivelli & Dupont, 1987; Waldman & Bailey, 1992; Kerby, 1993; Delling *et al.*, 2000). The success of hybridisation has often been considered as an indication of the evolutionary relationship between two species (Hester, 1970). Among fishes, hybridisation often occurs when one species experiences a substantial reduction in abundance, so that rare fish tend to mate with closely but much more abundant members of other species (Hubbs, 1955). Among the Eurasian *Salmo* species, hybrids between Atlantic salmon *Salmo salar* L. and brown trout *S. trutta* L. are reported frequently from the entire range of sympatry of the two species (McGowan *et al.*, 1992; Elo *et al.*, 1995). To date, there have been no published reports on natural hybridisation in the Sparid family and few have been published on laboratory-induced hybridisation (e.g. Dujaković & Glamusina, 1990). Nevertheless, the study of artificial Sparid hybrids is an important and interesting case study of the sympatric and genetically related species of this family, relying mainly on the biological analysis (genetic, morphology or growth) and in its commercial importance as a potentially high profitable and reliable resource.

The objective of this dissertation was to analyse the meristic and morphometric variability of eight species of the Sparidae family: gilthead seabream, *Sparus aurata* Linnaeus, 1758, (wild and reared samples), red porgy, *Pagrus pagrus* (Linnaeus, 1758),

white seabream, *Diplodus sargus* (Linnaeus, 1758), sharpsnout seabream, *Diplodus puntazzo* (Cetti, 1777), striped seabream, *Lithognathus mormyrus* (Linnaeus, 1758), red seabream, *Pagellus bogaraveo* (Brünnich, 1768), common dentex, *Dentex dentex* (Linnaeus, 1758), and black seabream, *Spondyliosoma cantharus* (Linnaeus, 1758) as well as the hybrid *Sparus aurata* x *Pagrus pagrus*.

This work tried not only to clarify the morphological characteristics of these eight species, but also to devise and answer new and relevant questions about them; 1) Are there differences between populations, and if so, could they be attributed to the same geographical barriers that were already known to constrain other species? 2) Could a geographical gradient be established between the studied populations, translating the geographical distance into morphological difference? 3) And from a technical perspective, were the chosen methods efficient enough to discriminate between populations? 4) Were the chosen characteristics adequate, assuring a conclusive sample differentiation? And finally, 5) what is the most reliable morphological technique for this type of study, the traditional morphometry or the truss network?

In order to give a clear answer to these questions, the dissertation was structured in three major areas; the meristic analysis of the eight Sparid species, the morphological analysis of those species and the hybrid analysis.

Beside the three major areas pointed above, three case studies were considered throughout this dissertation to complement the analysis; 1) the effect of the rearing conditions on the morphology of the gilthead seabream, *S. aurata*, 2) the study of the fluctuating asymmetry as a suitable indicator of stock condition and heterozygosity levels, and, 3) the study of an hybrid as a result of the cross of two sympatric species of a same family (in this case the species, *S. aurata* and *P. pagrus*), with a reference to the analysis of the expression of the morphological traits inherited from the progenitors.

The analysis of wild and reared samples of *Sparus aurata*, were carried out in order to verify if the rearing and reproductive conditions induced in the aquaculture facilities drive the morphology of those populations in other directions different from the wild animals. All artificial propagation and production affects the genetic composition of the cultured stock and even where no genetic selection is consciously practised, the change in the environmental conditions, increased food availability, higher densities, pathogens control and water quality have implications for survival and

hence for inheritance (Wilkins, 1987). Such conditions are often expressed as changes in the morphological characteristics of the fish.

The study of the fluctuating asymmetry (small random departures from bilateral symmetry) as a suitable indicator of stock condition and heterozygosity levels, through the comparison of the meristic information with genetics (allozyme and microsatellite analysis), was analysed in wild and reared samples of gilthead seabream. And finally, the hybrids analysis was extended from the previously referred (meristic and morphometry) to an osteological perspective (head morphology and mouth apparatus). Growth performance experiments were also carried out in comparative perspective with the parental species, but also as a potential candidate for aquaculture.

In the morphological analysis, the two different methods of analysis referred to above were used, the "traditional", and the truss method. These methods were compared in order to identify the most suitable for fish populations discrimination. This issue has been under discussion since more recent techniques (truss networks) have arisen. The meristic analysis tried to serve the same purpose but from a different angle of approach. Such analyses can be considered complementary.

Parts of the three chapters of this dissertation are identical to five papers already published or submitted for publication. The first paper (Chapter 1; Palma *et al.*, 2001b) is published in the *Journal of the Marine Biological Association of United Kingdom*. The second and third (Chapter 2; Palma & Andrade, 2001; 2002) are submitted in the *Journal of Fish Biology* and published in the *Fisheries Research*, respectively. The fourth and fifth (Chapter 3, Palma *et al.*, 1998; Palma *et al.*, 2001a) are published in the *Italian Journal of Zoology* and submitted to the journal *Aquaculture International*, respectively.

# CHAPTER 1 - MERISTIC ANALYSIS

## 1.1 - *Introduction*

Embryonic development generally progresses according to instructions laid down in the genetic blueprint, but timing and occurrence of specific developmental details are sensitive to environmental influences (Beacham, 1990; Helfman *et al.*, 1997), with the morphology of an individual being produced by the developmental process that transforms the genotype into the phenotype (Vøllestad & Hindar, 1997). Natural variations such as temperature, oxygen, salinity, light intensity, photoperiod or $CO_2$ can affect development. Non-natural phenomena such as anthropogenic influences can also induce changes in the aquatic habitat and result in larval abnormalities (Longwell *et al.*, 1992). Environmental changes or its manipulation, which occur in semi-intensive and intensive aquaculture facilities, can also produce similar distress factors in fish. The ability of development to produce a determined phenotype despite eventual disturbing factors is defined as developmental stability (Leary *et al.*, 1992).

Meristic traits such as number of fin rays, vertebrae, scales in the lateral line, and gill rakers are known to vary in relation to environmental conditions. The number of the different meristic traits is of considerable use in the classification of fishes (Lagler *et al.*, 1977). Historically, the morphology of fishes has been the primary source of information for taxonomic and evolutionary studies (Strauss & Bond, 1990). The practice of segregating groups, especially fishes, based on differences in vertebrae counts, fin ray counts, and morphometric distances is well entrenched in systematics (Sneath & Sokal, 1973) and fisheries biology (Royce, 1964).

Such differentiation can also be used in the discrimination between different populations of a same species, reflecting fairly different phenotypic differences among population subjected to different environmental conditions. As such, the basis for such analyses is that morphometric and meristic characteristics are essentially phenotypic expressions of environmental conditions during egg and larval development, which in an adequate spatial and temporal heterogeneity provide the basis for stock differentiation (Shepherd, 1991).

In paired structures, such as pectoral fins, gill rakers or number of scales of the lateral line, the distress caused by environmental changes can be assessed by the degree of asymmetry of the left and right side of the fish. Fluctuating asymmetry (FA), small

random departures from bilateral symmetry (Markow, 1995) has been used as a measure of developmental stability of bilateral, normally symmetrical morphological traits (Palmer & Strobeck, 1986; Zakharov, 1992). FA is a suitable indicator of stock condition and heterozygosity levels (Crozier, 1997) in salmonids and other fish (Leary *et al.*, 1984, 1985; Blanco *et al.*, 1990). In recent years, a number of studies have examined the relationship between enzyme heterozygosity and FA (e.g. Leary *et al.*, 1983; Crozier, 1997). The results of these studies have supported the hypothesis that individuals or populations with higher heterozygosity also display higher developmental homeostasis, which is reflected by lower degrees of bilateral asymmetry of meristic traits. FA quantifies the differences between the two sides in various morphological traits: the larger the FA, the lower the developmental stability. The concept is that individuals of low fitness cannot control their development precisely, and consequently more often develop different phenotypes on both sides (Windig & Nylin, 2000). Both intrinsic (genetic) and extrinsic (environmental) factors may influence developmental stability (Wilkins *et al.*, 1995). Their relative impact can be evaluated by means of FA, which can be easily used by aquaculturists, simply by counting meristic traits. In the present chapter the relationship between fluctuating asymmetry (FA) and multilocus heterozygosity, derived from allozyme and microsatellite markers, was studied for the specific case of wild and cultured stocks of gilthead seabream, *Sparus aurata.*

However, although using discriminative criteria such as FA and its relation with fish heterozygosity, the results are not always clear. The high within species variability of some traits are not easy to understand, and results must be analysed with precaution. In several species, sex is an important source of variation (e.g. Haddon & Hillis, 1995). However, in the Sparid species analysed in the present work, hermaphroditism (protogynous or protandrous) is widespread in the family (Whitehead *et al.*, 1986) and meristic sex differentiation is not referred in the literature.

In the South European Atlantic coast and Mediterranean Sea almost all Sparid fishes are highly valuable marketable fish. Their stock exploitation is often intensive and continuous throughout the year, so adequate stock management policies are required. Such measures can only be assured with a complete knowledge of the fish biology, which may provide the basis for separation and management of distinct populations (Pepin & Carr, 1993).

This study aims to identify eventual differences among the populations of eight species of the Sparid family, the gilthead seabream, *Sparus aurata* Linnaeus 1758, (wild

and reared samples), the red porgy, *Pagrus pagrus* (Linnaeus, 1758), the white seabream, *Diplodus sargus* (Linnaeus, 1758), the sharpsnout seabream, *Diplodus puntazzo* (Cetti, 1777), the striped seabream, *Lithognathus mormyrus* (Linnaeus, 1758), the red seabream, *Pagellus bogaraveo* (Brünnich, 1768), the common dentex, *Dentex dentex* (Linnaeus, 1758), and the black seabream, *Spondyliosoma cantharus* (Linnaeus, 1758), along the South European Atlantic coast and in the Mediterranean Sea, based on their meristic variability.

## 1.2 - MATERIALS AND METHODS

### 1.2.1 - *Sampling*

Meristic data from 1767 specimens of eight sparid species; *S. aurata*, (wild and reared samples), *P. pagrus*, *D. sargus*, *D. puntazzo*, *D. dentex*, *L. mormyrus*, *P. bogaraveo*, and *S. cantharus* (Annex I, Figures 1 to 8) from four European countries (Portugal, Spain, Italy and Greece) were used. Specimens were captured in the surrounding areas of the places presented in Figure 1 (see Table I for details).

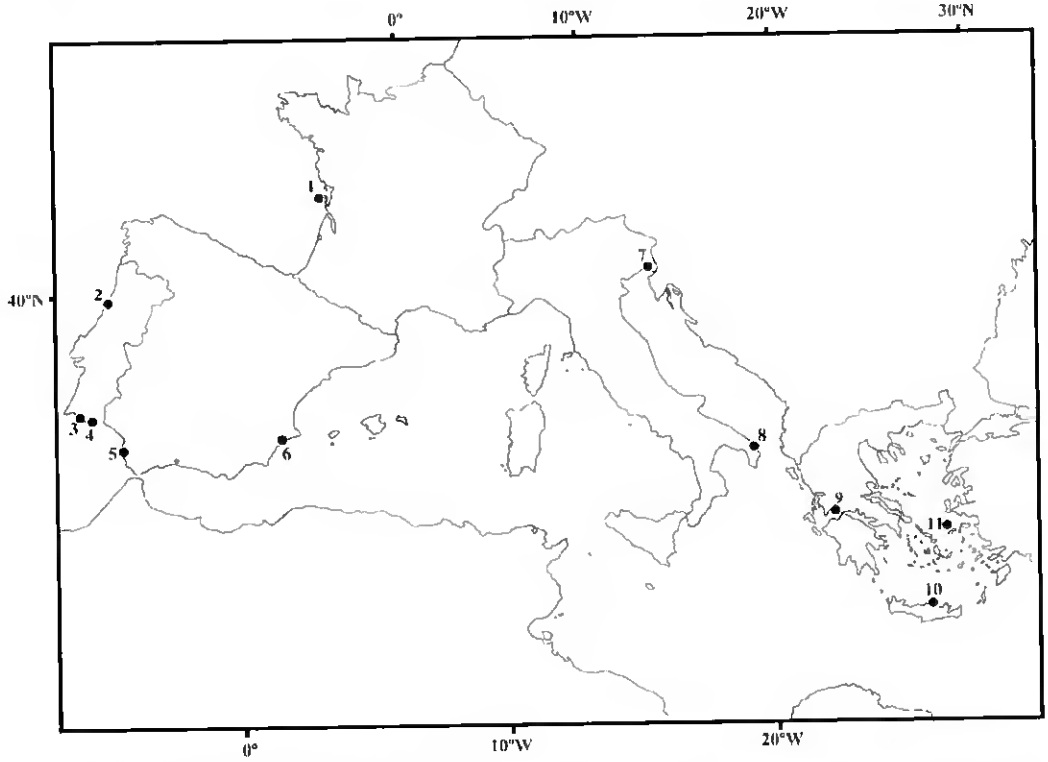



Figure 1 - Sampling locations; France (1 – Oleron Island, *S. aurata* reared sample), Portugal (2 - Ria de Aveiro lagoon (*S. aurata* wild sample), 3 - Faro (all samples except *P. bogaraveo* and *S. aurata*), 4 - Tavira (*S. aurata* reared sample)), Atlantic Spain (5 – Cadiz (both wild and reared samples of *S. aurata*)), Mediterranean Spain (6 – Murcia and Alicante (all Mediterranean Spanish samples)), Italy (7 – Trieste, (both wild and reared samples of *S. aurata*), 8 – Lecce, Italy (remaining species)), Greece (9 - Mesologgi lagoon, (*S.aurata* wild sample), 10 - Crete (all Greek samples, except *S. aurata*), 11 - Leros Island (*S. aurata* reared sample)).

Table I - Sampling location (geographic coordinates) and number of specimens in each sample for all the species in study, except *Sparus aurata* (both reared and wild).

| | Country | Sampling locations | N |
|---|---|---|---|
| *Pagrus pagrus* | Portugal | Quarteira (Algarve/South coast) Lat. 37° 04'N Long. 08° 06'W | 109 |
| | Spain | Tabarca/Alicante Lat. 38° 2'N Long. 0° 29'W | 37 |
| | Italy | Lecce Lat. 40° 13'N Long. 18° 10'W | 31 |
| | Greece | Iraklion Lat. 35° 20'N Long. 25° 08'E | 54 |
| *Diplodus sargus* | Portugal | Faro (Algarve/South coast) Lat. 37° 26'N Long. 90° 26'W | 114 |
| | Spain | Tabarca/Alicante Lat. 38° 2'N Long. 0° 29'W | 30 |
| | Italy | Lecce Lat. 40° 13'N Long. 18° 10'W | 33 |
| | Greece | Iraklion Lat. 35° 20'N Long. 25° 08'E | 30 |
| *Diplodus puntazzo* | Portugal | Faro (Algarve/South coast) Lat. 37° 26'N Long. 90° 26'W | 35 |
| | Spain | Tabarca/Alicante Lat. 38° 2'N Long. 0° 29'W | 41 |
| | Italy | Lecce Lat. 40° 13'N Long. 18° 10'W | 41 |
| | Greece | Iraklion Lat. 35° 20'N Long. 25° 08'E | 43 |
| *Lithognathus mormyrus* | Portugal | Faro (Algarve/South coast) Lat. 37° 26'N Long. 90° 26'W | 123 |
| | Spain | Tabarca/Alicante Lat. 38° 2'N Long. 0° 29'W | 32 |
| | Italy | Lecce Lat. 40° 13'N Long. 18° 10'W | 31 |
| | Greece | Iraklion Lat. 35° 20'N Long. 25° 08'E | 31 |
| *Pagellus bogaraveo* | Portugal | Horta (Faial/Açores) Lat. 38° 32'N Long. 28° 38'W | 73 |
| | Spain | Tabarca/Alicante Lat. 38° 2'N Long. 0° 29'W | 49 |
| | Italy | Lecce Lat. 40° 13'N Long. 18° 10'W | 32 |
| | Greece | Iraklion Lat. 35° 20'N Long. 25° 08'E | 32 |
| *Dentex dentex* | Portugal | Quarteira (Algarve/South coast) Lat. 37° 04'N Long. 08° 06'W | 38 |
| | Spain | Tabarca/Alicante Lat. 38° 2'N Long. 0° 29'W | 31 |
| | Italy | Lecce Lat. 40° 13'N Long. 18° 10'W | 30 |
| | Greece | Iraklion Lat. 35° 20'N Long. 25° 08'E | 45 |
| *Spondyliosoma cantharus* | Portugal | Faro (Algarve/South coast) Lat. 37° 26'N Long. 90° 26'W | 39 |
| | Spain | Tabarca/Alicante Lat. 38° 2'N Long. 0° 29'W | 30 |
| | Greece | Iraklion Lat. 35° 20'N Long. 25° 08'E | 3* |

* - Excluded from analysis due to the small sample size.

For *S. aurata*, additional samples were collected in the Atlantic area of Spain (Cadiz) (both wild and reared) and Atlantic area of France (reared sample) (Figure 1 and Table II for details). Hereon, *S. aurata* samples will be mentioned as, FrR (France - reared sample), PtW (Portugal - wild sample), PtR (Portugal - reared sample), SpAtW (Atlantic Spain - wild sample), SpAtR (Atlantic Spain - reared sample), SpMW (Mediterranean Spain - wild sample), SpMR (Mediterranean Spain - reared sample), ItW (Italy - wild sample), ItR (Italy - reared sample) and GrW (Greece - wild sample), GrR (Greece - reared sample). The remaining species will be identified by the species and country.

Table II - Sampling location, geographic coordinates and number of individuals (N) in each sample for *Sparus aurata* (both reared and wild).

| | Sampling locations | |
|---|---|---|
| | Reared | Wild |
| France | Oléron Island<br>Lat. 45° 55'N Long. 1° 16'W<br>N=40 | -<br>-<br>- |
| Portugal | Tavira<br>Lat. 37° 07'N Long. 7° 39'W<br>N=50 | Ria de Aveiro Lagoon<br>Lat. 40° 38'N Long. 8° 39'W<br>N=77 |
| Spain Atlantic | San Fernando/Cadiz<br>Lat. 36° 32'N Long. 6° 18'W<br>N=50 | San Fernando/Cadiz<br>Lat. 36° 32'N Long. 6° 18'W<br>N=48 |
| Spain Mediterranean | Mazarrón/Murcia<br>Lat. 37° 59'N Long. 1° 08'W<br>N=50 | Tabarca/Alicante<br>Lat. 38° 21'N Long. 0° 29'W<br>N=51 |
| Italy | Trieste<br>Lat. 45° 40'N Long. 13° 35'E<br>N=50 | Trieste<br>Lat. 45° 40'N Long. 13° 35'E<br>N=40 |
| Greece | Leros Island<br>Lat. 37° 10'N Long. 26° 50'E<br>N=54 | Mesologgi lagoon (Gulf of Patras)<br>Lat. 38° 20'N Long. 21° 20'E<br>N=40 |

Single samples were captured from each one of the referred locations. Portuguese sample of *P. bogaraveo* was captured in the Azorean waters around the Island of Faial. Reared samples of *S. aurata* were obtained during the spring of 1995, from well establish fish farms operating for several years. The broodstocks have been managed for several generations, and were formed with individuals collected from the wild in the surrounding sea areas of those fish farms. All the other species, including the *S. aurata* wild samples were captured during the spring of 1996, and spring of 1997. The Portuguese and Italian samples were obtained from commercial catches, and those

from Spain and Greece were collected from scientific cruises. All individuals evidenced adult morphology.

### 1.2.2 - *Meristic counts*

Characters recorded were: number of soft rays of the dorsal, anal, caudal and pectoral fins (this one in the left and right side), and number of gill rakers on the upper and lower first branchial left and right arches. Gill rakers on the first branchial arch were counted on the arch's upper (epibranchial) and lower (ceratobranchial) limb. The raker in the angle between the upper and lower limb was counted in the lower limb. Gill rakers were divided into upper and lower sectors, because they have been shown to vary independently (Leary *et al.*, 1983). All individuals of the cultured sample from Greece had an abnormality and did not present gill rakers in the first branchial arches. Thus, these were not scored for this character.

Pectoral fin rays and gill rakers counts were recorded on fresh specimens. When necessary a binocular microscope was used. These counts were performed by different readers (one in each country). After this, fish were X-rayed in a mammography X-ray device, which is much more sensitive than the standard X-ray device. An intensity between 25-27kV assured that all topographic landmarks were always visible, including the fleshy ones. Before taking the radiograph, this machine lowered down a plastic plate that hold the fish parallel to the X-ray source, thus minimizing distortions. Each fish was radiographed with the fins in the extended position and the resulting X-rays maintained their real size.

Counts of the dorsal fin, caudal fin, and anal fin were recorded from radiographs, and were performed by the same sampler for all samples. A previous meeting was held between samplers to standardize procedures.

Fluctuating asymmetry was assessed by counting three bilateral meristic characters: pectoral fin rays, and gill rakers on the upper and lower first branchial arches, and were counted as described above. These characters were chosen because they could be easily and accurately counted. Other bilateral characteristics such as the pelvic fins were not scored because they do not show variation in the Sparidae fishes.

### 1.2.3 - *Allozyme electrophoresis*

For allozyme electrophoresis, frozen tissues were subjected to no more than three freezing and thawing cycles to obtain a cell lysate, which was run through a

horizontal starch gel. Electrophoretic protocols, staining procedures and genetic interpretation of zymogram patterns and locus designation were done according to Reina *et al.* (1994). A total of 16 enzymatic systems were used in the sample analysis (Table III). The individual and sample heterozygosity (heterozygous loci in each animal/number of loci of each animal) was calculated for all samples.

Table III - List of enzyme systems. *- CTC - Continuous Tris-Citrate, pH 8.0, RID - Litium-citrate, pH 8.1

| | E.C. nº | Tissue | Buffers* | Nº of Loci |
|---|---|---|---|---|
| Adenilato Kinase (AK-1 and AK-2) | 4.2.1.3. | Muscle | CTC | 2 |
| Adenosine Desaminase (ADA) | 3.5.4.4. | Liver | RID | 1 |
| Alcohol Dehydrogenase (ADH) | 1.1.1.1. | Liver | CTC | 1 |
| Diaforase (DIA-1) | 1.6.2.2. | Liver | RID | 2 |
| Esterase (EST) | 3.1.1.*. | Liver | RID | 1 |
| Glucose-6-phosphate Dehydrogenase (GPI1 and GPI2) | 5.3.1.9. | Muscle | RID | 2 |
| Glicerol-3-phosphate Dehydrogenase (G3PDH) | 1.1.1.8. | Muscle | CTC | 1 |
| Iditol Dehydrogenase (IDDH) | 1.1.1.14. | Liver | CTC | 1 |
| Isocitrete Dehydrogenase (IDHP) | 1.1.1.42. | Liver | CTC | 1 |
| Lactate Dehydrogenase (LDH-1, LDH-2 and LDH-3) | 1.1.1.27. | Eye | RID | 3 |
| Malate Dehydrogenase (MDH-1, MDH-2 and MDH-3) | 1.1.1.37. | Muscle | CTC | 3 |
| Malic Enzyme (MEP-1and MEP-2) | 1.1.1.40. | Muscle | CTC | 2 |
| Phosphoglucomutase (PGM) | 5.4.2.2. | Muscle | RID | 1 |
| 6-phosphoglucunate Dehydrogenase (PGDH) | 1.1.1.44. | Liver | CTC | 1 |
| Superoxidase Dismutase (SOD-1 and SOD-2) | 1.15.1.1. | Liver | RID | 2 |
| Xantine Dehydrogenase (XDH) | 1.2.1.37. | Liver | CTC | 1 |

### 1.2.4 - *Microsatellite DNA analysis*

To estimate heterozygosity values three microsatellite loci were screened: (SA26, SA32 and SA41b, SA- *Sparus aurata*) with EMBL library accession numbers Y17266, Y17264 and Y17262, respectively.

DNA extraction from each individual was performed according either to the standard proteinase-K protocol (Sambrook *et al.*, 1989) or the salt-extraction technique of Miller *et al.* (1988). 10–100 ng of these DNA's were used in PCR reactions (vol. 10 µm), containing 0.2 mM of each dNTP, 1 mM $MgCl_2$, 0.5 µM of each primer, and 0.5 units of Taq polymerase. A small fraction of the reverse primer was end-labelled prior to amplification. Each amplification included seven cycles with denaturation at 94°C, annealing at 52°C and extension at 72°C, and 28 cycles under the same conditions, except that the denaturation temperature was 88°C. All cycles lasted for 30 seconds. An aliquot of the PCR products (5µl) was run in 6% polyacrylamide denaturing sequencing gels (2.5 h at 60W). DNA bands were visualised by autoradiography. The process was performed according to Batargias *et al.* (1999).

### 1.2.5 - *Statistical analysis*

The traditional way to present meristic data has been as mean values and total range (Reinert & Larstein, 1992). In the present work, beside the descriptive statistical analysis that was performed for each sample, the Mann-Whitney *U* test (Zar, 1984) was used to test the differences between samples. Although less powerful than the *t*-test or one-way ANOVA this test is less likely to find a significant result when there is no real difference (Dytham, 2000). Thus, the null hypothesis that two groups come from the same distribution was tested. The meristic variability was compared between all pairwise combinations of populations with the Mahalanobis distance ($D^2$).

Four steps were followed in the analysis of FA data: *i*) a two-way ANOVA tested the data, regardless of the right-left orientation. Larger values were placed in a first column and the smaller ones in a second. No statistical difference between columns indicates that asymmetry is not different from bilateral symmetry. A statistical difference indicates that asymmetry exists, *ii*) a two-way ANOVA tested the data in its original orientation with respect to right and left sides (Palmer & Strobeck, 1986). No statistical difference indicates non-directionality. Columns were tested for normality and homogeneity of variance (Sokal & Rohlf, 1981) *iii*) the distribution of the signed asymmetries was tested for normality (Palmer & Strobeck, 1986), with normality indicating no antisymmetry, *iv*) if the assumptions of the previous points were verified the FA was calculated and expressed as variance, var(A*i*), were A*i*=(R*i*-L*i*), (A*i*=Asymmetry of a particular character for individual *i*; R*i*= counts on the right side; L*i*= counts on left side). The above procedures were performed as suggested by Pomory (1997) for determining the pattern and type of asymmetry. The FA indices were correlated with the allozyme and microsatellite heterozygosity levels.

The Shapiro Wilks test (Conover, 1980; Zar, 1984) was used to assess normality of data.

Within-sample relationships between heterozygosity (allozyme and microsatellite loci) and asymmetry (FA) were examined at the individual level using the Spearman rank correlation test (Conover, 1980). Significance of correlation coefficients was corrected using the Bonferroni method (Snedecor & Cochran, 1982). A null hypothesis, stating that heterozygous individuals have the same level of bilateral symmetry as homozygous individuals was tested in this analysis.

FA and heterozygosity were compared among samples using the Wilcoxon signed rank test (Conover, 1980; Zar, 1984). Homogeneity among samples was tested using Chi-Square test ($\chi^2$) (Conover, 1980; Zar, 1984).

## 1.3 – RESULTS

Descriptive statistics for the species in study are presented in the Appendix I - Tables I to IX. The anal fin proved to be the least variable meristic trait. When no variability was registered, the anal fin accounted for 73.3% of the cases, the dorsal fin 20%, and the caudal fin 6.6%.

Sample variance (Appendix I - Table X) varied within a very narrow boundary, always presenting low values. Nevertheless, variance was higher for the pectoral fin (left and right side) and gill rakers, both upper and lower limb. Such occurrence was confirmed through the Man-Whitney *U* test, which showed no significant differences between samples for the majority of the pairwise combinations (Appendix I - Tables XI to XIX). In contrast, the pectoral fin (both right and left side) and gill rakers (both upper and lower limb) proved to be significantly different for the majority of the cases (Appendix I - Tables XI to XIX).

Left and right counts of the pectoral fin rays and gill rakers showed an independent variation between sides of the fish. Nevertheless, statistical analysis showed that both sides exhibit similar patterns of significance among the studied samples (Appendix I - Tables XI to XIX).

Despite the above stated differences between samples, these could not be translated through the Mahalanobis distances ($D^2$) into geographical differentiation between them (Table IV). The only evidence that can be retained from Mahalanobis distances is the major difference between paired samples, or between one sample and all the others, namely, the difference between the French sample of reared *S. aurata* and all the other samples; between the Italian and Portuguese samples of wild *S. aurata* from the others, and the Italian samples of *D. sargus* and *P. bogaraveo*, the Greek sample of *D. puntazzo* and the Spanish sample of *L. mormyrus* from all the others (Table IV). The pectoral fins rays (both left and right), and upper and lower gill rakers (both left and right) invariably made the largest contribution to this differentiation.

Table IV - Values of the squared Mahalanobis ($D^2$) distances for all the species (Atl. - Atlantic, Med. - Mediterranean).

| | | France | Portugal | Spain Atl. | Spain Med. | Italy |
|---|---|---|---|---|---|---|
| *Sparus aurata* (reared) | France | 0 | 11.81 | 11.74 | 9.16 | 10.36 |
| | Portugal | 11.81 | 0 | 2.86 | 10.39 | 3.07 |
| | Spain Atlantic | 11.74 | 2.86 | 0 | 6.68 | 5.13 |
| | Spain Mediterranean | 9.16 | 10.39 | 6.68 | 0 | 4.72 |
| | Italy | 10.36 | 3.07 | 5.13 | 4.72 | 0 |

| | | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|---|
| *Sparus aurata* (wild) | Portugal | 0 | 11.73 | 3.89 | 0.76 | 12.83 |
| | Spain Atlantic | 11.73 | 0 | 4.07 | 16.52 | 0.87 |
| | Spain Mediterranean | 3.89 | 4.07 | 0 | 5.80 | 3.78 |
| | Italy | 0.76 | 16.52 | 5.80 | 0 | 16.63 |
| | Greece | 12.83 | 0.87 | 3.78 | 16.63 | 0 |

| | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| *Pagrus pagrus* | Portugal | 0 | 3.77 | 0.79 | 2.56 |
| | Spain | 3.77 | 0 | 4.85 | 5.68 |
| | Italy | 4.85 | 0.79 | 0 | 1.08 |
| | Greece | 5.68 | 2.56 | 1.08 | 0 |

| | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| *Diplodus sargus* | Portugal | 0 | 4.54 | 10.23 | 2.80 |
| | Spain | 4.54 | 0 | 6.27 | 7.45 |
| | Italy | 6.27 | 10.23 | 0 | 10.56 |
| | Greece | 7.45 | 2.80 | 10.56 | 0 |

| | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| *Diplodus puntazzo* | Portugal | 0 | 18.88 | 12.44 | 11.79 |
| | Spain | 18.88 | 0 | 6.02 | 14.55 |
| | Italy | 6.02 | 12.44 | 0 | 17.81 |
| | Greece | 14.55 | 11.79 | 17.81 | 0 |

| | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| *Dentex dentex* | Portugal | 0 | 9.14 | 4.32 | 1.56 |
| | Spain | 9.14 | 0 | 3.70 | 6.83 |
| | Italy | 3.70 | 4.32 | 0 | 5.53 |
| | Greece | 6.83 | 1.56 | 5.53 | 0 |

| | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| *Pagellus bogaraveo* | Portugal | 0 | 2.54 | 3.77 | 4.89 |
| | Spain | 2.54 | 0 | 7.50 | 2.47 |
| | Italy | 7.50 | 3.77 | 0 | 9.28 |
| | Greece | 2.47 | 4.89 | 9.28 | 0 |

| | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| *Lithognathus mormyrus* | Portugal | 0 | 14.04 | 0.85 | 4.23 |
| | Spain | 14.04 | 0 | 15.89 | 24.27 |
| | Italy | 0.85 | 15.89 | 0 | 2.93 |
| | Greece | 4.23 | 24.27 | 2.93 | 0 |

When comparing the pooled counts of the meristic traits of all samples for each species with the values found in the literature, in 33.3% of the cases the values found in this study fall within the range found by other authors (Table V). When the counts extended beyond or below this range, in 63.3% of the cases it occurred only due to the presence of one to three fishes in the whole sample universe (Table VI). In the remaining cases, the number of individuals that exceeded the ranges varied between 4 and 50 (Table VI).

Table V - Comparison of the pooled values of all samples found in the present study with those obtained in the literature. (DF - dorsal fin; AF - anal fin; PF - Pectoral fin; UGR - upper gill rakers; LGR - lower gill rakers).

| | DF | CF | AF | PF | UGR | LGR |
|---|---|---|---|---|---|---|
| ***Sparus aurata*** | | | | | | |
| Albuquerque (1956) | XI+13 | n.ref. | III+11-12 | 16 | n.ref. | n.ref. |
| Fischer *et al*. (1981) | XI+13-14 | n.ref. | III+11-12 | n.ref. | 5-6 | 7-8 |
| Bauchot & Pras (1982) | XI+13-14 | n.ref. | III+11-12 | n.ref. | n.ref. | n.ref. |
| Bianchi (1984) | XI+12-13 | n.ref. | III+11-12 | n.ref. | 11-13* | |
| Whitehead *et al*. (1986) | XI+13-14 | n.ref. | III+11-12 | n.ref. | 5-6 | 7-8 |
| Present Study (reared) | X-XI+12-14 | 17-23 | III+10-13 | 12-18 | 3-8 | 6-10 |
| Present Study (wild) | XI+12-14 | 20-23 | III+10-12 | 13-18 | 3-7 | 6-10 |
| ***Pagrus pagrus*** | | | | | | |
| Albuquerque (1956) | XII+10-11 | n.ref. | III+8-9 | 15 | n.ref. | n.ref. |
| Fischer *et al*. (1981) | XI-XIII+9-12 | n.ref. | III+8-9 | n.ref. | 6-8 | 8-10 |
| Bauchot & Pras (1982) | XI-XIII+9-12 | n.ref. | III+7-9 | n.ref. | n.ref. | n.ref. |
| Bianchi (1984) | XI-XII+9-10 | n.ref. | III+7-8 | n.ref. | 14-17* | |
| Whitehead *et al*. (1986) | XI-XIII+9-10 | n.ref. | III+7-8 | n.ref. | 6-8 | 8-10 |
| Present Study | XI-XIII+9-11 | 20-24 | III+8-9 | 14-17 | 4-9 | 7-11 |
| ***Diplodus sargus*** | | | | | | |
| Albuquerque (1956) | XI-XII+12-15 | n.ref. | III+12-14 | 15-16 | n.ref. | n.ref. |
| Fischer *et al*. (1981) | XI-XII+12-15 | n.ref. | III+12-14 | n.ref. | 6-9 | 9-12 |
| Bauchot & Pras (1982) | XI-XII+13-15 | n.ref. | III+12-14 | n.ref. | n.ref. | n.ref. |
| Whitehead *et al*. (1986) | XI-XII+12-15 | n.ref. | III+12-14 | n.ref. | 6-9 | 9-12 |
| Pastor & Cuadros (1992) | XI-XII+12-17 | n.ref. | III+13-17 | 14-17 | n.ref. | n.ref. |
| Present Study | XI-XII+13-16 | 21-26 | III+12-15 | 11-17 | 6-10 | 8-11 |
| ***Diplodus puntazzo*** | | | | | | |
| Albuquerque (1956) | XI+13-14 | n.ref. | III+12 | 15-16 | n.ref. | n.ref. |
| Fischer *et al*. (1981) | XI+12-15 | n.ref. | III+11-13 | n.ref. | 5-7 | 7-11 |
| Bauchot & Pras (1982) | XI+12-15 | n.ref. | III+11-13 | n.ref. | n.ref. | n.ref. |
| Whitehead *et al*. (1986) | XI+12-15 | n.ref. | III+11-13 | n.ref. | 5-7 | 7-11 |
| Present Study | XI+12-14 | 21-26 | III+11-13 | 12-17 | 4-8 | 8-11 |
| ***Dentex dentex*** | | | | | | |
| Albuquerque (1956) | X-XI+11-12 | n.ref. | III+7-8 | 14-15 | n.ref. | n.ref. |
| Fischer *et al*. (1981) | XI+11-12 | n.ref. | III+7-9 | n.ref. | 8-9 | 9-10 |
| Bauchot & Pras (1982) | XI+11-12 | n.ref. | III+7-9 | n.ref. | n.ref. | n.ref. |
| Whitehead *et al*. (1986) | XI+11-12 | n.ref. | III+7-9 | n.ref. | 8-9 | 9-10 |
| Present Study | XI+10-12 | 20-23 | III+7-8 | 14-16 | 2-9 | 7-12 |
| ***Pagellus bogaraveo*** | | | | | | |
| Albuquerque (1956) | XII-XIII+11-12 | n.ref. | III+10-12 | 15-17 | n.ref. | n.ref. |
| Fischer *et al*. (1981) | XII-XIII+11-13 | n.ref. | III+11-13 | n.ref. | 11-13 | 18-19 |
| Bauchot & Pras (1982) | XII-XIII+11-13 | n.ref. | III+11-12 | n.ref. | n.ref. | n.ref. |
| Whitehead *et al*. (1986) | XII-XIII+11-13 | n.ref. | III+11-12 | n.ref. | 11-13 | 18-19 |
| Present Study | XII+10-13 | 20-24 | III+11-13 | 15-18 | 8-15 | 15-20 |
| ***Lithognathus mormyrus*** | | | | | | |
| Albuquerque (1956) | XI-XII+11-12 | n.ref. | III+10-11 | 16 | n.ref. | n.ref. |
| Fischer *et al*. (1981) | XI-XII+11-12 | n.ref. | III+10-11 | n.ref. | 9-11 | 14-17 |
| Bauchot & Pras (1982) | XI-XII+11-12 | n.ref. | III+10-11 | n.ref. | n.ref. | n.ref. |
| Whitehead *et al*. (1986) | XI+11-12 | n.ref. | III+10-11 | n.ref. | 9-11 | 14-17 |
| Present Study | XI+11-13 | 19-23 | III+9-11 | 14-17 | 7-12 | 12-19 |
| ***Spondyliosoma cantharus*** | | | | | | |
| Albuquerque (1956) | XI+11-12 | n.ref. | III+9-11 | 14-16 | n.ref. | n.ref. |
| Fischer *et al*. (1981) | XI+11-13 | n.ref. | III+9-11 | n.ref. | 8-9 | 14-16 |
| Bauchot & Pras (1982) | XI+11-13 | n.ref. | III+9-11 | n.ref. | n.ref. | n.ref. |
| Whitehead *et al*. (1986) | XI+11-13 | n.ref. | III+9-11 | n.ref. | 8-9 | 14-16 |
| Present Study | XI+11-12 | 21-24 | III+9-11 | 15-16 | 8-11 | 12-19 |

* - Author regard it as "Total gill rakers on first arch"

n. ref. - No references were found for this characteristic

Table VI - Pooled values of the number of fish in all samples with a number of counts different from those found in the literature. In parenthesis is the number of count that those fish presented.
(DF - dorsal fin; AF - anal fin; PF - Pectoral fin; UGR - upper gill rakers; LGR - lower gill rakers).

| | DF | AF | PF | UGR | LGR |
|---|---|---|---|---|---|
| *Sparus aurata* (reared) | 0 | 12(10)-5(13) | 4(12)-1(18) | 1(3)-1(8) | 2(6)-4(10) |
| *Sparus aurata* (wild) | 0 | 1(10) | 1(13)-1(18) | 1(3)-50(7) | 16(6)-1(10) |
| *Pagrus pagrus* | 0 | 0 | 12(14)-2(17) | 2(4)-2(9) | 1(7)-13(11) |
| *Diplodus sargus* | 0 | 0 | 1(11)-15(17) | 5(10) | 0 |
| *Diplodus puntazzo* | 0 | 0 | 1(12)-1(17) | 7(4)-3(8) | 0 |
| *Dentex dentex* | 8(10) | 0 | 14(16) | 1(2) | 1(7)-3(12) |
| *Pagellus bogaraveo* | 3(10) | 2(13) | 5(18) | 1(8)-1(15) | 3(15)-3(20) |
| *Lithognathus mormyrus* | 3(13) | 1(9) | 0 | 2(7)-27(12) | 2(12)-1(19) |
| *Spondyliosoma cantharus* | 0 | 0 | 0 | 7(11) | 2(12)-1(19) |

### 1.3.1 - *Asymmetries and fluctuating asymmetry*

#### 1.3.1.1 - *Asymmetries*

Table VII shows the percentage of fish with asymmetries and the percentage of fish with at least one asymmetry (A1). The Italian sample (R) was the most asymmetric (92%), while the Greek sample (W) was the least asymmetric (37.5%). The Spanish reared samples (SpAtR, SpMR) and the Italian wild sample (ItW) also presented high levels of asymmetry, respectively 68%, 64% and 65%.

Table VII - Percentage of fish that were asymmetric for each characteristic in each sample.
A1 is the percentage of asymmetric fish for at least one characteristic.

| | | FrR | PtR | PtW | SpAtR | SpAtW | SpMR | SpMW | ItR | ItW | GrR | GrW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P.F.R. | 21.8 | 20 | 18 | 26 | 25 | 22 | 9.8 | 60 | 20 | 48 | 7.5 |
| | U.G.R. | 13 | 20 | 16 | 24 | 31.3 | 30 | 21.6 | 50 | 32.5 | * | 7.5 |
| | L.G.R. | 39.1 | 20 | 20 | 36 | 33.3 | 40 | 25.5 | 42 | 40 | * | 22.5 |
| | A1 | 56.5 | 50 | 42 | 68 | 64 | 58.3 | 47.1 | 92 | 65 | 48** | 37.5 |
| FA | P.F.R. | 0.209 | 0.202 | 0.183 | 0.423 | 0.096 | 0.591 | 0.255 | 0.1318 | 0.4 | 0.499 | 0.154**** |
| | U.G.R. | 0.391 | 0.202 | 0.189 | 0.551 | 0.256 | 0.529 | 0.404 | 0.473 | 0.408 | * | 0.215 |
| | L.G.R. | 0.134 | 0.204 | 0.149*** | 0.219*** | 0.34 | 0.366 | 0.383 | 0.5 | 0.317 | * | 0.076**** |

* - these characteristics were not scored in the Gr R sample
** - based in just one characteristic
*** - failed the statistical procedure (first step)
**** - failed the statistical procedure (second step)

#### 1.3.1.2 - *Fluctuating asymmetry*

Pectoral fin rays (PFR) and the lower gill rakers (LGR) in the GrW failed the statistical procedure (first step), and were excluded from further analysis. The LGR of the SpAtR and PtW also failed, not presenting FA (second step), and were also excluded (Table VII).

FA varied between 0.096 (SpAtW) and 0.591 (SpMR) for PFR, 0.189 (PtW) and 0.551 (SpAtR) for UGR, and 0.134 (FrR) and 0.5 (ItR) for LGR (Table VII). Results of the Shapiro Wilks test indicated that meristic traits were normally distributed.

No significant correlations (Spearman rank correlation test; $p<0.005$) were found between the heterozygosity (both allozymes and microsatellites).

Wild samples were quite similar, with a marginal degree of heterogeneity. In contrast, there was a considerable difference in the degree of asymmetry among the reared samples. Chi-square tests for analysis of homogeneity among samples were barely significant (Table VIII).

Table VIII - Results obtained for Chi-square test for homogenity.

| | | Chi-square test | | |
|---|---|---|---|---|
| | | $\chi 2$ | DF | P |
| Reared | FrR | 5.655 | 6 | 0.463 |
| | PtR | 25.469 | 13 | 0.044 |
| | SpMR | 12.722 | 12 | 0.389 |
| | SpAtR | 25.469 | 15 | 0.044 |
| | ItR | 11.186 | 15 | 0.739 |
| | GrR | 7.789 | 6 | 0.254 |
| Wild | PtW | 11.426 | 8 | 0.179 |
| | SpMW | 11.426 | 8 | 0.179 |
| | SpAtW | 15.132 | 8 | 0.057 |
| | ItW | 11.186 | 15 | 0.739 |
| | GrW | 2.26 | 5 | 0.812 |

### 1.3.2 - *Allozyme heterozygosity*

Of the 24 loci screened, 11 proved polymorphic (EST, GPI-2, IDDH, IDHP, LDH-2, MDH-2, MDH-3, PGM, PGDH, SOD-1, SOD-2). Among these, five were polymorphic in all the samples (EST, GPI-2, IDHP, PGM, PGDH).

The percentage of heterozygous fish observed for each allozyme, as well as the observed heterozygosity (Ho), are presented in Table IX. Individually, fish were heterozygous to a maximum of six loci.

When the allozyme heterozygosity was compared in the reared individuals, the SpMR was significantly different from all the other samples (Wilcoxon signed rank test; $p<0.005$). No significant differences were found among the wild populations (Wilcoxon signed rank test; $p<0.005$). Similar results were obtained for the microsatellite heterozygosity in the reared animals. In the wild animals, the Greek sample was significantly different from the Portuguese and the Italian samples (Wilcoxon signed rank test; $p<0.005$).

Table IX - Percentages of heterozygous fish observed for each allozyme screened for all samples. Monomorphic loci are not presented. Ho – Observed heterozygosity

| | FrR | PtR | PtW | SpAtR | SpAtW | SpMR | SpMW | ItR | ItW | GrR | GrW |
|---|---|---|---|---|---|---|---|---|---|---|---|
| EST | 60.9 | 46 | 56 | 36 | 62.5 | 38 | 49 | 46 | 45 | 32 | 52.5 |
| GPI2 | 52.2 | 34 | 100 | 52 | 35.4 | 82 | 41.2 | 20 | 32.5 | 34 | 42.5 |
| IDDH | 0 | 0 | 0 | 0 | 0 | 0 | 3.9 | 0 | 2.5 | 0 | 0 |
| IDHP | 39.1 | 48 | 38 | 32 | 31.3 | 68 | 45.1 | 62 | 42.5 | 38 | 42.5 |
| LDH-2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| MDH2 | 0 | 0 | 0 | 0 | 0 | 0 | 5.9 | 8 | 0 | 0 | 0 |
| MDH3 | 0 | 0 | 0 | 0 | 2.1 | 0 | 2 | 0 | 0 | 0 | 0 |
| PGM | 56.5 | 36 | 56 | 68 | 52.1 | 82 | 74.5 | 44 | 55 | 38 | 65 |
| PGDH | 43.5 | 48 | 36 | 20 | 58.3 | 62 | 41.2 | 48 | 55 | 56 | 30 |
| SOD1 | 0 | 0 | 0 | 0 | 0 | 0 | 5.9 | 0 | 0 | 0 | 0 |
| SOD2 | 4.3 | 20 | 8 | 10 | 6.3 | 0 | 2 | 2 | 0 | 18 | 0 |
| Ho | 0.094 | 0.091 | 0.092 | 0.085 | 0.101 | 0.139 | 0.091 | 0.088 | 0.091 | 0.082 | 0.088 |

### 1.3.3 - *Microsatellites*

High percentages of heterozygous individuals were found for the majority of the samples (Table X). Nine samples presented 100% of heterozygous fishes for at least one MS locus.

The average observed heterozygosity values varied between 0.78 (PtR) and 0.91 (SpMR) in the reared samples, and between 0.61 (ItW) and 0.92 (GrW) in the wild samples (Table X).

Table X - NHF is the percentages of heterozygotic fish observed for each one of the three MS loci. MS1 is the percentage of fish in each sample that was heterozygotic for at least one MS loci. Ho - heterozygosity observed.

| | | FrR | PtR | PtW | SpAtR | SpAtW | SpMR | SpMW | ItR | ItW | GrR | GrW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NHF | Sa26 | 75 | 82.4 | 92.1 | 92.1 | 77.7 | 89.7 | 77.4 | 95.6 | 63 | 86 | 87.5 |
| | Sa32 | 93.8 | 94.1 | 39.3 | 76.3 | 77.7 | 96.6 | 67.7 | 86.7 | 51.9 | 97.2 | 87.5 |
| | Sa41b | 87.5 | 82.4 | 60.7 | 81.6 | 88.9 | 79.3 | 87.1 | 77.8 | 92.6 | 88.9 | 96.7 |
| MS1 | | 100 | 100 | 93 | 100 | 100 | 100 | 97.2 | 100 | 100 | 100 | 100 |
| Ho | Sa26 | 0.83 | 0.64 | 0.76 | 0.92 | 0.8 | 0.93 | 0.78 | 0.96 | 0.57 | 0.8 | 0.89 |
| | Sa32 | 0.97 | 0.98 | 0.55 | 0.85 | 0.78 | 0.96 | 0.63 | 0.85 | 0.41 | 0.98 | 0.89 |
| | Sa41b | 0.74 | 0.73 | 0.63 | 0.78 | 0.91 | 0.85 | 0.82 | 0.78 | 0.86 | 0.85 | 0.97 |
| pooled MS | | 0.85 | 0.78 | 0.65 | 0.85 | 0.83 | 0.91 | 0.74 | 0.86 | 0.61 | 0.88 | 0.92 |

## 1.4 – DISCUSSION

### 1.4.1 – *Meristic analysis*

Anatomical characteristics are hard to quantify but nevertheless important as species descriptors. They include shape, completeness, and position of the lateral line; position and size of internal organs; special anatomical features (such as air-breathing and electric organs); secondary characteristics (such as breeding tubercles on males); and the shapes, sizes, positions, and interrelationships of bones, nerves and muscles (Moyle & Cech, 1996). Most of these are yes-no characteristics: either a fish has them or it does not. As a consequence they can be definitive characteristics, useful for separating not only species but also higher taxa. However, even these characteristics are not always absolute, since occasionally individuals possess characteristics supposedly definitive for another, closely related species (Moyle & Cech, 1996).

In some cases, such characteristics can also ensure the differentiation between populations of a same species. Local environmental conditions can be quite different throughout the species geographic distribution and so influence throughout its lifetime, but especially during the early stages. Influences on ontogeny are more likely to induce transformations in specific morphological characteristics than others. However, because meristic characters generally vary within a relatively small range, it seems probable that unless there are consistent differences between groups, the amount they contribute to the total variance will be so small that they will be of relatively minor importance in stock separation (Schweigert, 1981). In the present study, in the majority of the cases, a geographic gradient in a West/East sequence (Portugal-Spain-Italy-Greece) with increasing values between samples could not be observed (exception for *P. pagrus*). Nevertheless, closeness between Italian and Portuguese samples was found for the *S. aurata* (both wild and reared), and *L. mormyrus* samples. In a first approach such a finding could be quite strange and eventual causes could not be explained. Yet, for the gilthead seabream if we cross the biological analysis with the geological characteristics and formation of the Mediterranean Sea some hypothesis can be formulated. It is assumed that a species diverts from a single population, deriving that into different populations. If these populations became completely isolated, they can suffer an evolutionary transformation and became different species, or maintain the original characteristics throughout time, if only partially isolated. Fryer & Iles (1972) pointed out geological factors for the development of species flocks of cichlids in the African

Great Lakes, and according to Eastman & McCune (2000) the Antarctic shelf supports a highly endemic fauna, which due to the geological movements of the Antarctic shelf was the centre of evolution for a variety of aquatic organisms. In the present study, the Portuguese samples were the only ones obtained from the Atlantic (except for *S. aurata*). The Italian samples of *S. aurata* were obtained in the most northern part of the Adriatic Sea, whereas the other ones were captured in the southern Adriatic. According to Bembo *et al.* (1996) and Naciri *et al.* (1999) there are considerable oceanographic differences between the Adriatic and the East and Western Mediterranean. In both cases, these differences can influence the early developmental stages and so provide a source of variation. Thus, if we look to those places as the most isolated ones that derived from the original populations, these populations could find themselves incapable of maintaining a constant contact with the neighbouring populations due to posterior geological events constraining the bulk of their migratory movements.

According to Bembo *et al.* (1996), there are three primary biogeographic regions in the Adriatic; the shallow waters of the northern Adriatic (<50 m), typically of moderate to high productivity, a western, similarly shallow coastal strip, with locally eutrophic conditions, and the central-southern oligotrophic open waters with depths over 75 m. The northern and coastal waters are heavily influenced by riverine input, whereas hydrography in more southern waters is affected by exchange with Ionian Sea. Independent circulatory patterns in the north and south Adriatic (Zore-Armanda, 1969) reinforce such latitudinal discontinuities, and induce the presence of several endemic fish species in the northern waters (Tortonese, 1983). Therefore, it is not surprising to find corresponding differences in the distribution of species and populations (Umani *et al.*, 1992). Thus, for the Italian *L. mormyrus* sample that was collected in the southern Adriatic, such constraints can also be applied due to the differences in the circulatory patterns between the southern Adriatic and the central Mediterranean.

In the other species, the differentiation between Atlantic and Mediterranean species could be observed. The Almeria-Oran oceanographic front (AOOF), is an effective boundary between Atlantic and Mediterranean surface waters, and is the major oceanographic discontinuity in the western Mediterranean (Beckers *et al.*, 1997). This corresponds to a major ecological break with drastic differences between different planktonic communities on either side (Estrada *et al.*, 1985). The contact of Atlantic surface water with higher density Mediterranean water of Almeria on the southern coast of Spain induces a jet towards North Africa. A part of the Atlantic water returns

westward to form the Alboran gyre and the other part flows eastward along the coast of North Africa (Naciri *et al.*, 1999). The presence of this gyre could provide an explanation for the Atlantic/Mediterranean ecological and genetic discontinuity for the species in study: larvae spawned in the Mediterranean, especially near the Alboran Sea would be retained in the Alboran gyre until recruitment. This is supported by the similarity between the Spanish Atlantic reared sample and the Portuguese reared sample (Table IV), the two closest samples in analysis, and between the former and the Italian reared sample.

The comparison between the studied populations can also be analysed from the environmental point of view, namely water temperature during the larval development. Northern latitudes usually produce colder water temperatures, that, in the present case would affect the Italian sample and the Atlantic samples due to colder water currents (see Brown *et al.*, 1995; Bigg, 1996). According to Tomczak & Godfrey (1994) there are meteorological factors that allow the water in the northern and central regions of the Adriatic to cool very fast during a bora event (characteristic wind in the northern Adriatic) and attain a density higher than water masses in the south. Therefore, the differences in meristics characters may reflect differences in the geographic origins of the larvae. The fact that these differences were still observed in the adult stage suggests that larvae, whose meristic counts developed under the influence of local environmental conditions, remained in the region of hatching and development throughout recruitment and maturity (Shepherd, 1991). According to Claytor *et al.* (1991), ecological features are more important than continental effects in explaining both morphometric and meristic variation, and again evidence the temperature as the most important ecological effect. Holčik & Jedlička (1994) extended the influencing factors to geographical latitude and longitude, elevation and fish size.

Beacham & Withler (1985a) also found a significant correlation between pairwise Mahalanobis distance based on meristic characters for pink salmon *Onchorhynchus gorbuscha*, and Ihssen *et al.* (1981) found that same positive correlation for whitefish *Coregonus clupeaformis*. Nevertheless, Beacham & Withler (1985a) found that the correlation between the genetic distance and the Mahalanobis distance based on gill raker number was greater than that based on morphometric characters. As will be seen further on (Chapter 2) the opposite occurred in this study, but as stated by other authors (Riddell & Leggett, 1981; Beacham, 1985) such a constraint is closely related with the homing behaviour of the salmonids. The life history characteristics of the

Salmonidae suggest that genetic variation may contribute significantly to geographic variation in population traits (Riddell & Leggett, 1981). The homing behaviour is unknown in sparids, and only certain species migrate to reproduction areas, usually in shallow waters. These behaviours do not induce major differences, since these areas usually share similar characteristics, in contrast to rivers, which in most of cases have their proper characteristics.

After the initial description of a fish species, they suffer throughout time re-examinations and revisions of their systematic position. Such studies often result in the reappraisal of Genus (e.g. Bianchi, 1984; Ben-Tuvia, 1990; Ivantsoff & Crowley, 1991; Crowley & Ivantsoff, 1992; Das & Nelson, 1996), new records of a species in determined areas (e.g. Klausewitz & Uiblein, 1994) and some times the description of new species (e.g. Akazaki, 1983; Randall & Guézé, 1984; Creech, 1992; Saeed *et al.*, 1993; Crowley *et al.*, 1995; Griffiths & Heemstra, 1995; Allen & Feinberg, 1998; Allen *et al.*, 1998; Randall, 1998; Ng & Freyhof, 2001a, 2001b). In the majority of cases, the redefinition or description of new fish species is based on meristic and morphological analysis. Nevertheless, in some cases even if there is not a redefinition of the species, the different authors present different ranges of variation of the fish characteristics.

The results obtained in this study and those reported to by other authors are summarised in Table V. This table shows that there are some differences between the results of the present study and those of other authors.

In some, such differences only happened for a small number of individuals, and two main reasons can provide explanations. The observed fishes suffered injuries during their life and in that case, the number of counts was much smaller than the value described for that species. Often, these cases are recognisable because the injury was extended to the supporting structure or even to the muscle. Furthermore, the possibility of sampler error is not to be excluded.

In fact, the definition of counting is an important step to normalize the criteria of different samplers. Still, major misunderstandings arise from the analysis of more complex repetitive counts. For example, Kruse *et al.* (1996), found significant different mean counts of pyloric caecae and scales above the lateral line among different samplers, but did not found significant differences for gill rakers between those same samplers. In this study, the sampling criteria were established *a priori* between samplers, and it was predictable that eventual errors would be reduced. The occurrence

of pooled counts of the four countries, both in the pectoral fin and gill rakers, within the range found by other authors reinforces this assumption.

The organisms analysed in this study presented, for the same characteristics, systematic differences (higher or lower counts) when compared with the range of values reported in the literature. These differences were not due to few extreme organisms, but were present in a large number of the individuals, suggesting that these counts were valid, and, therefore, constitute a diagnostic criterion for species discrimination. Furthermore, in some cases, these findings were recorded in all the four countries.

### 1.4.2 - *Fluctuating asymmetry and heterozygosity analysis*

In this study, allozyme heterozygosity showed high values, and in contrast, the FA levels were quite low which is in agreement with the results reported by Palmer & Strobeck (1986) and Crozier (1997) for Atlantic salmon. Nevertheless, the comparative analysis between reared and wild populations suggests that developmental stability appears to be weaker in the reared samples. All reared samples (except for SpMR), presented lower allozyme heterozygosity values and higher values of FA (except for pooled FA in ItW) than the wild samples. This result might indicate a loss of genetic variability of the reared populations. Although microsatellite values were always higher for the reared samples (except for the Greek samples) this could be attributed to the mixed origin of founding populations and/or the renewal practice of the stocks. The allozyme loci usually have only a few alternative states (alleles), they are involved in the functioning of the biochemical pathways of the cell and they are candidate targets of natural selection. Large differences at allozyme loci are not usual among conspecifics samples and when seen, they imply very strong selective differences exacted on the population by virtue of difference in their environments, or (more likely) that the populations have been in reproductive isolation from each other for a very long period of time. In contrast, microsatellites are small stretches of DNA made by tandem repeats of a pair of nucleotide bases and vary from each other because the number of pairs varies widely among homologous sites. As a result, the two copies an individual receives from each of its parents usually vary, with the consequence that the majority of individuals are heterozygous for each microsatellite (Zouros *et al.*, 1998). Thus there is a big difference between allozymes and microsatellites in the amount of variation among individuals in population.

These differences between natural and cultivated samples are justified by the dominance of the rare alleles in the wild populations, which have disappeared in the reared ones through genetic drift effect (Zouros *et al.*, 1998). This fact is related to breeding techniques used in aquaculture, which rely on the same parental brood stock for several generations. Samples showed a predictable result of a high degree of homogeneity among samples.

The majority of the meristic traits did not show high values of FA, which indicates that the mechanisms of developmental stability were present. No destabilisation in development seems to be occurring, except for the Spanish reared samples (both Atlantic and Mediterranean) and the Italian samples (both wild and reared). The higher asymmetry obtained in these samples, could be related to environmental stress. This hypothesis is also related to the fact that the heterozygosity values were very similar for all the samples. The higher levels of asymmetry of the Italian samples can be affected by particular abiotic conditions in the Northern Adriatic (e.g. temperature, salinity and freshwater inflow) (Tomczak & Godfrey, 1994). The values presented by the ItR (highest A1 of all the samples) seem to be enhanced under artificial conditions (due to breeding techniques) what seems to be the case in the wild populations. The high and similar FA obtained for both Spanish cultivated samples might result from exchanges between hatcheries, probably reflecting common origins. Moreover, animals might be experiencing different rearing conditions when compared to other countries. In contrast, the wild populations from Portugal, Spain (Atlantic and Mediterranean) and Greece as well as the cultivated populations from France, Portugal and Greece might reflect more stable environmental and culturing conditions.

The present study does not clearly indicate a distinct pattern of geographic differentiation for both wild and cultivated samples. In fact, non-significant differences were found for the comparison of the same data. The results obtained for the cultivated samples suggest two main hypotheses: firstly, the exchange of eggs and breeders between aquaculture facilities within countries (as mentioned above) and also between neighbouring countries which is a common practice; and secondly, the production of *Sparus aurata* is still a quite recent technique, and until now, it is not responsible for considerable changes in the genetic pool of cultivated populations.

In the case of the wild samples, the migration of individuals and consequent crossing between neighbouring populations is the main reason for their shared meristic

characteristics. Considering the dispersal abilities and the high fecundity of the gilthead sea bream, only slight genetic differentiation could *a priori* be expected.

# CHAPTER 2 – MORPHOMETRIC ANALYSIS

## 2.1 – INTRODUCTION

Species are the fundamental unit of classification schemes. What is a species, and how should species be arranged in a phylogenetic classification? The British ichthyologist C. Tate Reagan defined species as "a group of organisms with distinctive enough morphological characteristics that, in the opinion of a competent systematist, are sufficiently definite to entitle them to a specific name" (Norman, 1948). This practical, but somewhat circular, definition of a species, now termed as morphospecies, does not depend on evolutionary concepts (Helfman *et al.*, 1997).

Studies of morphological development have contributed significantly to our understanding of the evolution of form and function in vertebrates (Gans, 1969; Lauder, 1981; Liem & Wake, 1985; Splechtna & Hilgers, 1989). The discipline of biological morphometrics has had a reticulate evolution. From the 1960s until early 1980s the term was generally held to be synonymous with numerical taxonomy or phenetics, and the aim of it was to relate organisms objectively in terms of their overall similarity, assessed by measurements of body form and other quantitative traits (Strauss, 1990). In particular, ontogenic studies have focused attention on the relationships between allometric scaling, function and ecological role (Strauss & Altig, 1992). Morphological approaches to the study of community relationships are based on the premise that morphological differences among organisms reflect in large part their ecological relationship and that morphological "space" can be mapped onto ecological "space" (Strauss, 1987).

A central problem in evolutionary biology is whether a single population contains one or more optimum phenotypes (Wilson, 1989). Polymorphism in ecological or morphological traits has frequently been misinterpreted as speciation due to the lack of knowledge about the range and nature of phenotypic variation within population (Sage & Selander, 1975). The quantification of specific characteristics of an individual or group of coexistent individuals can be a measure of the degree of differentiation induced by both biotic and abiotic conditions. Those characteristics contribute to the definition of different stocks within a species. A general definition of the term stock generally involves a group of individuals that sustains itself over time, but precise definitions vary among disciplines (Booke, 1981). For example, morphometric variation

can be used to discriminate "phenotypic stocks" defined as groups with similar growth, mortality, and reproductive rates (Cadrin, 2000). Species rarely reproduce randomly with conspecifics throughout their geographic range, but form a serious of stocks that are reproductively isolated in space or time (Jennings *et al.*, 2001). The development of a stock distinction scheme that facilitates the logistical management of a fishery and the monitoring of biological and genetic integrity of its population units is crucial to the long-term survival of the fish stocks and to the fishery itself (Schweigert, 1990). In this process, it is also meaningful to identify the geographic ranges of the stock units (Ihssen *et al.*, 1981) or even in the estimation of biomass (Beddow & Ross, 1996; Hockaday *et al.*, 2000).

Multivariate statistical analysis of morphometric characters is a powerful technique to investigate the geographical variation of populations. It has provided useful results for assessing the stock structure of several species of marine fishes, because it can yield information complementary to that derived from biochemical, physiological and life history studies, (Schaefer, 1989). Biological morphometry has progressed in the past few years, however, owing primarily to the infusion of a geometric perspective on form and to a renewed emphasis on explicit assumptions about anatomical homologies and on allometric models on growth and size-variation. Such model assumptions are necessary for biological comparisons (as opposed to purely statistical contrasts) because they furnish a firm biological context within which to interpret results (Strauss, 1990).

The use of body landmarks, which are the points at which one's explanations of biological processes are grounded, have eased this task. They are signposts the organism conveniently erects to ease the task of functional or evolutionary biologist while remaining biometricians (Bookstein, 1991). There are three principal types of points that are frequently used as landmarks, and are, discrete juxtapositions of tissues, maxima curvature or other local morphogenetic processes and extremal points. The space of this "configurations" in which landmark locations are recorded are at the same time, the space of possible depictions of effects upon form and the space of statistics of deformations relating those forms. Thus, landmark-based morphometrics is the embodiment within biometrics of the functional form of biological explanation (Bookstein, 1991).

The landmark-based techniques of geometric morphometrics pose no restrictions on the directions of variation and localization of changes in shape, and are very effective in capturing information about the shape of an organism (Cavalcanti *et al.*,

1999). The emphasis on geometric aspects of the form dates back to the seminal work of D'Arcy Thompson, the father of the deformation grid, which graphically depicts the point-for-point geometric transformation of one form to another. Since then, many morphologists have taken a geometric approach in comparing life-history stages. These comparisons are facilitated in Strauss & Bookstein's (1982) truss of morphometric measurements. The truss consists of distances between homologous landmarks on the outline of a two-dimensional projection of a form. Such conformation revelled to be advantageous over the traditional morphometric data sets, because;

- it provides a geometric protocol for morphometric character selection;
- it archives the configuration of the landmarks so that the form of an individual specimen can be reconstructed
- measurements can be performed in a digitising board
- it enables construction of a composite, average form that represents a given population at a given age or size
- it allows visualisation of multivariate trends of growth and allometry within populations (Strauss & Bookstein, 1982).

More recently, the image analysis systems played a major role in the development of morphometric techniques, boosting the utility of morphometric research in the fish stock identification (Cadrin & Friedland, 1999).

Although extensive information about population discrimination in salmonids has been published in the last few years (e.g. Bailey & Ervine, 1991; Swain *et al.*, 1991; Beeman *et al.*, 1994; Bronte *et al.*, 1999), none has been produced concerning Sparids, a highly important fishing resource in the South European Atlantic coasts and the Mediterranean Sea. Aside from natural exploitation, the gilthead seabream is an example of a species that is extensively reared in hatcheries to supplement natural production. In recent years, sharpsnout seabream has also been successfully produced (Sarà *et al.*, 1999). A widespread concern is that selection in the novel hatchery environment may result in an evolutionary response away from the wild type (Swain *et al.*, 1991).

In this chapter, eventual differences among the populations will be studied in eight species of the Sparid family, the gilthead seabream, *Sparus aurata* Linnaeus 1758, the red porgy, *Pagrus pagrus* (Linnaeus 1758), the white seabream, *Diplodus sargus* (Linnaeus 1758), the sharpsnout seabream, *Diplodus puntazzo* (Cetti, 1777), the striped seabream, *Lithognathus mormyrus* (Linnaeus 1758), the red seabream, *Pagellus*

*bogaraveo* (Brünnich, 1768), the common dentex, *Dentex dentex* (Linnaeus 1758), and the black seabream, *Spondyliosoma cantharus* (Linnaeus 1758), along the South European Atlantic coast and in the Mediterranean Sea, based on their morphological variability. The traditional morphometrics and the truss networks will be used. Results between methods will be compared, since the discussion over which method best describes the morphological variation of a species is far from over, and both methods are commonly used.

## 2.2 - Material and Methods

### 2.2.1 - *Sampling*

Morphological analysis was carried out on the same samples referred to in Chapter 1. A total of 1767 specimens of eight sparid species; *S. aurata*, *P. pagrus*, *D. sargus*, *D. puntazzo*, *L. mormyrus*, *P. bogaraveo*, *D. dentex*, and *S. cantharus* from four European countries; Portugal, Spain, Italy and Greece were used (Table I). For *S. aurata*, additional samples of reared animals were collected in the Atlantic area of Spain (Cadiz) (both wild and reared) and Atlantic area of France (reared sample), as also described in chapter one (Table II).

For the black seabream the morphological comparison was only undertaken between the Portuguese and the Spanish samples. It was not possible to collect specimens from Italian waters during the sampling period, and only three were collected from Greece. Due to the small sample size, those animals were not included in the analysis.

### 2.2.2 – *Morphometric analysis*

#### *2.2.2.1 – Traditional morphometrics*

Traditional morphometrics was recorded on the left side of the fish. Thirteen morphometrics measures were taken (Figure 2); standard length (SL), maximum fish height (MFH), head length (HL), head depth (HD), preorbital length (POL), orbital length (OOL), postorbital length (PSL), lower jaw length (LJL), upper jaw length (UJL), dorsal fin length (DFL), caudal peduncle length (CPL), length between the pelvic and the anal fins (LPA), and the anal fin length (AFL). In the Portuguese samples, data were collected from fresh fish. In all the others samples measurements were performed on the X-rays. All measurements were taken with a digital calliper (MITUTOYO). In order to avoid biases due to different procedures, ten fishes were

selected and measured in the fresh, after which they were X-rayed and re-measured. Image acquisition was carried out following Creech (1992). X-rays were taken as described in Chapter 1 (section 1.2.2 of Material and Methods). Due to the precautions taken during the fish X-raying, no significant differences ($p<0.05$) were found between the two procedures. Recordings were always performed by the same sampler.

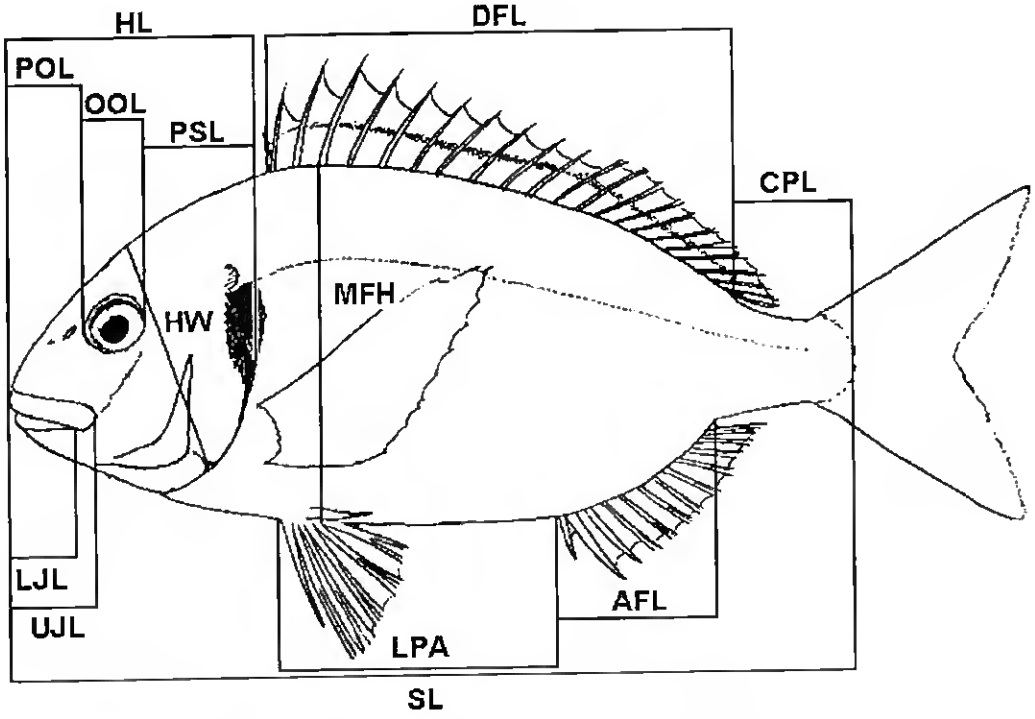



Figure 2 – Location of the 13 measurements used for traditional morphometry.

#### 2.2.2.2 - *Truss measurements*

The truss morphometrics protocol (Strauss & Bookstein, 1982; Bookstein *et al.*, 1985) was used to describe the shape of each fish using a set of morphometric characters. The landmark data were transferred to computer from the X-rays with an X-Y coordinate digitalizing table (WACOM Ultra Pad) properly calibrated. Measurements were taken to the nearest 0.01 mm. The Euclidian distances were obtained with the software program Image Pro Plus 3.0. For each specimen, 31 distance measurements were taken between the 14 landmark points (Figure 3) on the left side of the fish. The landmarks were, in clockwise direction: (1) anterior tip of the snout, (2) dorsal surface of the head at the nearest point to the eye globe, (3) posterior point of the neurocranium, (4) origin of the dorsal fin, (5) origin of the $8^{th}$ ray of the dorsal fin, (6) origin of the $16^{th}$ ray of the dorsal fin, (7) posterior end of the dorsal fin, (8) dorsal origin of the caudal

fin, (9) ventral origin of the dorsal fin, (10) insertion of the anal fin, (11) origin of the anal fin, (12) insertion of the pelvic fin, (13) origin of the pelvic fin and (14) posterior insertion of the sub-operculum. The choice of these measurements assured that those points were identified with consistent features of the local morphology, covered the entire body form and the inter-landmark distances were as short as possible, since short measurements contain more localised information about shape (Strauss & Bookstein, 1982). Additionally, six area measurements were also taken (Fig. 3).

In order to verify the precision of the method, all measurements were repeated ten times over a chosen individual. The standard deviation of each one of the 31 truss measurements varied between ±0.02 mm and ±0.07 mm, and between 0.06 $mm^2$ and 0.14 $mm^2$ for the six areas.

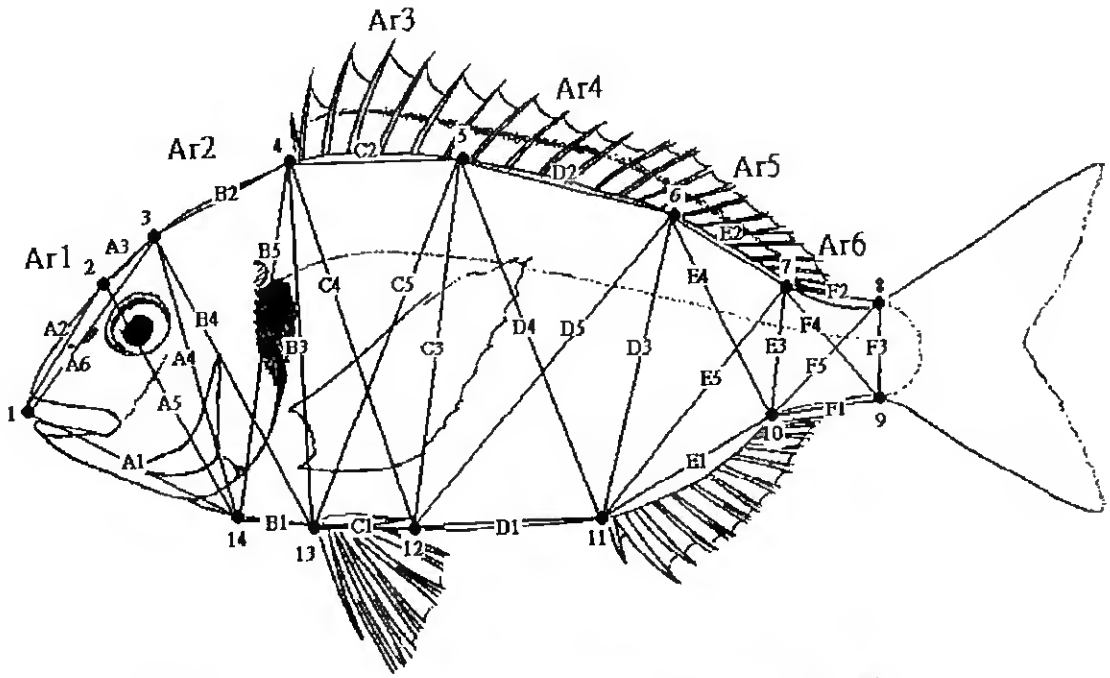



Figure 3 – Location of the 14 landmarks used to calculate the truss network (lines).

### 2.2.3 - *Data analysis*

Both the traditional measurements and the truss networks were transformed to common logarithms in order to increase linearity and multivariate normality (Pimentel, 1979). Outliers were detected by regression analyses of morphometric characters against the standard length and by scatter plots of residuals *vs.* predicted values (Cook & Weisburg, 1982).

In organisms characterised by determinate growth, variation in absolute size within or between taxa can be interesting, and such variables can be used to quantify

similarity or dissimilarity. However, for organisms with indeterminate growth, the absence of well defined stages of growth and of fixed sizes of adults both necessitate the transformation of absolute sizes of body parts to some estimates of relative size (shape) of those parts (Reist, 1985, 1986). Size and shape covary, and unless isometry pertains, such covariation implies a changing relationship between size and shape (Gould, 1966). The terms size and shape have been used in various and sometimes conflicting ways (see Humphries *et al.*, 1981, for references). Size and shape can be interpreted not as measured variables, but as general factors, with linear combinations parsimoniously accounting for the association among the distance measures. Size, in particular, is not a single variable such as biomass or a standard length, but a factor which, when called upon to predict all the distance measures in the population, leaves the smallest mean squared residual (Humphries *et al.*, 1981). Shape, can be defined as the geometry of the organism after information about position, scale and orientation has been removed (Bookstein, 1978). As such, a basic problem in the field of multivariate morphometrics is that of quantifying shape differences among form separately from size differences (Rohlf & Bookstein, 1987). In general, populations or species under study are considered to be groups defined *a priori* and linear measurements of morphological characters are obtained from the specimens within each sample. However, when applying this procedure it is important that the within-sample sources of variation be controlled in such a manner that the possible variation among samples will not be masked or result from sampling errors (Reis *et al.*, 1990).

Size-dependent variation was removed using an allometric approach (Reist, 1985). An *a priori* statistical analysis (ANOVA on the basic size variation between samples) was performed. Significant size differences ($p<0.05$) were found between all the samples, with the exceptions occurring between the Italian and Portuguese reared samples of *S. aurata*, the Greek and the Spanish Mediterranean reared samples of *S. aurata*, and between the Spanish and Greek samples of *P. bogaraveo*. Thus, data was transformed to eliminate size-dependency using the formula:

$$M_{trans}=\log M - \beta (\log SL - \log SL_{mean})$$

were $M_{trans}$ is the transformed measurement, $M$ the original measurement, $\beta$ the within-group slope regressions of the log $M$ vs. log $SL$, $SL$ the standard length of the fish and $SL_{mean}$ the overall mean of the standard length.

Principal components analysis (PCA) of a covariance matrix transforms a set of variables into orthogonal (uncorrelated) components. In order to elucidate the requirements of pertinent techniques related to PCA, the discussion is based on orthogonal size and shape components derived from linear measurements taken from some aspects of the morphology, with this being one of the common uses of PCA and related techniques (Thorpe, 1988).

As a first approach, PCA was used: *i*) to analyse the separation between populations through the matrix variability; *ii*) to identify collinear variables; and, *iii*) to choose the right subset of variables to be used in the discriminant analysis, which verified the level of distinction between samples. Correlations between the five principal components (PC1 to PC5) and the standard length (SL) were evaluated with Pearson's product-moment correlation.

After checking for homoscedasticity of the variance-covariance matrices, variables were evaluated on the basis of: *i*) the percentage of correct classification in discriminant analysis (PCS); *ii*) the Mahalanobis distance ($D^2$) between centroids and its associated probability; *iii*) the values of Wilks' λ, and *iv*) the consistency of the variable selection by stepwise procedures. A multivariate variance analysis of the canonical variable obtained from the previous sequence of analysis was used to evaluate the spatial separation between the centroids of the samples (Johnson & Wichern, 1992).

The PCS is expressed as the percent of correctly classified cases in each group. The classification of cases will be based on *a priori* classification probabilities that all cases belong to the same group. The squared Mahalanobis distances of each case from each group centroid are analysed. These distances are similar to the squared Euclidean distances of the respective case from the centroids for each group (the point defined by the means for all variables in the respective group). However, unlike the Euclidean distance, the Mahalanobis distance takes into account the intercorrelations between the variables in the model (which define the multivariate space). To access the discriminatory effectiveness of the analysis, Wilks' λ was used (Wilks, 1932), which is a multivariate analysis of variance statistic that tests the equality of group means for the variable(s) in the discriminant function. The smaller the λ, the bigger the difference among groups, ranging from zero to one. The consistency of the variable selection by stepwise procedures depends mainly on the criterion for the choice of F to remove. In this study the statistical model assigned the initial F and the stepwise procedure was "guided" by the respective F to enter and F to remove values. The F value for a variable

indicates its statistical significance in the discrimination between groups, that is, a measure of the extent to which a variable makes a unique contribution to the prediction of group membership.

In general, the statistical model will continue to choose variables to be included, as long as the respective F values for those variables are larger than the user-specified F to enter. Variables will be removed from the model if their significance is less that the user-specified F to remove.

Statistical differences were considered significant for the $p<0.05$ level.

Methods of manipulating truss configurations for quantitative comparisons were performed as described by Strauss & Bookstein (1982). Substantial measurements will be exposed when the form is drawn, while more subtle errors may be revealed by measures of the mutual inconsistence of data. A useful measure of such "strain" adjusted for the varying sizes of the cells, is the relative discrepancy between measured and reconstructed distances. The total strain value for each sample was also obtained. These values are a measure of the mutual lack of fit of the original distance measurements and it is the square root of the sum of the squared deviations of the distances in the mapped form from those originally measured (Strauss & Bookstein, 1982).

In a posterior analysis, the truss measurements were identified with the body areas were they were recorded. Four distinct body regions were chosen; head related characteristics (A1, A2, A3, A4, A5, A6), longitudinal measurements along the trunk of the fish (B3, B4, B5, C3, C4, C5, D3, D4, D5, E4, E5), transversal measurements along the trunk of the fish (B1, B2, C1, C2, D1, D2, E1, E2) and caudal related measurements (E3, F1, F2, F3, F4, F5). Such differentiation will help further on to discriminate the characteristics that gave a higher contribution to the sample discrimination.

## 2.3 – RESULTS

No outliers were detected in the samples of each species. A basic statistical description of the sample size (standard length) is shown in Annex II (Tables I-IX) for each species. Portuguese samples presented a higher variance for all species, except for *S. aurata* reared samples, and *S. cantharus*, fact that was associated with the larger sample sizes. Standard error and standard deviation varied accordingly.

For the principal components analysis (PCA), the first five components (PC1 to PC5) were analysed. PC1 loadings were similar in size and sign indicating it was a general measure of fish size (Bookstein *et al.*, 1985), such findings occurred in both methods and for all the species in study. This component was always correlated significantly with the standard length (SL) (values for each one of the species will be presented further on). PC2 to PC5 accounted for much smaller percentages of variation not correlating with SL and were used to interpret the nature of the morphometric variation. Correlations between the morphological measurements and the remaining principal components occurred sporadic, and usually with PC2. Only one (in a universe of twelve and thirty-one morphological characteristics for the traditional morphometrics and truss networks, respectively) correlated with one of those components. The loadings of each one of these components varied in magnitude, indicating that they were shape related components. For PC2 the largest loadings were usually head related from the traditional morphometry and head and the caudal peduncle related in truss analysis, which is indicative of differential growth of these body regions compared to rest of the body. The remaining components, PC3 to PC5 were similar to PC2, but with lower magnitudes.

The number of characteristics that contributed to the sample discrimination varied between four and twelve, and between five and twenty-two for the traditional morphometry and truss networks, respectively. In the traditional morphometry, from the characteristics that contributed to the sample discrimination the number of head related ones was always higher than the number of the number of body related characteristics. For the truss networks the transversal measurements along the trunk of the fish usually accounted for the higher percentage of contribution. The head related characteristics gave a smaller contribution to the sample discrimination.

The chosen F to enter, F to remove values were three and two, respectively, for the majority of the species (except for *D. sargus* and L. *mormyrus*). These values were chosen because when higher values of F to enter and F to remove were tested they did not produce higher degrees of discrimination between samples.

Under a geographical perspective of sample discrimination a geographic gradient in a West/East sequence (Portugal-Spain-Italy-Greece) with increasing values between samples was observed for the *D. puntazzo* and *L. mormyrus* through the traditional morphometry and for *D. sargus*, *D. puntazzo*, and *D. dentex* through the truss networks.

### 2.3.1 – *Traditional morphometry*

#### 2.3.1.1 – Gilthead seabream (*S. aurata*)

A total of 98.51% and 97.43% percent of the total variation associated with the 12-morphometric measurements was accounted for by the first five principal components for the reared and wild samples, respectively. PC1 loadings explained 89.2% and 94.9% of the total variation for the reared and wild samples, respectively (Tables XI and XII).

Table XI - Loadings from principal component analysis of the 12 morphometric characters for *S. aurata* reared samples. Loadings are listed for the five principal components. Character descriptors refer to Figure 2. (Exp. Var. - Explained Variance).

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| MFH | 0.978 | -0.112 | -0.104 | -0.052 | 0.014 |
| POL | 0.986 | 0.022 | -0.050 | -0.009 | -0.066 |
| OOL | 0.904 | -0.275 | 0.252 | 0.204 | 0.037 |
| PSL | 0.955 | 0.161 | 0.171 | -0.092 | -0.053 |
| HL | 0.988 | 0.076 | 0.034 | 0.006 | -0.059 |
| HD | 0.962 | -0.021 | 0.213 | -0.106 | -0.017 |
| UJL | 0.972 | 0.190 | -0.019 | 0.005 | -0.046 |
| LJL | 0.957 | 0.076 | -0.204 | 0.126 | -0.107 |
| CPL | 0.956 | 0.207 | -0.063 | 0.025 | 0.144 |
| DFL | 0.687 | -0.711 | -0.117 | -0.063 | 0.000 |
| LPA | 0.968 | 0.179 | -0.103 | 0.007 | 0.079 |
| AFL | 0.983 | -0.013 | -0.026 | -0.055 | 0.077 |
| Exp. Var. | 10.71 | 0.74 | 0.22 | 0.09 | 0.06 |
| % of total | 89.23 | 6.2 | 1.86 | 0.73 | 0.49 |

Table XII - Loadings from principal component analysis of the 12 morphometric characters for *S. aurata* wild samples. Loadings are listed for the five principal components. Character descriptors refer to Figure 2. (Exp. Var. - Explained Variance).

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| MFH | 0.984 | 0.067 | 0.025 | 0.085 | -0.013 |
| POL | 0.982 | -0.087 | -0.025 | 0.050 | -0.069 |
| OOL | 0.952 | 0.261 | -0.016 | -0.087 | -0.039 |
| PSL | 0.976 | -0.034 | 0.132 | 0.010 | -0.116 |
| HL | 0.988 | -0.003 | 0.013 | 0.074 | -0.081 |
| HD | 0.963 | -0.114 | 0.142 | -0.181 | 0.026 |
| UJL | 0.961 | -0.236 | -0.079 | -0.003 | -0.007 |
| LJL | 0.959 | -0.029 | -0.256 | -0.056 | 0.021 |
| CPL | 0.971 | -0.023 | 0.097 | 0.077 | 0.170 |
| DFL | 0.991 | 0.057 | 0.011 | -0.032 | 0.040 |
| LPA | 0.979 | 0.057 | -0.018 | 0.093 | 0.038 |
| AFL | 0.985 | 0.081 | -0.030 | -0.036 | 0.030 |
| Exp. Var. | 11.39 | 0.16 | 0.12 | 0.08 | 0.06 |
| % of total | 94.93 | 1.37 | 1.02 | 0.06 | 0.05 |

In the reared samples only the dorsal fin length (DFL) did not correlate with PC1. PC1 correlated significantly with the standard length (SL) (reared, $r$ = 0.99; $p$<0.05; N =294; wild, $r$ = 0.97; $p$<0.05; N =256).

Stepwise discriminant function analysis on wild and reared animals indicated that all morphological characteristics in study contributed to the discrimination between samples (Table XIII). The role of those characteristics was quite high with the majority of them with F remove values over 10. These results are corroborated by the low values of Wilks' λ, especially in the reared samples (Table XIII).

Table XIII - Stepwise Discriminant Analysis for the traditional morphometry of the *S. aurata* samples (Wilks' λ= 0.00004; F (60,1300)=163.36; $p$<0.0001) and wild (Wilks' λ= 0.0049; F (48,926)=57.565; $p$<0.0001).

| reared | | | | | wild | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Descriptor | Wilks λ | Partial λ | F-remove | p-level | Descriptor | Wilks λ | Partial λ | F-remove | p-level |
| LPA | 0.000 | 0.268 | 151.276 | 0.01 | LPA | 0.009 | 0.557 | 47.809 | 0.001 |
| HD | 0.000 | 0.354 | 101.213 | 0.01 | OOL | 0.008 | 0.622 | 36.398 | 0.001 |
| MFH | 0.000 | 0.524 | 50.273 | 0.01 | MFH | 0.007 | 0.690 | 26.914 | 0.001 |
| IIL | 0.000 | 0.799 | 13.939 | 0.001 | CPL | 0.007 | 0.719 | 23.437 | 0.001 |
| DFL | 0.000 | 0.764 | 17.154 | 0.001 | DFL | 0.006 | 0.788 | 16.146 | 0.001 |
| CPL | 0.000 | 0.718 | 21.782 | 0.001 | HD | 0.006 | 0.792 | 15.718 | 0.001 |
| OOL | 0.000 | 0.786 | 15.047 | 0.001 | LJL | 0.006 | 0.832 | 12.078 | 0.001 |
| LJL | 0.000 | 0.821 | 12.119 | 0.001 | UJL | 0.006 | 0.833 | 12.014 | 0.001 |
| POL | 0.000 | 0.875 | 7.930 | 0.001 | HL | 0.006 | 0.853 | 10.325 | 0.001 |
| AFL | 0.000 | 0.883 | 7.370 | 0.001 | POL | 0.006 | 0.858 | 9.957 | 0.001 |
| PSL | 0.000 | 0.868 | 8.457 | 0.001 | PSL | 0.005 | 0.905 | 6.295 | 0.001 |
| UJL | 0.000 | 0.907 | 5.693 | 0.001 | AFL | 0.005 | 0.926 | 4.828 | 0.001 |

The overall percent-correct classification success (PCS) was 90.2% and 98% for the wild and reared samples, respectively. Generalised Mahalanobis distances ($D^2$), $F$-statistics, and particularised PCS for each sample for wild and reared samples are presented in Tables X, XI and XII of Annex II. These parameters indicated a large difference between the French sample and all the other reared samples. Consequently, the canonical discriminant-factor scores of the remaining samples were highly superposed (Spain Atlantic, Spain Mediterranean, Portugal and Greece) (Figure 4). When the French sample was withdrawn from the analysis a more clear differentiation between the remaining samples was obtained (Figure 5). For the wild samples it was not possible to establish a clear discrimination between the Portuguese, Greek and Spanish Mediterranean samples. Only the Spanish Atlantic and the Italian samples became separate from the others (Figure 6).

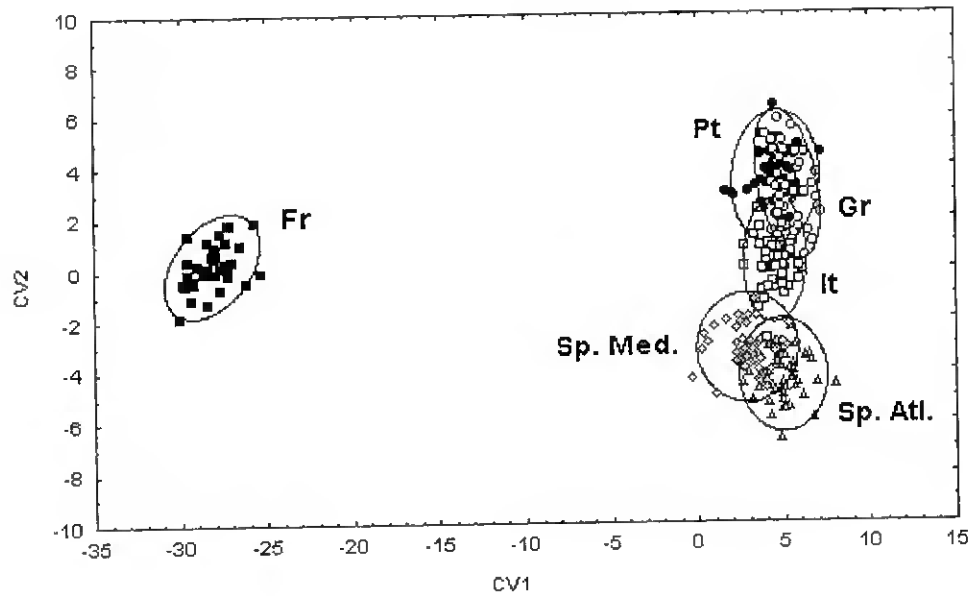



Figure 4 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the reared samples of *S. aurata* based on the traditional morphometry; France (▪), Portugal (•), Sp. Atl. (Δ), Sp. Med. (◇), Italy (□), Greece (○).

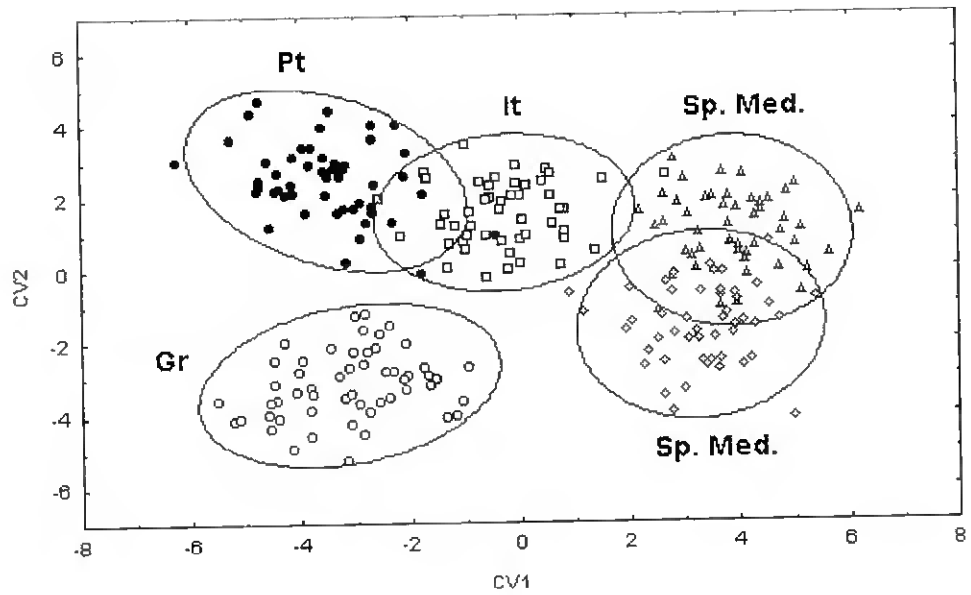



Figure 5 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the reared samples of *S. aurata* based on the traditional morphometry, without the French sample; Portugal (•), Sp. Atl. (Δ), Sp. Med. (◇), Italy (□), Greece (○).

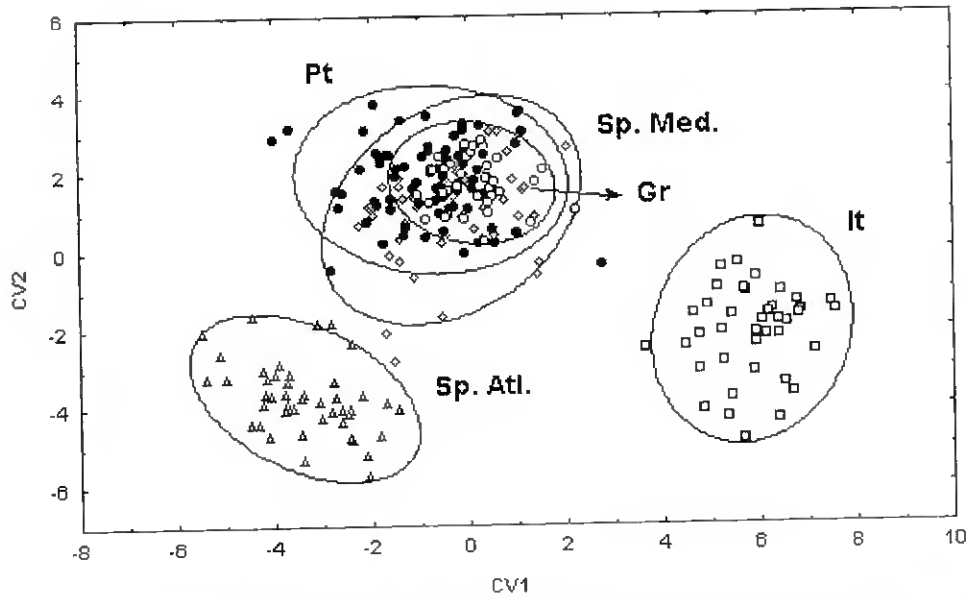



Figure 6 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the wild samples of *S. aurata* based on the traditional morphometry; Portugal (•), Sp. Atl. (Δ), Sp. Med. (◇), Italy (□), Greece (○).

Table XIV - Means of Canonical Variables for the reared and wild *S. aurata* samples (CV - Canonical variables). (Atl. - Atlantic, Med. -Mediterranean).

| | Reared | | | |
|---|---|---|---|---|
| | CV1 | CV2 | CV3 | CV4 |
| Samples | | | | |
| Portugal | 4.843 | 2.934 | 2.708 | 1.330 |
| Spain Atl. | 4.569 | -4.286 | 0.785 | 1.271 |
| Spain Med. | 2.469 | -2.839 | -1.707 | -0.625 |
| Italy | 4.193 | 0.644 | 1.674 | -2.520 |
| Greece | 5.407 | 3.016 | -3.227 | 0.417 |
| France | -27.391 | 0.362 | 0.030 | 0.117 |
| | Wild | | | |
| | CV1 | CV2 | CV3 | CV4 |
| Samples | | | | |
| Portugal | 0.004 | -1.827 | 0.354 | -1.013 |
| Spain Atl. | -3.488 | 3.918 | 0.203 | -0.112 |
| Spain Med. | -1.031 | -1.515 | -1.781 | 0.552 |
| Italy | 5.710 | 2.460 | -0.203 | 0.095 |
| Greece | -0.218 | -1.713 | 1.548 | 1.286 |

This fact is supported by the means of the canonical variables (Table XIV). For the reared samples, the first function discriminated the French sample from all the others, while the second function discriminated the Italian and Portuguese samples from the remaining samples. For the wild samples, the first canonical variable (CV1)

discriminated the Italian sample from all the others, and the CV2 separated the Atlantic samples from the remaining Mediterranean ones.

#### 2.3.1.2 - Red porgy (*P. pagrus*)

A total of 97.9% percent of the total variation associated with the 12-morphometric characters was accounted for by the first five principal components (Table XV).

Table XV - Loadings from principal component analysis of the 12 morphometric characters for *P. pagrus* samples. Loadings are listed for the five principal components. Character descriptors refer to Figure 2. (Exp. Var. - Explained Variance).

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| MFH | 0.955 | 0.263 | -0.008 | -0.013 | -0.052 |
| POL | 0.980 | 0.086 | -0.025 | 0.004 | -0.035 |
| OOL | 0.920 | 0.057 | 0.334 | -0.026 | 0.176 |
| PSL | 0.932 | -0.235 | 0.042 | -0.248 | -0.107 |
| HL | 0.988 | 0.069 | 0.068 | -0.049 | 0.010 |
| HD | 0.924 | 0.184 | -0.274 | -0.073 | 0.135 |
| UJL | 0.969 | -0.058 | -0.165 | 0.008 | 0.052 |
| LJL | 0.882 | -0.447 | -0.011 | 0.090 | 0.081 |
| CPL | 0.956 | -0.068 | -0.210 | 0.081 | -0.037 |
| DFL | 0.957 | 0.060 | 0.140 | 0.112 | -0.090 |
| LPA | 0.974 | 0.011 | 0.036 | 0.061 | -0.091 |
| AFL | 0.980 | 0.040 | 0.075 | 0.048 | -0.023 |
| Exp. Var. | 10.87 | 0.39 | 0.29 | 0.10 | 0.09 |
| % of total | 90.6 | 3.22 | 2.43 | 0.86 | 0.77 |

PC1 loadings explained 90.6% of the total variation. PC1 correlated significantly with the standard length (SL) ($r = 0.99$; $p<0.05$; N =231).

Nine morphometric characteristics contributed to the sample differentiation (MFH, PSL, UJL, POL, AFL, CPL, HD, OOL and LJL) with six of those characteristics

Table XVI - Stepwise Discriminant Analysis for the traditional morphometry comparison between *P. pagrus* samples. (Wilks' $\lambda$= 0.0412; F (27,640)=46.970; $p$ <0.0001).

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| MFH | 0.077 | 0.532 | 64.173 | 0.001 |
| PSL | 0.074 | 0.556 | 58.257 | 0.001 |
| UJL | 0.065 | 0.633 | 42.241 | 0.001 |
| POL | 0.055 | 0.754 | 23.840 | 0.001 |
| AFL | 0.053 | 0.783 | 20.172 | 0.001 |
| CPL | 0.050 | 0.820 | 16.004 | 0.001 |
| HD | 0.046 | 0.902 | 7.904 | 0.001 |
| OOL | 0.044 | 0.939 | 4.779 | 0.003 |
| LJL | 0.043 | 0.958 | 3.175 | 0.025 |

with F to remove over 16 (Table XVI).

The overall percent-correct classification success (PCS) was 94.4%. Generalised Mahalanobis distances ($D^2$), $F$-statistics, and particularised PCS for each sample are presented in Annex II, Tables X, XI and XII. All these parameters indicated significant differences between samples (Figure 7).

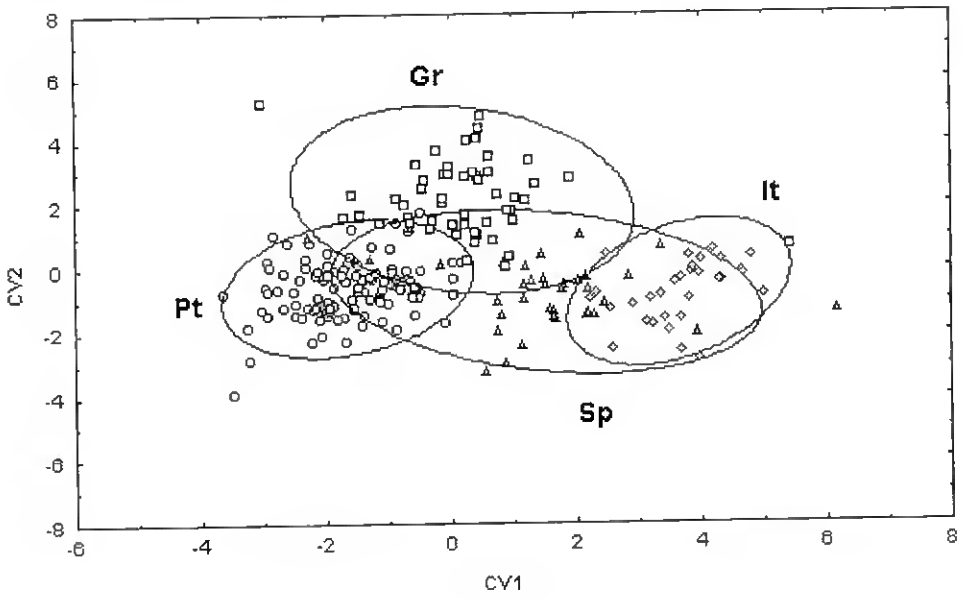



Figure 7 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the *P. pagrus* samples based on the traditional morphometry; Portugal (○), Spain (Δ), Italy (◇), Greece (□).

The value of CV1 obtained for the Italian sample confirmed this result discriminating it from all the others (Table XVII).

Table XVII - Means of Canonical Variables for *P. pagrus* samples (CV - Canonical variables)

| Samples | CV1 | CV2 | CV3 |
|---|---|---|---|
| Portugal | -1.652 | -0.597 | 0.307 |
| Spain | 1.531 | -0.746 | -2.211 |
| Italy | 3.645 | -0.776 | 1.599 |
| Greece | 0.193 | 2.161 | -0.022 |

### 2.3.1.3 – White seabream (*D. sargus*)

A total of 97.2% percent of the total variation associated with the 12-morphometric characters was accounted for by the first five principal components

(Table XVIII). PC1 loadings explained 83% of the total variation. PC1 correlated significantly with the standard length (SL) ($r = 0.99$; $p<0.05$; N =207).

Table XVIII - Loadings from principal component analysis of the 12 morphometric characters for *D. sargus* samples. Loadings are listed for the five principal components. Character descriptors refer to Figure 2. (Exp. Var. - Explained Variance).

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| MFH | 0.969 | 0.018 | -0.033 | 0.110 | -0.002 |
| POL | 0.973 | -0.033 | -0.065 | -0.148 | -0.054 |
| OOL | 0.830 | 0.449 | -0.227 | 0.081 | 0.087 |
| PSL | 0.944 | -0.178 | 0.030 | 0.155 | -0.127 |
| HL | 0.988 | 0.043 | -0.056 | 0.039 | -0.061 |
| HD | 0.937 | -0.270 | 0.056 | 0.082 | -0.108 |
| UJL | 0.960 | -0.083 | -0.062 | -0.192 | -0.089 |
| LJL | 0.904 | 0.199 | -0.209 | -0.282 | 0.015 |
| CPL | 0.785 | -0.544 | 0.150 | -0.112 | 0.188 |
| DFL | 0.681 | 0.396 | 0.612 | -0.074 | -0.002 |
| LPA | 0.947 | 0.015 | -0.030 | 0.125 | 0.215 |
| AFL | 0.963 | 0.066 | 0.001 | 0.173 | -0.015 |
| Exp. Var. | 9.96 | 0.81 | 0.51 | 0.25 | 0.13 |
| % of total | 83.02 | 6.77 | 4.25 | 2.1 | 1.1 |

Nine morphometric characteristics contributed to the sample differentiation (CPL, UJL, HD, LPA, OOL, LJL, POL, HL and MFH) (Table XIX).

Table XIX - Stepwise Discriminant Analysis for the traditional morphometry comparison between *D. sargus* samples. (Wilks' $\lambda$= 0.0907; F (27,570)=26.919; $p<0.0001$)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| CPL | 0.145 | 0.625 | 39.020 | 0.001 |
| UJL | 0.123 | 0.738 | 23.102 | 0.001 |
| HD | 0.121 | 0.750 | 21.717 | 0.001 |
| LPA | 0.116 | 0.784 | 17.856 | 0.001 |
| OOL | 0.101 | 0.902 | 7.067 | 0.001 |
| LJL | 0.109 | 0.836 | 12.779 | 0.001 |
| POL | 0.104 | 0.872 | 9.518 | 0.001 |
| HL | 0.096 | 0.941 | 4.089 | 0.008 |
| MFH | 0.095 | 0.952 | 3.303 | 0.021 |

The overall percent-correct classification success (PCS) was 89.4%. The Spanish sample presented the lower PCS of all the species in study (Annex II, Table XII). Generalised Mahalanobis distances ($D^2$), F-statistics, and particularised PCS for each sample are presented in Annex II, Tables X, XI and XII. As a consequence of the low PCS of the Spanish sample, its individuals appeared scattered with the remaining

samples (Figure 8). Nevertheless, a geographic gradation was found between them (Annex II, Table X).

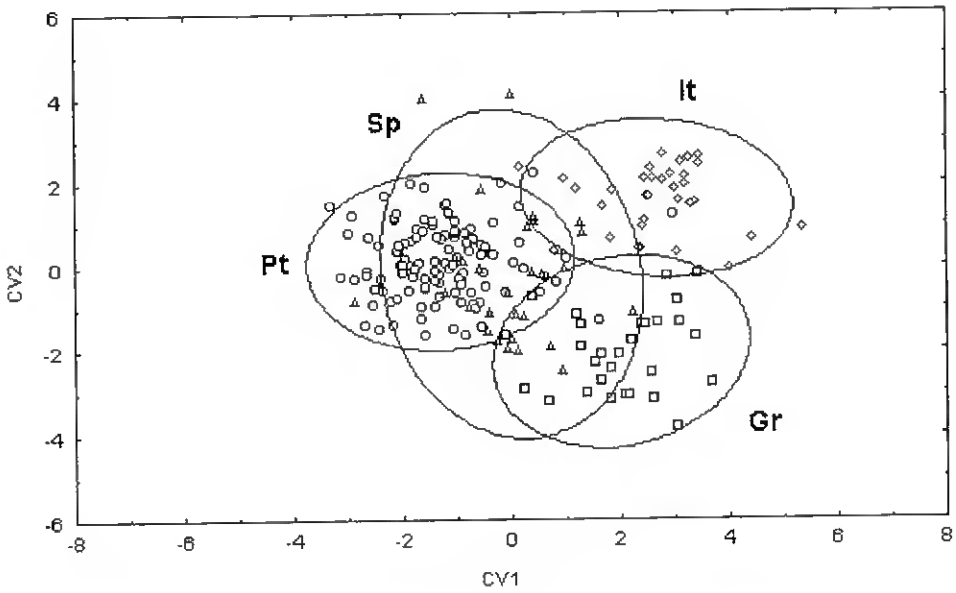



Figure 8 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the *D. sargus* samples based on the traditional morphometry; Portugal (○), Spain (Δ), Italy (◇), Greece (□).

The CV1 values clustered the Italian and Greek samples together, which is a clear evidence of the difference between these samples and the Atlantic sample (Table XX).

Table XX - Means of Canonical Variables for *D. sargus* samples (CV - Canonical variables)

| Samples | CV1 | CV2 | CV3 |
|---|---|---|---|
| Portugal | -1.302 | 0.123 | -0.270 |
| Spain | -0.002 | -0.186 | 1.618 |
| Italy | 2.675 | 1.597 | -0.195 |
| Greece | 2.007 | -2.039 | -0.378 |

### 2.3.1.4 – Sharpsnout seabream (*D. puntazzo*)

A total of 96% of the total variation was accounted for by the first five principal components (Table XXI). PC1 loadings explained 82.6% of the total variation. Only the CPL measurement did not correlate with PC1, correlating with PC2 instead. PC1 correlated significantly with the standard length (SL) ($r = 0.97$; $p<0.05$; N =160).

Ten morphometric characteristics contributed to the sample separation (AFL, POL, UJL, OOL, DFL, LPA, HD, LJL, HL and MFH) (Table XXII).

Table XXI - Loadings from principal component analysis of the 12 morphometric characters for *D. puntazzo* samples. Loadings are listed for the five principal components. Character descriptors refer to Figure 2. (Exp. Var. - Explained Variance).

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| MFH | 0.746 | -0.197 | -0.631 | 0.016 | 0.009 |
| POL | 0.961 | -0.057 | 0.131 | 0.062 | 0.098 |
| OOL | 0.958 | -0.054 | 0.099 | 0.042 | -0.083 |
| PSL | 0.920 | 0.019 | 0.008 | -0.295 | 0.109 |
| HL | 0.982 | -0.045 | 0.090 | -0.002 | 0.079 |
| HD | 0.944 | 0.015 | 0.112 | -0.185 | 0.011 |
| UJL | 0.940 | -0.073 | 0.117 | 0.120 | 0.147 |
| LJL | 0.895 | -0.134 | 0.062 | 0.367 | -0.090 |
| CPL | 0.649 | 0.741 | -0.131 | 0.107 | 0.032 |
| DFL | 0.959 | 0.017 | 0.033 | -0.057 | -0.216 |
| LPA | 0.942 | -0.071 | -0.061 | 0.002 | 0.156 |
| AFL | 0.947 | 0.026 | -0.006 | -0.128 | -0.240 |
| Exp. Var. | 9.91 | 0.63 | 0.49 | 0.31 | 0.19 |
| % of total | 82.59 | 5.22 | 4.05 | 2.56 | 1.62 |

Table XXII - Stepwise Discriminant Analysis for the traditional morphometry comparison between *D. puntazzo* samples. (Wilks' $\lambda$= 0.0393; F (30,432)=28.999; $p$<0.0001)

| Descriptor | Wilks $\lambda$ | Partial $\lambda$ | F-remove | p-level |
|---|---|---|---|---|
| AFL | 0.055 | 0.717 | 19.333 | 0.001 |
| POL | 0.051 | 0.777 | 14.067 | 0.001 |
| UJL | 0.049 | 0.798 | 12.377 | 0.001 |
| OOL | 0.047 | 0.828 | 10.175 | 0.001 |
| DFL | 0.047 | 0.837 | 9.561 | 0.001 |
| LPA | 0.046 | 0.856 | 8.261 | 0.001 |
| HD | 0.045 | 0.881 | 6.588 | 0.001 |
| LJL | 0.044 | 0.884 | 6.450 | 0.001 |
| HL | 0.043 | 0.903 | 5.286 | 0.002 |
| MFH | 0.042 | 0.939 | 3.161 | 0.026 |

The overall percent-correct classification success (PCS) was 90.6%. Generalised Mahalanobis distances ($D^2$), $F$-statistics, and particularised PCS for each sample are presented in Annex II, Tables X, XI and XII. The $D^2$ values were rather high and discriminated a geographic gradient between samples (Annex II, Table X). This result is corroborated by the canonical discriminant-factor scores between samples (Figure 9).

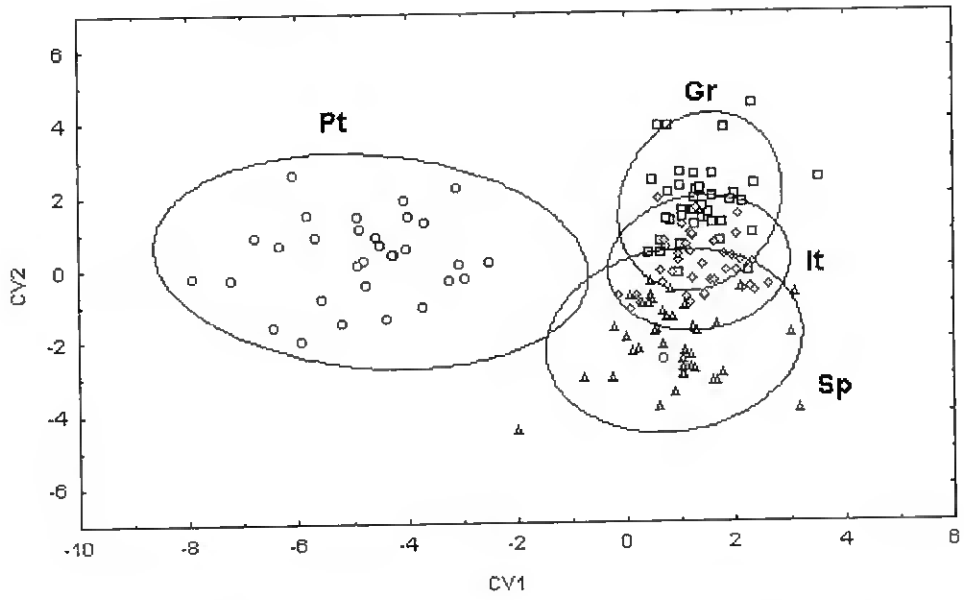



Figure 9 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the *D. puntazzo* samples based on the traditional morphometry; Portugal (○), Spain (Δ), Italy (◇), Greece (□).

The mean canonical values showed a clear differentiation between the Atlantic (Portuguese) sample and all the others (Table XXIII).

Table XXIII - Means of Canonical Variables for *D. puntazzo* samples (CV - Canonical variables)

| Samples | CV1 | CV2 | CV3 |
|---|---|---|---|
| Portugal | -3.531 | 0.781 | -0.307 |
| Spain | -0.125 | -2.240 | 0.412 |
| Italy | 1.233 | 1.345 | 1.029 |
| Greece | 1.818 | 0.218 | -1.124 |

### 2.3.1.5 – Striped seabream (*L. mormyrus*)

A total of 96.6% of the total variation was accounted for by the first five principal components (Table XXIV). PC1 loadings explained 87.2% of the total variance, with PC1 correlated significantly with the standard length (SL) ($r$ = 0.98; $p$<0.05; N =217).

Eight morphometric characteristics contributed to the sample individualization (LPA, OOL, HD, HL, LJL, AFL, PSL and CPL) (Table XXV). The overall percent-correct classification success (PCS) was high (96.8%), and it was similar among samples (Table XII, Annex II). Generalised Mahalanobis distances ($D^2$), $F$-statistics, and particularised PCS for each sample are presented in Annex II, Tables X, XI and XII.

Table XXIV - Loadings from principal component analysis of the 12 morphometric characters for *L. mormyrus* samples. Loadings are listed for the five principal components. Character descriptors refer to Figure 2. (Exp. Var. - Explained Variance).

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| MFH | 0.971 | 0.087 | 0.137 | -0.053 | -0.005 |
| POL | 0.952 | -0.023 | -0.164 | -0.154 | -0.135 |
| OOL | 0.807 | 0.495 | -0.251 | 0.174 | 0.090 |
| PSL | 0.978 | -0.012 | -0.089 | -0.047 | -0.001 |
| HL | 0.974 | 0.082 | -0.163 | -0.071 | -0.040 |
| HD | 0.909 | -0.325 | -0.042 | -0.040 | 0.192 |
| UJL | 0.930 | -0.265 | -0.139 | 0.112 | -0.021 |
| LJL | 0.956 | -0.166 | -0.139 | 0.018 | -0.073 |
| CPL | 0.919 | -0.249 | 0.175 | 0.194 | -0.007 |
| DFL | 0.961 | 0.166 | 0.088 | -0.043 | -0.016 |
| LPA | 0.904 | 0.140 | 0.345 | 0.065 | -0.133 |
| AFL | 0.929 | 0.122 | 0.230 | -0.115 | 0.167 |
| Exp. Var. | 10.46 | 0.59 | 0.39 | 0.14 | 0.12 |
| % of total | 87.15 | 4.89 | 3.28 | 1.13 | 0.1 |

Table XXV - Stepwise Discriminant Analysis for the traditional morphometry comparison between *L. mormyrus* samples. (Wilks' $\lambda$= 0.02403; F (33,598)=46.180; $p$ <0.0001)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| LPA | 0.047 | 0.516 | 63.487 | 0.001 |
| OOL | 0.041 | 0.589 | 47.206 | 0.001 |
| HD | 0.032 | 0.760 | 21.362 | 0.001 |
| LJL | 0.028 | 0.848 | 12.172 | 0.001 |
| AFL | 0.028 | 0.867 | 10.411 | 0.001 |
| PSL | 0.027 | 0.875 | 9.677 | 0.001 |
| HL | 0.027 | 0.877 | 9.504 | 0.001 |
| MFH | 0.026 | 0.922 | 5.692 | 0.001 |
| POL | 0.026 | 0.939 | 4.387 | 0.005 |
| CPL | 0.025 | 0.949 | 3.671 | 0.013 |
| UJL | 0.025 | 0.956 | 3.149 | 0.026 |

Through the $D^2$ values it was also possible to establish a geographic gradient between samples of *L. mormyrus* (Table X, Annex II). The canonical discriminant-factor scores between samples are presented in Figure 10.

Table XXVI - Means of Canonical Variables for *L. mormyrus* samples (CV - Canonical variables).

| Samples | CV1 | CV2 | CV3 |
|---|---|---|---|
| Portugal | 0.765 | 1.234 | 0.023 |
| Spain | -0.892 | -1.738 | 2.275 |
| Italy | 2.482 | -2.770 | -1.297 |
| Greece | -4.597 | -0.332 | -1.141 |

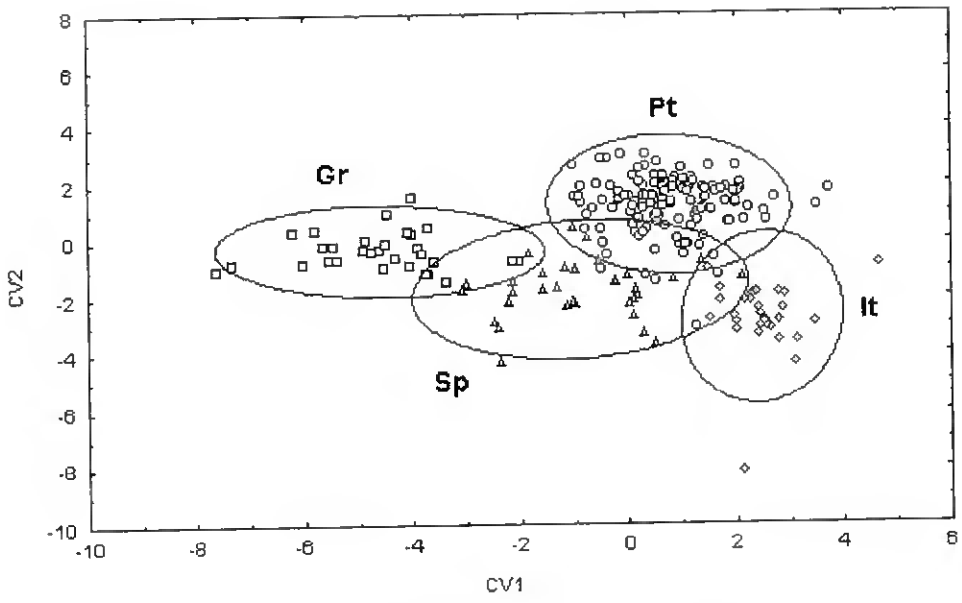



Figure 10 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the *L. mormyrus* samples based on the traditional morphometry; Portugal (), Spain (△), Italy ( ◇), Greece (□).

The canonical mean values for the *L. mormyrus* samples discriminated the two most eastern samples (Italian and Greek samples) from the most Western ones (Portuguese and Spanish samples) (Table XXVI).

### 2.3.1.6 – Red seabream (*P. bogaraveo*)

A total of 96% of the total variation was accounted for by the first five principal components (Table XXVII).

Table XXVII - Loadings from principal component analysis of the 12 morphometric characters for *P. bogaraveo* samples. Loadings are listed for the five principal components. Character descriptors refer to Figure 2. (Exp. Var. - Explained Variance).

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| MFH | 0.963 | 0.134 | -0.131 | -0.028 | -0.035 |
| POL | 0.975 | -0.013 | 0.078 | -0.016 | -0.049 |
| OOL | 0.978 | 0.036 | 0.025 | -0.080 | -0.003 |
| PSL | 0.960 | 0.004 | 0.109 | 0.068 | -0.100 |
| HL | 0.654 | -0.741 | -0.148 | -0.028 | 0.014 |
| HD | 0.912 | 0.021 | 0.043 | 0.193 | -0.316 |
| UJL | 0.934 | -0.027 | 0.268 | -0.105 | 0.004 |
| LJL | 0.914 | 0.066 | 0.145 | -0.306 | 0.091 |
| CPL | 0.875 | -0.024 | 0.212 | 0.332 | 0.271 |
| DFL | 0.857 | 0.182 | -0.400 | 0.068 | 0.097 |
| LPA | 0.963 | 0.097 | -0.121 | -0.051 | 0.043 |
| AFL | 0.968 | 0.048 | -0.134 | -0.023 | 0.011 |
| Exp. Var. | 10.09 | 0.62 | 0.39 | 0.27 | 0.21 |
| % of total | 84.05 | 5.16 | 3.25 | 2.27 | 1.72 |

PC1 loadings explained only 84.1% of the total variation, and from the twelve characteristics only HL did not correlate with PC1. Nonetheless, PC1 was significantly correlated with the standard length (SL) ($r = 0.99$; $p<0.05$; N =186).

Nine of the morphometric characteristics contributed to the sample separation (OOL, MFH, PLA, UJL, HD, AFL, LJL, PSL and POL) (Table XXVIII). Mean PCS was 98.9%, with 100% correct classification of the Portuguese and Spanish samples (Table XXVIII).

Table XXVIII - Stepwise Discriminant Analysis for the traditional morphometry comparison between *P. bogaraveo* samples. (Wilks' λ= 0.004; F (27,508)=106.09; $p<0.0001$)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| OOL | 0.008 | 0.503 | 57.214 | 0.001 |
| MFH | 0.008 | 0.512 | 55.243 | 0.001 |
| LPA | 0.006 | 0.641 | 32.487 | 0.001 |
| UJL | 0.006 | 0.705 | 24.257 | 0.001 |
| HD | 0.005 | 0.843 | 10.803 | 0.001 |
| AFL | 0.005 | 0.846 | 10.531 | 0.001 |
| LJL | 0.004 | 0.887 | 7.397 | 0.001 |
| PSL | 0.004 | 0.892 | 7.011 | 0.001 |
| POL | 0.004 | 0.911 | 5.647 | 0.001 |

Generalised Mahalanobis distances ($D^2$), *F*-statistics, and particularised PCS for each sample are presented in Annex II, Tables X, XI and XII. No geographic gradient was found among samples (Annex II, Tables X), although marked differences were found between the Atlantic (Portuguese) sample and the Mediterranean samples.

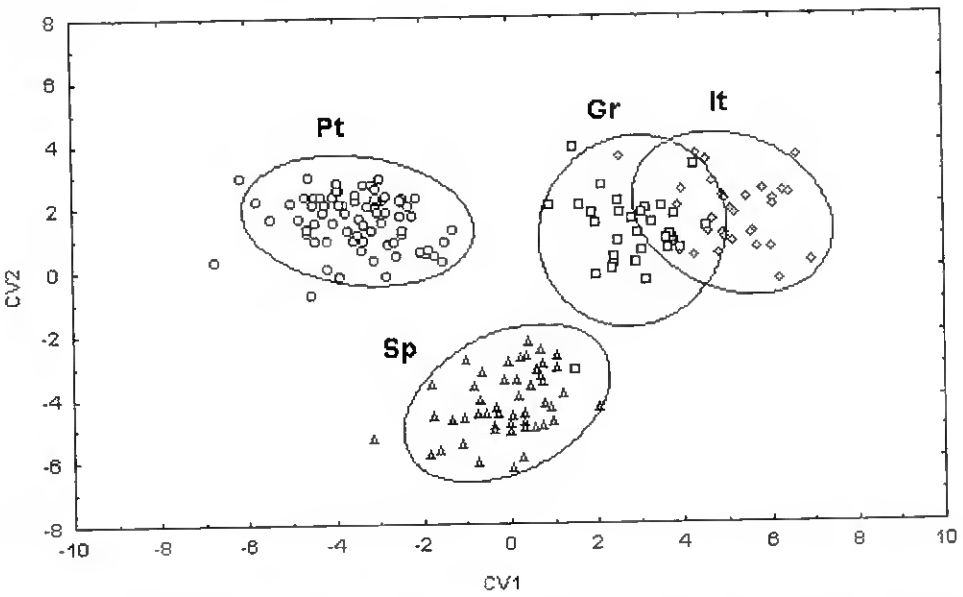



Figure 11 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the *P. bogaraveo* samples based on the traditional morphometry; Portugal(○), Spain (△), Italy ( ◇), Greece (□).

Such findings are well evidenced through the canonical discriminant-factor scores (Figure 11). Again, the mean canonical values confirmed the results reported above (TableXXIX).

Table XXIX - Means of Canonical Variables for *P. bogaraveo* samples (CV - Canonical variables).

| Samples | CV1 | CV2 | CV3 |
|---|---|---|---|
| Portugal | -3.453 | 1.587 | 0.247 |
| Spain | -0.086 | -4.216 | 0.143 |
| Italy | 5.159 | 1.674 | 1.819 |
| Greece | 2.851 | 1.160 | -2.601 |

### 2.3.1.7 – Common Dentex (*D. dentex*)

A total of 96% of the total variation was accounted for by the first five principal components (Table XXX) with PC1 loadings explaining 83.6% of the total variation. PC1 correlated significantly with the standard length (SL) ($r = 0.98$; $p<0.05$; N =144).

Table XXX - Loadings from principal component analysis of the 12 morphometric characters for *D. dentex* samples. Loadings are listed for the five principal components. Character descriptors refer to Figure 2. (Exp. Var. - Explained Variance).

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| MFH | 0.979 | -0.038 | -0.056 | 0.003 | -0.027 |
| POL | 0.909 | -0.056 | 0.002 | 0.290 | -0.176 |
| OOL | 0.937 | -0.051 | -0.027 | -0.114 | 0.072 |
| PSL | 0.961 | -0.027 | -0.040 | 0.036 | -0.074 |
| HL | 0.960 | -0.041 | -0.148 | 0.169 | -0.087 |
| HD | 0.681 | 0.731 | -0.033 | -0.013 | 0.007 |
| UJL | 0.920 | 0.005 | 0.348 | -0.124 | -0.007 |
| LJL | 0.916 | -0.060 | 0.358 | -0.066 | -0.029 |
| CPL | 0.866 | -0.061 | -0.039 | 0.124 | 0.458 |
| DFL | 0.852 | -0.092 | -0.261 | -0.365 | -0.056 |
| LPA | 0.966 | -0.056 | -0.070 | -0.015 | 0.025 |
| AFL | 0.980 | -0.062 | -0.045 | 0.045 | -0.072 |
| Exp. Var. | 10.03 | 0.57 | 0.35 | 0.30 | 0.27 |
| % of total | 83.55 | 4.72 | 2.95 | 2.48 | 2.25 |

Eight morphometric characteristics contributed to the sample separation (LJL, POL, UJL, LPA, MFH, OOL, HL and AFL) (Table XXXI). The PCS was 89.6% (Annex II, Table XII). Generalised Mahalanobis distances ($D^2$), *F*-statistics, and particularised PCS for each sample are presented in Annex II, Tables X, XI and XII. For

*D. dentex* it was not also possible to establish a geographic gradient (Annex II, Table X) between samples.

Table XXXI - Stepwise Discriminant Analysis for the traditional morphometry comparison between *D. dentex* samples. (Wilks' $\lambda$= 0.032; F (24,386)=36.644; $p$<0.0001)

| Descriptor | Wilks $\lambda$ | Partial $\lambda$ | F-remove | p-level |
|---|---|---|---|---|
| LJL | 0.064 | 0.504 | 43.639 | 0.001 |
| POL | 0.058 | 0.552 | 35.939 | 0.001 |
| UJL | 0.043 | 0.746 | 15.080 | 0.001 |
| LPA | 0.041 | 0.781 | 12.442 | 0.001 |
| MFH | 0.041 | 0.787 | 11.966 | 0.001 |
| OOL | 0.041 | 0.788 | 11.929 | 0.001 |
| HL | 0.037 | 0.868 | 6.767 | 0.001 |
| AFL | 0.035 | 0.923 | 3.689 | 0.014 |

There was also a marked difference between the Atlantic (Portuguese) sample and the Mediterranean samples. Canonical discriminant-factor scores are presented in Figure 12.

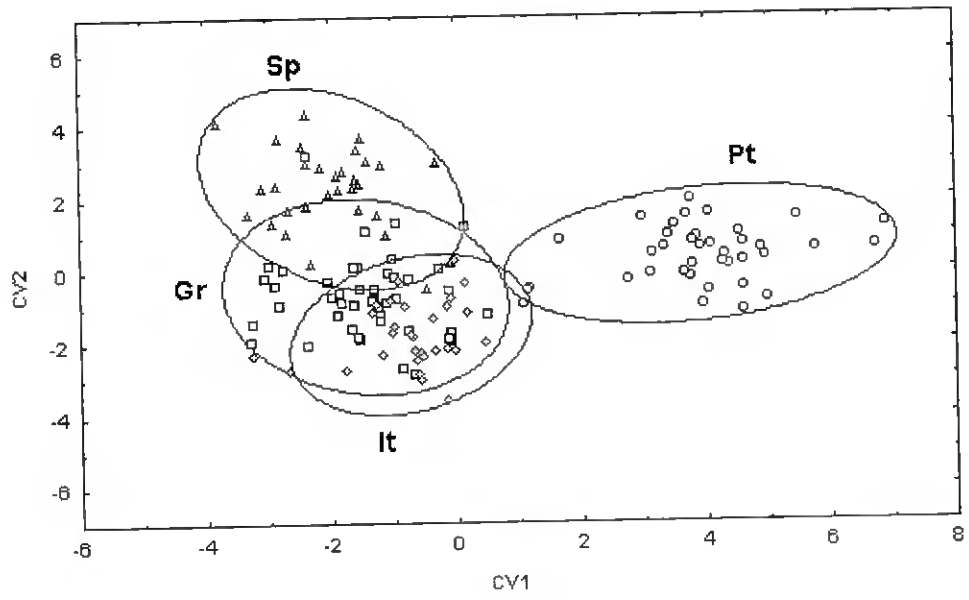



Figure 12 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the *D. dentex* samples based on the traditional morphometry; Portugal (○), Spain (Δ), Italy (◇), Greece (□).

Mean canonical values evidenced a clear separation of the Atlantic (Portuguese) sample from all the Mediterranean (Spanish, Italian and Greek) samples (Table XXXII).

Table XXXII - Means of Canonical Variables for *D. dentex* samples (CV - Canonical variables).

| Samples | CV1 | CV2 | CV3 |
|---|---|---|---|
| Portugal | 3.912 | 0.369 | -0.078 |
| Spain | -1.977 | 2.286 | 0.386 |
| Italy | -0.734 | -1.764 | 1.033 |
| Greece | -1.452 | -0.711 | -0.888 |

### 2.3.1.8 – Black seabream (*S. cantharus*)

A total of 97.2% of the total variation was accounted for by the first five principal components (Table XXXIII) with PC1 loadings explaining 84.2% of the total variation. HD did not correlate with PC1, but was correlated with PC2. Like all the other species PC1 correlated significantly with the standard length (SL) ($r$ = 0.97; $p$<0.05; N =66).

Table XXXIII - Loadings from principal component analysis of the 12 morphometric characters for *S. cantharus* samples. Loadings are listed for the five principal components. Character descriptors refer to Figure 2. (Exp. Var. - Explained Variance).

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| MFH | 0.978 | -0.066 | 0.053 | 0.135 | -0.074 |
| POL | 0.962 | -0.056 | 0.035 | -0.031 | 0.003 |
| OOL | 0.958 | -0.095 | 0.165 | -0.079 | -0.015 |
| PSL | 0.945 | 0.176 | -0.011 | 0.098 | 0.039 |
| HL | 0.747 | -0.373 | -0.547 | 0.033 | -0.033 |
| HD | 0.945 | 0.220 | -0.065 | 0.152 | 0.056 |
| UJL | 0.928 | -0.004 | -0.019 | -0.266 | 0.230 |
| LJL | 0.865 | -0.430 | 0.171 | -0.125 | 0.029 |
| CPL | 0.898 | 0.334 | -0.054 | 0.138 | 0.145 |
| DFL | 0.986 | 0.022 | 0.071 | 0.023 | -0.072 |
| LPA | 0.933 | -0.184 | 0.185 | 0.159 | -0.101 |
| AFL | 0.836 | 0.406 | -0.105 | -0.273 | -0.221 |
| Exp. Var. | 10.10 | 0.73 | 0.42 | 0.26 | 0.15 |
| % of total | 84.19 | 6.09 | 3.48 | 2.21 | 1.25 |

With only four morphometric characteristics contributing to the sample differentiation (DFL, HL, LJL and CPL), *S. cantharus* was the species that had the

Table XXXIV - Stepwise Discriminant Analysis for the traditional morphometry comparison between *S. cantharus* samples. (Wilks' λ= 0.0258; F (5,60)=453.48; $p$ <0.0001)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| DFL | 0.048 | 0.533 | 52.489 | 0.001 |
| HL | 0.033 | 0.771 | 17.866 | 0.001 |
| LJL | 0.029 | 0.881 | 8.106 | 0.006 |
| CPL | 0.027 | 0.946 | 3.415 | 0.070 |

smallest number of discriminatory characteristics between samples (Table XXXIV). Nonetheless, the overall percent-correct classification success (PCS) was 100% for both Portuguese and Spanish samples. The generalised Mahalanobis distance ($D^2$) was 156.33, with an *F*-statistics F(5,6)=452.5 for $p<0.001$.

From the canonical analysis, both samples correlated significantly with CV1 (5.04 and –7.25, for the Portuguese and Spanish samples, respectively), which invalidated the graphical image of the difference between samples. However, the obtained $D^2$ and PCS values ensure a high degree of differentiation between samples.

### 2.3.2 – *Truss networks*

#### 2.3.2.1 – Gilthead seabream (*S. aurata*)

A total of 95.4% and 94.2% of the total variation associated with the 31-truss morphometric characters was accounted for by the first three principal components for the reared and wild samples, respectively. PC1 loadings explained 92.9% and 89.4% of the total variation for the reared and wild samples, respectively (Table XXXV). This component was significantly correlated with the standard length (SL) (reared, $r = 0.93$; $p<0.05$; N =294; wild, $r = 0.87$; $p<0.05$; N =256). For PC2 the largest loadings were for the head and the caudal peduncle related measurements, which are indicative of differential growth of these body regions compared to the rest of the body. Nevertheless, this occurred mainly in reared fish, and was less visible in the wild fish.

Stepwise discriminant function analysis on truss data from reared and wild animals indicated highly significant differences in the truss element distances among the different countries (reared, Wilks' $\lambda$ =0.0021, F(25, 87)=48.192; $p<0.0001$; wild, Wilks' $\lambda$ =0.00371, F(88, 911)=32.325; $p<0.0001$) Tables XXXVI and XXXVII. A total of 18 truss elements were selected for the reared fish (A1, A2, A3, A6, B1, B2, B5, C1, C2, D1, D2, D4, D5, E2, E5, F1, F3, F5) and 22 for the wild fish (A1, A2, A4, B1, B2, B3, B4, B5, C1, C3, C4, C5, D1, D3, D4, D5, E1, E4, E5, F1, F2, F5).

The PCS was 96.5% and 98.7% for the reared and wild samples, respectively. Particularised PCS for each sample generalised Mahalanobis distances ($D^2$) and *F*-statistics for wild and reared samples are presented in Annex II, Tables XIII, XIV and XV. The French and the Spain Atlantic and the Italian reared and wild samples were 100% correctly classified.

Table XXXV - Loadings from the principal components analysis of the 31 morphometric characters for the reared and wild samples. Loadings are listed for the five principal components. Characters descriptors refer to Figure 3. (Exp. Var. - Explained Variance).

| | Reared | | | | | Wild | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 | PC1 | PC2 | PC3 | PC4 | PC5 |
| A1 | 0.920 | 0.273 | 0.056 | 0.036 | 0.042 | 0.941 | 0.154 | 0.013 | -0.035 | 0.018 |
| A2 | 0.965 | 0.057 | -0.013 | -0.055 | -0.004 | 0.971 | -0.065 | -0.077 | -0.028 | -0.056 |
| A3 | 0.877 | 0.378 | -0.174 | 0.117 | -0.033 | 0.711 | 0.436 | 0.445 | 0.260 | 0.163 |
| A4 | 0.991 | 0.060 | 0.044 | 0.032 | 0.024 | 0.982 | 0.117 | 0.029 | -0.015 | -0.021 |
| A4 | 0.986 | 0.059 | 0.071 | 0.026 | 0.042 | 0.980 | 0.090 | -0.005 | -0.040 | -0.013 |
| A6 | 0.972 | 0.150 | -0.064 | 0.008 | 0.007 | 0.975 | 0.084 | 0.055 | 0.039 | -0.017 |
| B1 | 0.863 | -0.203 | -0.417 | 0.050 | 0.130 | 0.787 | -0.548 | 0.108 | 0.123 | -0.041 |
| B2 | 0.935 | -0.143 | 0.103 | -0.191 | -0.046 | 0.967 | -0.082 | -0.113 | -0.066 | -0.054 |
| B3 | 0.990 | -0.071 | -0.015 | 0.051 | 0.080 | 0.991 | 0.026 | -0.009 | -0.037 | -0.014 |
| B4 | 0.988 | 0.010 | -0.071 | 0.044 | 0.057 | 0.989 | -0.001 | -0.026 | -0.023 | 0.020 |
| B5 | 0.987 | -0.072 | 0.058 | 0.005 | 0.032 | 0.991 | 0.041 | -0.006 | -0.028 | -0.037 |
| C1 | 0.914 | 0.015 | 0.235 | 0.153 | 0.039 | 0.791 | 0.442 | 0.020 | -0.017 | -0.358 |
| C2 | 0.967 | 0.018 | -0.012 | -0.033 | -0.080 | 0.764 | 0.022 | -0.354 | 0.536 | -0.017 |
| C3 | 0.895 | -0.156 | -0.021 | 0.230 | -0.331 | 0.989 | 0.031 | -0.008 | -0.042 | 0.004 |
| C4 | 0.990 | -0.070 | -0.002 | 0.051 | 0.051 | 0.991 | 0.029 | -0.013 | -0.039 | -0.028 |
| C5 | 0.990 | -0.080 | 0.002 | 0.036 | 0.018 | 0.989 | 0.051 | -0.011 | -0.044 | -0.014 |
| D1 | 0.964 | -0.084 | -0.059 | -0.109 | -0.093 | 0.924 | -0.287 | 0.100 | 0.010 | 0.036 |
| D2 | 0.988 | -0.007 | 0.017 | -0.019 | 0.003 | 0.983 | -0.028 | -0.026 | -0.033 | 0.012 |
| D3 | 0.994 | -0.045 | 0.024 | 0.013 | 0.028 | 0.992 | 0.006 | -0.017 | -0.033 | 0.011 |
| D4 | 0.992 | -0.041 | 0.035 | 0.020 | 0.042 | 0.994 | 0.015 | -0.015 | -0.043 | 0.002 |
| D5 | 0.989 | -0.076 | -0.013 | -0.012 | -0.012 | 0.981 | -0.002 | -0.020 | -0.043 | 0.039 |
| E1 | 0.980 | -0.008 | 0.034 | -0.008 | 0.039 | 0.984 | 0.006 | -0.060 | -0.016 | 0.006 |
| E2 | 0.943 | -0.075 | 0.134 | 0.061 | 0.128 | 0.947 | -0.022 | -0.027 | -0.028 | -0.118 |
| E3 | 0.986 | -0.044 | -0.027 | -0.029 | 0.003 | 0.975 | -0.048 | -0.040 | -0.041 | 0.108 |
| E4 | 0.988 | -0.065 | 0.049 | 0.011 | 0.066 | 0.991 | -0.018 | -0.024 | -0.035 | 0.003 |
| E5 | 0.992 | -0.033 | 0.031 | 0.001 | 0.035 | 0.992 | -0.009 | -0.046 | -0.025 | 0.030 |
| F1 | 0.945 | 0.152 | -0.037 | -0.108 | -0.056 | 0.937 | 0.051 | -0.077 | -0.052 | 0.190 |
| F2 | 0.949 | 0.066 | -0.031 | -0.173 | -0.076 | 0.742 | -0.035 | 0.001 | -0.026 | 0.110 |
| F3 | 0.970 | -0.019 | 0.046 | -0.017 | -0.049 | 0.808 | -0.398 | 0.324 | 0.107 | -0.188 |
| F4 | 0.977 | 0.016 | -0.035 | -0.087 | -0.024 | 0.977 | -0.013 | 0.010 | -0.036 | 0.047 |
| F5 | 0.980 | 0.060 | 0.006 | -0.076 | -0.041 | 0.970 | -0.029 | -0.004 | -0.025 | 0.120 |
| Exp. Var. | 28.81 | 0.42 | 0.33 | 0.22 | 0.2 | 27.7 | 1.01 | 0.49 | 0.41 | 0.3 |
| % of total | 92.9 | 1.38 | 1.1 | 0.7 | 0.64 | 89.4 | 3.2 | 1.58 | 1.34 | 0.96 |

Table XXXVI - Stepwise Discriminant Analysis for the truss comparison between the reared *S. aurata* samples. (Wilks' λ =0.0021, F(25,87)=48,192; $p$ <0.0001)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| B5 | 0.000 | 0.425 | 73.453 | 0.001 |
| A2 | 0.000 | 0.561 | 42.403 | 0.001 |
| A1 | 0.000 | 0.600 | 36.108 | 0.001 |
| D4 | 0.000 | 0.630 | 31.842 | 0.001 |
| D5 | 0.000 | 0.668 | 26.915 | 0.001 |
| D2 | 0.000 | 0.698 | 23.442 | 0.001 |
| B1 | 0.000 | 0.699 | 23.335 | 0.001 |
| A6 | 0.000 | 0.726 | 20.478 | 0.001 |
| B2 | 0.000 | 0.749 | 18.141 | 0.001 |
| C2 | 0.000 | 0.752 | 17.842 | 0.001 |
| A3 | 0.000 | 0.782 | 15.152 | 0.001 |
| D1 | 0.000 | 0.804 | 13.177 | 0.001 |
| E5 | 0.000 | 0.828 | 11.238 | 0.001 |
| E2 | 0.000 | 0.839 | 10.429 | 0.001 |
| F5 | 0.000 | 0.842 | 10.198 | 0.001 |
| C1 | 0.000 | 0.865 | 8.433 | 0.001 |
| F3 | 0.000 | 0.880 | 7.418 | 0.001 |
| F1 | 0.000 | 0.890 | 6.728 | 0.001 |

Table XXXVII - Stepwise Discriminant Analysis for the truss comparison between the wild *S. aurata* samples. (Wilks' λ= 0.0037; F (88,911)=32.325, $p$ <0.0001)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| B4 | 0.005 | 0.758 | 18.334 | 0.001 |
| B5 | 0.005 | 0.762 | 17.925 | 0.001 |
| C4 | 0.005 | 0.786 | 15.648 | 0.001 |
| C5 | 0.005 | 0.821 | 12.529 | 0.001 |
| D4 | 0.004 | 0.833 | 11.489 | 0.001 |
| D5 | 0.004 | 0.863 | 9.105 | 0.001 |
| E4 | 0.004 | 0.884 | 7.514 | 0.001 |
| E5 | 0.004 | 0.891 | 7.042 | 0.001 |
| F5 | 0.004 | 0.897 | 6.631 | 0.001 |
| A2 | 0.004 | 0.897 | 6.618 | 0.001 |
| B2 | 0.004 | 0.898 | 6.514 | 0.001 |
| F2 | 0.004 | 0.902 | 6.278 | 0.001 |
| F1 | 0.004 | 0.905 | 6.062 | 0.001 |
| E1 | 0.004 | 0.911 | 5.641 | 0.001 |
| D1 | 0.004 | 0.912 | 5.544 | 0.001 |
| C1 | 0.004 | 0.916 | 5.261 | 0.001 |
| B1 | 0.004 | 0.918 | 5.107 | 0.001 |
| A1 | 0.004 | 0.919 | 5.069 | 0.001 |
| A4 | 0.004 | 0.935 | 3.972 | 0.004 |
| B3 | 0.004 | 0.937 | 3.881 | 0.005 |
| C3 | 0.004 | 0.938 | 3.792 | 0.005 |
| D3 | 0.004 | 0.939 | 3.738 | 0.006 |

The results of the truss analysis were similar to those of the traditional morphometry, stressing the large difference between the French sample and all the other reared samples, with considerable overlap of the canonical discriminant-factor scores of the remaining samples (Spain Atlantic, Spain Mediterranean, Portugal and Greece) (Figure 13).

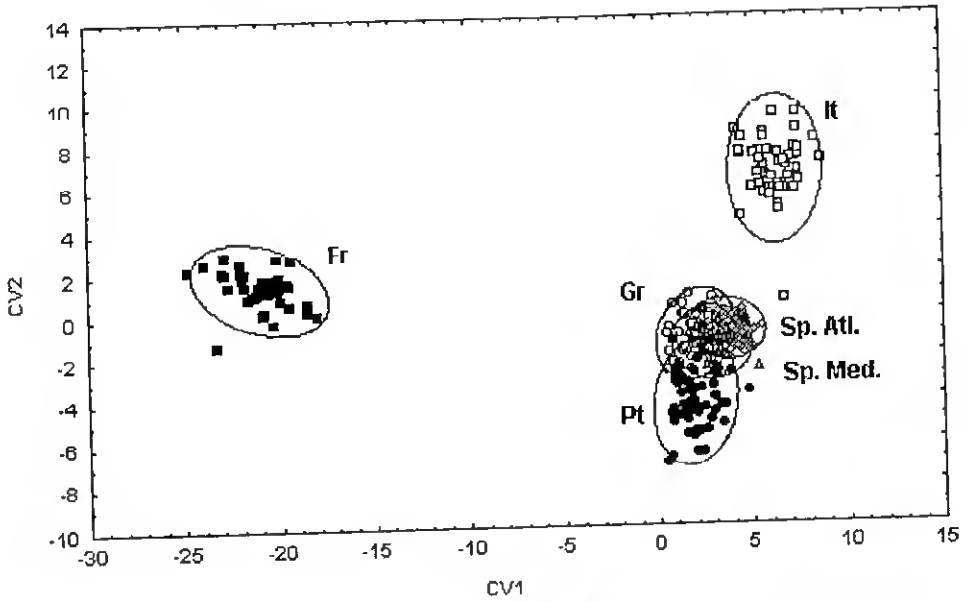



Figure 13 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the reared samples of *S. aurata* based on the truss networks; France (•), Portugal (•), Sp. Atl. (Δ), Sp. Med. (◇), Italy (□), Greece (○).

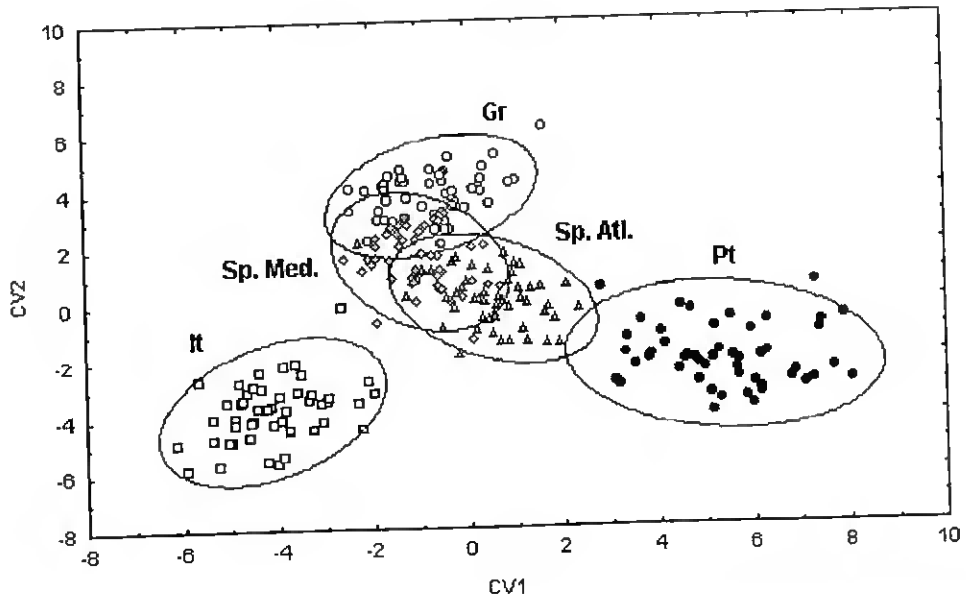



Figure 14 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the reared samples of *S. aurata* based on the truss networks without the French sample; Portugal (•), Sp. Atl. (Δ), Sp. Med. (◇), Italy (□), Greece (○).

Again, when the French sample was withdrawn from the analysis a more clear differentiation between the remaining samples was obtained (Figure 14).

The truss protocol revealed a differentiation between samples, giving a reasonable degree of individualization, with a significant separation of the Italian sample (Figure 15).

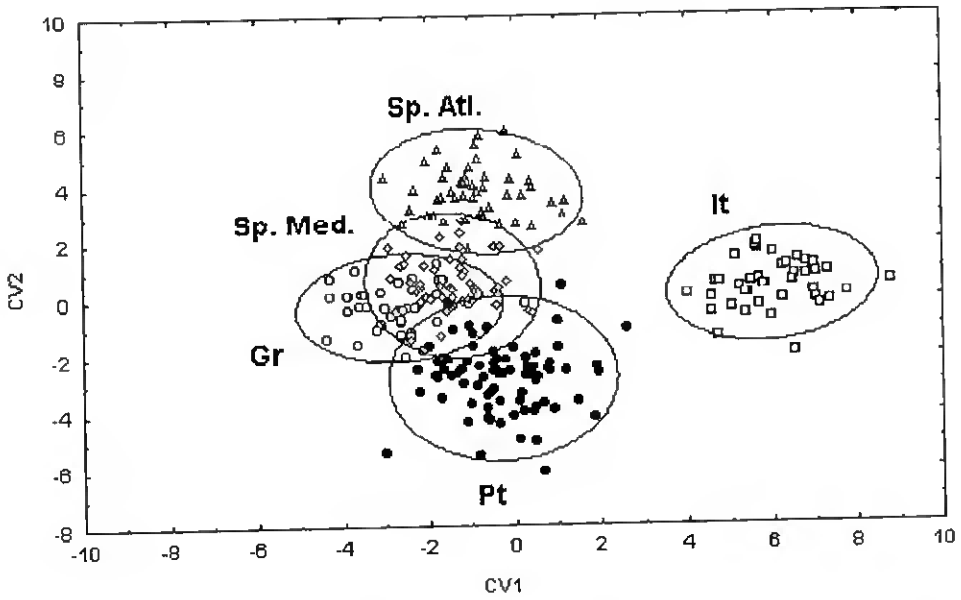



Figure 15 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the wild samples *S. aurata* based on the truss networks; Portugal (•), Sp. Atl. (Δ), Sp. Med. ( ◇), Italy (□), Greece (○).

Atlantic samples (Portugal and Spain Atlantic) also showed a distance from the Mediterranean ones (Spain Mediterranean, Italy and Greece). This fact is evidenced by the means of the canonical variables (Table XXXVIII). For the reared samples, the first function discriminated the French sample from all the others, while the second function discriminated the Italian and Portuguese samples from the remaining samples.

For the wild samples, the first canonical value (CV1) discriminated the Italian sample from all the others, and the CV2 separated the Atlantic samples from the

Table XXXVIII - Means of Canonical Variables for the reared and wild samples (CV - Canonical variables, Atl. - Atlantic, Med. - Mediterranean)

| | Reared | | | Wild | | |
|---|---|---|---|---|---|---|
| | CV1 | CV2 | CV3 | CV1 | CV2 | CV3 |
| Samples | | | | | | |
| France | 20.103 | -1.198 | 0.268 | - | - | - |
| Portugal | -1.795 | 4.727 | 3.232 | 0.162 | 3.425 | -0.927 |
| Spain Atl. | -2.738 | 1.126 | -0.325 | 1.935 | -3.092 | -2.327 |
| Spain Med. | -3.552 | 0.398 | -1.550 | 1.453 | -0.994 | 1.620 |
| Italy | -5.780 | -5.738 | 2.178 | -5.863 | -1.386 | 0.201 |
| Greece | -2.051 | 0.411 | -3.470 | 1.675 | -0.258 | 2.588 |

remaining Mediterranean ones.

### 2.3.2.2 – Red porgy (*P. pagrus*)

A total of 97.1% of the total variation associated with the 31 truss morphometric characters was accounted for by the first five principal components for all samples. PC1 loadings explained 84% of the total variation (Table XXXIX). This component correlated significantly with the standard length (SL) ($r = 0.84$; $p<0.05$; N =231).

Table XXXIX - Loadings from principal component analysis of the 31 truss morphometric characters for *P. pagrus* samples. Loadings are listed for the five principal components. Character descriptors refer to Figure 3. (Exp. Var. - Explained variance).

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| A1 | 0.994 | -0.012 | -0.039 | -0.007 | -0.014 |
| A2 | 0.959 | 0.202 | -0.010 | -0.062 | -0.038 |
| A3 | 0.995 | -0.014 | -0.002 | 0.030 | -0.017 |
| A4 | 0.960 | -0.007 | 0.091 | 0.019 | 0.062 |
| A5 | 0.970 | -0.038 | 0.084 | 0.043 | -0.049 |
| A6 | 0.931 | 0.263 | -0.016 | -0.141 | -0.032 |
| B1 | 0.992 | -0.036 | -0.032 | -0.001 | -0.020 |
| B2 | 0.994 | -0.005 | -0.009 | -0.007 | 0.033 |
| B3 | 0.930 | -0.196 | 0.075 | -0.101 | -0.095 |
| B4 | 0.080 | 0.861 | 0.464 | -0.091 | -0.017 |
| B5 | 0.966 | -0.038 | 0.183 | 0.060 | -0.036 |
| C1 | 0.986 | -0.049 | 0.013 | 0.004 | -0.008 |
| C2 | 0.996 | -0.004 | 0.009 | 0.023 | -0.016 |
| C3 | -0.104 | -0.877 | 0.248 | -0.359 | -0.088 |
| C4 | 0.982 | 0.065 | 0.038 | -0.043 | -0.023 |
| C5 | 0.857 | -0.132 | 0.451 | 0.110 | -0.036 |
| D1 | 0.930 | 0.227 | -0.124 | 0.073 | -0.009 |
| D2 | 0.933 | 0.104 | -0.211 | -0.015 | -0.166 |
| D3 | 0.982 | -0.013 | -0.001 | -0.050 | -0.055 |
| D4 | 0.868 | -0.036 | -0.317 | -0.151 | 0.272 |
| D5 | 0.784 | -0.034 | -0.574 | -0.101 | 0.067 |
| E1 | 0.985 | -0.018 | -0.068 | -0.027 | -0.019 |
| E2 | 0.743 | -0.447 | 0.052 | 0.479 | 0.050 |
| E3 | 0.984 | -0.007 | 0.074 | 0.011 | 0.004 |
| E4 | 0.994 | -0.005 | -0.019 | 0.000 | 0.024 |
| E5 | 0.991 | -0.033 | -0.039 | -0.030 | 0.062 |
| F1 | 0.983 | 0.019 | -0.075 | 0.026 | -0.047 |
| F2 | 0.991 | -0.033 | -0.016 | 0.021 | -0.017 |
| F3 | 0.996 | -0.009 | -0.020 | 0.000 | -0.011 |
| F4 | 0.991 | 0.069 | -0.043 | -0.043 | -0.002 |
| F5 | 0.704 | -0.080 | 0.621 | -0.091 | 0.225 |
| Exp. Var. | 26.03 | 1.97 | 1.44 | 0.48 | 0.20 |
| % of total | 84 | 6.34 | 4.63 | 1.53 | 0.60 |

Stepwise discriminant function analysis between samples indicated highly significant differences in the truss element distances among the different countries.

Wilks' λ and *F* values are presented in Table XL. A total of 18 truss elements were selected for sample discrimination (A1, A2, A3, A4, A6, B1, B2, B4, C1, C3, D1, D2, D5, E1, E2, E4, F3 and F5).

Table XL - Stepwise Discriminant Analysis for the truss comparison between *P. pagrus* samples. (Wilks' λ= 0.00013; F(54,626)=220.43, $p$ <0.0001)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| D2 | 0.001 | 0.195 | 289.482 | 0.001 |
| F3 | 0.000 | 0.286 | 174.613 | 0.001 |
| A3 | 0.000 | 0.653 | 37.127 | 0.001 |
| A2 | 0.000 | 0.762 | 21.889 | 0.001 |
| D1 | 0.000 | 0.770 | 20.874 | 0.001 |
| E4 | 0.000 | 0.800 | 17.449 | 0.001 |
| A1 | 0.000 | 0.846 | 12.697 | 0.001 |
| B1 | 0.000 | 0.854 | 12.011 | 0.001 |
| A6 | 0.000 | 0.855 | 11.855 | 0.001 |
| E1 | 0.000 | 0.870 | 10.497 | 0.001 |
| E2 | 0.000 | 0.876 | 9.918 | 0.001 |
| C1 | 0.000 | 0.920 | 6.070 | 0.001 |
| C3 | 0.000 | 0.924 | 5.764 | 0.001 |
| B4 | 0.000 | 0.925 | 5.711 | 0.001 |
| D5 | 0.000 | 0.940 | 4.433 | 0.005 |
| B2 | 0.000 | 0.952 | 3.523 | 0.016 |
| F5 | 0.000 | 0.957 | 3.139 | 0.026 |
| A4 | 0.000 | 0.958 | 3.080 | 0.028 |

$D^2$, *F*-statistics and particularised PCS for each sample are presented in Annex II, Tables XIII, XIV and XV. The overall PCS was 99.6%, with three of the samples (Portugal, Spain and Greece) presenting a correct classification of 100%. The Italian samples had a predicted classification of 96.8%, but this value was due to one individual that shared a higher resemblance with fish from the Spanish sample.

Figure 16 show, a clear differentiation of the Portuguese and Greek samples (confirmed by the mean canonical values (Table XLI)). Another extremely relevant fact revealed in Figure 16 is the high similarity of the individuals within samples.

Table XLI - Means of Canonical Variables for *P. pagrus* samples (CV - Canonical variables).

| | CV1 | CV2 | CV3 |
|---|---|---|---|
| Samples | | | |
| Portugal | -8.112 | -3.391 | 0.046 |
| Spain | 1.277 | 9.613 | 1.880 |
| Italy | 0.962 | 8.446 | -2.460 |
| Greece | 14.947 | -4.590 | 0.031 |

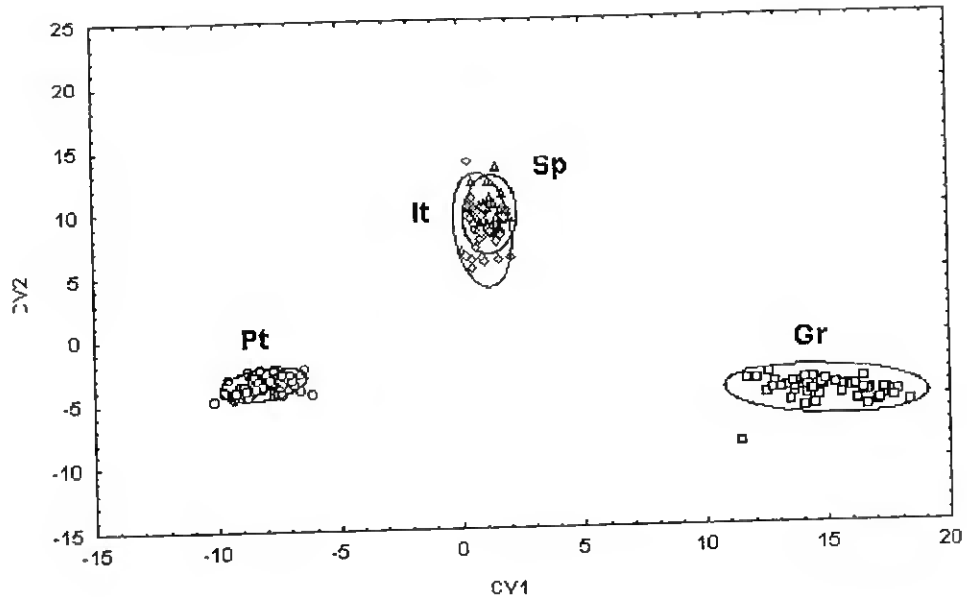



Figure 16 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the *P. pagrus* samples based on the truss networks; Portugal (○), Spain (Δ), Italy (◇), Greece (□).

### 2.3.2.3 – White seabream (*D. sargus*)

A total of 95.5% of the total variation associated with the 31 truss morphometric characters was accounted for by the first five principal components for all samples. PC1 loadings explained 85.1% of the total variation (Table XLII). This component correlated significantly with the standard length (SL) ($r = 0.36$; $p<0.05$; N =207).

Stepwise discriminant function analysis between samples indicated highly significant differences in the truss element distances among the different countries, which showed a geographic gradient. Wilks' λ and F values are presented in Table XLIII. Only five truss elements were selected for sample discrimination (A3, A6, B5, D2 and F3). The white seabream was the species where sample discrimination was achieved with the fewest number of morphological characteristics.

$D^2$, *F*-statistics and particularised PCS for each sample are presented in Annex II, Tables XIII, XIV and XV. The overall PCS was 93.1%, with the Greek and the Portuguese samples presenting the highest PCS values.

Table XLII - Loadings from principal component analysis of the 31 morphometric characters for *D. sargus* samples. Loadings are listed for the five principal components. Character descriptors refer to Figure 3. (Exp. Var. - Explained variance).

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| A1 | 0.907 | 0.156 | 0.076 | -0.051 | 0.214 |
| A2 | 0.921 | -0.231 | -0.035 | 0.028 | 0.214 |
| A3 | 0.508 | 0.723 | 0.216 | 0.393 | -0.045 |
| A4 | 0.954 | 0.157 | 0.048 | 0.023 | 0.187 |
| A5 | 0.966 | 0.076 | 0.016 | -0.057 | 0.188 |
| A6 | 0.951 | 0.032 | 0.064 | 0.153 | 0.184 |
| B1 | 0.874 | -0.243 | -0.028 | 0.138 | -0.206 |
| B2 | 0.934 | -0.264 | -0.072 | -0.095 | 0.014 |
| B3 | 0.993 | -0.061 | -0.016 | 0.014 | 0.014 |
| B4 | 0.987 | -0.025 | 0.005 | 0.050 | -0.005 |
| B5 | 0.983 | -0.064 | -0.010 | -0.011 | 0.095 |
| C1 | 0.178 | -0.214 | 0.951 | -0.107 | 0.000 |
| C2 | 0.961 | 0.052 | 0.002 | -0.089 | 0.010 |
| C3 | 0.992 | 0.017 | -0.028 | 0.016 | -0.005 |
| C4 | 0.993 | -0.068 | -0.004 | 0.014 | 0.016 |
| C5 | 0.993 | 0.014 | -0.008 | 0.002 | -0.003 |
| D1 | 0.946 | 0.027 | -0.148 | 0.042 | 0.018 |
| D2 | 0.960 | -0.066 | -0.122 | -0.002 | 0.027 |
| D3 | 0.985 | -0.125 | 0.012 | 0.033 | -0.047 |
| D4 | 0.992 | -0.078 | -0.023 | 0.014 | -0.011 |
| D5 | 0.991 | -0.024 | -0.049 | 0.027 | -0.021 |
| E1 | 0.965 | -0.143 | -0.031 | 0.005 | -0.055 |
| E2 | 0.825 | -0.356 | 0.091 | 0.197 | -0.179 |
| E3 | 0.972 | -0.068 | -0.025 | -0.055 | -0.038 |
| E4 | 0.958 | -0.207 | 0.037 | 0.056 | -0.096 |
| E5 | 0.982 | -0.117 | -0.039 | -0.006 | -0.032 |
| F1 | 0.806 | 0.376 | 0.002 | -0.259 | -0.115 |
| F2 | 0.835 | 0.344 | 0.060 | -0.165 | -0.134 |
| F3 | 0.933 | 0.236 | -0.023 | -0.040 | -0.096 |
| F4 | 0.951 | 0.185 | 0.036 | -0.069 | -0.095 |
| F5 | 0.953 | 0.176 | -0.054 | -0.131 | -0.080 |
| Exp. Var. | 26.37 | 1.44 | 1.03 | 0.40 | 0.36 |
| % of total | 85.1 | 4.64 | 3.33 | 1.3 | 1.15 |

Table XLIII - Stepwise Discriminant Analysis for the truss comparison between *D. sagrus* samples.
(Wilks' $\lambda$= 0.078, F(21,566)=38.481 $p$<0.0001)

| Descriptor | Wilks $\lambda$ | Partial $\lambda$ | F-remove | p-level |
|---|---|---|---|---|
| B5 | 0.159 | 0.494 | 67.367 | 0.0001 |
| F3 | 0.141 | 0.554 | 52.843 | 0.0001 |
| D2 | 0.112 | 0.703 | 27.759 | 0.0001 |
| A3 | 0.089 | 0.885 | 8.491 | 0.0001 |
| A6 | 0.087 | 0.896 | 7.584 | 0.0001 |

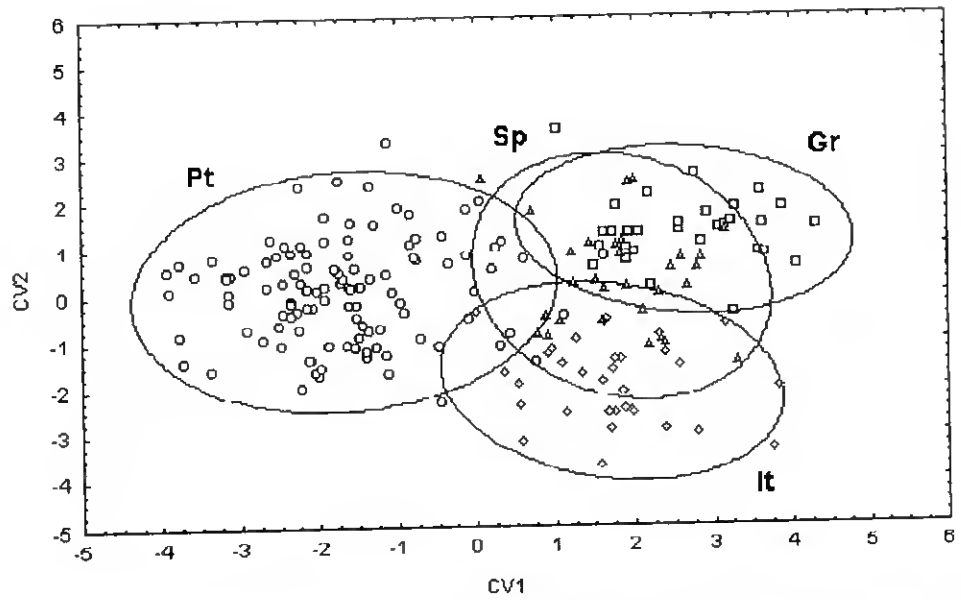



Figure 17 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the *D. sagrus* samples based on the truss networks; Portugal (○), Spain (Δ), Italy (◇), Greece (□).

The mean canonical values did not show a clear differentiation between samples, and all values can be considered to be of the same magnitude. The strongest evidence of differentiation between samples is the negative correlation of the Atlantic (Portuguese) sample with CV1 (Table XLIV).

Table XLIV - Means of Canonical Variables for *D. sagrus* samples (CV - Canonical variables)

| Samples | CV1 | CV2 | CV3 |
|---|---|---|---|
| Portugal | -1.674 | 0.104 | -0.051 |
| Spain | 1.842 | 0.361 | 1.485 |
| Italy | 1.702 | -1.918 | -0.342 |
| Greece | 2.648 | 1.355 | -0.914 |

### 2.3.2.4 – Sharpsnout seabream (*D. puntazzo*)

A total of 95.5% of the total variation was associated with the 31 truss morphometric characters and was accounted for by the first five principal components for all samples. PC1 loadings explained 90.1% of the total variation (Table XLV). This component correlated significantly with the standard length (SL) ($r$ = 0.69; $p$<0.05; N =160).

Table XLV - Loadings from principal component analysis of the 31 truss morphometric characters for *D. puntazzo* samples. Loadings are listed for the five principal components. Character descriptors refer to Figure 3. (Exp. Var. - Explained variance).

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| A1 | 0.955 | -0.104 | 0.074 | -0.032 | 0.096 |
| A2 | 0.950 | -0.002 | -0.079 | -0.058 | 0.113 |
| A3 | 0.822 | -0.386 | -0.132 | -0.329 | -0.033 |
| A4 | 0.958 | -0.141 | 0.074 | -0.033 | 0.112 |
| A5 | 0.978 | -0.043 | 0.032 | -0.018 | 0.065 |
| A6 | 0.961 | -0.118 | -0.102 | -0.138 | 0.071 |
| B1 | 0.918 | 0.126 | -0.164 | 0.148 | -0.060 |
| B2 | 0.966 | 0.077 | -0.042 | 0.057 | -0.002 |
| B3 | 0.988 | 0.048 | 0.017 | 0.056 | -0.023 |
| B4 | 0.991 | 0.027 | -0.016 | 0.050 | -0.016 |
| B5 | 0.993 | 0.013 | -0.032 | -0.002 | 0.008 |
| C1 | 0.822 | -0.274 | 0.351 | 0.155 | -0.267 |
| C2 | 0.966 | -0.080 | 0.011 | -0.030 | 0.078 |
| C3 | 0.794 | 0.329 | 0.163 | -0.392 | -0.206 |
| C4 | 0.988 | 0.059 | 0.012 | 0.055 | -0.030 |
| C5 | 0.990 | 0.019 | 0.033 | 0.047 | -0.031 |
| D1 | 0.956 | 0.081 | -0.113 | 0.041 | 0.107 |
| D2 | 0.976 | 0.060 | -0.063 | 0.020 | 0.041 |
| D3 | 0.907 | 0.156 | -0.146 | -0.001 | -0.171 |
| D4 | 0.992 | 0.051 | -0.013 | 0.036 | -0.016 |
| D5 | 0.991 | 0.039 | -0.021 | 0.041 | -0.001 |
| E1 | 0.976 | -0.068 | -0.050 | 0.010 | -0.081 |
| E2 | 0.933 | 0.041 | -0.104 | -0.015 | -0.125 |
| E3 | 0.964 | 0.128 | 0.006 | 0.068 | 0.049 |
| E4 | 0.984 | 0.071 | -0.027 | 0.013 | -0.025 |
| E5 | 0.991 | -0.015 | -0.014 | 0.023 | -0.054 |
| F1 | 0.894 | -0.211 | -0.093 | 0.081 | 0.004 |
| F2 | 0.893 | 0.113 | 0.233 | -0.045 | 0.279 |
| F3 | 0.961 | -0.035 | 0.116 | 0.037 | -0.064 |
| F4 | 0.955 | 0.089 | 0.180 | -0.006 | 0.141 |
| F5 | 0.964 | -0.090 | -0.037 | 0.066 | -0.025 |
| Exp. Var. | 27.93 | 0.56 | 0.39 | 0.37 | 0.34 |
| % of total | 90.1 | 1.79 | 1.26 | 1.2 | 1.11 |

Stepwise discriminant function analysis between samples indicated highly significant differences in the truss element distances among countries, evidencing a geographic gradient between them. Wilks' λ and F values are presented in Table XLVI. Seven truss elements were selected for sample discrimination (A2, A5, A6, B1, C1, D4 and F3).

$D^2$, *F*-statistics and particularised PCS for each sample are presented in Annex II, Tables XIII, XIV and XV. The overall PCS was 90.8%, the Italian and Greek samples showed values below 90%, with 87.8 and 88.4 percent, respectively. Such fact

is evidenced in Figure 18, were those samples appeared to be almost superimposed and therefore share a high degree of similarity.

Table XLVI - Stepwise Discriminant Analysis for the truss comparison between *D. puntazzo* samples.
(Wilks' λ=0.0230; F(27,432)=42.297; $p$<0.0001)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| B1 | 0.037 | 0.625 | 29.595 | 0.001 |
| A5 | 0.033 | 0.701 | 21.055 | 0.001 |
| D4 | 0.032 | 0.717 | 19.476 | 0.001 |
| A6 | 0.030 | 0.770 | 14.697 | 0.001 |
| C1 | 0.029 | 0.796 | 12.618 | 0.001 |
| A2 | 0.029 | 0.798 | 12.457 | 0.001 |
| F3 | 0.026 | 0.886 | 6.359 | 0.001 |

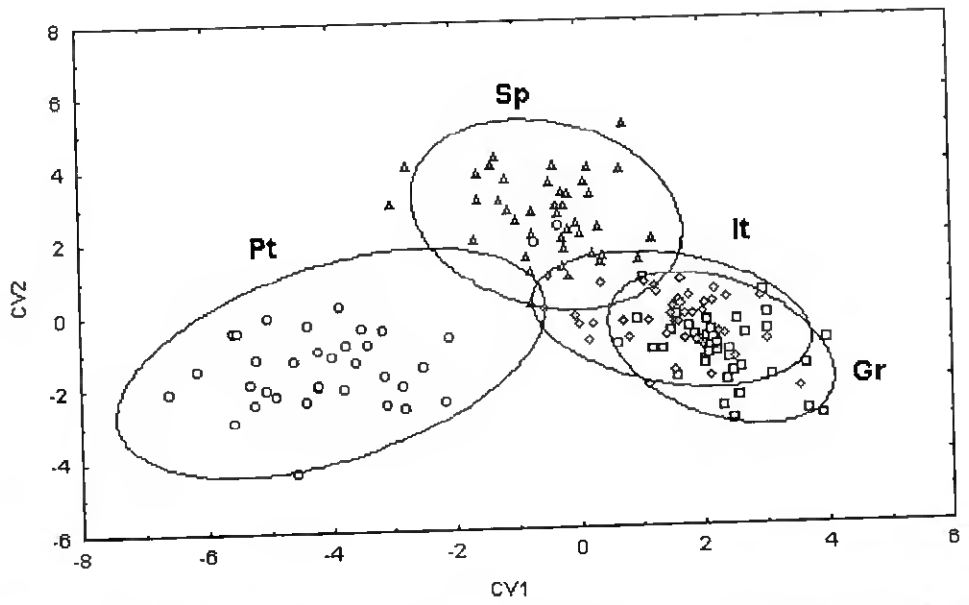



Figure 18 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the *D. puntazzo* samples based on the truss networks; Portugal (○), Spain (Δ), Italy (◇), Greece (□).

In contrast, the Portuguese sample was well differentiated from all the others, as confirmed by the mean canonical values (Table XLVII).

Table XLVII - Means of Canonical Variables for *D. puntazzo* samples (CV - Canonical variables)

| Samples | CV1 | CV2 | CV3 |
|---|---|---|---|
| Portugal | 3.804 | -1.414 | 0.362 |
| Spain | 0.061 | 2.904 | 0.604 |
| Italy | -0.560 | -0.015 | -1.751 |
| Greece | -2.620 | -1.604 | 0.798 |

### 2.3.2.5 – Striped seabream (*L. mormyrus*)

A total of 94.5% of the total variation was associated with the 31 truss morphometric characters and was accounted for by the first five principal components. PC1 loadings explained 85.1% of the total variation (Table XLVIII). This component correlated significantly with the standard length (SL) ($r$ = 0.31; $p$<0.05; N =217), however the obtained $r$ for the *L. mormyrus* sample comparison was the lowest between all studied samples.

Table XLVIII - Loadings from principal component analysis of the 31 truss morphometric characters for *L. mormyrus* samples. Loadings are listed for the five principal components. Character descriptors refer to Figure 3. (Exp. Var. - Explained variance).

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| A1 | 0.955 | 0.019 | -0.043 | 0.056 | -0.182 |
| A2 | 0.921 | 0.018 | 0.131 | 0.075 | -0.293 |
| A3 | 0.791 | 0.176 | -0.488 | 0.087 | -0.008 |
| A4 | 0.977 | 0.041 | -0.130 | 0.043 | -0.087 |
| A5 | 0.974 | 0.031 | -0.052 | 0.041 | -0.120 |
| A6 | 0.947 | 0.079 | -0.076 | 0.081 | -0.222 |
| B1 | 0.835 | -0.061 | 0.326 | -0.057 | -0.078 |
| B2 | 0.840 | -0.093 | 0.348 | -0.026 | -0.249 |
| B3 | 0.990 | 0.012 | -0.030 | -0.024 | 0.020 |
| B4 | 0.986 | 0.015 | 0.019 | 0.003 | -0.061 |
| B5 | 0.990 | 0.011 | -0.003 | -0.006 | -0.061 |
| C1 | 0.169 | -0.956 | -0.226 | -0.035 | -0.038 |
| C2 | 0.933 | 0.007 | -0.148 | -0.010 | -0.027 |
| C3 | 0.980 | 0.035 | -0.072 | -0.048 | 0.112 |
| C4 | 0.991 | -0.006 | -0.020 | -0.028 | 0.022 |
| C5 | 0.982 | 0.011 | -0.076 | -0.037 | 0.085 |
| D1 | 0.930 | 0.102 | 0.158 | -0.064 | 0.087 |
| D2 | 0.625 | -0.113 | 0.169 | 0.730 | 0.185 |
| D3 | 0.975 | -0.006 | -0.025 | -0.075 | 0.166 |
| D4 | 0.990 | 0.000 | 0.004 | -0.033 | 0.083 |
| D5 | 0.979 | 0.043 | 0.037 | -0.058 | 0.111 |
| E1 | 0.975 | -0.033 | 0.045 | -0.046 | 0.043 |
| E2 | 0.825 | -0.141 | 0.313 | -0.134 | 0.157 |
| E3 | 0.968 | 0.012 | -0.040 | -0.052 | 0.147 |
| E4 | 0.962 | -0.046 | 0.109 | -0.086 | 0.151 |
| E5 | 0.983 | -0.017 | 0.005 | -0.066 | 0.118 |
| F1 | 0.908 | -0.012 | -0.036 | -0.002 | -0.037 |
| F2 | 0.899 | -0.062 | -0.029 | -0.004 | -0.077 |
| F3 | 0.961 | 0.071 | -0.152 | 0.021 | 0.014 |
| F4 | 0.967 | 0.019 | -0.053 | 0.003 | -0.005 |
| F5 | 0.974 | 0.005 | -0.040 | -0.026 | 0.027 |
| Exp. Var. | 26.37 | 1.03 | 0.79 | 0.62 | 0.47 |
| % of total | 85.1 | 3.31 | 2.56 | 2 | 1.52 |

Stepwise discriminant analysis between samples indicated highly significant differences in the truss element distances among the different countries. Wilks' λ and F values are presented in Table XLIX. Nine truss elements were selected for sample discrimination (A1, A2, A6, B2, B3, C5, D1, F2 and F3).

$D^2$, *F*-statistics and particularised PCS for each sample are presented in Annex II, Tables XIII, XIV and XV. The overall PCS was 97.8%, with the Spanish sample presenting the lowest PCS among the *L. mormyrus* samples, with 93.75%. The remaining samples showed values over 96%. Canonical discriminant-factor scores are presented in Figure 19.

Table XLIX - Stepwise Discriminant Analysis for the truss comparison between *L. mormyrus* samples.
(Wilks' λ=0.03791; F(27,599)=45.87; $p$ <0.0001)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| C5 | 0.065 | 0.579 | 49.647 | 0.001 |
| F3 | 0.061 | 0.623 | 41.263 | 0.001 |
| B3 | 0.048 | 0.785 | 18.674 | 0.001 |
| F2 | 0.047 | 0.813 | 15.767 | 0.001 |
| A1 | 0.046 | 0.821 | 14.941 | 0.001 |
| A2 | 0.046 | 0.825 | 14.451 | 0.001 |
| B2 | 0.046 | 0.830 | 13.968 | 0.001 |
| D1 | 0.044 | 0.854 | 11.689 | 0.001 |
| A6 | 0.043 | 0.890 | 8.432 | 0.001 |

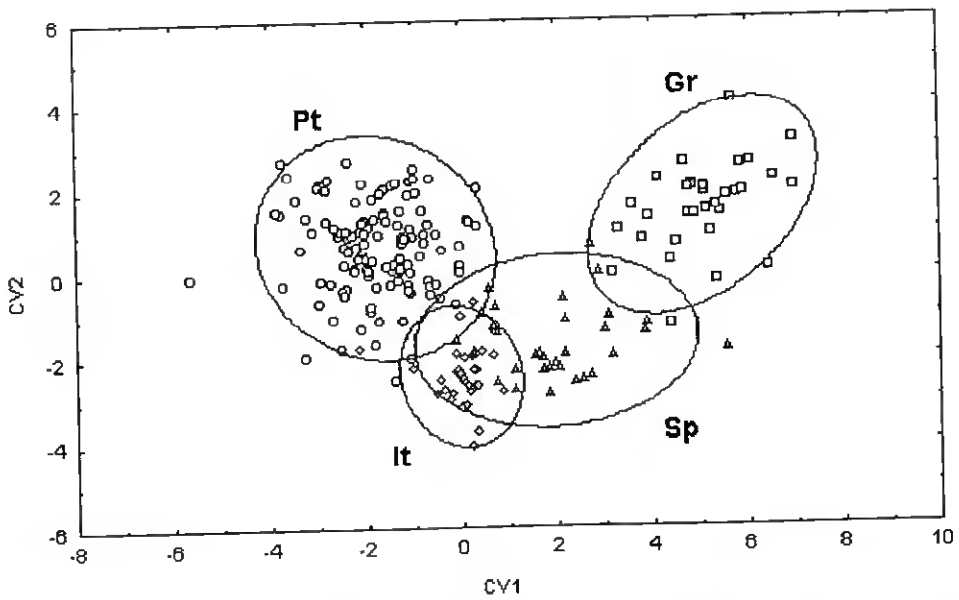



Figure 19 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the *L. mormyrus* samples based on the truss networks; Portugal (○), Spain (Δ), Italy (◇), Greece (□).

Mean canonical values correlate the Greek sample with CV1, untying it from all the others. Due to its negative correlation with CV1 the Portuguese sample is also separated (Table L).

Table L - Means of Canonical Variables for *L. mormyrus* samples (CV - Canonical variables)

| Samples | CV1 | CV2 | CV3 |
|---|---|---|---|
| Portugal | -1.783 | 0.645 | 0.056 |
| Spain | 1.962 | -1.618 | 1.288 |
| Italy | -0.023 | -2.422 | -1.109 |
| Greece | 5.072 | 1.534 | -0.441 |

### 2.3.2.6 – Red seabream (*P. bogaraveo*)

A total of 97% of the total variation was associated with the 31 truss

Table LI - Loadings from principal component analysis of the 31 truss morphometric characters for *P. bogaraveo* samples. Loadings are listed for the five principal components. Character descriptors refer to Figure 3. (Exp. Var. - Explained variance).

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| A1 | 0.953 | -0.184 | 0.097 | -0.077 | 0.021 |
| A2 | 0.966 | 0.063 | 0.062 | -0.110 | -0.015 |
| A3 | 0.887 | -0.141 | -0.398 | 0.112 | -0.054 |
| A4 | 0.994 | -0.021 | -0.023 | -0.025 | 0.022 |
| A5 | 0.988 | -0.068 | 0.039 | -0.061 | 0.017 |
| A6 | 0.984 | -0.017 | -0.094 | -0.031 | -0.025 |
| B1 | 0.871 | 0.365 | -0.165 | -0.223 | -0.093 |
| B2 | 0.930 | 0.112 | 0.300 | -0.004 | 0.004 |
| B3 | 0.995 | 0.040 | -0.014 | -0.015 | 0.030 |
| B4 | 0.993 | 0.052 | -0.004 | -0.067 | -0.005 |
| B5 | 0.995 | 0.049 | -0.007 | -0.011 | 0.033 |
| C1 | 0.845 | -0.479 | -0.062 | -0.158 | 0.052 |
| C2 | 0.984 | -0.029 | 0.036 | -0.013 | 0.019 |
| C3 | 0.994 | 0.036 | -0.018 | 0.030 | 0.047 |
| C4 | 0.996 | 0.011 | -0.015 | -0.022 | 0.032 |
| C5 | 0.994 | 0.012 | -0.018 | 0.019 | 0.049 |
| D1 | 0.952 | 0.188 | -0.007 | 0.160 | 0.052 |
| D2 | 0.986 | -0.025 | 0.014 | 0.036 | 0.006 |
| D3 | 0.996 | 0.022 | -0.004 | 0.009 | 0.007 |
| D4 | 0.997 | -0.002 | -0.002 | 0.006 | 0.020 |
| D5 | 0.991 | 0.077 | -0.006 | 0.075 | 0.043 |
| E1 | 0.982 | 0.040 | 0.021 | 0.028 | -0.035 |
| E2 | 0.965 | 0.125 | -0.002 | -0.030 | 0.014 |
| E3 | 0.980 | 0.131 | -0.040 | 0.052 | 0.013 |
| E4 | 0.990 | 0.074 | -0.020 | 0.017 | 0.044 |
| E5 | 0.992 | 0.064 | 0.007 | 0.027 | -0.023 |
| F1 | 0.938 | -0.139 | 0.113 | 0.050 | -0.282 |
| F2 | 0.948 | -0.159 | 0.058 | 0.096 | 0.054 |
| F3 | 0.983 | -0.094 | 0.032 | -0.016 | 0.022 |
| F4 | 0.981 | -0.074 | 0.032 | 0.042 | 0.065 |
| F5 | 0.977 | -0.075 | 0.049 | 0.073 | -0.155 |
| Exp. Var. | 29.13 | 0.60 | 0.33 | 0.17 | 0.14 |
| % of total | 93.4 | 1.93 | 1.06 | 0.56 | 0.47 |

morphometric characters and was accounted for by the first five principal components, with PC1 loadings explaining 93.4% of the total variation (Table LI).

This component correlated significantly with the standard length (SL) ($r$ = 0.99; $p$<0.05; N =186).

Stepwise discriminant analysis between samples indicated highly significant differences in the truss element distances among the different countries. Wilks' λ and F values are presented in Table LII. A total 14 truss elements were selected for sample discrimination (A2, A3, A6, B1, B3, B4, C1, D2, D3, D5, E2, E3, F2 and F5).

Table LII - Stepwise Discriminant Analysis for the truss comparison between *P. bogaraveo* samples.
(Wilks' λ=0.0002; F(42,502)=213.19; $p$<0.0001)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| F5 | 0.000 | 0.685 | 25.940 | 0.001 |
| B1 | 0.000 | 0.719 | 22.057 | 0.001 |
| F2 | 0.000 | 0.724 | 21.423 | 0.001 |
| A2 | 0.000 | 0.809 | 13.288 | 0.001 |
| C1 | 0.000 | 0.814 | 12.869 | 0.001 |
| B4 | 0.000 | 0.825 | 11.947 | 0.001 |
| D5 | 0.000 | 0.827 | 11.758 | 0.001 |
| B3 | 0.000 | 0.853 | 9.717 | 0.001 |
| A6 | 0.000 | 0.859 | 9.249 | 0.001 |
| E2 | 0.000 | 0.884 | 7.427 | 0.001 |
| E3 | 0.000 | 0.889 | 7.057 | 0.001 |
| D2 | 0.000 | 0.896 | 6.532 | 0.001 |
| A3 | 0.000 | 0.917 | 5.100 | 0.002 |
| D3 | 0.000 | 0.943 | 3.377 | 0.020 |

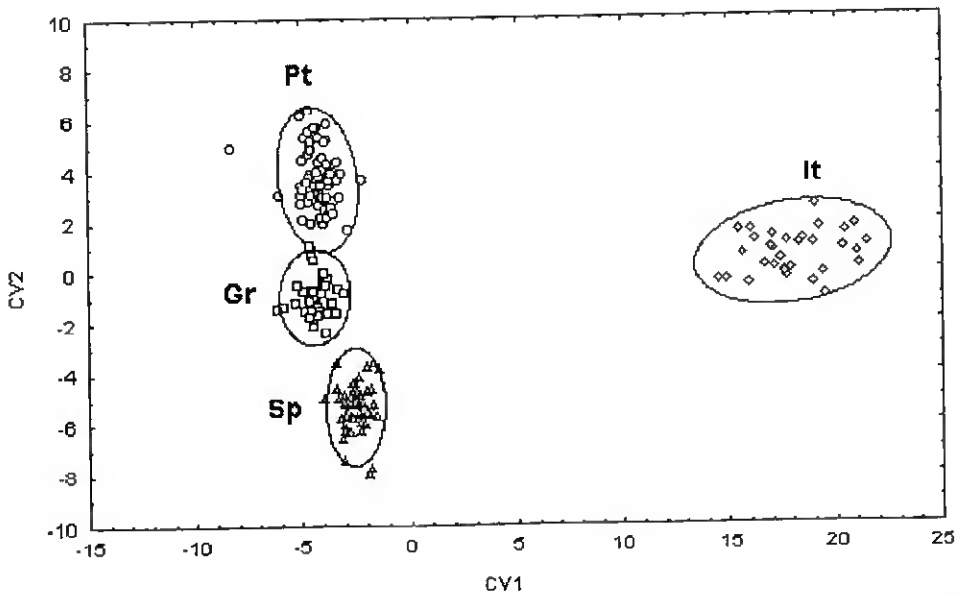



Figure 20 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the *P. bogaraveo* samples based on the truss networks; Portugal (○), Spain (Δ), Italy (◇), Greece (□).

$D^2$, *F*-statistics and particularised PCS for each sample are presented in Annex II, Tables XIII, XIV and XV. The overall PCS was 100%, with all samples correctly identified. Based on the above stated findings it was possible to establish a clear differentiation among samples (Figure 20, and Table LIII for the mean canonical values), especially between the Italian sample and all the others.

Table LIII - Means of Canonical Variables for *P. bogaraveo* samples (CV - Canonical variables).

| Samples | CV1 | CV2 | CV3 |
|---|---|---|---|
| Portugal | -4.226 | 3.716 | 1.055 |
| Spain | -2.568 | -5.310 | 1.627 |
| Italy | 17.983 | 0.600 | -0.225 |
| Greece | -4.410 | -0.946 | -4.673 |

### 2.3.2.7 – Common Dentex (*D. dentex*)

A total of 98% of the total variation was associated with the 31 truss morphometric characters and was accounted for by the first five principal components, with 92,1% being explained by PC1 (Table LIV). PC1 was significantly correlated with the standard length (SL) ($r = 0.88$; $p<0.05$; N =144).

Stepwise discriminant analysis between samples indicated highly significant differences in the truss element distances among the different countries. Wilks' λ and F values are presented in Tables LV. A total of 14 truss elements were selected for sample discrimination (A1, A2, A4, A5, B1, B4, C2, C4, D1, D2, D4, E1, E2 and F2).

$D^2$, *F*-statistics and particularised PCS for each sample are presented in Annex II, Tables XIII, XIV and XV. The $D^2$ values discriminated a geographic gradient between samples, with a clear difference between the Atlantic sample (Portugal) and the Mediterranean ones. This result was corroborated through the PCS values, with the Portuguese sample presenting a correct classification of 100%. All the others showed PCS's over 96%, with an overall PCS of 97.9%. Based on the above findings the canonical discriminant-factor scores established a clear differentiation between the Atlantic and the Mediterranean samples (Figure 21, and Table LVI for mean canonical values).

Table LIV - Loadings from principal component analysis of the 31 truss morphometric characters for *D. dentex* samples. Loadings are listed for the five principal components. Character descriptors refer to Figure 3. (Exp. Var. - Explained variance).

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| A1 | 0.959 | 0.125 | -0.011 | -0.058 | -0.156 |
| A2 | 0.959 | 0.121 | -0.080 | -0.150 | -0.083 |
| A3 | 0.649 | 0.710 | -0.181 | 0.153 | 0.044 |
| A4 | 0.973 | 0.202 | -0.001 | -0.041 | -0.041 |
| A5 | 0.981 | 0.132 | 0.017 | -0.052 | -0.071 |
| A6 | 0.924 | 0.346 | -0.100 | -0.057 | -0.052 |
| B1 | 0.831 | -0.423 | -0.144 | 0.260 | -0.100 |
| B2 | 0.901 | -0.346 | 0.015 | -0.189 | -0.018 |
| B3 | 0.993 | -0.023 | 0.033 | 0.000 | 0.021 |
| B4 | 0.989 | -0.075 | 0.011 | 0.061 | -0.018 |
| B5 | 0.978 | 0.077 | 0.003 | -0.094 | -0.003 |
| C1 | 0.838 | 0.221 | 0.477 | 0.104 | -0.045 |
| C2 | 0.985 | -0.061 | 0.028 | 0.029 | 0.020 |
| C3 | 0.994 | -0.008 | 0.022 | -0.015 | 0.029 |
| C4 | 0.994 | -0.025 | 0.039 | 0.010 | 0.025 |
| C5 | 0.994 | 0.005 | 0.036 | -0.023 | 0.024 |
| D1 | 0.963 | -0.117 | -0.100 | 0.002 | -0.027 |
| D2 | 0.990 | -0.048 | -0.014 | -0.002 | 0.029 |
| D3 | 0.993 | -0.076 | 0.002 | 0.007 | 0.039 |
| D4 | 0.994 | -0.069 | 0.008 | 0.010 | 0.038 |
| D5 | 0.995 | -0.035 | -0.023 | -0.021 | 0.017 |
| E1 | 0.988 | -0.053 | -0.005 | -0.032 | -0.019 |
| E2 | 0.966 | -0.050 | -0.014 | -0.051 | 0.104 |
| E3 | 0.989 | -0.070 | -0.003 | 0.028 | 0.026 |
| E4 | 0.992 | -0.055 | -0.004 | 0.007 | 0.074 |
| E5 | 0.993 | -0.073 | -0.015 | -0.015 | 0.003 |
| F1 | 0.955 | -0.089 | 0.011 | 0.115 | -0.129 |
| F2 | 0.958 | -0.007 | -0.021 | 0.052 | 0.199 |
| F3 | 0.985 | 0.045 | 0.001 | -0.025 | -0.011 |
| F4 | 0.983 | -0.018 | 0.017 | 0.023 | 0.134 |
| F5 | 0.985 | -0.053 | -0.027 | 0.052 | -0.071 |
| Exp. Var. | 28.55 | 1.14 | 0.32 | 0.21 | 0.16 |
| % of total | 92.10 | 3.67 | 1.02 | 0.68 | 0.51 |

Table LV - Stepwise Discriminant Analysis for the truss comparison between *D. dentex* samples.
(Wilks' $\lambda$= 0.0086; F(42,377)=35.734; $p$<0.0001)

| Descriptor | Wilks $\lambda$ | Partial $\lambda$ | F-remove | p-level |
|---|---|---|---|---|
| D4 | 0.017 | 0.518 | 39.369 | 0.001 |
| B4 | 0.013 | 0.673 | 20.540 | 0.001 |
| E1 | 0.012 | 0.695 | 18.563 | 0.001 |
| A1 | 0.012 | 0.708 | 17.481 | 0.001 |
| A5 | 0.010 | 0.818 | 9.433 | 0.001 |
| A4 | 0.010 | 0.822 | 9.146 | 0.001 |
| B1 | 0.010 | 0.827 | 8.861 | 0.001 |
| C4 | 0.010 | 0.828 | 8.789 | 0.001 |
| D2 | 0.010 | 0.833 | 8.464 | 0.001 |
| C2 | 0.010 | 0.858 | 7.023 | 0.001 |
| D1 | 0.010 | 0.878 | 5.873 | 0.001 |
| F2 | 0.010 | 0.892 | 5.136 | 0.002 |
| E2 | 0.010 | 0.901 | 4.665 | 0.004 |
| A2 | 0.009 | 0.905 | 4.422 | 0.005 |

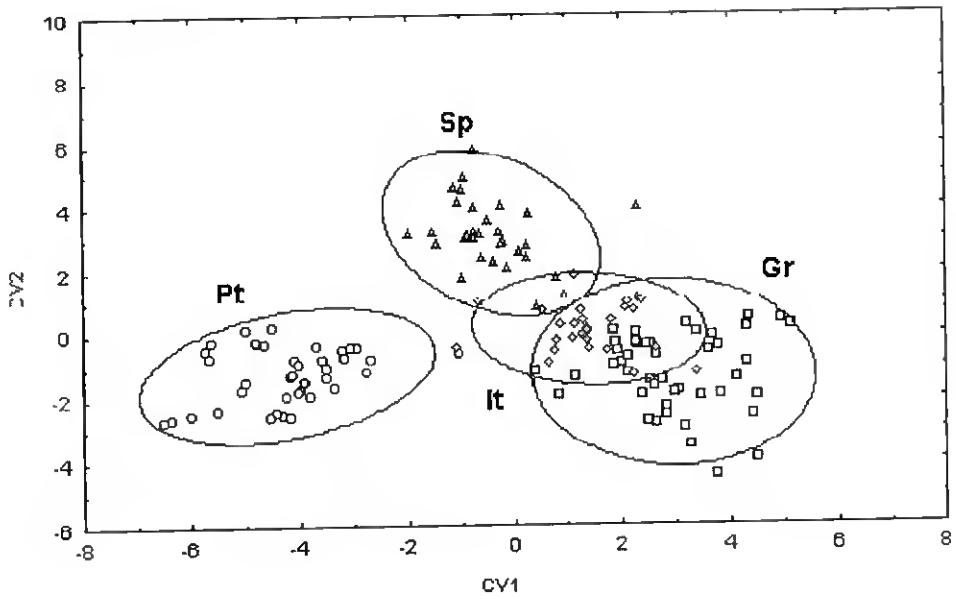



Figure 21 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the *D. dentex* samples based on the truss networks; Portugal (○), Spain (Δ), Italy (◇), Greece (□).

Table LVI - Means of Canonical Variables for *D. dentex* samples (CV - Canonical variables)

| Samples | CV1 | CV2 | CV3 |
|---|---|---|---|
| Portugal | -4.233 | -1.239 | 0.153 |
| Spain | -0.413 | 3.183 | 0.814 |
| Italy | 1.374 | 0.145 | -2.684 |
| Greece | 2.943 | -1.243 | 1.100 |

### 2.3.2.8 – Black seabream (*S. cantharus*)

Due to the sampling problems referred to in section 2.2.1, the truss morphometric analysis was only carried out between the Portuguese and the Spanish samples A total of 96.8% of the total variation was associated with the 31 truss morphometric characters and was accounted for by the first five principal components for all samples, with PC1 loadings explaining 88.2% of the total variation (Table LVII). PC1 was significantly correlated with the standard length (SL) ($r$ = 0.84; $p$<0.05; N =66).

Stepwise discriminant analysis between samples indicated highly significant differences between truss elements for samples from the two countries. Wilks' λ and F

values are presented in Table LVIII. Ten truss elements were selected for sample discrimination (A1, A4, A5, B2, C2, D2, D3, D5, E1 and E3).

Table LVII - Loadings from principal component analysis of the 31 morphometric characters for *S. cantharus* samples. Loadings are listed for the five principal components. Character descriptors refer to Figure 3. (Exp. Var. - Explained variance).

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| A1 | 0.970 | 0.052 | 0.008 | -0.054 | -0.046 |
| A2 | 0.947 | -0.077 | -0.103 | -0.069 | -0.156 |
| A3 | 0.681 | 0.535 | -0.270 | 0.381 | 0.154 |
| A4 | 0.985 | 0.103 | 0.016 | 0.044 | -0.063 |
| A5 | 0.989 | 0.049 | 0.013 | -0.007 | -0.074 |
| A6 | 0.959 | 0.135 | -0.137 | 0.070 | -0.065 |
| B1 | 0.837 | -0.377 | -0.188 | -0.104 | 0.133 |
| B2 | 0.948 | -0.170 | 0.019 | -0.056 | -0.156 |
| B3 | 0.990 | -0.045 | -0.048 | 0.032 | -0.060 |
| B4 | 0.988 | -0.081 | -0.078 | -0.009 | -0.021 |
| B5 | 0.992 | 0.026 | 0.011 | 0.036 | -0.080 |
| C1 | 0.128 | 0.922 | -0.056 | -0.348 | -0.009 |
| C2 | 0.936 | 0.135 | 0.020 | 0.054 | -0.178 |
| C3 | 0.994 | -0.020 | 0.009 | 0.034 | -0.046 |
| C4 | 0.994 | -0.013 | -0.041 | 0.015 | -0.049 |
| C5 | 0.991 | 0.016 | 0.007 | 0.043 | -0.077 |
| D1 | 0.928 | -0.241 | -0.013 | -0.019 | -0.013 |
| D2 | 0.932 | -0.100 | -0.037 | -0.124 | 0.203 |
| D3 | 0.994 | -0.013 | 0.028 | 0.028 | -0.018 |
| D4 | 0.995 | -0.034 | -0.006 | 0.006 | 0.006 |
| D5 | 0.988 | -0.087 | 0.014 | 0.005 | -0.001 |
| E1 | 0.967 | -0.081 | -0.076 | -0.095 | 0.098 |
| E2 | 0.896 | -0.151 | -0.213 | -0.108 | 0.203 |
| E3 | 0.986 | 0.032 | 0.062 | 0.039 | -0.072 |
| E4 | 0.989 | -0.010 | -0.022 | 0.003 | 0.045 |
| E5 | 0.996 | -0.037 | 0.005 | -0.017 | 0.004 |
| F1 | 0.922 | 0.081 | 0.283 | -0.005 | 0.095 |
| F2 | 0.887 | 0.101 | 0.324 | 0.098 | 0.200 |
| F3 | 0.956 | 0.104 | 0.023 | -0.097 | 0.035 |
| F4 | 0.963 | 0.138 | 0.136 | 0.024 | 0.045 |
| F5 | 0.969 | 0.008 | 0.178 | -0.016 | 0.060 |
| Exp. Var. | 27.34 | 1.53 | 0.45 | 0.36 | 0.31 |
| % of total | 88.20 | 4.94 | 1.44 | 1.17 | 1.01 |

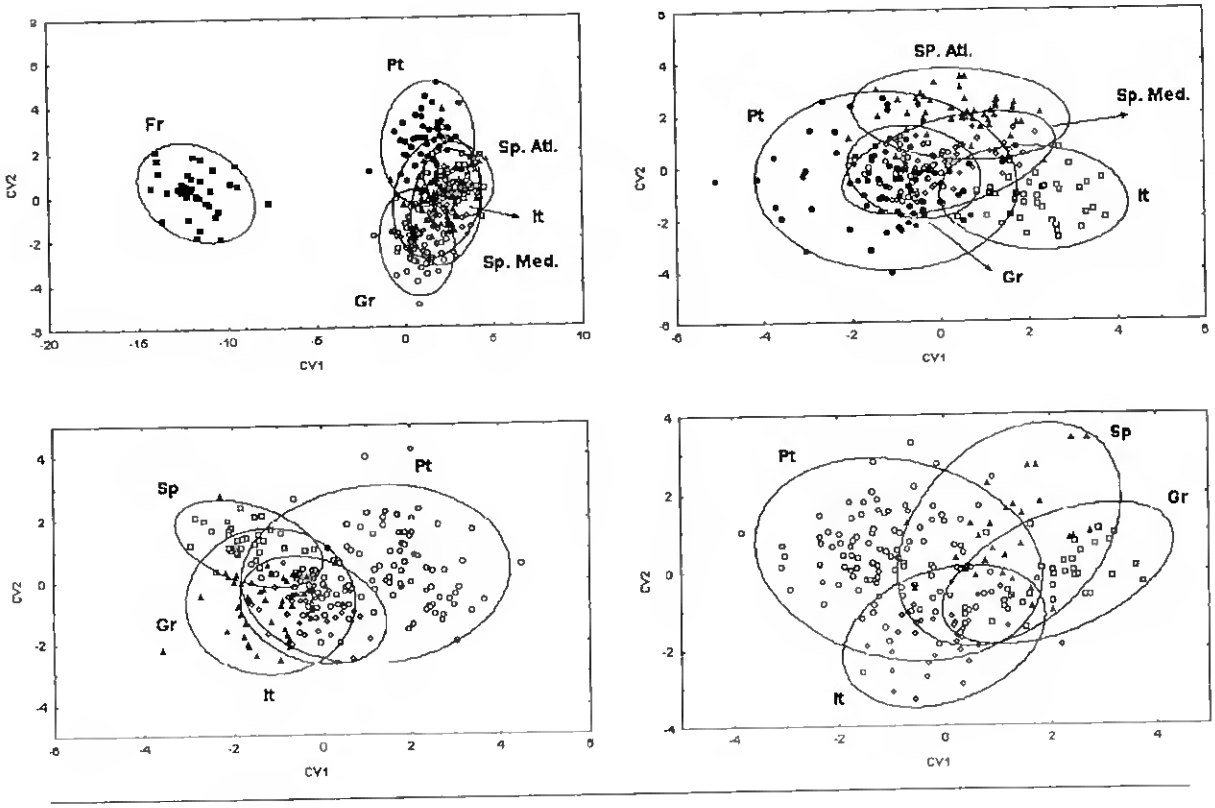
As was the case with the traditional morphology analysis, the overall percent-correct classification success (PCS) through the truss protocol was 100%. Generalised Mahalanobis distances ($D^2$) was 31.4, the $F$-statistics 41.65 for $p<0.001$. From the canonical analysis, both samples correlated significantly with CV1, invalidating the graphical presentation of the differences between samples. Nevertheless, the obtained $D^2$ and PCS values ensure a good degree of differentiation between samples.

Table LVIII - Stepwise Discriminant Analysis for the truss comparison between *S. cantharus* samples. (Wilks' λ=0.1164; F(10,55)=41.741; $p$<0.0001)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| D2 | 0.153 | 0.763 | 17.061 | 0.000 |
| D3 | 0.152 | 0.766 | 16.818 | 0.000 |
| A1 | 0.150 | 0.776 | 15.869 | 0.000 |
| D5 | 0.139 | 0.838 | 10.652 | 0.002 |
| B2 | 0.135 | 0.864 | 8.645 | 0.005 |
| C2 | 0.131 | 0.887 | 7.007 | 0.011 |
| A4 | 0.131 | 0.887 | 6.991 | 0.011 |
| A5 | 0.129 | 0.903 | 5.900 | 0.018 |
| E1 | 0.129 | 0.905 | 5.748 | 0.020 |
| F3 | 0.126 | 0.923 | 4.558 | 0.037 |

### 2.3.3 – *Area analysis*

In five of the eight studied species, all six-area measurements were selected for sample differentiation (*S. aurata* (both wild and reared), *D. sargus*, *D. dentex*, *L. mormyrus*, *P. bogaraveo*), five for *P. pagrus*, four for *D. puntazzo*, and three for *S. cantharus*. Wilks' λ and F values are presented for each of the species in Annex II, Tables XVI-XXIV. The Wilks' λ values for all species were always higher, than the ones obtained through the traditional and truss measurements, while the canonical discriminant-factor scores appear scattered, providing worse sample differentiation (Figure 22).



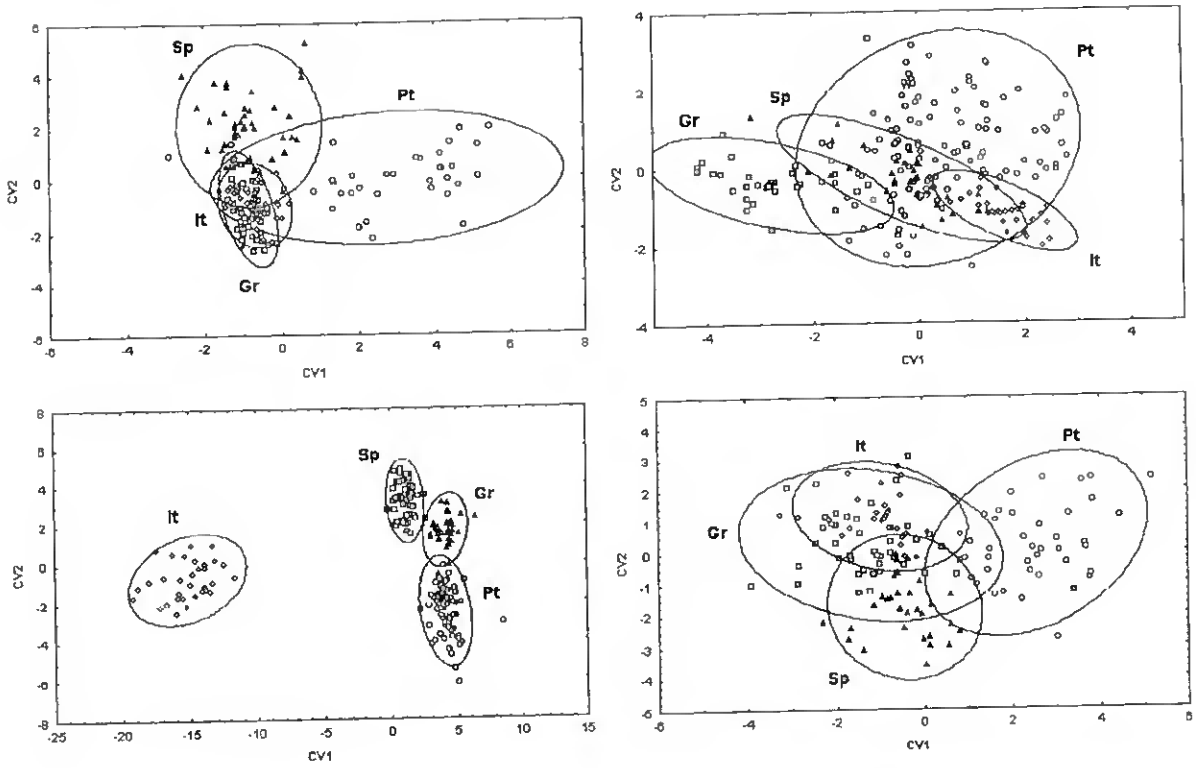



Figure 22 - Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the *D. dentex* samples based on the area measurements; a) *S. aurata* wild, b) *S. aurata* reared, c) *P. pagrus*, d) *D. sargus*, e) *D. puntazzo*, f) *L. mormyrus*, g) *P. bogaraveo*, and h) *D. dentex*. Portugal (○), Spain (Δ), Italy (◇), Greece (□).

### 2.3.4 – *Mapped configurations*

Mapped landmark configurations for wild and reared samples of *S. aurata* are shown in Figure 23. No apparent distortions are noticeable, with the differences between the reconstructed forms for each of the samples being attributed to dissimilarity among samples. This is re-enforced by the small values obtained of total strain values of each sample (Figure 23).

The remaining species in study did not show any relevant distortions in the overall sample configuration. These results confirmed the precautions undertaken during data acquisition. As for the *S. aurata* samples, all the remaining species showed smaller total strain values of each of the samples (Figures 24 to 27).

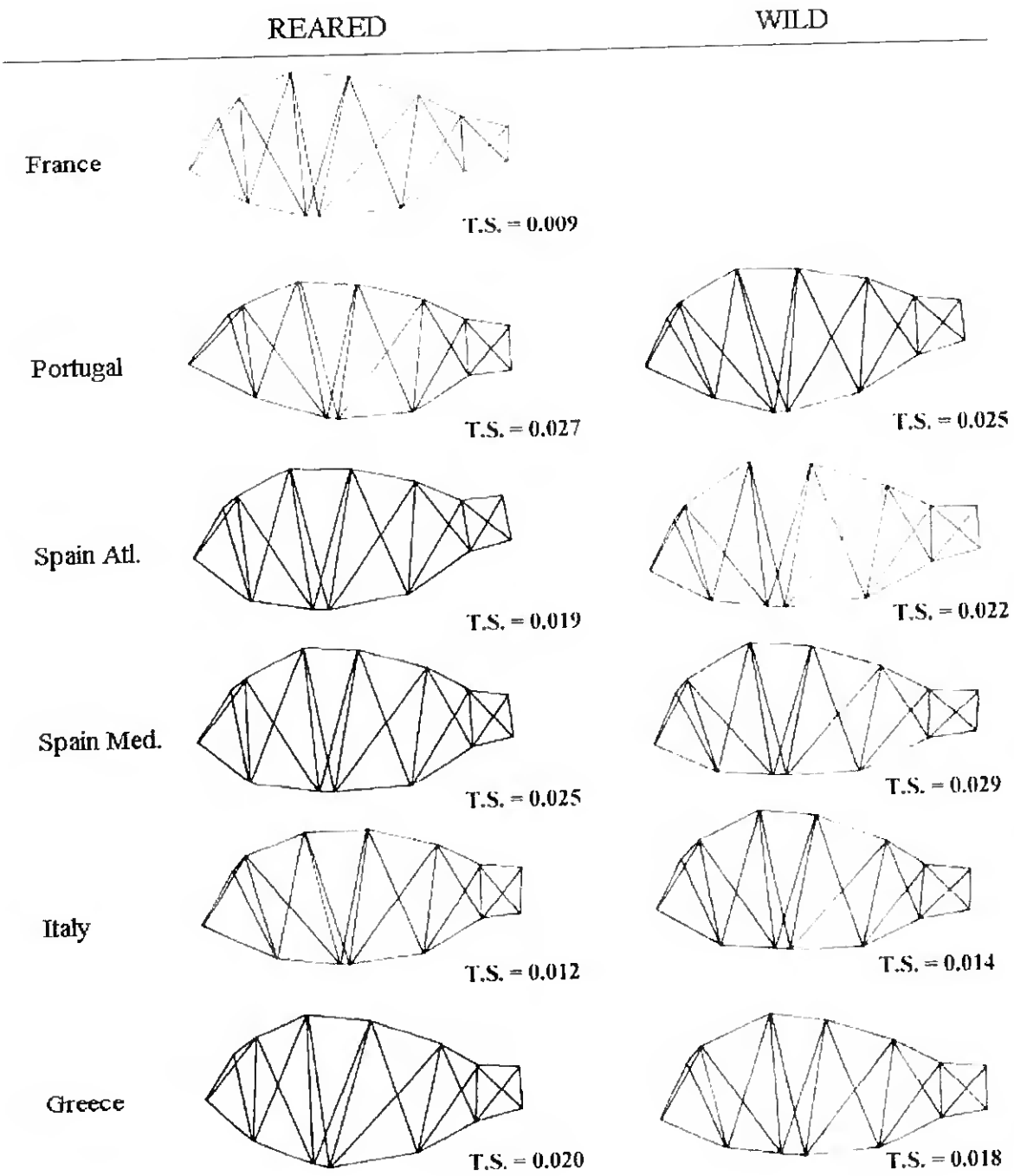



Figure 23 – Mapped configurations for each of the wild and reared samples of *S. aurata*. T.S. – total strain value.

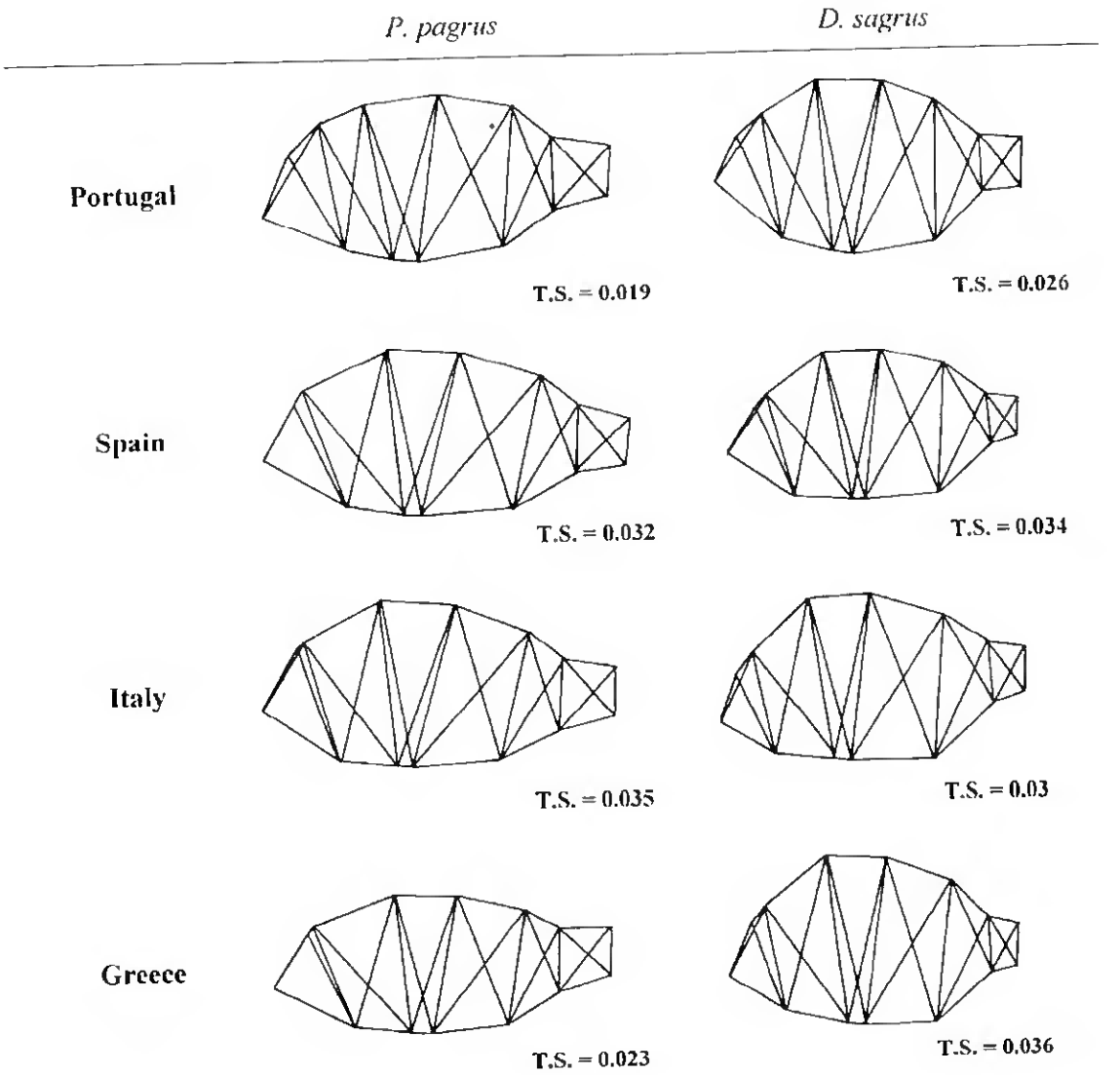



Figure 24 – Mapped configurations for each of the samples of *P. pagrus* and *D. sargus*. T.S. – total strain value.

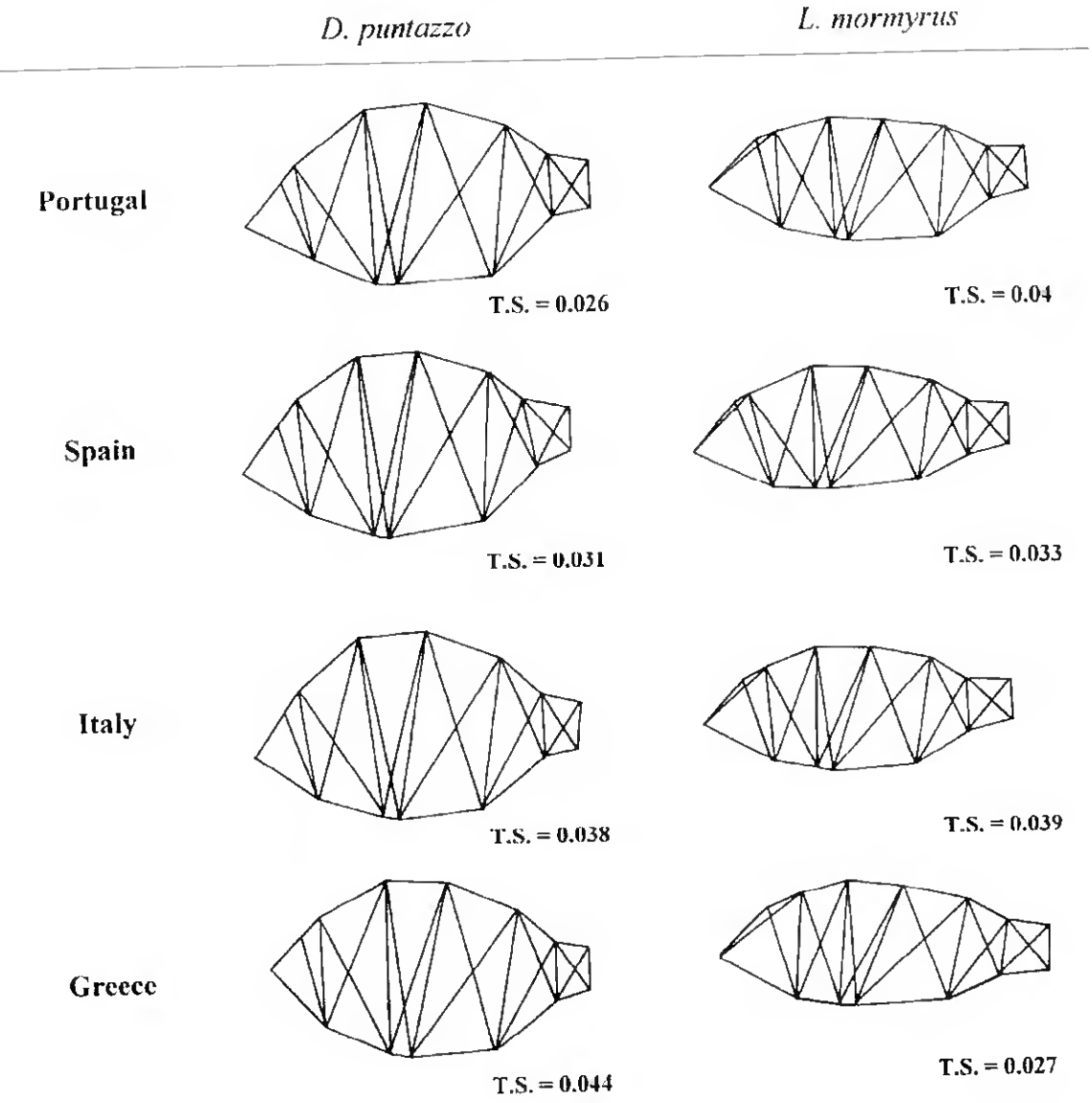



Figure 25 – Mapped configurations for each of the samples of *D. puntazzo* and *L. mormyrus*. T.S. – total strain value.

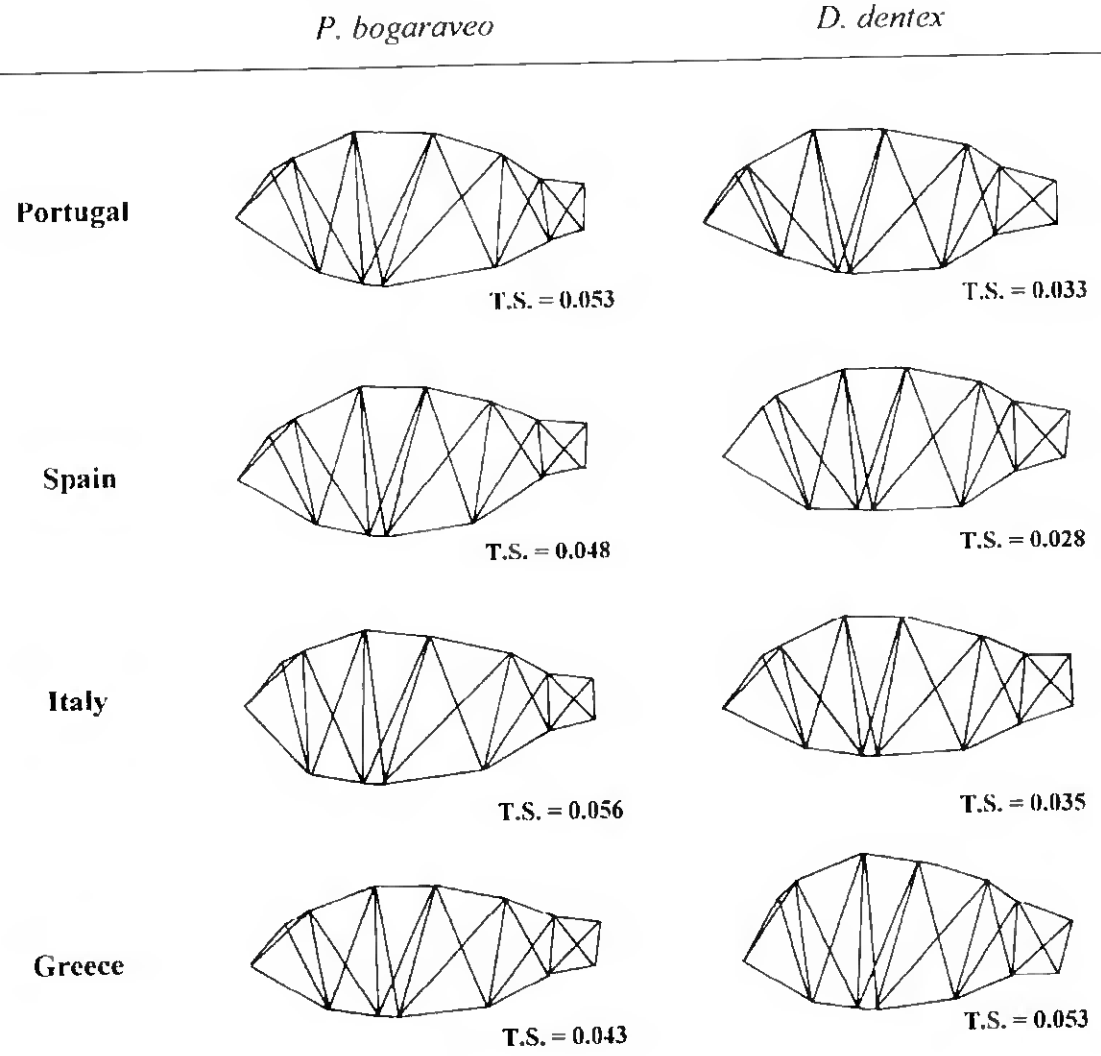



Figure 26 – Mapped configurations for each of the samples of *P. bogaraveo* and *D. dentex*. T.S. – total strain value.

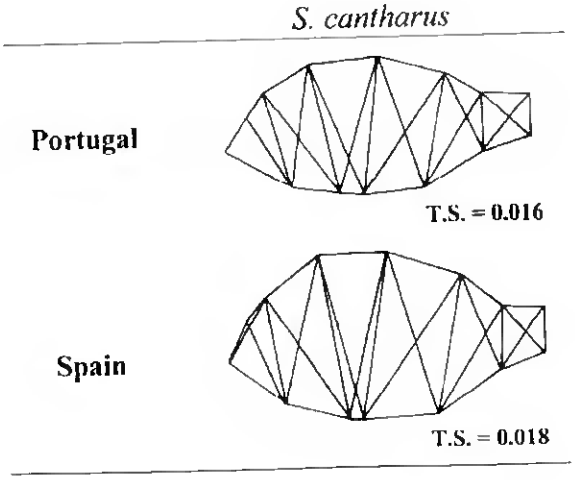



Figure 27 – Mapped configurations for each of the samples of *S. cantharus*. T.S. – total strain value.

## 2.4 - DISCUSSION

From an historical point of view, many nongenetic markers have been used in order to manage fish stocks and understand the population structure and the movement of the fish (Garcia de Léon *et al.*, 1997). The definition and identification of discrete fish stocks still remains an important issue in the fisheries management scheme. The dilemma from both biological and management perspective is how to determine the correct spatial scale to employ when identifying populations of fish (Larkin, 1981).

Isolation can induce both genotypic and phenotypic differentiation between stocks. Thus, fish stocks are identified as natural breeding units that are reproductively isolated from other similar intraspecific populations (Haddon & Willis, 1995). Among the several factors that contribute to the divergence of animal populations, geographic partitioning assumes a major importance, particularly if that separation is due to natural barriers. In the present study, and as already referred in Chapter 1, the phylogeographic break created by the Strait of Gibraltar between the Northeast Atlantic and the Mediterranean Sea (as referred by Borsa *et al.*, 1997; Naciri *et al.*, 1999) can be responsible for the separation between populations of the same species. Its influence will be discussed further on. In freshwater fishes, rivers and lakes are usually considered as geographical barriers. This definition is widely confirmed by morphological findings (e.g. Melvin, *et al.*, 1992; Garavello *et al.*, 1992; Jerry & Cairns, 1998; Keenlyne *et al.*, 1994a; Golubtsov & Berendzen 1999; Treer *et al.*, 2000).

Reared fish can also be considered as isolated populations. The reproduction in these "units" is artificially manipulated and based only on selected specimens. As so, it is interesting to compare them with the wild populations. Nevertheless, these populations are originally derived from the native population. Thus, it is of major interest to assess, both qualitatively and quantitatively, the differentiation or proximity from the wild individuals.

In recent years, morphological studies have been used not only to discriminate populations of a same species, but also to discriminate species. Species identification seams to be a rather strait forward concept, but in specific habitats, where niche variability is limited, species tend to adapt similarly, and so to reduce the phenotypic differences between them, with lack of discrete morphological features, and uncertain homologies of pigmentation patterns. In some cases, morphological analysis is complemented with colour analysis (e.g. Garavello *et al.*, 1991). However,

pigmentation patterns may be of some phylogenetic value once ontogenic sequences have been described in representative species and putative homologous elements have been identified (Strauss, 1985).

Under similar stimulation, species can evidence rather different reactions to environment, with diverse morphologies providing different adaptations. But, is supposed that species of a same family have similar responses, and so, their morphology be affected in a similar way? The answer to this will be analysed and discussed further on.

### 2.4.1 – *Traditional measurements vs. truss networks*

It is often referred by authors that the so-called "traditional morphometry" provides worse results than the modern procedures, where the truss analysis is included. Such differences rely on the fact that the traditional morphometry is based on measurements that are usually chosen along the longitudinal axis of the animal, limiting information over it. Another problem, has to do with the redundancy of measurements based on determined parts of the body morphology, the selection of which is mainly due to the scarcity of conspicuous points in body parts or the tendency of the sampler to choose the points in a cognitive way and so, give preference to particular areas, usually, head measurements.

In the present study, for the traditional morphometry, twelve measurements were chosen (excluding standard length). The established criteria that guided this choice were the use of traits evenly distributed throughout the fish body. Yet, seven of those characteristics were head related. For the truss networks, and following the Strauss & Bookstein (1982) protocol, measurements were evenly distributed, avoiding any subjective landmark choice.

When comparing both procedures, results were similar and proportional, but with the truss protocol providing a better distinction between samples, based on higher discriminant values. Although the finding of a geographical gradient was not the goal in the sample discrimination, the results obtained through the truss protocol can be seen as an improvement in the sample discrimination. Through the traditional morphometry, only two species evidenced a geographic gradient (*D. puntazzo* and *L. mormyrus*), whereas, through the truss protocol this was the case of three species (*D. sargus*, *D. puntazzo* and *D. dentex*), with only one of in common (*D. puntazzo*).

Such a discrepancy can be related to two major factors: the difference of the acquired measurements by both methods, and the greater number of analysed traits in the truss protocol. Good examples are the samples of *D. sargus*, *P. pagrus* and *P. bogaraveo*. In the first case, through the traditional morphometry nine morphometric characteristics contributed to sample discrimination, whereas, through the truss network it was only based on five (the lowest for all species). However, those five characteristics showed the existence of a geographic gradient among samples. For red porgy, sample discrimination was much more inefficient with the traditional morphometry than with the truss protocol, where the sample individualisation was very high (confront Figures 7 and 16). For red seabream, although sample discrimination based on traditional morphology, was quite good a high deviation of the Italian sample was detected through the truss protocol.

However, if the truss method enhances the possibility of finding differences between populations due to the greater number of analysed traits, the specificity of the measurements of the traditional method can also provide an adequate basis for differentiation. In this study, in two of the species (*L. mormyrus* and *S. cantharus*), the sample discrimination based on the traditional procedure overlapped the results obtained with the truss networks.

Due to the limitations pointed out in the Material and Methods, the results obtained for *S. cantharus* cannot be as conclusive as those obtained for the other species (only two samples were compared). On the other hand, the samples of *L. mormyrus* are a good example, since a geographic gradient was found among samples. For this species, morphological characteristics such eye diameter, length of the upper or lower jaws can be more important for sample discrimination than the distances between landmarks. Moreover, sample discrimination in *L. mormyrus* was based on eight morphological characteristics (both traditional and truss analysis), which taking in to account that the truss analysis was based on a greater number of measurements, reinforces the adequacy of the traditional morphometry in the description of this particular species. According to Akazaki (personal communication) within one fish family or even between species of a same genus it is necessary to consider particular characteristics, and to avoid looking upon the family as an entity. Similar findings were reported by Schaefer (1990), who reported an adequacy of 11 out of 12 morphometric characteristics when discriminating populations of yellow fin tunas (*Thunnus albacares*), which is an indicator of the adequacy of the chose morphological

characteristics. Jerry & Cairns (1998) also noted that conventional characters were better at discriminating populations of the catadromous Australian bass (*Macquaria novemaculeata*) than the truss network.

Thus, the above-formulated question starts to be answered. If each one of the methods is found to be more adequate in the characterisation of a species, it is because the discriminatory characteristics must be analysed from a different perspective, even if (like the present case) they belong to the same family. Thus, the results obtained with the traditional morphology do not reject it as a morphometric procedure, since it provided a fairly good discrimination between samples. Its use can be helpful when other procedures that involve more demanding logistic procedures are not available, or when the truss networks do not produce relevant findings. The main concern for its use should be the choice of the measurements. These results can only be addressed to the methodological differences between procedures, since the statistical analysis was the same for both methods. However, summing the pros and cons of both methods the results obtained through the truss protocol overcame those obtained by the traditional morphometry. Truss results were based on solid statistical findings, with no contradictions between the statistical parameters. $D^2$ values below 10 were only found in a reduced number of pair-wise sample comparison, and always between Mediterranean samples.

### 2.4.2 – *Species analysis*

Analysis of the morphometric characters suggested that each of the areas considered in this study is inhabited by discrete groups of gilthead seabream. The existence of different populations of *S. aurata* along South European Atlantic coasts and Mediterranean Sea was demonstrated. The correct classification rates of the samples herein (always higher than 94%) as well as the results obtained in the discriminant function analysis reinforce this assumption. Therefore, differences or similarities must be attributes of within-sample characteristics and not of an incorrect sample classification.

The Mahalanobis distances ($D^2$) were calculated as units of standard deviation about the group means, so they are good descriptors of the differences between stocks. In the present study, these differences (cf. Table XIII, Annex II) were very clear. Considering the reared samples, the westernmost samples (France and Portugal) were quite different from the Mediterranean ones. The Spanish Atlantic sample completes the

gradient between Atlantic and Mediterranean samples, occupying an intermediate position between the Portuguese and the Spanish Mediterranean samples. Nevertheless, it showed a higher proximity to the Spanish Mediterranean sample, due to the higher geographic proximity.

Considering the wild samples, the Italian sample was quite different from all the others. This dissimilarity might be due to a higher geographic isolation. According to Bembo *et al.* (1996), there are three primary biogeographic regions in the Adriatic: 1) the shallow waters of the northern Adriatic (<50m deep), with moderate to high productivity; 2) a western shallow coastal strip, but with locally eutrophic conditions; and 3) the open oligotrophic waters of the central-southern area with depths exceeding 75m. Independent circulatory patterns in the north and south Adriatic (Zore-Armanda, 1969) reinforce such latitudinal discontinuities, as evidenced by the presence of several endemic fish species in the northern waters (Tortonese, 1983). Therefore, it is not surprising to find corresponding differences in the distribution of species and populations (Umani *et al.*, 1992), which for gilthead seabreams corresponded to a different morphotype, distinguishable from the other Mediterranean stocks.

On the other hand, populations that inhabit the areas around the sampling places of Spain and Greece could migrate throughout the Central Mediterranean and maintain a closer contact between bordering populations.

The remaining samples did not display a clear geographic pattern, despite the existence of a good differentiation among them. The analysis of the mean canonical values, cluster the Portuguese and Spanish Atlantic samples (in the CV2, after discrimination of the Italian samples by the CV1). Nevertheless, the Mahalanobis distance between these two samples was smaller than those between Portugal/Spain Mediterranean and Portugal/Greece.

When wild and reared samples were compared, there was a significant degree of dissimilarity between them. $D^2$ clearly increased, showing an evident sample differentiation (cf. Table XIII, Annex II). This fact is particularly clear between the Italian samples, where the reared morphotype increased the difference already observed between the wild population and all the other samples.

The differences between wild and reared stocks were also evident when a within species comparison was performed. Reared samples showed a higher and positive correlation to the body height and caudal related characteristics. Conversely, the samples of wild fishes correlated negatively with those characteristics. On the other

hand, the samples of wild fish correlated positively with the head and longitudinal characteristics, whereas the samples of reared fish were negatively correlated with those characteristics. Our findings translate to a more robust body in the reared populations, and a more elongated body in the wild ones (Figure 23). In contrast, Fleming & Gross (1989) predicted the evolution of a more streamlined body shape in hatchery populations of Pacific salmon. This is due to reduced selection for burst swimming performance, because juvenile rearing occurs in a controlled predator-free environment and spawning is artificial. These authors stated that this difference indicates an evolutionary response of reared populations to novel selection pressures in the hatchery environment. Attending to the Sparidae morphology, a stronger body with reinforced capacity for constant swimming, rather than a more elongated body, is the physiological response to the intensive rearing conditions with constant water flow. The observed morphology of the wild populations provides fish a higher capability for predator avoidance. Differences between wild and reared population seem to mainly depend upon the rearing conditions. However, differences between wild and reared populations are not always detectable. Ellis *et al.* (1997) also found differences between populations of turbot, *Scophthalmus maximus*, whereas Johnson *et al.* (1986) did not find any differences between wild and reared populations of American shad (*Alosa sapidissima*).

Red porgy evidenced the greatest difference between the results obtained from the two morphological procedures. In the traditional method the sample discrimination, although reasonable did not achieve the degree of separation attained by the truss protocol (compare Figures 7 and 16). Thus, it can be speculated that non-related head characteristics are more relevant than the head related. Based on truss measurements, *P. pagrus* was one of the species were sample discrimination was based on a large number of characteristics (eighteen). Of those, eleven were trunk related, proving that *P. pagrus* was one of the studied species were the body form adjustment is more visible. Beside the slight adjustment of the head morphology, the body adjustment has an important role in their adaptability. Like common dentex, red porgy is an active predator, which is not limited to slower or sessile preys. Thus, the burst speed is a necessity for this species. Similar findings were observed in cyprinids, which displayed morphological gradients that correspond to the degree of adaptation to moving waters. The individual forms that inhabit rivers with stronger currents displayed a more streamlined body as well as longer, and more curved fins (Barlow, 1961). In this work a streamlining of the body was also found between the wild and reared samples of *S. aurata*.

When analysing the only two species that belong to the same sub-family (Diplodinae), *D. sargus* and *D. puntazzo*, results confirmed a differentiation between the Atlantic and the Mediterranean populations, with a clear geographic pattern for both of them.

Although sharing the same habitats, *D. sargus* and *D. puntazzo* (Macpherson *et al.*, 1997) have dissimilar morphological characteristics. According to Macpherson (1998) the distinction between these two species is perceptible since the early stages of development as they avoid overlap by using the shallow crannies at different times of year, *D. puntazzo* in autumn and *D. sargus* in the spring. After attaining larger sizes they begin to disperse to zones outside the nursery areas, and the juveniles of *D. sargus* join shoals of adult conspecifics. Due to the low density of conspecific adults, juvenile *D. puntazzo* tend to remain solitary. Low densities of *D. puntazzo* were quantified by Vigliola *et al.* (1998) in a study over the settlement of juveniles of *D. vulgaris*, *D. sargus* and *D. puntazzo*. In that study, *D. vulgaris* accounted with 71%, *D. sargus*, 25%, and *D. puntazzo* 4%. The higher occurrence of *D. sargus* can ensure a more effective dispersion capacity, increasing the probability of combination between neighbour populations, reducing high dissimilarities between them.

Differences between populations of *D. sargus* were based on a smaller number of morphological characteristics than in *D. puntazzo*. In both cases, more than 50% of the characteristics were head-related, which suggests the influence of habitats differences between the populations. In fact, feeding is a well-known factor that influences head morphology (e.g. Gerking, 1994; Hyndes *et al.*, 1997; Delariva & Agostinho, 2001). Thus, if different populations of a same species show discordant pattern of head morphology, this is often due to the exploitation of different ecological niches, especially constrained by the availability and type of prey. These differences are based on hydrographic and physical characteristics of each habitat (e.g. water temperature, currents) induced by geographical dissimilarities. Similar results regarding the head morphology were obtained by Sarà *et al.* (1999) in cultivated *D. puntazzo* reared under different conditions, and Schaefer (1992) between Pacific samples of yellowfin tuna (*Thunnus albacares*).

Genetically analysis (Bargelloni, unpublished data) of these same samples produced similar results, with higher degree of homogeneity for *D. sargus* than for *D. puntazzo*. Furthermore, this study also found a genetic differentiation between Atlantic and Mediterranean samples. A similar constraint, although with a lower significant

differentiation appeared between East and Eastern Mediterranean samples. Lenfant & Planes (1996) also reported a genetic differentiation between samples of *D. sargus* from the Gulf of Lion (France) and the Ligurian Sea (Italy). According to these authors, the break in the costal continuum and the complexity of local current systems, which may retard larval dispersal, could determine the degree of heterogeneity referred to above.

In the case of *L. mormyrus*, where population isolation is also relevant, the Atlantic population is closer to the Italian sample than to the Spanish one (truss analysis). This degree of similarity is probably supported by the above stated environmental conditions of the southern Adriatic (open waters with depths exceeding 75m), which share higher similarities with the Atlantic oligotrophic conditions. The grouping of the Portuguese and the Italian samples indicates a greater degree of isolation with regards to the other samples. With the Portuguese sample being the geographically most isolated population, the closeness of the Italian sample, reinforces the assumption that the Spanish and Greek samples have diverted from them, and so from an initial global population, the initial characteristics of which are retained in the Portuguese and the Italian samples. A similar constraint for differentiation such as head morphology found for *D. sargus* and *D. Puntazzo* cannot be applied, since only 30% of the differentiating characteristics are head related. Such a fact can be closely associated to the choice of feeding grounds.

Red seabream provided a rather enigmatic result similar to red porgy. Although good sample discrimination was obtained by both methods, the results were quite different. Truss measurements evidenced a high distinction between samples (PCS was 100% for all species). Initially, it was expected that the greatest difference would be found between the Portuguese sample and all the others, since this sample was captured in Azorean waters. Although it as been found a significant difference between the Atlantic and the Mediterranean samples (Figures 11 and 20), the difference between the Italian sample and all the others was even greater. Such a difference cannot be easily explained in light of the present facts. Only a more interdisciplinary analysis can supply answers. Nevertheless, the sample variation must be dependent on constraints similar to those pointed out for the striped seabream. According to the genetic data obtained by Bargelloni (unpublished data) for these same samples, no relevant genetic differences were found. Such findings are not at all surprising and new, morphological differentiation is often clear and statistically significant, whereas differences in gene frequency are commonly less powerful in discriminating among populations (Lewontin,

1984). Other authors have reported absence of correlation between morphological and genetic analysis in a wide variety of species, *Oncorhynchus gorbuska* (Salmonidae), Beacham & Withler (1985a), *Oncorhynchus keta* (Salmonidae), Beacham & Withler (1985b), *Gephyrocharax valencia* (Characidae), Rivero & Rojas (1995), *Leuciscus cephalus* (Cyprinidae), Hänfling & Brandl (1998), *Gadus morhua* (Gadidae), Pepin & Carr (1993), and *Hoplostethus atlanticus* (Trachichthydae), Elliott *et al.* (1995).

Elliott *et al.* (1995) found considerable morphometric variability among 10 samples of orange roughy *H. atlanticus*, despite the genetic data indicating appreciable levels of gene flow between them. In a study of artic charr (*Salvelinus alpinus*) Hindar & Jonsson (1993), argued thatbased on a comparison of charr life history in captivity and in the wild, ecological polymorphism of this species is chiefly a result of variation in growth conditions between different habitats.

The common dentex was the third species (according to the truss protocol) where a geographic gradient could be identified. Such a fact is a direct consequence of the observed dissimilarity between the Atlantic and the Mediterranean samples. The Atlantic sample, was the only one among the four were the PCS was 100%. All the other samples although with a high degree of distinction between them (over 96.7%) registered one individual in each that shared similar characteristics with the neighbouring population (except the Italian sample) (Table XV, Annex II). The isolation between the Atlantic sample and the Mediterranean ones is also well evidenced in the mean canonical values (Table LVI) were the Atlantic sample correlates negatively and significantly with CV1.

Differentiations between samples were mainly based on two types of characteristics, the head related (four distance measurements) and longitudinal characteristics (seven distance measurements). These two types represented eleven of the fourteen chosen characteristics for sample discrimination. These findings call attention to a similar explanation pointed out for the differentiation between the *P. pagrus* samples, since as referred above these two species share many similarities in their feeding behaviour.

In contrast to the other species, the importance of sample comparison of the black seabream is smaller since samples were available from only two places. As described in the Material and Methods sampling was not possible during the specified periods. However, sampling was carried out during the same period of the year in order to minimize bias induced by seasonal differences due to the influence of reproductive

period, or stomach fullness. Mamuris *et al.* (1998), argued that he could not establish a clear morphological differentiation between populations red mullet (*Mullus barbatus*) from different geographical places in the Ionian and Aegean sea, which according to the author was due to the fact that the samples were collected in different periods of the year. Saborido-Rey & Nedreaas (2000) reported the same problem with samples of *Sebastes mentella*, and Schaefer (1992) with *T. albacares*.

Nevertheless, when comparing the Portuguese and Spanish samples of black seabream, all the statistical findings suggest a high degree of dissimilarity between them. 100% PCS was found for both species, and a $D^2$ value of 31.4 can be considered high. These findings, sustain the hypothesis of a high differentiation between Atlantic and Mediterranean samples of black seabream. Such a possibility is supported by the genetic findings of Bargelloni (unpublished data) based on these same samples.

These results shed light on the differentiation between the Atlantic and Mediterranean species based on morphological analysis. In an overall perspective, such separation was observed for all species, although with a stronger degree of differentiation for *P. pagrus*, *D. puntazzo*, *D. dentex*, *P. bogaraveo* and *S. cantharus* samples. In the remaining species, *S. aurata* (both wild and reared), *D. sargus* and *L. mormyrus*, the degree of individualization although present was less pronounced.

The Mediterranean Sea is a relatively young system that has been subjected to extensive changes in configuration and climate change over the last six million years (Pérès, 1967; Blanc, 1968). The combination of events such as the opening and closing of the Strait of Gibraltar, advances and contractions of glaciations and changes in current patterns, have apparently made the north-east Atlantic/Mediterranean area a remarkable generator of diversity.

It can be hypothesised that the history of the Mediterranean Sea, combined with the present hydrographic pattern, might have promoted and maintained the differentiation of the Mediterranean samples. Following the Messinian salinity crisis (ca 5.5 million years ago) (Hsü *et al.*, 1977; McCullach & De-Deckker, 1989), communication between the Atlantic and Mediterranean was re-established, and fully marine conditions were restored during the Pliocene (5.4 to 1.8 million years ago). In the Pliocene (1.8 to 0 million years ago), and particularly during the Quaternary, a series of glaciations and interglacial periods with associated marine regressions affected the area (Blanc, 1968). Atlantic and Mediterranean species of *P. pagrus*, *D. puntazzo*, *D.*

*dentex*, *P. bogaraveo* and *S. cantharus* may have become physically isolated during these cycles and differentiated morphologically.

Present day hydrographic barriers, in particular the previously mentioned Almeria-Oran oceanographic front (AOOF), may be implicated in the observed differentiation. This front is a zone of turbulence in the Alboran Sea (Tintore *et al.*, 1988) that may restrict larval dispersal in both directions (Pannacciulli *et al.*, 1997). This author supports the hypothesis that the AOOF is the major barrier to the gene flow between the Atlantic and Mediterranean populations of two *Chthamalus* species. Genetic discontinuity due to the AOOF is also reported by Naciri *et al.* (1999) for population of sea bass (*Dicentrarchus labrax*). Likewise, the AOOF seam to be an effective boundary between the Atlantic and Mediterranean populations for the majority of the sparid species studied in the present work, both morphologically and genetically, since the morphological results obtained in the present work are in strong correlation with the genetic findings of Bargelloni (unpublished data) carried out on these same samples. Genetic and morphological results were only uncorrelated for the samples of *P. pagrus* and *P. bogaraveo*.

As referred above, such a finding is not unique, since some morphological adaptations are not dependent on genetic mutations. The novelties of some morphological adaptations are not immediately expressed in the species gene pool. As with all stock identification techniques including genetic factors, the lack of differences in one study does not preclude the existence of a stock differentiation (Schweigert, 1991). According to Bargelloni (unpublished data) the lack of genetic differentiation between the *P. pagrus* populations is caused by large demographic fluctuations. In this case, if a dramatic reduction in population size brought the Mediterranean population to extinction, then the lack of differentiation is the exceptional result of recolonization of the Mediterranean from the Atlantic, quite recently. Under this scenario, there was not enough time for populations of the two basins to diverge again, even if under "normal" conditions migration across the Gibraltar Strait is low, as is the case with other sparid species. However, from a morphological point of view, changes already exist, which demonstrate that morphological differentiations are expressed earlier than genetic ones and are a direct consequence of environmental adaptation.

According to the same author, the distribution of *P. bogaraveo* suggests that the observed genetic pattern might be the legacy of historical events, rather than a consequence of the present-day oceanographic situation, and might be related to a

temperature gradient across the Mediterranean Sea. Such a constraint indicates that the presence of this species in this marine basin has been (re-)established only recently, without the possibility of accumulating detectable divergence between Atlantic and Mediterranean populations.

Throughout the data analysis, the Italian samples were the most atypical, since for some species there were no significant differences between the neighbouring populations, and in other cases they evidenced a high degree of differentiation. The explanation of such findings seem to be in direct relation with the different adaptability of the analysed species. The hydrodynamic and competitive constraints imposed by the aquatic environment do not necessarily lead to morphological similarity among assemblages by way of evolutionary convergence, at least with regard to patterns of variation among species. The teleost "morphotype" seems to be sufficiently flexible that morphological structure need not be highly constrained in similar ways in different habitats. Instead, each characteristic body form exploits the aquatic environment somewhat differently in terms of its own morphology and ecological design, and resultant differences among species (in magnitude and direction) are highly variable. Thus phylogenetic patterns of form diversity are responsible both for consistency among different fish assemblages within the same biogeographic regions and not for noncongruence among assemblages having little faunal similarity (Strauss, 1987).

Although the results obtained in the present work, not all morphometric studies were been able to distinguish differences among the studied populations (e.g. Guénette *et al.*, 1992; Krzykawski *et al.*, 1994; Gutiérrez *et al.*, 1995; Rivero & Rojas, 1995; Almeida, 1996; Velasco *et al.*, 1996). Nevertheless the majority of morphometric studies have been able to identify differences and these achievements emphasize morphometric studies as helpful tools for the discrimination of fish populations. Truss analysis combined with image analysis is a step ahead to produce a better understanding of stock structuring of fish species.

# CHAPTER 3 – HYBRID ANALYSIS

## 3.1 – INTRODUCTION

Theoretically, species are genetically isolated even if they have the same number of chromosomes. In Teleosts, however, hybridisation is a common phenomenon (Crivelli & Dupond, 1987) irrespective of chromosome number, and hybridization is considered as an inter-specific insemination (Dujaković & Glamusina, 1990) as well as a basic type of genetic combination in which traits of two or more parental species are intermingled in the hybrid progeny (Kerby & Harrel, 1990). Different species contain different gene combinations, which means that a hybrid brings together genes that have not undergone such fine-tuned co-evolution (Helfman *et al.*, 1997).

Among fishes, hybridization is more common in freshwater species and less well documented among marine species (Schwartz, 1981). Schwartz (2001) reported hybridization in 95 fish families (34 freshwater, 53 marine, and 8 intermediate representatives), which represent 19.7% of the 482 fish families worldwide (Nelson, 1994). Freshwater fish families such as Acipenseridae, Salmonidae, Esocidae, Ictaluridae, Cichlidae, Cyprynidae, Centrarchidae, and Poeciliidae dominated the hybrid related publications until 1981. They have now been joined by marine and intermediate fish families, such as: Atherinidae, Gasterosteidae, Moronidae, Sparidae, Sciaenidae, Pomacanthidae, Scombridae, Pleuronectidae, and a variety of other marine fish families (Schwartz, 2001).

Hybridization in fishes has been studied since the 1800's, but only recently, was recognised and used as a tool to enhance certain desirable characteristics and for management purposes (Kerby, 1993). Natural hybridization has been recognized in several fish families namely the Cyprinidae (Child & Solomon, 1977; Crivelli & Dupond, 1987; Bianco, 1982; 1988; Dupond & Crivelli, 1988; Martins *et al.*, 1998), the Moronidae (Waldman & Baily, 1992) the Salmonidae (Chevassus, 1987; Wilkins *et al.*, 1993), the Esosidae (Reist & Crossman, 1987) and Acipenseridae (Keenlyne *et al.*, 1994b) among others.

Knowledge about hybrids, for any fish family, is vital not only for identification purposes (in the case of the natural hybrids), or for control (in the case of the artificially produced hybrids), but also to ensure that the so-called new species will not compete with the native species contributing to their decrease. Biological constraints such as

reproduction, age and growth, meristics and morphology are very important for defining the viability of the hybrids.

Artificial hybridisation has served a variety of purposes, and during the past 25 years, advances in culture and rearing techniques have opened new management opportunities in inland lakes and reservoirs where the production of some new "species" from the original inhabitants (e.g. Salmonidae and Moronidae) has resulted in extensive new recreational fisheries for sport fishing (Kerby, 1980, 1993). Research during the 1980s, also demonstrated that hybrids can be commercially cultured as new, high-value food fish, resulting in the development of a new commercial industry (see Kerby (1993) for references). Today at least 179 countries are hybridising fishes to meet sport and commercial food needs, with China, Japan, and India among the ones that produce most hybrids (Pedini, 2000). Although a number of freshwater fish hybrids have been described or produced, dominant hybridization efforts concentrate on sturgeons (Russia), salmonids (USA), carp-goldfish-grass carp (Israel and Europe) and tilapia/Cichlidae (a freshwater family often raised in marine environments, Asia and many countries of the world). Most of the marine hybrids involve members of the Moronidae (Schwartz, 2001).

Fish farming, in contrast to modern agriculture, is still concerned largely with the domestication of wild stocks (Wilkins, 1987). Throughout the world, this has been achieved with several wild species, but the continued demand for new products puts a constant pressure on the fish farmers. Thus, the crosses of species where the breeding techniques are well established with others that are virtually impossible to successfully produce in aquaculture are constantly been attempted. In the South European Atlantic and Mediterranean Sea aquaculture production has traditionally been based in a small number of species, with an emphasis on gilthead seabream (*S. aurata*).

Since Roman times this species has had an important role in the Mediterranean diet due to it refined taste, and has been fished from the south European waters. Nonetheless, in the last decades the increased exploitation has decreased their numbers, boosting their artificial production as a complement to the natural production. Because of its commercial value, relative ease of adjustment to environmental conditions, fast growth and low mortality rate, the gilthead seabream, has always been extensively reared in brackish water lagoons along the South European coasts (Ardizzone *et al.*, 1988; Gouveia, 1995). With the control of the reproductive cycle, extensive rearing has

been transformed to semi-intensive and intensive rearing in earth and concrete ponds, and sea cages, yielding increased production.

Red porgy, *Pagrus pagrus* (L.), has been recently introduced in aquaculture in order to diversify the production of fish in South European countries (Colombani, 1993), using rearing technologies similar to those applied in gilthead seabream culture (Kentouri *et al.*, 1995a). Thus, the production of the hybrid, *S. aurata* × *P. pagrus* (*Sa*×*Pp*), arises as a new step in this diversification. Using these parental species, it was expected not only that the red porgy rearing difficulties would be reduced by the qualities of adaptation of the gilthead sea bream to aquaculture, but also the develop a highly profitable resource for aquaculture.

Growth rate is an important criterion for the selection of a new species for intensive aquaculture because it indicates the time required for a fish to reach market size (Divanach *et al.*, 1993). Growth can be calculated as changes in biomass (weight), energy (calories), carbon or nitrogen, during an interval or measurement and includes somatic (protein and lipid) growth and development of gonads (Adams & Breck, 1990).

The quantitative needs of food depend mainly on its composition (Hepher, 1988), and optimal feeding levels are obtained when the food supplied contains the proper proportions of essential nutrients required by the fish, both for maintenance and growth. In intensive marine aquaculture, the quality of food has a strong influence in the growth rate, amount of food supplied, transformation index and organoleptic characteristics of fish. In fact, it also has a strong influence on the cost-effective production (Kentouri *et al.*, 1993a).

This hybrid raises some new and interesting questions about its biology and behaviour. It's study can be addressed from two different perspectives: strictly biological or as an aquaculture product. Both provide answers that together can produce a greater understanding. Thus, in this chapter, in addition to the biological analysis used in the previous chapters to characterize the above mentioned sparid species ther biological analyses, such as head morphology, both quantitative, with the counts of different tooth types, as well as qualitative, with the osteological characterization were carried out. Detailed analysis of the hybrids head ensured not only that all the morphology of the animal was taken in to account, but also that this analysis permitted a comparison with the parental species. Differences between them are well known (Bianchi, 1984). Under the above stated aquaculture perspective its growth performance was also analysed, and again compared with the parental species.

## 3.2 - MATERIALS AND METHODS

### 3.2.1 - *Meristic Analysis*

#### 3.2.1.1 – *Experimental procedure*

Meristic analysis of the hybrids was extended beyond the analysis carried out in Chapter 1 for the other sparid species. Beside the above stated meristic characteristics, special attention was paid to the head morphology, both quantitatively and qualitatively. For the quantitative analysis the tooth type were identified and counted as; canines (big or small (when present)), intermediate tooth and molars in each row in the upper and lower jaw, both in the right and left side of the jaw. Characters were counted both in hybrid and parental species. In the dentary analysis samples were divided in two length classes, below 150 mm, and more than 150 mm.

For the qualitative analysis the head morphology was analysed from an osteological perspective, where relevant bones were identified and described, namely the neurocranium, in which other sub-structures were studied (supraoccipital, vomer, ethmoid, parasphenoid, basioccipital, condyle of the hyomandibula, exoccipital, basisphenoid), lacrimal bones, opercle, sub-opercle, inter-opercle, pre-opercle, maxilla, articular, premaxilla and dentary.

This analysis (both meristic and osteological) was carried out using 354 specimens of *Sa*×*Pp*. Parental species analysis was based on the Portuguese wild specimens of these species (*S. aurata*, n=92, and *P. pagrus* n=106). Sample size is slightly different from chapters one and two, because specimens were added to the *S. aurata* sample and three individuals were taken out from the *P. pagrus* sample.

A meristic analysis similar to the one described in Chapter 1 was carried out with 80 hybrids and compared with the pooled wild samples of *S. aurata* and *P. pagrus*.

#### 3.2.1.2 –*Data analysis*

Firstly, one-way ANOVAs were used to compare differences between hybrids and progenitors. Significant relationships were tested using the Tukey HSD test. Subsequently, a principal components analysis was performed, and when justifiable a discriminant function analysis was also applied.

### 3.2.2 - *Morphological analysis*

#### 3.2.2.1 – *Experimental procedure and data analysis*

Morphometric data from 80 specimens of *Sa×Pp* were analysed and compared with the pooled wild samples of *S. aurata* and *P. pagrus*, using the same procedure described in the Chapter 2 for both methods. Statistical analysis was performed accordingly. The traditional morphological was also compared with the truss method.

### 3.2.3 – *Growth experiment*

#### 3.2.3.1 - *Experimental procedure*

The growth experiments of the hybrid *Sa×Pp* were performed in the facilities of the Institute of Marine Biology of Crete (Dep. of Aquaculture), were the hybrids were also produced.

From the initial available stock, the hybrids were manually graded for size uniformity and randomly assigned into the tanks, creating six groups (T1 to T6). Each fish was marked with a subcutaneous injection of alcian blue solution for individual identification. A total of 50 fish was placed in each tank (2 $m^3$ water capacity, with permanent water recycling), with an average initial stocking density of 1.3 $kg.m^{-3}$. In the first cycle the initial length and weight was 13.65±1.45cm and 51.48±16.31g, and in the second was 16.99±15.6cm and 94.14±16.31g. The tanks were placed under natural conditions for both temperature and photoperiod. Fish were fed under self-feeding conditions (Divanach, *et al.*, 1993; Kentouri *et al.*, 1995b) with three commercial foods (Table LIX). Differences among these were only due to the content in proteins and lipids, with all the other components, such as vitamins, ash, and water in identical percentages. Each type of food was supplied as follows: food A in tank 1 and tank 2, food B in tank 3 and tank 4, and food C in tank 5 and tank 6.

Table LIX - Content in proteins and lipids for food A, B and C (in percentage)

| | Protein | Lipids |
|---|---|---|
| FOOD A | 48.1 | 11.9 |
| FOOD B | 47.8 | 16.8 |
| FOOD C | 50 | 16 |

Growth experiments were carried out between September 1995 and December 1995 (1st period) and between January 1996 and March 1996 (2nd period). A total of 10 fish were sampled in the beginning of each period and 30 fish (5 in each tank) at the end.

For each fish, liver was weighed to the nearest 0.1 g, and a blood sample was taken from the caudal vein and placed in 1.5 ml Eppendorf tubes, using a sterile syringe. Blood was allowed to clot and centrifuged at 2500 g. The concentration in protein, glucose and total lipids was assessed using Biosis kits®. Carcasses were frozen for further studies of body composition on ashes and water content.

At the end of the 1st period (December 1995), fish from all tanks were mixed and then allocated to the six tanks and fed with a different food from the one that they had been eating until then (based on their individual identification).

Every two weeks, fish were anaesthetised (0.3 $ml.l^{-1}$ etylenglycol-monophenylether, Merck), measured (total length, to the nearest 1 mm) and weighed (total weight, to the nearest 0.1 g).

Temperature ($^0C$), oxygen ($mg.l^{-1}$), and weight (g) of food consumed were recorded daily, and salinity ($^o/_{oo}$) was measured weekly. The average temperature decreased from 23.49±0.23 °C to 18.87±0.04 °C in the first period, and from 18.23±0.05 °C to 15.89±0.04 °C in the second (Figure 24). Salinity was approximately 40±1.2$^o/_{oo}$ throughout the experiments.

The average dissolved oxygen varied between 5.15±0.2 $mg.l^{-1}$ and 6.35±0.05 $mg.l^{-1}$ in the first period, and between 6.68±0.08 $mg.l^{-1}$ and 6.65±0.23 $mg.l^{-1}$ in the second (Figure 28).

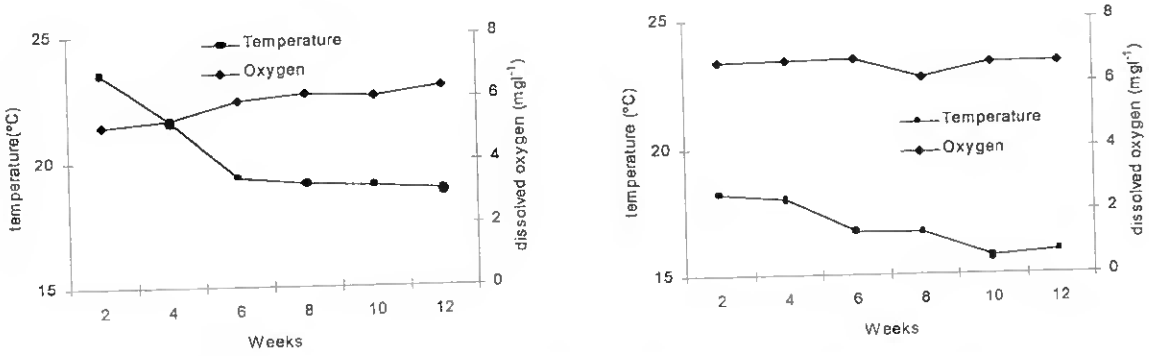



Figure 28 - Average temperature and dissolved oxygen in a) the first period and b) in the second period.

### 3.2.3.2 - *Data analysis*

The overall growth performance of the hybrids was described using the following indices and ratios:

Specific Growth Rate (SGR)

$SGR=|(W_f-W_i)^{1/\Delta T}-1| \times 100$

Growth rate (GR)

$GR=((W_f/W_i)/\Delta T-1) \times 100$

Conversion index (CI)

$CI=C_f/W_g$

where, $W_i$ is the initial mean body weight, $W_f$ is the final mean body weight, $\Delta t$ is the duration (in days) of the experiment, $C_f$ is the food consumed during the experiment and $W_g$ is the weight increase ($W_f$-$W_i$). Food utilisation was calculated using the following indices:

Daily Feeding Rate (DFR)

$DFR=|(F_d/(B_f+B_i)/2)/\Delta T| \times 100$

Hepatosomatic Index (HIS)

$HSI=(L_w/F_w) \times 100$

Mesenteric Fat Index (MFI)

$MI=M_f \times 100/F_w$

where $F_d$ is the food demanded, $B_i$ initial biomass, $B_f$ the final biomass, $L_w$ is the liver weight, $F_w$ is the gutted fish weight, $M_f$ the mesenteric fat weight, $F_w$ the gutted fish weight and $\Delta T$ the number of days of the experiment.

### 3.2.3.3 - *Statistical Analysis*

One-way ANOVAs were used to compare differences within and among diets. Two replicates were considered for each diet: tanks 1 and 2 for food A, tanks 3 and 4 for food B, and tanks 5 and 6 for food C. Significant relationships were tested using the Tukey HSD test. Growth curves were fit by regression analysis, and regression parameters were compared using the *t*-Student test (Snedecor & Cochran, 1982).

## 3.3 – RESULTS

### 3.3.1 – *Meristic analysis (fins and gill rakers)*

Descriptive statistics of the meristic characteristics of the hybrid and parental species are presented in Annex III, Table I. Intermediate mean values were recorded for the dorsal fin and anal fin, with the other meristic counts being equal to at least one of the progenitors. The range of the counts also fell within the ones observed for the parental species. Only one exception occurred in the pectoral fin, where a count of 12 rays occurred (Table LX).

Table LX - Comparison of the meristic range values of the hybrids and parental species (DF - dorsal fin; AF - anal fin; PF - Pectoral fin; UGR - upper gill rakers; LGR - lower gill rakers).

| | DF | CF | AF | PF | UGR | LGR |
|---|---|---|---|---|---|---|
| ***Sparus aurata*** | XI+12-14 | 20-23 | III+10-12 | 13-18 | 3-7 | 6-10 |
| **Hybrid *Sa* x*Pp*** | XII-XIV+10-12 | 20-23 | III-IV+8-11 | 12-16 | 3-8 | 7-10 |
| ***Pagrus pagrus*** | XI-XIII+9-11 | 20-24 | III+8-9 | 14-17 | 4-9 | 7-11 |

The observed variance for each of the traits (Table LXI), showed similar patterns to the parental species. In three cases (upper right gill rakers and lower gill rakers (right and left)) they were even lower than both progenitors.

Table LXI - Sample variance of the meristic traits recorded for the hybrid *Sa* x*Pp* and the parental species (DF- dorsal fin, CF- caudal fin, AF- anal fin, RPF- right pectoral fin, LPF- left pectoral fin, URGR- upper right gill raker, ULGR- upper right gill raker, LRGR- lower right gill raker, LLGR- lower right gill raker)

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| ***Sparus aurata*** | 0.33 | 0.57 | 0.23 | 0.74 | 0.86 | 0.41 | 0.48 | 0.56 | 0.61 |
| **Hybrid *Sa* x*Pp*** | 0.42 | 0.90 | 0.53 | 0.37 | 0.33 | 0.16 | 0.91 | 0.26 | 0.24 |
| ***Pagrus pagrus*** | 0.04 | 1.00 | 0.00 | 0.24 | 0.20 | 0.65 | 0.56 | 0.39 | 0.45 |

### 3.3.2 - *Meristic analysis (teeth counts)*

Although counted separately in the left and right side of the jaw, all counts were symmetrical, presenting the same number of teeth in the left and right sides of the mouth. The only exception was recorded for the molars; in the second and third rows of the hybrids and in *S. aurata* respectively (Annex III, Tables II, III, and IV). The sample variance varied within small ranges with no major difference between *Sa*×*Pp* and

progenitors. Again, the exceptions occurred in the molars of the fourth row of the upper jaw (both sides) and in the molars of the second row of the lower jaw (both sides) (Annex III, Tables V and VI). No significant differences in the teeth counts ($p<0.05$) were found in fish of different length classes. The only visible difference was the higher number of molar rows, both in hybrids and the parental species. *S. aurata* had five rows, *Sa×Pp* four, and *P. pagrus* only two. Thus, the hybrids had an intermediate position between progenitors. Consequently, the statistical analysis was carried out only for the first two rows in each sample. Other difference arises from the fact that no small canines were found in the lower jaw of hybrids.

Non-significant differences in the upper jaw were found in the number of big canine teeth (both left and right side) and molar teeth in the first row (both left and right side) between the hybrids and *S. aurata*. Small canines, intermediate teeth and molars in the second row (all in both and right side) were significantly different (Annex III, Table VII). When compared with *P. pagrus*, non-significant differences were only found for the small canine teeth (both and right side) (Annex III, Table VII).

In the lower jaw, non-significant differences between hybrids and *S. aurata*, were found only in the canines (both left and right side), and molars in the second row (both left and right side) (Annex III, Table VIII) between hybrids and *P. pagrus*.

Due to the high occurrence of significant differences in character counts between the hybrid and the parental species, it would be expected that such characteristics would provide a differentiation between samples. Through the principal components analysis, a total of 79.5% and 90.08% of the variation was accounted for by the five principal components for the upper and lower jaws, respectively (Tables LXII and LXIII). For the upper jaw, none of these components correlated with the bulk of characters. In contrast, characters correlated with the first two components in the lower jaw.

Table LXII - Loadings from principal component analysis of the tooth counts for the hybrids and parental species samples in the upper jaw. Loadings are listed for the five principal components. (Exp. Var. - Explained Variance)

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| BRC | -0.466 | 0.299 | 0.128 | 0.701 | -0.144 |
| BLC | -0.620 | 0.332 | 0.149 | 0.372 | 0.202 |
| SRC | -0.064 | -0.498 | -0.547 | 0.251 | 0.605 |
| SLC | 0.012 | -0.234 | -0.739 | 0.236 | -0.548 |
| ITR | -0.765 | -0.043 | -0.057 | -0.356 | -0.127 |
| ITL | -0.807 | -0.112 | -0.010 | -0.207 | -0.014 |
| RM1R | 0.672 | 0.479 | -0.288 | -0.010 | 0.002 |
| RM2R | -0.668 | 0.374 | -0.311 | -0.188 | 0.092 |
| LM1R | 0.602 | 0.577 | -0.297 | -0.130 | 0.155 |
| LM2R | -0.697 | 0.325 | -0.342 | -0.129 | 0.054 |
| Exp. Var. | 3.59 | 1.33 | 1.27 | 0.99 | 0.78 |
| % of total | 35.87 | 13.25 | 12.73 | 9.86 | 7.79 |

Table LXIII - Loadings from principal component analysis of the tooth counts for the hybrids and parental species samples in the lower jaw. Loadings are listed for the five principal components. (Exp. Var. - Explained Variance)

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| RC | -0.152 | 0.785 | 0.233 | -0.544 | -0.085 |
| LC | -0.125 | 0.801 | -0.106 | 0.573 | -0.039 |
| ITR | -0.723 | -0.128 | 0.514 | 0.199 | -0.043 |
| ITL | -0.751 | -0.128 | 0.482 | 0.071 | -0.113 |
| RM1R | 0.811 | 0.046 | 0.353 | 0.044 | 0.271 |
| RM2R | -0.850 | 0.029 | -0.158 | -0.079 | 0.386 |
| LM1R | 0.813 | 0.088 | 0.286 | 0.067 | 0.355 |
| LM2R | -0.858 | 0.062 | -0.120 | -0.035 | 0.366 |
| Exp. Var. | 3.90 | 1.31 | 0.81 | 0.68 | 0.51 |
| % of total | 48.79 | 16.32 | 10.11 | 8.54 | 6.32 |

The above stated similarity between the left and right side of the fish, is clearly shown with the clustering of the characters in figures 29 and 30.

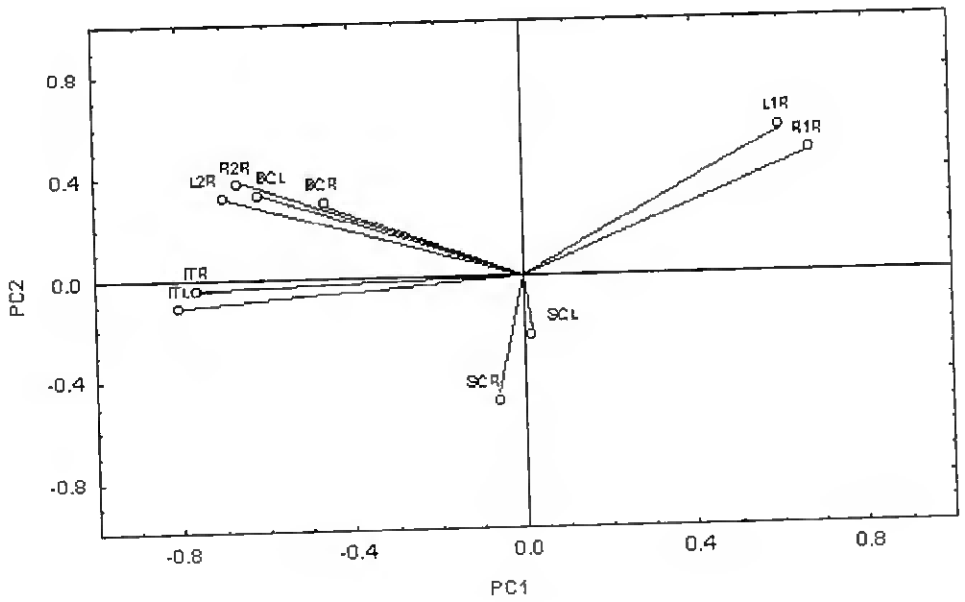



Figure 29 – Factor loadings for the PCA analysis for the left and right counts of teeth in the upper jaw of the hybrid *Sa×Pp*. (BCR – Big canine right; BCL – Big canine left; SCR – Small canine right; SCL – Small canine left; ITR – Intermediate tooth right; ITL – Intermediate tooth left; L1R – Left molars in first row; R1R – Right molars in first row; L2R – Left molars in second row; R2R – Right molars in second row).

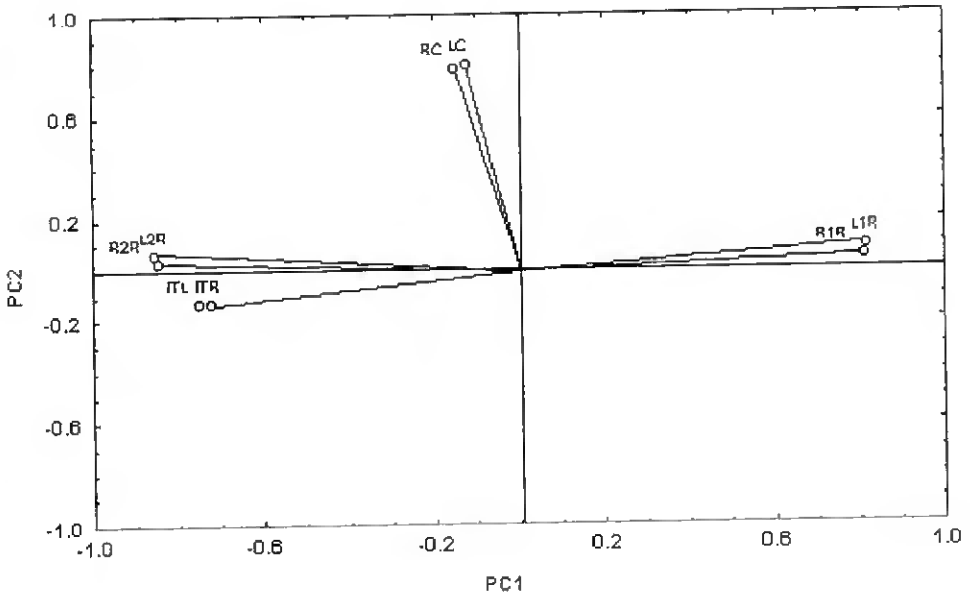



Figure 30 – Factor loadings for the PCA analysis for the left and right counts of teeth in the lower jaw of the hybrid *Sa*×*Pp*. (RC – Right canine; LC – Left canine; ITR – Intermediate tooth right; ITL – Intermediate tooth left; L1R – Left molars in first row; R1R – Right molars in first row; L2R – Left molars in second row; R2R – Right molars in second row).

Under a criterion of F to remove equal to 3 (*cf.* Results in Chapter 2, pp. 36), all characters accounted for the differentiation between samples (Table LXIV) in the discriminant function analysis for the upper jaw (Wilks' $\lambda$ =0.288, F(20,1022)=44.17, $p$<0.001), and seven for the lower jaw (Table LXV) (Wilks' $\lambda$ =0.285, F(14,107)=66.7, $p$<0.001).

Table LXIV - Stepwise Discriminant Analysis for the comparison of tooth counts between the hybrid and the parental species for the upper jaw. (Wilks' $\lambda$= 0.288, F (20,1022)=44.173, and $p$ <0.0001).

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| ITR | 0.314 | 0.916 | 23.320 | 0.001 |
| BCL | 0.313 | 0.919 | 22.370 | 0.001 |
| R1R | 0.308 | 0.934 | 18.135 | 0.001 |
| L1R | 0.303 | 0.950 | 13.480 | 0.001 |
| SCR | 0.298 | 0.965 | 9.223 | 0.001 |
| R2R | 0.298 | 0.966 | 8.959 | 0.001 |
| BCR | 0.296 | 0.972 | 7.372 | 0.001 |
| L2R | 0.296 | 0.972 | 7.233 | 0.001 |
| ITL | 0.296 | 0.973 | 6.998 | 0.001 |
| SCL | 0.292 | 0.984 | 4.025 | 0.018 |

Table LXV - Stepwise Discriminant Analysis for the comparison of tooth counts between the hybrid and the parental species for the lower jaw. (Wilks' λ= 0.288, F (20,1022)=44.173, and $p$ <0.0001).

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| RM2R | 0.322 | 0.885 | 34.745 | 0.001 |
| LM1R | 0.314 | 0.906 | 27.844 | 0.001 |
| ITL | 0.301 | 0.945 | 15.578 | 0.001 |
| LM2R | 0.301 | 0.947 | 14.961 | 0.001 |
| ITR | 0.295 | 0.965 | 9.756 | 0.001 |
| LC | 0.291 | 0.977 | 6.230 | 0.002 |
| RC | 0.288 | 0.988 | 3.204 | 0.041 |

Particularised PCS for each sample, $F$-statistics and generalised Mahalanobis distances ($D^2$) are presented in Annex III, Table IX for the upper and lower jaws, respectively. From the PCS analysis the poor discrimination of the *S. aurata* sample in both jaws, presenting in both cases lower discriminant values than the hybrids and red porgy can be clearly seen. Mahalanobis distances showed a distinct pattern of proximity for the upper and lower jaws. $D^2$ values between hybrids and *S. aurata* were smaller (3.22) than between hybrids and *P. pagrus* (11.35) for the upper jaw, but were higher between hybrids and *S. aurata* (9.83) and lower between hybrids and *P. pagrus* (1.87) for the lower jaw. The upper jaw canonical discriminant-factor scores provided a better sample discrimination (Figure 31) than those scored for the lower jaw (Figure 32).

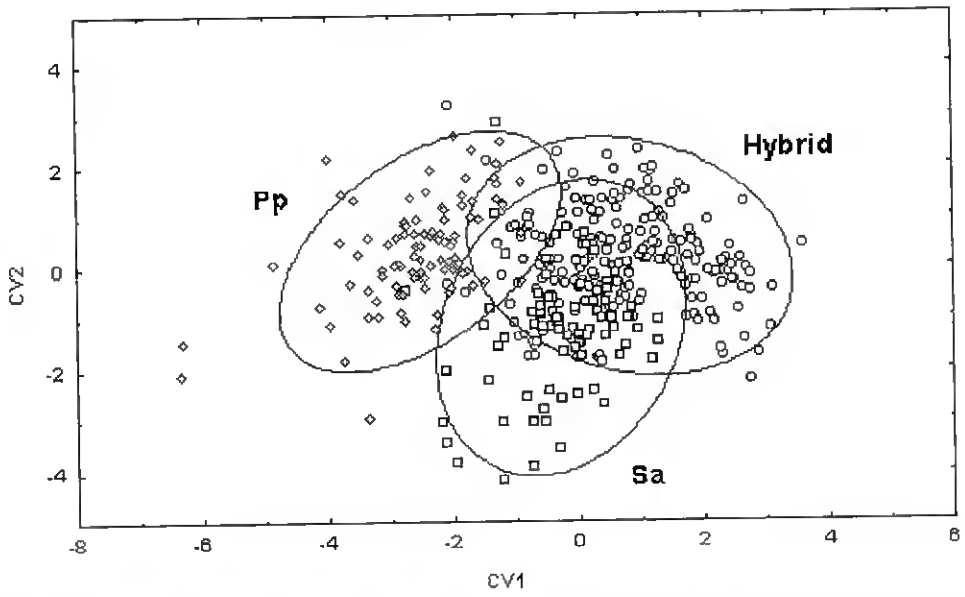



Figure 31 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the hybrid *Sa×Pp* and parental species samples based on the tooth counts in the upper jaw; hybrid *Sa×Pp* (○), *S. aurata* (□), *P. pagrus* (◇).

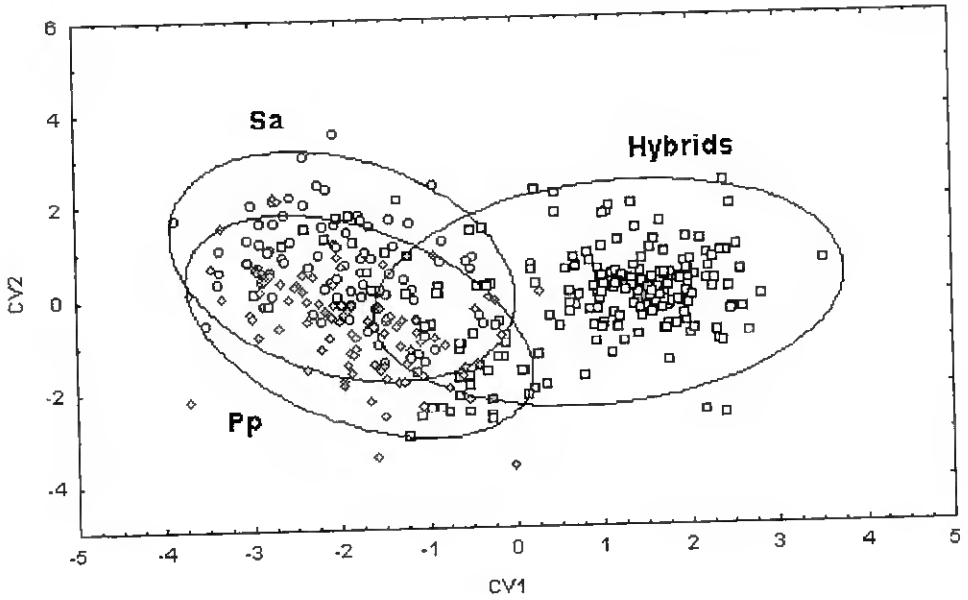



Figure 32 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the hybrid *Sa×Pp* and parental species samples based on the tooth counts in the lower jaw; hybrid *Sa×Pp* (○), *S. aurata* (□), *P. pagrus* (◇).

The mean canonical values, separate *P. pagrus* from the other two for the upper jaw, but clustered the three samples for the lower jaw (same magnitude, but negatively for *S. aurata* and *P. pagrus* and positively for *Sa×Pp*) (Table LXVI).

Table LXVI - Mean Canonical Values between hybrids and parental species for the upper and lower jaws.

Upper jaw

| | CV1 | CV2 |
|---|---|---|
| *S. aurata* | -0.278 | -1.197 |
| *Sa x Pp* | 0.836 | 0.202 |
| *P. pagrus* | -2.521 | 0.331 |

Lower jaw

| | CV1 | CV2 |
|---|---|---|
| *S. aurata* | -1.979 | -0.696 |
| *Sa x Pp* | 1.071 | -0.014 |
| *P. pagrus* | -1.768 | 0.649 |

### 3.3.3 – *Osteological analysis*

#### 3.3.3.1- *Neurocranium*

The neurocranium was more elongated in *P. pagrus* than in *S. aurata* and in the hybrid (both similar in size) (Figures 33, 34, and 35). The supraoccipital crest was more pronounced in *S. aurata* than in *P. pagrus* and *Sa×Pp*. The ethmoid was similar between the hybrid and *S. aurata*, while in *P. pagrus* it is more elongated and less concave. The

ventral profile of the neurocranium was formed by the vomer, parasphenoid and basioccipital.

The vomer was especially pronounced in *P. pagrus* and less so in *S. aurata*, with the hybrid showing an intermediate shape. The parasphenoid was more pronounced in *S. aurata* than in the *P. pagrus* and *Sa×Pp*. The basioccipital was similar in both parental species and the hybrid. The articulating facet for the anterior condyle of hyomandibula shared similarities between the parental species but was less pronounced in the hybrid. The exoccipital, although similar between samples, shared a higher degree of proximity between *S. aurata* and *Sa×Pp*. While the basisphenoid was more pronounced in *S. aurata*, it was only vestigial in the *P. pagrus* and *Sa×Pp*.
From a frontal view the articulated facet for the palatine and the olfactory foramen were very close, both in the hybrids and *S. aurata*. In *P. pagrus* there was a greater distance between these two structures. Similarities between the hybrid and the parental species are summarized in Table LXVII.

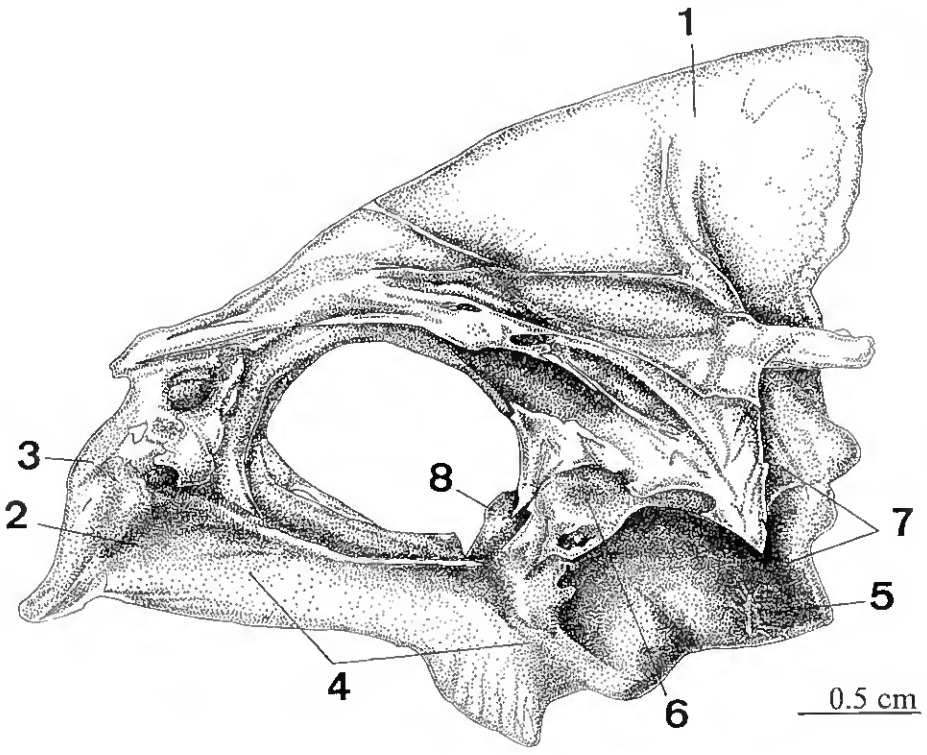



Figure 33 – Gilthead seabream neurocranium. (1) supraoccipital, (2) vomer, (3) ethmoid, (4) parasphenoid, (5) basioccipital, (6) condyle of the hyomandibula, (7) exoccipital, (8) basisphenoid.

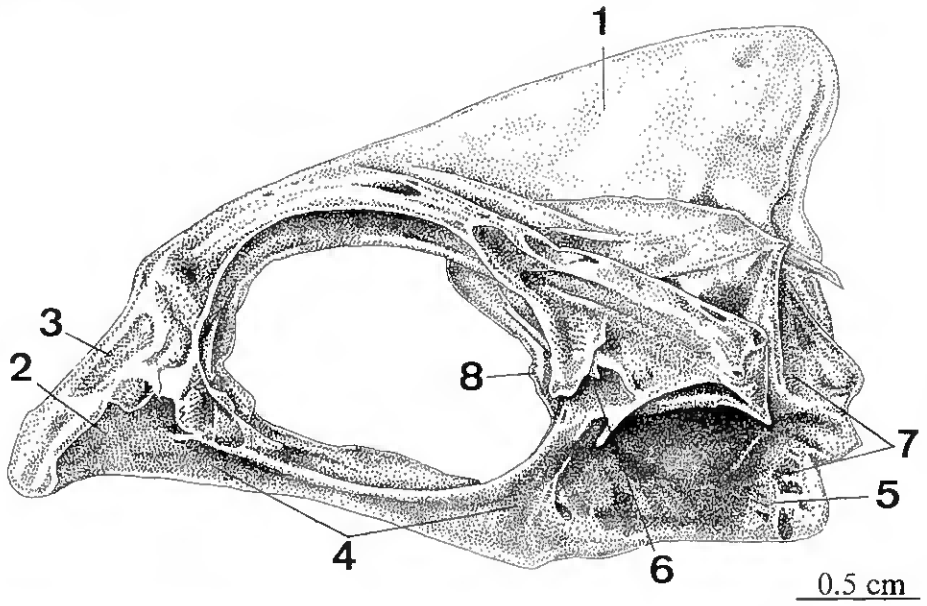



Figure 34 – Red porgy neurocranium. (1) supraoccipital, (2) vomer, (3) ethmoid, (4) parasphenoid, (5) basioccipital, (6) condyle of the hyomandibula, (7) exoccipital, (8) basisphenoid.

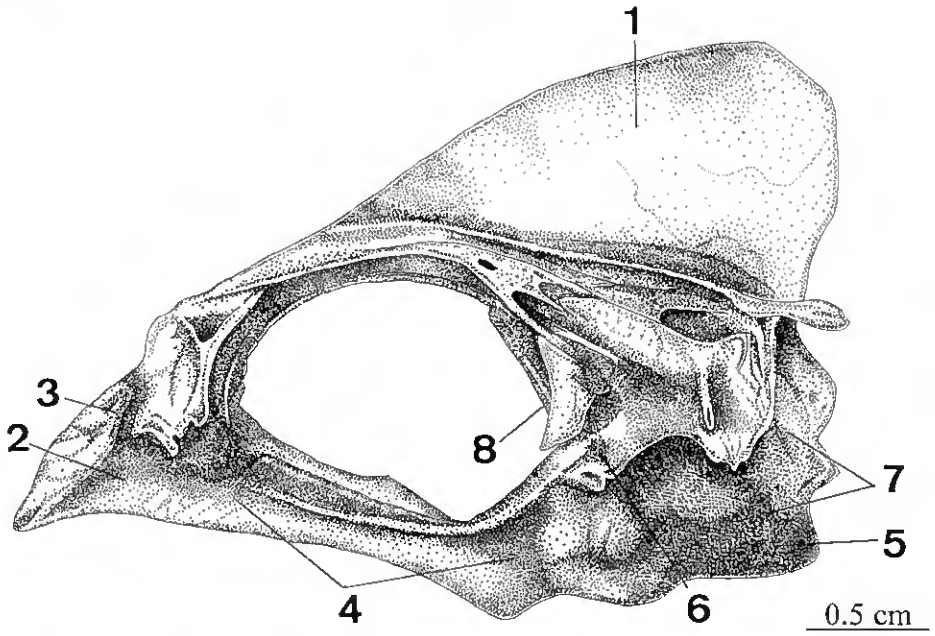



Figure 35 – Hybrid *Sa×Pp* neurocranium. (1) supraoccipital, (2) vomer, (3) ethmoid, (4) parasphenoid, (5) basioccipital, (6) condyle of the hyomandibula, (7) exoccipital, (8) basisphenoid.

Table LXVII - Shared similarities of the neurocranial sub-structures between the parental species and hybrids. + - Denote higher similarity.

| | *S. aurata* | *Sa×Pp* | *P.pagrus* |
|---|---|---|---|
| Neurocranium | | + | + |
| Supraocippital | | + | + |
| Vomer | | + | + |
| Ethmoid | + | + | |
| Parasphenoid | | + | + |
| Basiooccipital | - | - | - |
| Condyle of hyomandibula | + | | + |
| Exoccipital | + | + | |
| Basisphenoid | | + | + |

### 3.3.3.2 - *Lacrimal bones*

The lacrimal is the first of the infraorbital bones, forming the anterior part of the maxillary sheath. It is rectangular in shape, deeper than wide, with a projection at the upper anterior corner, which is attached to the lateral ethmoid. The upper part of this bone is usually well ossified and the lower half consists of a thin lamina. The shape of this bone is very different in *S. aurata* and *P. pagrus* (Figure 36).

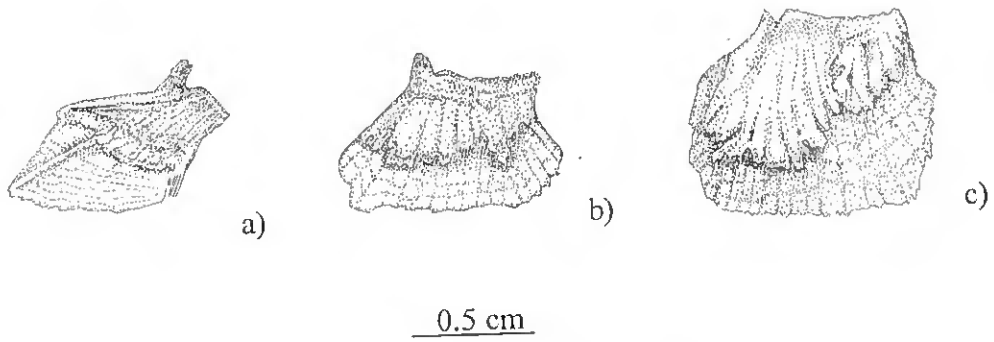



Figure 36 – Lacrimal bones of: a) *S. aurata*, b) Hybrid *Sa×Pp*, c) *P. pagrus*

The hybrid incorporates the two major differences presented by the progenitors in that it is well ossified like *P. pagrus* and the anterior tip is quite elongated like in the *S. aurata*.

### 3.3.3.3 – *Opercle*

The opercle presented a greater degree of similarity between *P. pagrus* and *Sa×Pp*. The upper two left crests are very similar (Figure 37). The areas A and B are also very similar.

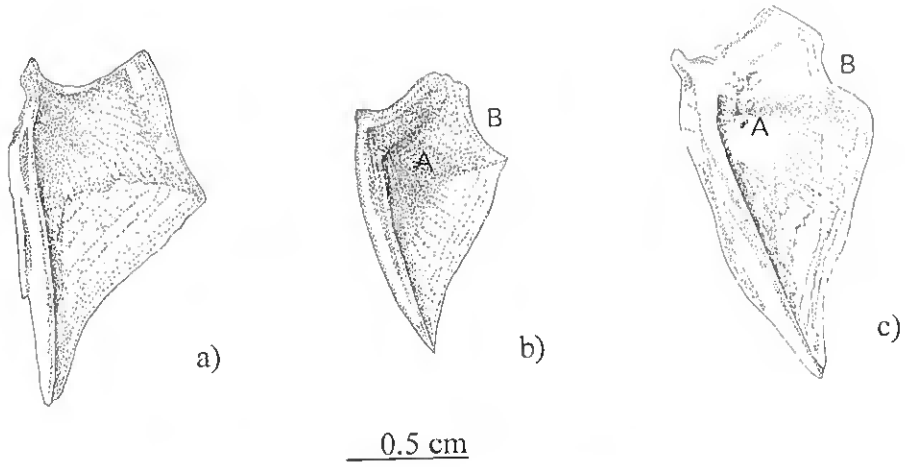



Figure 37 – Opercle bones of: a) *S. aurata*, b) Hybrid *Sa×Pp*, c) *P. pagrus*.

### 3.3.3.4 – *Subopercle*

This bone is similar in *P. pagrus* and *Sa×Pp* (Figure 38). The greater ossification in area A is an evidence of it.

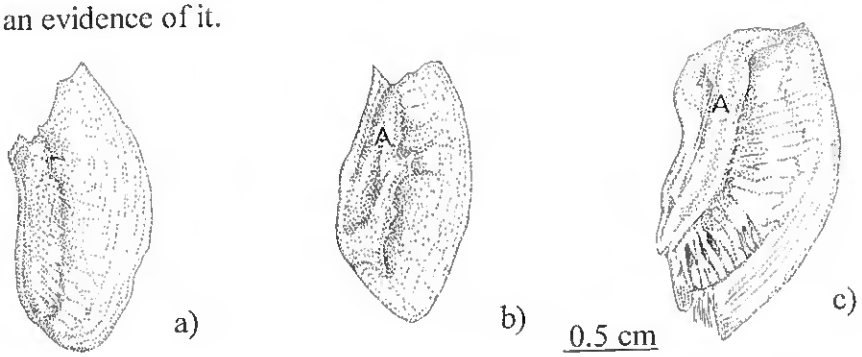



Figure 38 – Subopercle bones of: a) *S. aurata*, b) Hybrid *Sa×Pp*, c) *P. pagrus*

### 3.3.3.5 – *Interopercle*

The interopercle shows a higher degree of similarity between hybrids and *P. pagrus* (Figure 39).

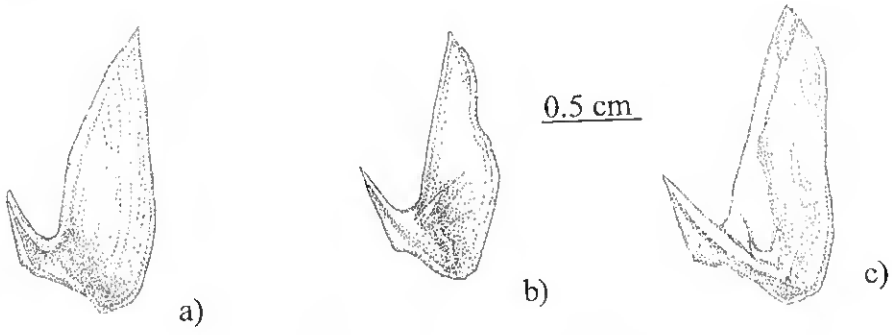



Figure 39 – Interopercle bones of: a) *S. aurata*, b) Hybrid *Sa×Pp*, c) *P. pagrus*.

### 3.3.3.6 – *Preopercle*

As in the case of the previous structures, there is a higher degree of similarity between hybrids and *P. pagrus* (Figure 40).

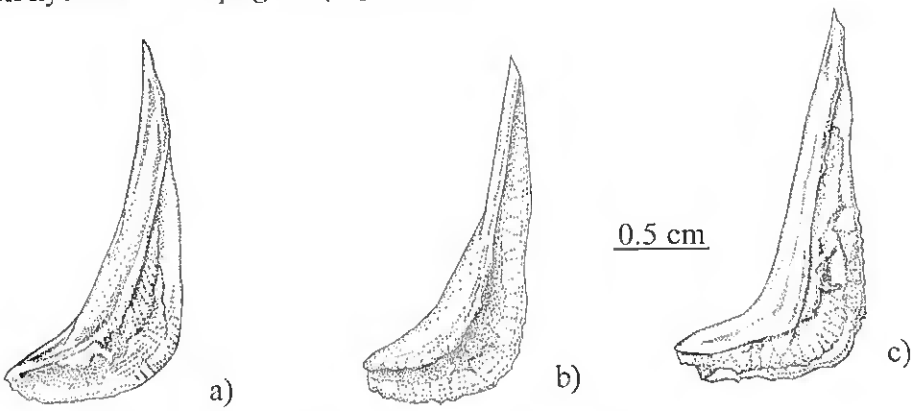



Figure 40 – Preopercle bones of: a) *S. aurata*, b) Hybrid *Sa×Pp*, c) *P. pagrus*.

### 3.3.3.7 - *Maxilla*

It is almost concealed by the subocular sheath and lies along the upper half of the premaxilla ramus, posteriorly extending beyond it. It is formed by an outer lamina and inner upper and inner lower projections, anteriorly located, forming respectively a groove for the articulation with the palatine and one for the premaxilla. These processes are quite strong both in *P. pagrus* and hybrids, but are weaker in *S. aurata* (Figure 41).

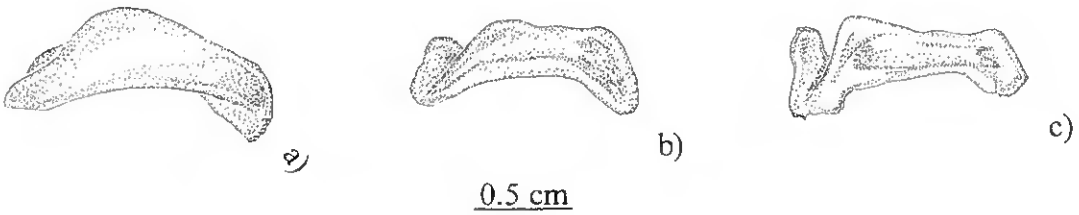



Figure 41 – Maxilla bones of: a) *S. aurata*, b) Hybrid *Sa×Pp*, c) *P. pagrus.*

### 3.3.3.8 – *Premaxilla*

It is composed of a horizontal ramus bearing teeth and vertical peticel, the latter usually shorter than or as long as the former. In *S. aurata* the ramus is particularly stout when compared with *P. pagrus* and *Sa×Pp* (Figure 42), because of the large number of molars and the presence of one very large tooth posteriorly on each side.

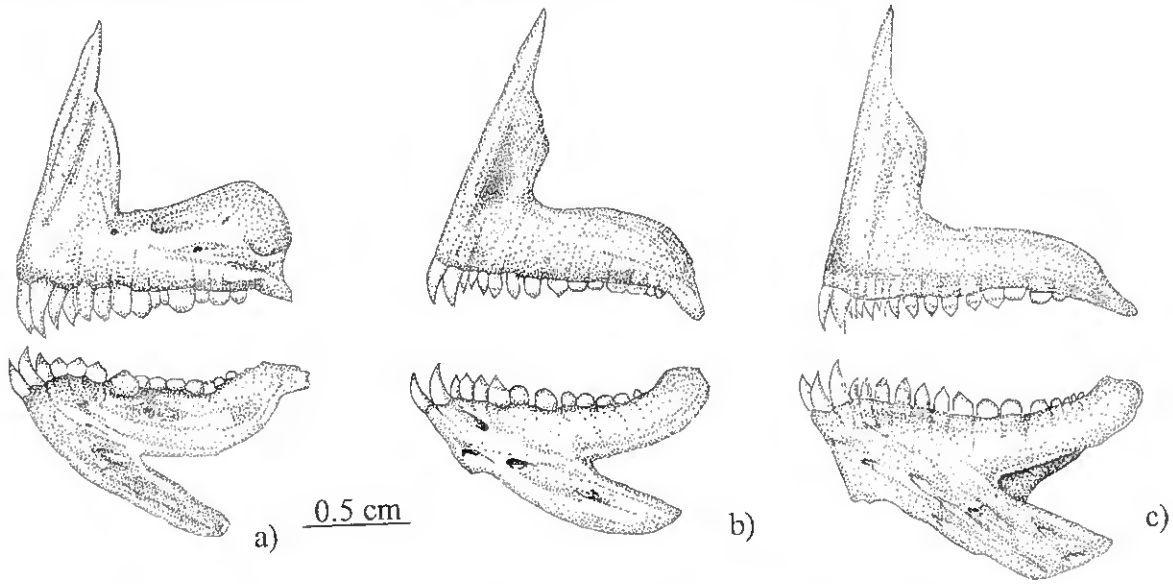



Figure 42 – Premaxilla (upper) and dentary (lower) bones of: a) *S. aurata*, b) Hybrid *Sa×Pp*, c) *P. pagrus.*

**3.3.3.9 – *Dentary***

It is formed by an upper, horizontal dentigerous branch and oblique ventral branch. At the corner formed by the two arches a pocket fits the triangular anterior projection of the angular. Again there is a higher degree of similarity between *P. pagrus* and *Sa×Pp* (Figure 42). Such similarity lies in the number of molar rows, which is smaller in *P. pagrus* and hybrids.

**3.3.3.10 - *Articular***

This bone fits in the pocket formed between the horizontal dentigerous branch and oblique ventral branch of the dentary. The inferior tip (a) of the articular bones is similar in *Sa×Pp* and *P. pagrus* (Figure 43). Such tips have sharpened configurations than *S. aurata*.

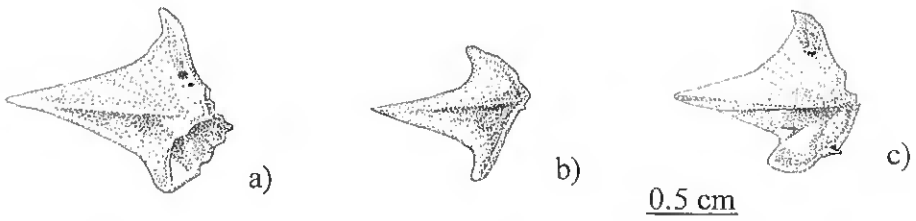



Figure 43 – Articular bones of: a) *S. aurata*, b) Hybrid *Sa×Pp*, c) *P. pagrus*.

The above-described characteristics are summarized in Table LXIII. It is clear that all the head bones (apart from the neurocranium) share a higher degree of similarity with *P. pagrus*.

Table LXVIII - Shared similarities of the osteological analysis between the parental species and hybrids. + - Denote greater similarity.

| | *S. aurata* | *Sa×Pp* | *P.pagrus* |
|---|---|---|---|
| Lacrimal bones | | + | + |
| Opercle | | + | + |
| Subopercle | | + | + |
| Interopercle | | + | + |
| Pre-opercle | | + | + |
| Maxilla | | + | + |
| Premaxilla | | + | + |
| Dentary | | + | + |
| Articular | | + | + |

### 3.3.4 – *Morphometric analysis*

#### 3.3.4.1 – *Traditional method*

Descriptive statistics for each of the characteristics is presented in Annex III, Table X. Mean character values of the hybrids were intermediate for 7 of the 12 characters (PSL, HD, UJL, DFL, AFL and LPA).

A total of 97.61% of the total variation associated with the 14-morphometric characters was accounted for by the first five principal components, both for hybrid and parental species. PC1 loadings explained 88.85% of the total variation (Table LXIX).

Table LXIX - Loadings from principal component analysis of the 12 morphometric characters for both hybrids and parental species. Loadings are listed for the five principal components. Character descriptors refer to figure 2. (Exp. Var. - Explained variance).

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| MFH | 0.983 | 0.004 | 0.007 | 0.109 | -0.022 |
| POL | 0.975 | 0.046 | 0.123 | -0.012 | -0.022 |
| OOL | 0.813 | -0.577 | 0.000 | -0.043 | 0.002 |
| PSL | 0.963 | 0.045 | -0.036 | 0.055 | 0.191 |
| HL | 0.979 | -0.139 | -0.013 | 0.013 | 0.043 |
| HD | 0.955 | 0.111 | -0.079 | -0.134 | 0.148 |
| UJL | 0.955 | 0.216 | 0.030 | -0.057 | 0.013 |
| LJL | 0.914 | 0.050 | 0.340 | -0.156 | -0.105 |
| CPL | 0.889 | 0.059 | -0.412 | -0.105 | -0.136 |
| DFL | 0.963 | 0.071 | 0.075 | 0.150 | -0.043 |
| LPA | 0.966 | 0.035 | -0.051 | 0.153 | -0.083 |
| AFL | 0.974 | 0.032 | -0.046 | 0.136 | -0.052 |
| Exp. Var. | 9.77 | 0.43 | 0.32 | 0.12 | 0.10 |
| % of total | 88.85 | 3.89 | 2.88 | 1.09 | 0.90 |

As in the case of the previous species analysis, the PC1 loadings were similar in size and sign indicating it was a general measure of fish size (Bookstein *et al.*, 1985). This component was significantly correlated with the standard length (SL) ($r$ = 0.99; $p$<0.05; N =566).

Nine measurements assured sample differentiation (LJL, OOL, DFL, LPA, POL, UJL, MFH, CPL and HL) (Table LXX).

Stepwise discriminant function analysis indicated highly significant differences based on the chosen measurements among the three samples (*Sa×Pp*, *S. aurata* and *P. pagrus*) (Wilks' $\lambda$ =0.027, F(18, 1106)=310.1; $p$<0.001). Five of the characteristics contributed largely to the sample differentiation with F-remove values over 50.

Graphically, the canonical discriminant-factor scores also display a clear sample differentiation (Figure 44).

Table LXX - Stepwise Discriminant Analysis for the traditional morphology comparison between the hybrid and the parental species. (Wilks' $\lambda$= 0.027, $F(18,1106)=310.10$ $p<0.001$).

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| LJL | 0.047 | 0.584 | 197.037 | 0.001 |
| OOL | 0.038 | 0.716 | 109.570 | 0.001 |
| DFL | 0.037 | 0.745 | 94.752 | 0.001 |
| LPA | 0.034 | 0.800 | 69.211 | 0.001 |
| POL | 0.033 | 0.828 | 57.625 | 0.001 |
| UJL | 0.032 | 0.861 | 44.814 | 0.001 |
| MFH | 0.031 | 0.891 | 33.763 | 0.001 |
| CPL | 0.030 | 0.924 | 22.682 | 0.001 |
| HL | 0.028 | 0.977 | 6.481 | 0.002 |

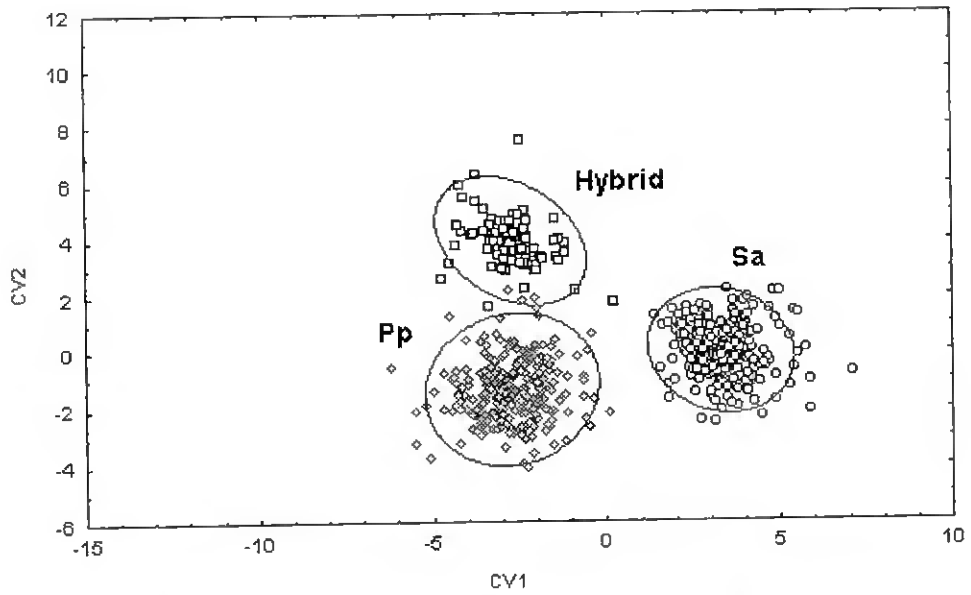



Figure 44 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the hybrids *Sa*×*Pp*, and parental species samples based on the traditional morphometry; *S. aurata* (○), hybrid *Sa*×*Pp* (□), *P. pagrus* (◇).

The overall percent-correct classification success (PCS) was 100%, for *S. aurata* and hybrid, and 98.7% for *P. pagrus* (Table LXXI).

Table LXXI - Percent classification success (PCS) for the linear discriminant functions for all traditional morphometric characters for the hybrid and parental species.

| | PCS | *S. aurata* | Hybrid *Sa*x*Pp* | *P. pagrus* | N |
|---|---|---|---|---|---|
| *S. aurata* | 100 | 256 | 0 | 0 | 256 |
| Hybrid *Sa*x*Pp* | 100 | 0 | 80 | 0 | 80 |
| *P. pagrus* | 98.70 | 0 | 3 | 228 | 231 |
| Mean | 99.47 | | | | |

Generalised Mahalanobis distances ($D^2$) and F-statistics are presented in Table LXII. $D^2$ values between hybrids and *S. aurata* were higher (48.97) than between hybrids and *P. pagrus* (28.1). The smaller distance between hybrids and *P. pagrus* is reflected in the mean canonical values, with those two samples correlating similarly with CV1 (negatively and with similar magnitude) (Table LXXIII).

Table LXXII - Generalised Mahalanobis distances ($D^2$), and the F-statistics for the linear discriminant functions for all truss characters for the hybrid and parental species. Asterisks denote *- $p<0.01$ (F-values; df = 9,553).

| | Statistic | *S. aurata* | Hybrid *Sa* x *Pp* | *P. pagrus* |
|---|---|---|---|---|
| *S. aurata* | $D^2$ | - | | |
| | *F* | - | | |
| Hybrid *Sa* x *Pp* | $D^2$ | 48.97 | - | |
| | *F* | 314.04* | - | |
| *P. pagrus* | $D^2$ | 36.09 | 28.08 | - |
| | *F* | 477.96* | 175.67* | - |

Table LXXIII - Means of canonical variables for the hybrids and the parental species.

| | CV1 | CV2 |
|---|---|---|
| *S. aurata* | 3.178 | -0.031 |
| Hybrid *Sa* x *Pp* | -2.529 | 3.987 |
| *P. pagrus* | -2.679 | -1.295 |

### 3.3.4.2 – *Truss method*

A total of 94.28% of the total variation associated with the 31-morphometric characters was accounted for by the first five principal components, both for hybrid and parental species. PC1 loadings explained 81.48 % of the total variation (Table LXXIV). Again, PC1 loadings were similar in size and sign indicating it was a general measure of fish size (Bookstein *et al.*, 1985). Only three measurements (A3, D2 and F3) did not correlate with PC1, instead correlating with PC2. This component was significantly correlated with the standard length (SL) ($r$ = 0.91; $p<0.05$; N =566). PC2 and PC3 accounted for a much smaller percentage of variation (Table LXVIII), not correlating with SL, and were used to interpret the nature of the morphometric variation. The loadings of each of these components varied in magnitude, indicating that they were

shape related components. For PC2 the largest loadings were in the head and the caudal peduncle related measurements, which are indicative of differential growth of these body regions compared to rest of the body. The remaining components, PC3-PC5, was similar to PC2, but with lower magnitudes.

Table LXXIV- Loadings from principal component analysis of the 31 morphometric characters for both hybrids and parental species. Loadings are listed for the five principal components. Character descriptors refer to Figure 3. (Exp. Var. - Explained variance).

| Descriptor | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| A1 | 0.946 | 0.097 | 0.097 | -0.001 | 0.130 |
| A2 | 0.937 | 0.196 | -0.064 | 0.037 | -0.137 |
| A3 | 0.080 | 0.924 | 0.179 | 0.063 | -0.203 |
| A4 | 0.970 | 0.050 | 0.153 | -0.051 | 0.009 |
| A5 | 0.973 | 0.128 | 0.039 | 0.004 | 0.020 |
| A6 | 0.891 | -0.065 | 0.336 | -0.118 | -0.095 |
| B1 | 0.745 | -0.157 | -0.335 | 0.295 | -0.187 |
| B2 | 0.850 | -0.188 | -0.346 | -0.022 | -0.120 |
| B3 | 0.991 | -0.023 | -0.019 | -0.041 | -0.010 |
| B4 | 0.988 | -0.005 | -0.034 | 0.019 | 0.039 |
| B5 | 0.984 | 0.041 | -0.017 | -0.027 | -0.086 |
| C1 | 0.700 | 0.116 | 0.580 | -0.011 | -0.121 |
| C2 | 0.829 | 0.235 | -0.048 | 0.080 | -0.030 |
| C3 | 0.991 | -0.005 | -0.011 | -0.037 | 0.037 |
| C4 | 0.991 | -0.026 | -0.010 | -0.039 | -0.014 |
| C5 | 0.992 | 0.005 | 0.004 | -0.039 | 0.024 |
| D1 | 0.925 | 0.002 | -0.215 | 0.091 | -0.001 |
| D2 | 0.688 | -0.513 | 0.088 | -0.423 | 0.055 |
| D3 | 0.990 | -0.014 | -0.021 | -0.015 | 0.047 |
| D4 | 0.992 | -0.035 | -0.024 | -0.026 | -0.001 |
| D5 | 0.981 | 0.015 | -0.068 | -0.020 | 0.048 |
| E1 | 0.953 | -0.150 | -0.027 | -0.060 | -0.166 |
| E2 | 0.905 | -0.037 | -0.139 | -0.208 | -0.256 |
| E3 | 0.932 | 0.139 | -0.025 | 0.109 | 0.261 |
| E4 | 0.989 | -0.038 | -0.034 | -0.014 | 0.016 |
| E5 | 0.993 | -0.012 | -0.037 | 0.000 | 0.018 |
| F1 | 0.931 | 0.124 | 0.039 | 0.045 | 0.192 |
| F2 | 0.928 | -0.073 | 0.080 | 0.170 | 0.062 |
| F3 | 0.238 | -0.773 | 0.376 | 0.392 | -0.107 |
| F4 | 0.973 | 0.057 | 0.004 | 0.087 | 0.061 |
| F5 | 0.960 | 0.116 | 0.022 | 0.063 | 0.159 |
| Exp. Var. | 25.26 | 2.00 | 0.99 | 0.57 | 0.41 |
| % of total | 81.48 | 6.45 | 3.19 | 1.83 | 1.33 |

Stepwise discriminant function analysis on truss data indicated highly significant differences in the truss element distances among the three samples (*Sa*×*Pp*, *S. aurata* and *P. pagrus*) (Wilks' $\lambda$ =0.0424, F(32.109)=131.3; $P$<0.001). Sixteen truss elements

were selected for sample differentiation (A1, A2, A6, B1, B2, B4, B5, C1, C4, D1, D3, D4, E1, E2, E3, E5, F3) (Table LXXV). Although graphical visualisation based on the discriminant analysis proved to be slightly less precise than the traditional morphometry, it is still recommendable (Figure 45).

Table LXXV - Stepwise Discriminant Analysis for the truss comparison between the hybrid and the parental species.
(Wilks λ=0.04243; F (32,109)=131.30; $p$ <0.001).

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| B5 | 0.065 | 0.652 | 145.728 | 0.001 |
| E1 | 0.059 | 0.723 | 104.204 | 0.001 |
| E3 | 0.050 | 0.845 | 49.914 | 0.001 |
| D4 | 0.048 | 0.878 | 37.919 | 0.001 |
| C4 | 0.047 | 0.896 | 31.763 | 0.001 |
| A6 | 0.047 | 0.904 | 28.980 | 0.001 |
| E2 | 0.046 | 0.929 | 20.747 | 0.001 |
| A1 | 0.045 | 0.940 | 17.403 | 0.001 |
| F3 | 0.045 | 0.947 | 15.350 | 0.001 |
| A2 | 0.045 | 0.949 | 14.769 | 0.001 |
| D1 | 0.044 | 0.967 | 9.175 | 0.001 |
| E5 | 0.044 | 0.973 | 7.637 | 0.001 |
| C1 | 0.044 | 0.974 | 7.281 | 0.001 |
| D3 | 0.044 | 0.975 | 6.916 | 0.001 |
| B2 | 0.043 | 0.980 | 5.655 | 0.004 |
| B4 | 0.043 | 0.980 | 5.500 | 0.004 |

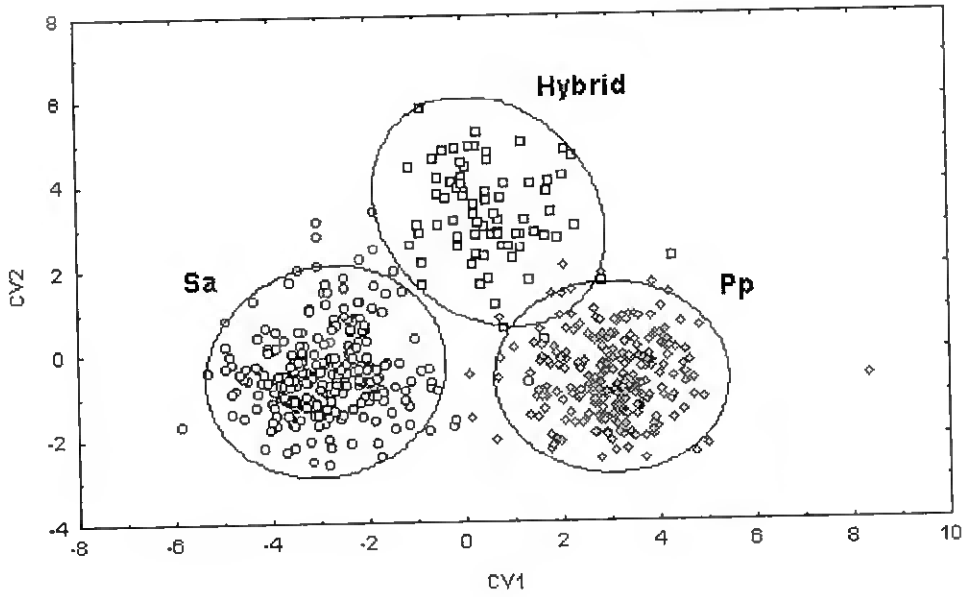



Figure 45 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the hybrids *Sa*×*Pp*, and parental species samples based on the truss network; *S. aurata* (○), hybrid *Sa*×*Pp* (□), *P. pagrus* (◇).

Percent-correct classification success (PCS) was higher for the progenitors, 98.83 and 99.19% for *S. aurata* and *P. pagrus*, respectively. Hybrids PCS was slightly worse with 94.74 % (Table LXXVI).

Table LXXVI - Percent classification success (PCS) for the linear discriminant functions for all truss network for the hybrid and parental species.

| | PCS | *S. aurata* | Hybrid *Sa*x*Pp* | *P. pagrus* | N |
|---|---|---|---|---|---|
| *S. aurata* | 98.83 | 253 | 2 | 1 | 256 |
| Hybrid *Sa*x*Pp* | 95.00 | 0 | 76 | 4 | 80 |
| *P. pagrus* | 99.13 | 1 | 1 | 229 | 231 |
| Mean | 98.4 | | | | |

Generalised Mahalanobis distances ($D^2$) were slightly smaller between hybrids and *S. aurata* (25.73) than between the hybrids and *P. pagrus* (21.91). F-statistics varied accordingly (Table LXXVII).

Table LXXVII - Percent classification success (PCS), generalised Mahalanobis distances ($D^2$), and the F-statistics for the linear discriminant functions for all truss characters for the hybrid and parental species. Asterisks denote *- $p<0.01$ (F-values; df = 16,545 ).

| Country | Statistic | *S. aurata* | *Sa*x*Pp* | *P. pagrus* |
|---|---|---|---|---|
| *S. aurata* | $D^2$ | - | | |
| | *F* | - | | |
| Hybrid *Sa* x *Pp* | $D^2$ | 25.73 | - | |
| | *F* | 90.69* | - | |
| *P. pagrus* | $D^2$ | 35.16 | 21.91 | - |
| | *F* | 258.59* | 75.38* | - |

CV1 separated the *P. pagrus* sample from the other two, whereas CV2 isolated the hybrids from the parental species (Table LXXVIII).

Table LXXVIII - Means of canonical variables for the hybrids and the parental species.

| | CV1 | CV2 |
|---|---|---|
| *S. aurata* | -2.886 | -0.415 |
| Hybrid *Sa*x*Pp* | 0.530 | 3.316 |
| *P. pagrus* | 3.024 | -0.631 |

Sample differentiation provided worse results, with the percent of correct classification of the hybrids sample dropping to 65.79%, and the *S. aurata* and *P. pagrus* to 90.63 and 92.64%, respectively.

In the stepwise discriminant analysis, two body areas Ar5 and Ar6 contributed largely to the sample differentiation, (Table LXXIX). $D^2$ varied in the same proportion as the truss elements, with a shorter distance between *Sa*×*Pp* and *S. aurata* (Table LXXX).

Table LXXIX - Stepwise Discriminant Analysis for area comparison between the hybrid and the parental species.
(Wilks λ= 0.212; F (12.111)=108.25; $p$ <0.001).

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| A5 | 0.362 | 0.587 | 195.562 | 0.01 |
| A6 | 0.346 | 0.613 | 175.058 | 0.01 |
| A2 | 0.276 | 0.769 | 83.453 | 0.01 |
| A1 | 0.249 | 0.853 | 47.751 | 0.001 |
| A4 | 0.235 | 0.904 | 29.457 | 0.001 |
| A3 | 0.215 | 0.985 | 4.154 | 0.016 |

This low differentiation between can be clearly seen in the graphical representation of the discriminant analysis (Figure 46).

Table LXXX - Generalised Mahalanobis distances ($D^2$), and the F-statistics for the linear discriminant functions for the area measurements for the hybrid and parental species. Asterisks denote *- $p$ <0.01 (F-values; df = 6.555).

| | Statistic | *S. aurata* | *Sa* x *Pp* | *P. pagrus* |
|---|---|---|---|---|
| *S. aurata* | $D^2$ | - | | |
| | *F* | - | | |
| Hybrid *Sa* x *Pp* | $D^2$ | 4.28 | - | |
| | *F* | 41* | - | |
| *P. pagrus* | $D^2$ | 11.02 | 8.66 | - |
| | *F* | 220.17* | 80.92* | - |

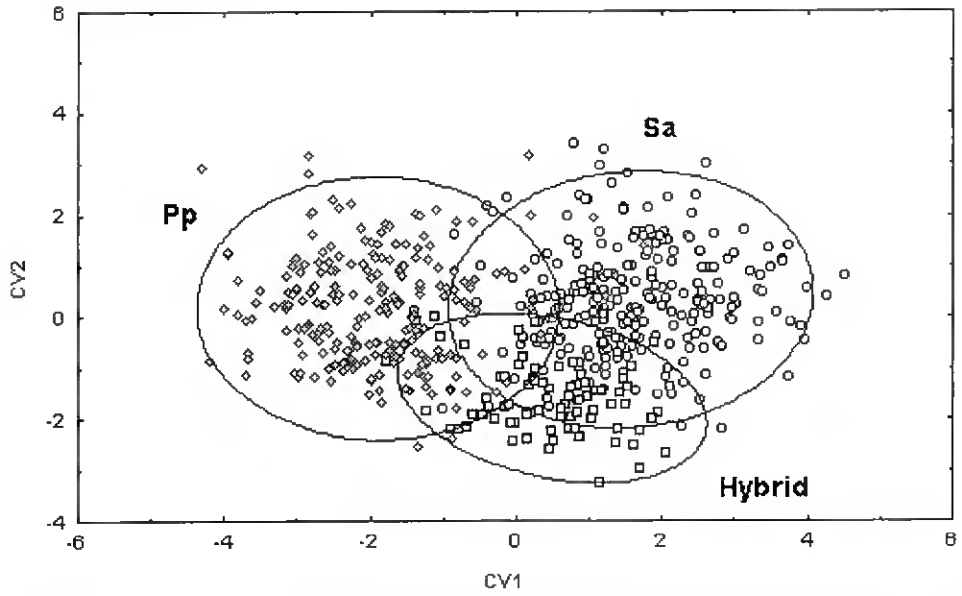



Figure 46 – Bivariate mean 95% confidence ellipsoids for the canonical discriminant-factor scores of the hybrids *Sa*×*Pp*, and parental species samples based on the area analysis; *S. aurata* (○), hybrid *Sa*×*Pp* (□), *P. pagrus* (◊).

### 3.3.5 – *Growth experiments*

#### 3.3.5.1 - *Biological performance parameters*

No statistical differences were found within tanks of the same food (ANOVA, $p>0.05$).

Best results for weight increase were obtained with food A in the first period (38.43±11.68g), and with food B in the second (32.67±12.31g). Lowest values were obtained with Food C in both periods: 33.81±13.35g and 18.01±6.41g, respectively (Figure 41).

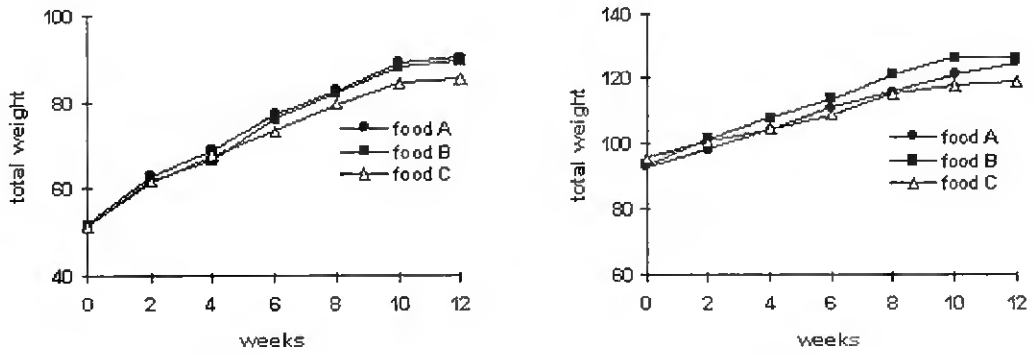



Figure 41 - Total weight increase for each diet during a) the first and b) the second period of experiment.

The analysis of the values calculated for specific growth rate (SGR), growth rate (GR), conversion index (CI) and daily feeding rate (DFR) (Table LXXXI) indicates that food A resulted in better growth at the end of the experiments while presented the best results at the beginning of the experiments were obtained with B. Nevertheless, these were similar to results obtained with food A at the end. Food C always presented the worst results.

Table LXXXI - Mean values and standard deviations calculated for growth rate (GR), specific growth rate (SGR), daily feeding rate (DFR) and conversion index (CI) for foods A, B and C.

1st Period

| | Food A | | Food B | | Food C | |
|---|---|---|---|---|---|---|
| | beggining | end | beggining | end | beggining | end |
| GR | 9.41±0.84 | 7.8±0.24 | 9.46±0.92 | 7.86±0.23 | 9.33±0.71 | 7.79±0.37 |
| SGR | 1.41±0.44 | 0.19±0.21 | 1.45±0.64 | 0.28±0.2 | 1.36±0.54 | 0.16±0.34 |
| DFR | 2.2±0.3 | 0.99±0.16 | 2.53±0.06 | 0.82±0.11 | 2.14±0.41 | 0.86±0.4 |
| CI | 1.61±0.2 | 5.47±2.6 | 1.85±0.26 | 3.16±1.07 | 1.63±0.09 | 8.3±5.96 |

2nd Period

| | Food A | | Food B | | Food C | |
|---|---|---|---|---|---|---|
| | beggining | end | beggining | end | beggining | end |
| GR | 8.2±0.51 | 7.84±0.13 | 8.36±0.36 | 7.77±0.17 | 8.08±0.35 | 7.79±0.37 |
| SGR | 0.44±0.49 | 0.25±0.21 | 0.57±0.31 | 0.15±0.15 | 0.35±0.32 | 0.12±0.14 |
| DFR | 0.84±0.05 | 0.44±0.03 | 1.05±0.04 | 0.47±0.01 | 0.88±0.03 | 0.41±0.05 |
| CI | 2.18±0.83 | 3.41±0.42 | 1.9±0.6 | 6.72±1.7 | 2.82±1.53 | 4.89±1.19 |

The similarity between food A and B was shown by the lack of significant differences between them ($p>0.876$ and $p>0.298$ in the first and in the second periods, respectively). In contrast, food A and food B were significantly different from food C (food A/food C, $p<0.009$ and $p<0.001$, food B/food C, $p<0.02$ and $p<0.01$ in the first and second periods of analysis, respectively). This analysis is corroborated by the results of Tukey HSD test: food A/food C with $p<0.045$ and food B/food C with $p<0.042$.

The regressions of the length/weight relationships at the beginning and at the end of each period were significantly different for all the foods in the first period ($t<0.05$) and significantly different for food A ($t<0.05$) during the second period. Opposite results were obtained for food B and food C during the same experiments (Figure 47 and Figure 48).

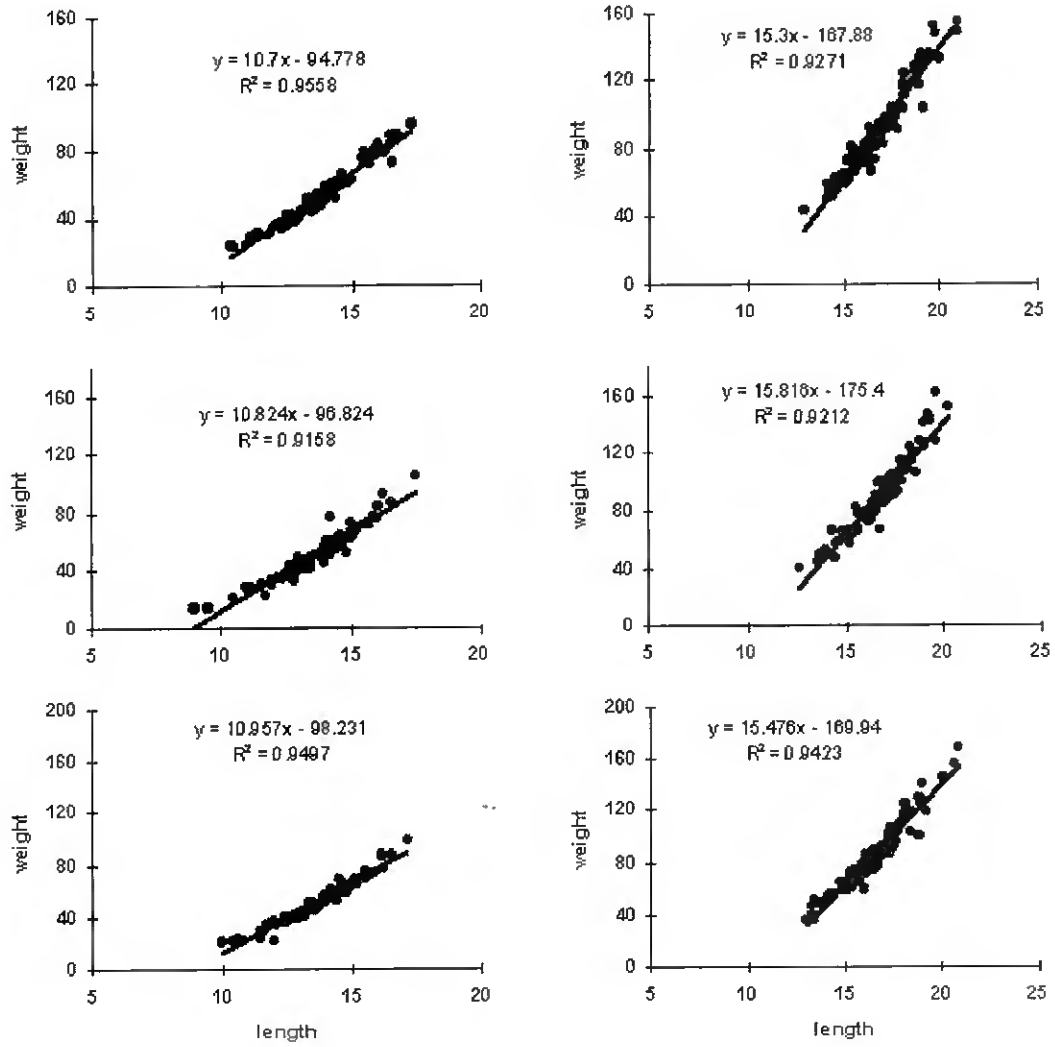



Figure 47 - Comparison of the regressions of the length/weight relationships at the start and at the end of the first period for food A ($a_1$,$a_2$), food B ($b_1$,$b_2$) and food C ($c_1$,$c_2$).

The analysis of carcass composition indicated a marked decrease of the water content and ashes in the tissues for the three diets. In contrast, there was an increase of the body content in proteins (except for food C/first period) and lipids (except for food B/first period) in both experiments (Table LXXXII).

The HSI increased throughout both experiments, and MI decreased with all food types (except for food B/second period) (Table LXXXII).

The results of the blood analysis are presented in Table LXXXIII. Protein levels showed significant differences between the initial and final values in both experiments ($p$<0.05), except for food C, where no significant differences were found ($p$>0.05). Significant differences were found between food A and food C, and between food B and

food C ($p$<0.05) in the first period. In the second period no significant differences were found between foods ($p$>0.05).

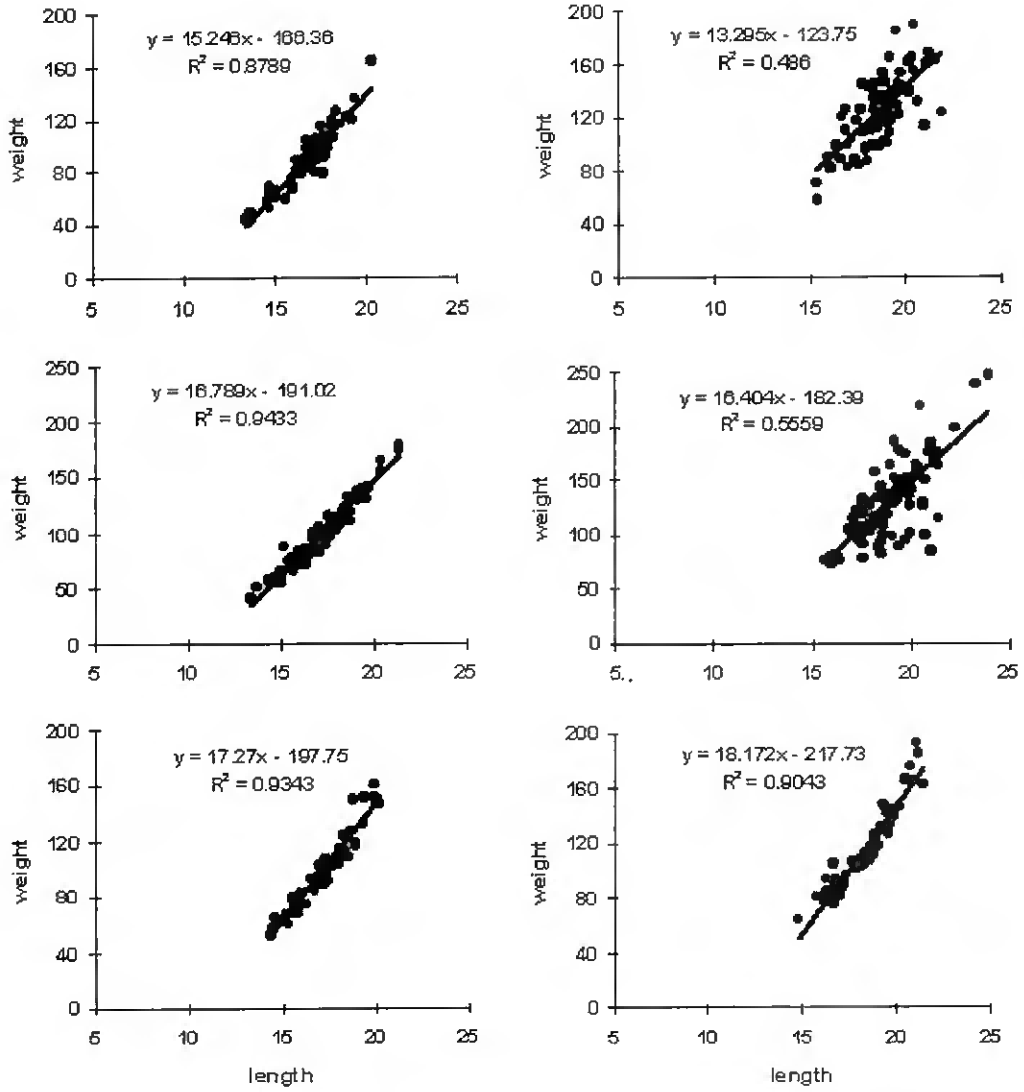



Figure 48 - Comparison of the regressions of the length/weight relationships at the start and at the end of the second period for food A ($a_1$,$a_2$), food B ($b_1$,$b_2$) and food C ($c_1$,$c_2$).

Glucose levels were significantly different in the beginning and at the end of the experiments, for both periods ($p$<0.05). However, no significant differences were found between food types, in both periods ($p$>0.05). Lipid concentration in the blood showed no significant differences between the beginning of the experiments and food A ($p$>0.05). Significant differences were found between food A and B and A and C at the end of the first period ($p$<0.05). In the second period, no significant differences were found between the initial and the final values for each food type and between food types ($p$>0.05).

Table LXXXII - Carcass composition of the sampled fish at the start and at the end of each period, for each food.

1st Period

| | | Food A | Food B | Food C |
|---|---|---|---|---|
| | beginning | end | end | end |
| Water | 68.8 | 63.62 | 60.73 | 67.01 |
| Proteins | 22 | 22.2 | 25.7 | 20.1 |
| Lipids | 9.2 | 11 | 9.1 | 9.85 |
| Ashes | 3.25 | 3.06 | 3.06 | 3 |
| HIS | 2.53 | 2.8 | 3 | 3.19 |
| MI | 0.78 | 0.66 | 0.41 | 0.67 |

2nd Period

| | | Food A | Food B | Food C |
|---|---|---|---|---|
| | beginning | end | end | end |
| Water | 69.97 | 67.2 | 66.19 | 65.39 |
| Proteins | 20.5 | 22.7 | 22.1 | 21.7 |
| Lipids | 6.2 | 7.2 | 8.8 | 9.9 |
| Ashes | 3.33 | 2.91 | 2.99 | 3.09 |
| HIS | 3.09 | 4.11 | 4.51 | 4.1 |
| MI | 0.51 | 0.3 | 0.58 | 0.51 |

Table LXXXIII – Levels of proteins, glucose and lipids in the blood of the hybrids

| | | beginning | final | | |
|---|---|---|---|---|---|
| | | | Food A | Food B | Food C |
| 1st period | proteins (g%) | 4.17 | 6.31 | 5.95 | 5.09 |
| | glucose (mmoles/l) | 1.44 | 3.14 | 3.38 | 2.38 |
| | lipids (mg%) | 1224.86 | 1592.69 | 2427.63 | 2356.85 |

| | | beginning | final | | |
|---|---|---|---|---|---|
| | | | Food A | Food B | Food C |
| 2nd period | proteins (g%) | 2.63 | 3.92 | 4.01 | 3.9 |
| | glucose (mmoles/l) | 2.64 | 4.36 | 3.35 | 3.95 |
| | lipids (mg%) | 2805.82 | 2662.1 | 3315.41 | 2540.18 |

### 3.4 – DISCUSSION

It is considered as common knowledge, that the hybrids present intermediate characteristics when compared to parental species. In fact, Hubbs (1955) stated that: "It has proved to be an almost universally valid rule that natural interspecific hybrids are intermediate between their parental species in all characters in which those species differ…". Nevertheless, this is a straightforward perspective, which reduces the importance of the genetic heritage of the parents, and most of all, level it to similar proportions, that further on will be transmitted to the hybrid. In some cases, the transmitted genes of one of the progenitors that regulate the phenotypic occurrence of a characteristic overlap the genes of the other progenitor with the same results as a dominant/recessive gene within a species, increasing the hybrid similarity with one of the progenitors, suggesting that codominance of genetic input to these variables is unlikely. This opinion is corroborated by several authors (e.g. Alm, 1955; Simon & Noble, 1968; Kerby, 1979) where in some hybrids particular characters more closely resemble those of one parent or the other, or are unlike those of either parents. In this study, depending on the characters analysed, similar results were obtained.

From the meristic point of view, the hybrid *Sa*×*Pp* did not express a strong phenotypic dissimilarity to the progenitors. The meristic analysis of the number of rays in each of the analysed fins as well as the gill rakers only exposed intermediate mean values in two characteristics, while the others were skewed towards the characteristic of the parental species. Most of the time, dissimilarity between parental species induces high variance rates in the hybrids. In the present case, due to the relative similarity of the meristic traits of the progenitors, the hybrids did not express high variance. In some of the cases, it was even smaller than the parental species. As a consequence of the meristic similarity between hybrids and progenitors, it was not possible to establish a clear differentiation between them.

On the contrary, the meristic analysis of the mouth morphology revealed more interesting results. In the upper jaw, hybrids shared a higher degree of similarity with *S. aurata*. Of the four tooth types, the hybrid only presented similarities with *P. pagrus* in one (small canines), and presented an intermediate number of molar teeth rows between progenitors, which washowever, closer to *S. aurata*. This fact is reinforced by the low values of PCS, where a relatively high number of specimens of the *S. aurata* sample (42) presented similar characteristics to the ones evidenced by the hybrids. In this case, the

poor discrimination of the *S. aurata* sample is a direct consequence of its similarity with the hybrids.

In the lower jaw, the hybrids shared similarities. The number of canines was alike in *S. aurata*, and the number of molars in the second row was similar to *P. pagrus*. Like the upper jaw they also presented an intermediate number of molar rows. Thus, when analysing the mouth apparatus as a whole, the major similarities are shared with *S. aurata*. In spite of this similarity, it was possible to establish a differentiation between hybrids and parental species based on the dentition, and more specifically in the number of molar rows. Similar sample isolation based on dentition was also found by Waldman (1986) for several *Morone* crosses.

The ability to capture a prey depends not only on the prey but also on the predator (Drenner & Comas, 1980). Some fish species are specifically adapted to feed on their prey. A specialist feeder is morphologically suited to exploit its prey habitats, whereas the generalist has developed structures allowing feeding in different habitats (Morris, 1981). With such a mouth apparatus, the hybrid *Sa×Pp* might have similar feeding capabilities as *S. aurata*. However, the smaller number of molar rows can determine a certain incapability to consume those same preys. Gilthead seabream feed mainly on bivalves, crustaceans and polychaetes (Andrade *et al.*, 1996), while the red porgy feeds mainly on crustaceans and bivalves (Reis, 1998). Nevertheless, the bivalve species consumed in each case are different, due to the greater crushing capability of *S. aurata*, increasing the capability to ingest larger and harder shelled bivalves. When artificially produced, the crushing capability also assures a higher and faster absorption of artificial diets based on pelleted food. The lower adequacy of the hybrids dentition (smaller number of molar rows) can influence the growing capacity under artificial condition. Thsis fact will be discussed further on.

On the contrary, the remaining analysed structures of the head morphology shared a higher degree of similarity with *P. pagrus*. Only two neurocranium sub-structures shared similarities with *S. aurata* (ethmoid and exoccipital), and none of the analysed bones of the head structures shared resemblances with that progenitor. Therefore, it is interesting to note that under a generic perspective the analysed structures in the head morphology are highly similar to one of the progenitors. The tooth structure with *S. aurata*, and the head structure with *P. pagrus*. Intermediate characteristics were scarce and reduced in number, skewing the hybrids morphology to

one of the progenitors, and not supporting the assumption of intermediate characteristics between the hybrids and the parental species.

The morphological analysis also proved very interesting results. Based on the traditional morphometry, 7 of the 12 characters revealed to be intermediate with the ones presented by the parental species. Thus, the mean values calculated for the hybrids using body shape related characters were much closer to those calculated for the gilthead seabream, with the head related ones being more close to the red porgy. Such findings show that characters did not manifest a random influence of parental gene pool. Morphological relatedness was constrained to specific body regions, reinforcing the above stated theory that specific genes of one of the progenitor overlap the genes of the other.

The morphological analysis based on the traditional measurements differentiated the three samples (Figure 44). Only a small hybrid sample fraction shared similar characteristics with the parental species. Such discrimination is again based on the similarity of one of the body regions with one of the parents. The differences of the body morphology between the hybrid and *P. pagrus* were enough to discriminate them, with the same occurring between the hybrid and *S. aurata* for the head related characteristics. Sample differentiation was based on solid statistical findings, where nine measurements contributed to sample differentiation. Wilks' $\lambda$ presented a low value, and the F to remove values was consistently high (eight characteristics with values over 20). The obtained Mahalanobis differences showed a higher proximity between the hybrid and *P. pagrus*.

The truss method produced a less clear differentiation (Figure 45), with sixteen truss measurements contributing to the sample separation. Although with an inferior degree of discrimination the statistical analysis provided significant indications for sample separation. The only incongruous analysis with the traditional morphometry was the Mahalanobis distance, which indicated a higher proximity with *S. aurata*. In a first analysis, these results may seem discordant, leading to inconclusive findings, but they are far from being unexplainable. In the traditional method, seven out of twelve measurements were head related dimensions. Of these twelve measurements, nine contributed to the sample differentiation, with six being head related. In the truss analysis, the morphological traits are evenly distributed in the fish morphology, with none of the body areas overcoming the others. Therefore, although with slightly worse

results, the truss methods described in a more evenly weighted way the dissimilarity between samples.

These findings reinforce the theory that the results obtained with the truss methods give a more clear perspective of sample differentiation than those obtained with the traditional one. Nevertheless, the traditional method still is a powerful tool in sample discrimination, although it must be used with certain precautions. The truss method eliminates the subjectivity of the choice, and so the over emphasis of specific body areas. Several other authors have addressed this differentiation between morphological methods (e.g. Creech, 1992; Almeida, 1996; Velasco *et al.*, 1996; Bronte *et al.*, 1999) but most did so it only in the context of justifying the use of one or other method. Only a few have demonstrated the difference between methods in practical terms.

Thus, when pooling together the meristic and morphological analysis, it is clear that the influence of each of the parental species is a constraint to specific parts of the hybrid body morphology. In each one of the analyses, the hybrids characteristics are consistently close to one of the parents, even when the obtained results revealed the existence of intermediate values to the ones of the progenitors.

When the resulting hybrids become intermediate in the majority of the studied characteristics it can be rather difficult to discriminate them from the progenitors. Waldman *et al.* (1988) obtained 70-80% precision for striped bass (*Morone saxatilis*) stock delineation using various discriminant techniques, and Harrel & Dean (1988) reported 83% resolution for discriminant morphometric analysis of juveniles of several *Morone* crosses, and Procarione *et al.* (1988) reported 90.8% correct classification between spotted seatrout (*Cynoscion nebulus*), orangemouth corvine (*C. xantalus*) and their hybrids. Using truss networks Muoneke *et al.* (1991) found a resolution of 85% in the discrimination between the striped bass (*Morone saxatilis*), white bass (*Morone chrysops*) and their hybrids. In the present study, the percent classification success increased to 98.7% and 94.7% for the traditional morphology and truss networks, respectively. Thus, both methods assured a high degree of separation between the hybrids and progenitors due to the above stated reasons.

However, such differentiation may also be based on the fact that the parental species do not hybridise naturally. Several authors agree that closer taxonomic relationships between the parental species produce hybrids with a higher number of intermediate characteristics, resulting in higher degrees of resemblance with the progenitors. In such cases, the presence or absence of definitive hybrid features rather

than in intermediate values is likely to be more reliable (Child & Solomon, 1977). According to Crevelli & Dupond (1987) the frequency of interspecific and intergeneric hybridization in cyprinids occurring in European waters creates a taxonomical problem, especially at the genus level. Dubois (1981) suggested that genera should be defined as evolutionary units, and Crevelli & Dupond (1987) emphasized this concept, stating that two species that are able to produce viable adult hybrids should be included in the same single genus.

The progenitors used in the present study belong to different genera (*Sparus* and *Pagrus*). In their first identification these species were considered as belonging to two different genera, *Sparus* Linnaeus, 1758 and *Pagrus* Cuvier, 1816. The genus *Sparus* was established by Linnaeus (type-species: *Sparus aurata* Linnaeus, 1758) and included 22 species from various parts of the word. With the recognition of many new species, the original Linnean genus was split into a number of well-defined genera (Jordan & Fesler, 1893; Regan, 1913; Fowler, 1933; Smith, 1938).

Cuvier established the genus *Pagrus* in 1817 (type species: *Sparus argenteus*) Bloch & Scheneider (1801)= *Sparus pagrus* Linnaeus, 1758) (Bianchi, 1984) and distinguished it from *Sparus* by the presence of two rows of molars instead of three or four. Over time their taxonomical differentiation was redefined and they were placed in the same genera. The primary key for their differentiation was based in the number of teeth. Nevertheless, Jordan & Fesler (1893) did not agree on this separation, placing both species in the same genera and arguing that there were no important differences in the skull and skeleton. As so, besides the morphological and genetic separation of both species, the results obtained with their hybridization provides new evidence for their demarcation in two genera and so, reinforces the present classification.

Due to the taxonomic difference between *Sparus aurata* and *Pagrus pagrus*, the characteristics found in the hybrid *Sa*×*Pp* fall entirely within the concepts stated above, with intermediate values in some of the studied characteristics but with higher degrees of similarity with one of the parents, easy morphological discrimination between them and the progenitors and lack of reproductive capability (Paspatis, personal communication).

Most knowledge concerning fish dietary requirements arises from experimental nutrition studies carried out on cultured species, primarily in salmonids. These studies have demonstrated the relative importance of dietary proteins, lipids and carbohydrates for growth (Moyle & Cech, 1996).

During the last few years, similar work has been developed with Sparids, mainly gilthead sea bream (e.g. Anthouard *et al.*, 1996; Kentouri *et al.*, 1993a, 1993b, 1994a, 1995b) and, more recently, with red porgy (e.g. Colombani, 1993; Kentouri *et al.*, 1994b, 1995a; Rueda *et al.*, 1995a, 1995b, 1997). The present study indicated that the growth performance of the hybrid *Sa×Pp* is a rough expression of growth rates of the parental species. Results calculated for GR, SGR and CI were quite similar to those reported to by Kentouri *et al.* (1993b) for *S. aurata* using fish of similar sizes and temperature range. Nevertheless, the best results have been obtained under relatively high temperature, because when exposed to lower temperature the growth performance was also lower. The hybrid showed similar or lower values than the ones attained by *P. pagrus*. According to Kentouri *et al.* (1995a) red porgy shows higher growth rates during winter, a fact that could not be validated in the present study, confirming the findings of Paspatis *et al.* (1999) for this hybrid. Two main reasons may account for this; firstly, the experimental design did not provide adequate rearing conditions, or, secondly, the hybrid did not acquire the growth capacity of *P. pagrus* during winter. Water quality is a limiting factor for *Pagrus* growth (Kentouri *et al.*, 1994b). However, considering that high concentrations of dissolved oxygen were recorded during the experiment, it does not seem that it played a major role in the present study.

Diet composition seems to be one of the main factors affecting the growth performance. Fish fed food C always presented worse results than those fed food A and food B. The statistical difference between food C and the other two supported this finding. It was expected that food C, with higher protein content (50%), would give better results, but the protein utilisation by the fish was not as efficient as for food A and food B. This fact might be due to a partial vegetal origin of the protein. Again, the effect of food composition is particularly evident when the temperature decreased to certain limits. In this situation, food A seemed to be slightly better than food B. This was statistically supported by the significant differences between the length/weight relationship at the start and at the end of the second experiment.

Results from blood analysis were similar to those obtained for the carcass composition. The pooled information (muscle and blood) showed significant protein retention efficiency as a percentage of the digestible intake. The lipid levels in the second period were higher than in the first. This might indicate a reduction in the growth performance and consequently a decrease in the energy budget. Glucose levels in the

blood did not vary among food types, indicating that carbohydrates of the three diets covered the nutritional requirements.

Under the same temperature range, the protein levels in the blood were higher than those found for gilthead sea bream by Pavlidis *et al.* (1997), but lower than glucose levels. Similar results were found by Pavlidis *et al.* (1999) for red porgy.

This study indicated that a diet formulation for optimal growth of the hybrid *Sa*×*Pp* must be based on the shared characteristics of food A and food B.

## FINAL CONSIDERATIONS

Throughout this dissertation, it was possible to answer, or at least to clarify the questions previously formulated. Results varied among species, and a general constraint applicable to this fish family was not found.

The meristic analysis presented less visible differences among samples than the morphometric analysis. Such results are not at all unexpected due to the lower plasticity of the meristic traits. A geographical gradation was not attained among samples. The analysis of the fluctuating asymmetry in *S. aurata* helped to clarify these assumptions. Results proved that although existing, the FA values were low, showing that the developmental stability processes were present. Since there is a straight relation between higher values of FA and environmental stress, it was expected that reared populations would evidence higher FA values. This fact was confirmed because reared populations presented higher values of FA. This supported the assumption that in the wild, species meristic variability tends to diverge but in a progressively diminishing process. Environmental extreme circumstances that diverge from the optimal conditions (considered to be the main reason for meristic dissimilarities) do not seem to have occurred constantly, leading species to divert markedly.

The meristic analysis, however, lead to one important issue that must be taken in consideration, since the divergence between the number of counts in some of the characteristics analysed and those proposed in the bibliography was rather different. In some of the cases, the counts found in this work overlapped the referenced ones, in all the samples, and in a significant number of specimens. Due to the large geographical distance between some of the samples it can be argued that it is the actual range value for the overall population, and the referred counts in the bibliography must be revised.

The morphometric analysis provided rather different and interesting results. Based on solid statistical results, the existence of significant differences between populations of the studied species has been demonstrated. A geographical gradient was found in three of them *D. sargus*, *D. puntazzo* and *D. dentex* (through the truss networks). The morphological plasticity seems to occur more pronouncedly in the morphometric than in meristic characteristics. In most of the cases, the morphological variability is driven by the feeding habits inherent to the capture of the preys that occur in the exploited ecological niches. Different environments provide different types of prey, implying an adaptation to the conditions, whenever they occur. Yet, in particular

cases, even if the environmental conditions forced the species to diverge into different populations, the neighbouring populations can maintain contact between them, thereby reducing their dissimilarity. This occurred in some of the studied species, between certain pairwise sample comparisons, and more often between the central Mediterranean/South Adriatic species, and in some cases the Greek samples.

On the other hand, it was possible in most of the cases to distinguish between Atlantic and Mediterranean samples, a fact that might be related to the geographical barrier represented by the Strait of Gibraltar in conjunction with the Almeria-Oran Oceanographic Front. Nonetheless, such differentiation cannot be seen in a straightforward perspective for all species. The inherent dispersal abilities of some of them, assured that after the successive changes in the water levels of the Mediterranean and the Atlantic during the last few million years, they could again establish a contact between populations, thereby reducing their dissimilarity.

The present study indicated that the Strait of Gibraltar is not the only geographical barrier causing the dissimilarity between the studied populations. The difference between the Adriatic Sea and the central Mediterranean, Alboran and Ligurian Sea in one side and the Ionian and Aegean Sea on the other, lead to significant differences between populations.

As proved throughout the dissertation, findings were based in solid statistical results, with the chosen methods providing conclusive evidences of similarity of dissimilarity between samples. In any case, the statistical analyses lead to dubious or contradictory findings, enabling the formulation of the required answers.

The use of two morphological procedures, besides serving the purpose of comparison between them (determining the most accurate) also confirmed the above stated differences between populations, since both methods lead to the same results. Nonetheless, in six of the eight species, the truss networks improved the results obtained with the traditional morphometry, showing that in most of the cases it is a more accurate and balanced procedure than the traditional morphometry.

Finally, the study of the hybrid *S. aurata*×*P. pagrus* extended the analysis of the Sparid family beyond the morphological analysis. Beside the unequivocal biological interest of the hybrid, the cladistic relationship between the parental species could also be studied. According to the bibliography, species that do not hybridise naturally produce hybrids with characteristics that in the majority of the cases are not intermediate to the ones presented by the progenitors. In the present study, the results of

the morphological analysis of the hybrid corroborate this assumption, and can be considered as conclusive evidence of the difference between the genus *Sparus* and the genus *Pagrus*, corroborating their phylogenetic position.

The dietary analysis can also be seen as an extension of these findings since the growing capacity of the hybrid during warm periods was similar to one of the progenitors (*S. aurata*). However in the coldest period when it was expected that they would have a similar growth to the other progenitor (*P. pagrus*), this did not occur, which bring together the growth capacity of the hybrid and the gilthead seabream. Therefore, the hybrid offers a good commercial perspective if grown under restricted temperature ranges.

As a proposition for further studies on this subject, several measures could be taken to ensure more extensive and decisive assumptions on population discrimination of these Sparid species. A greater number of samples should be collected, not only in the North of the Mediterranean, in order to clearly establish the borders of the neighbouring populations, but also to collect them throughout the South Mediterranean. The collection of Atlantic samples could help not only to clarify the divergence between Atlantic/Mediterranean populations, but also to establish eventual differences or similarities between Atlantic populations. But most of all, sample analysis should be extended to other biological information, such as genetic and parasitological markers in order to compare them with the morphological analysis, helping to define ranges of the different populations within species.

In the case of the black seabream, beside the measures referred to above, the collection of Italian and Greek samples would help to clarify the differences already seen between the Portuguese and the Spanish samples.

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# ANNEX I

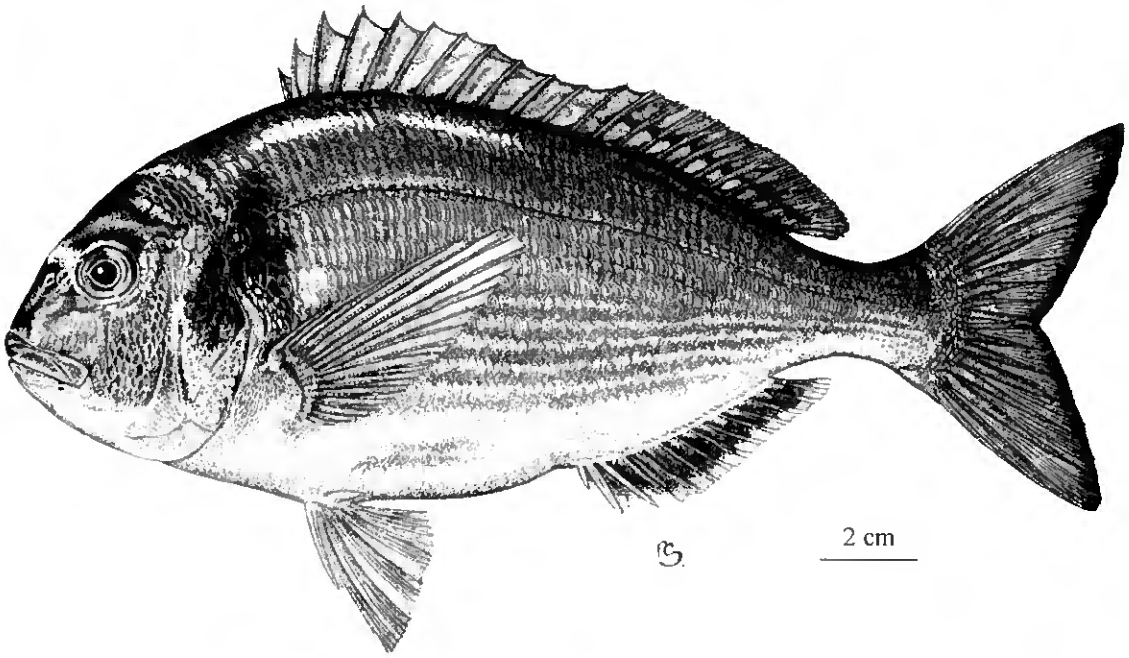


Figure 1 – Gilthead seabream, *Sparus aurata* Linnaeus, 1758.

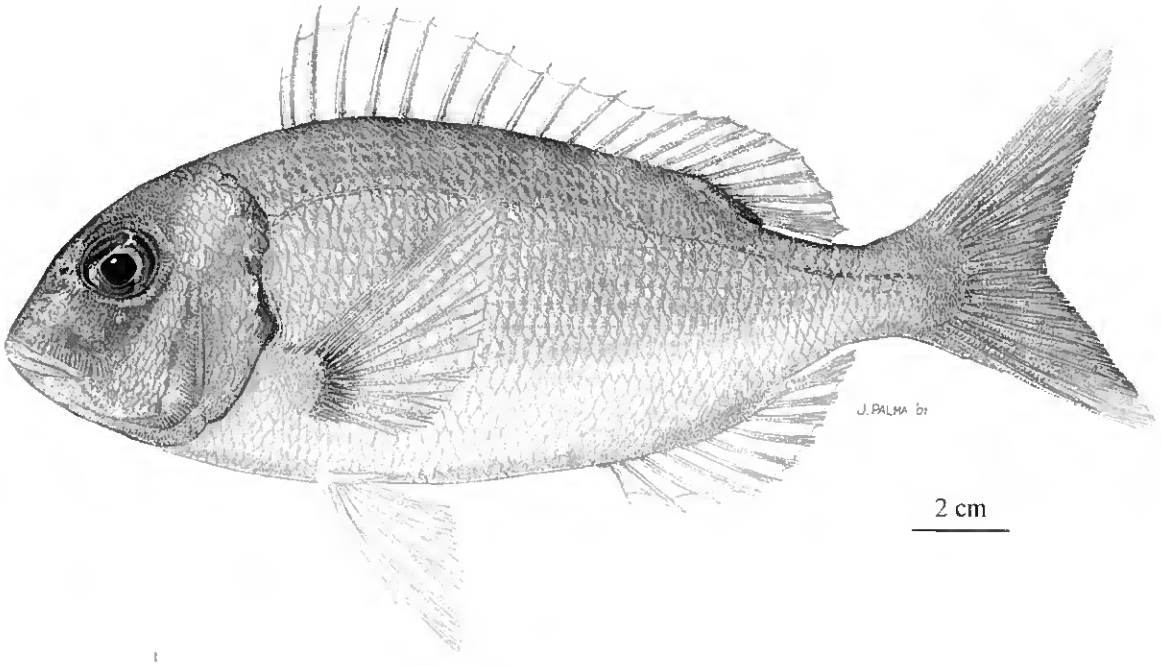


Figure 2 – Red porgy, *Pagrus pagrus* (Linnaeus, 1758).

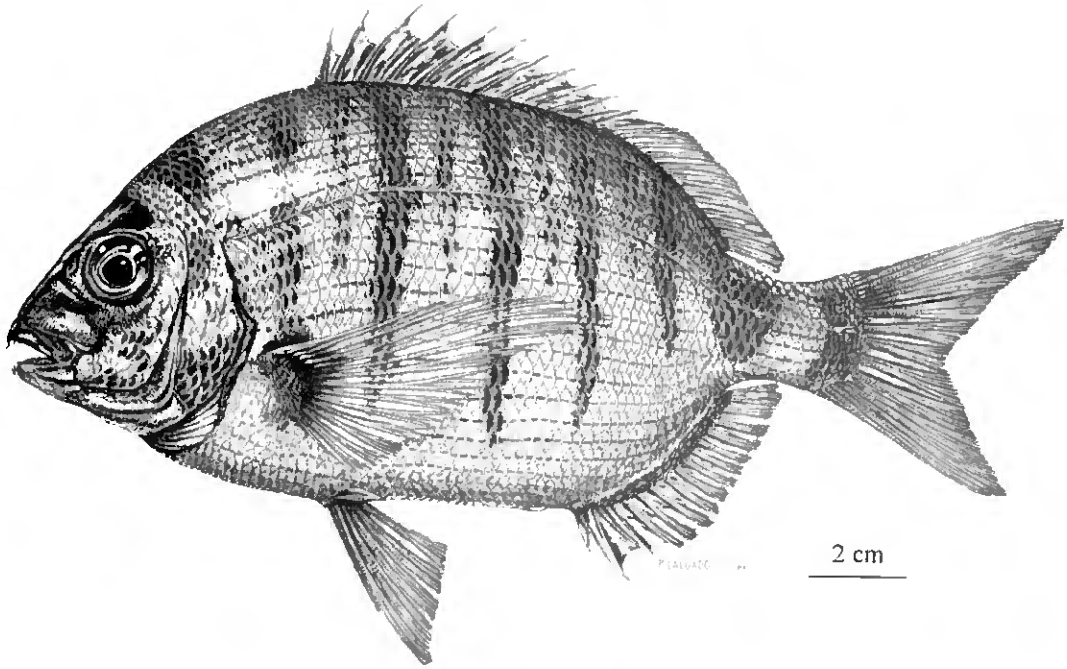


Figure 3 – White seabream, *Diplodus sargus* (Linnaeus, 1758).

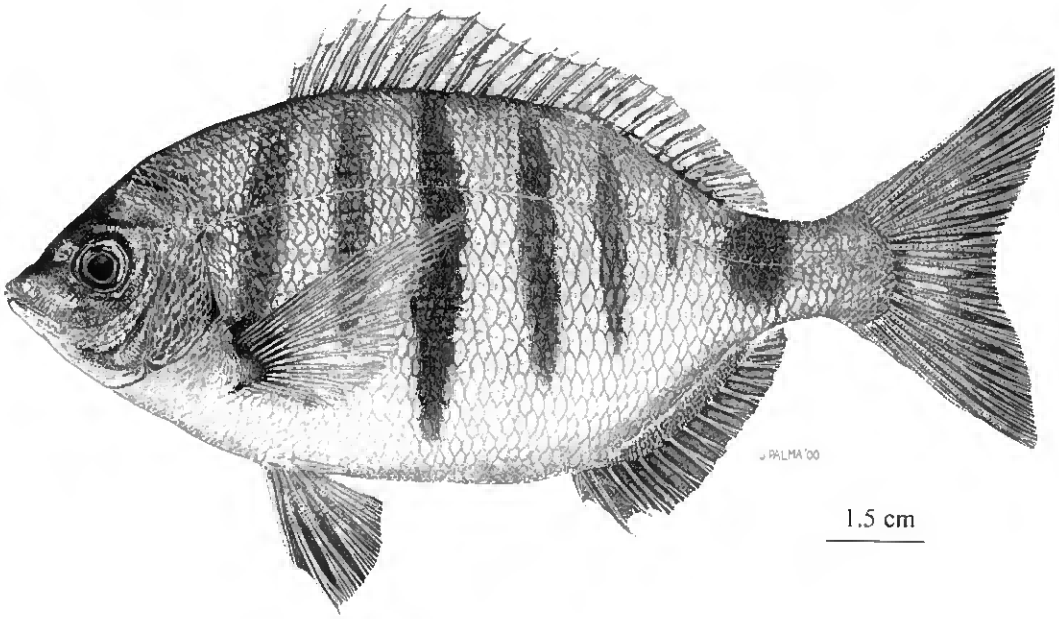


Figure 4 – Sharpsnout seabream, *Diplodus puntazzo* (Cetti, 1777).

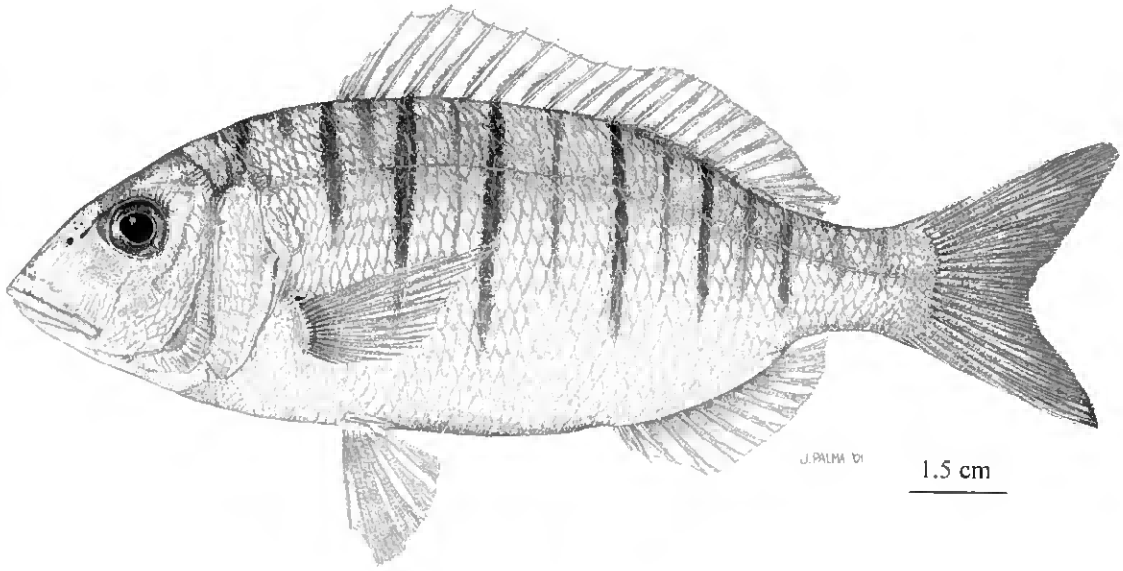


Figure 5 – Striped seabream, *Lithognathus mormyrus* (Linnaeus, 1758).

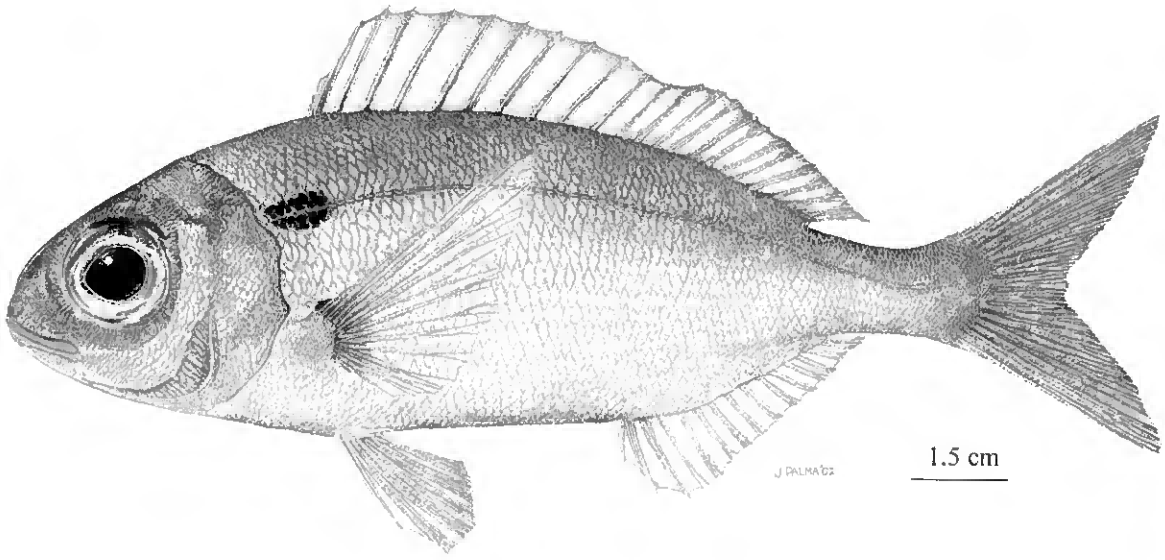


Figure 6 – Red seabream, *Pagellus bogaraveo* (Brünnich, 1768).

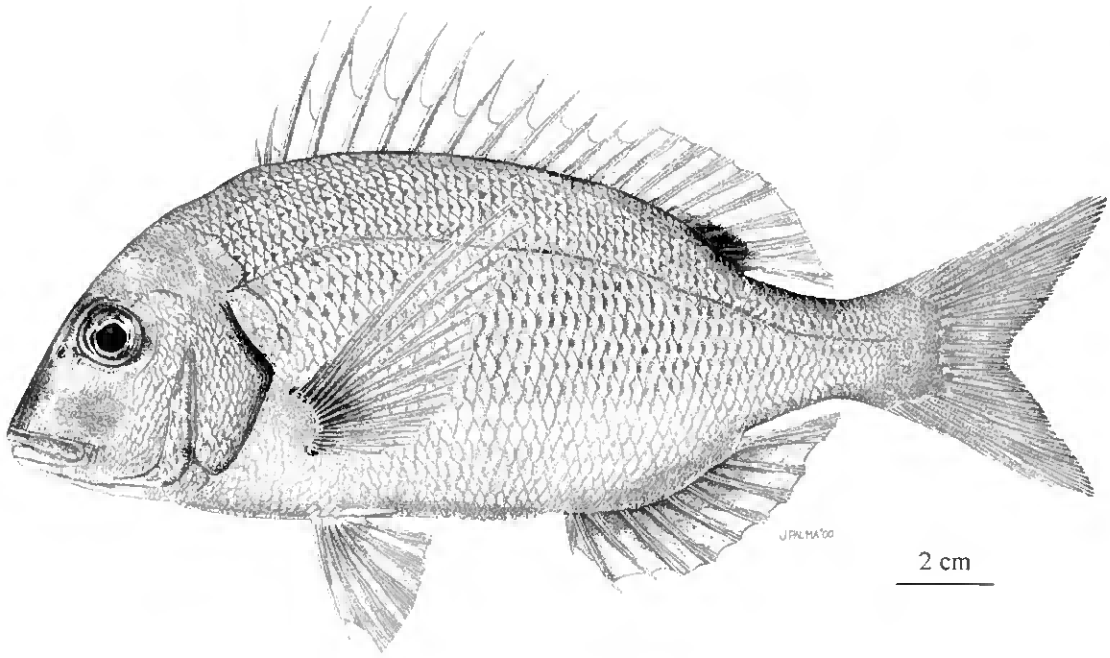


Figure 7 – Common dentex, *Dentex dentex* (Linnaeus, 1758).

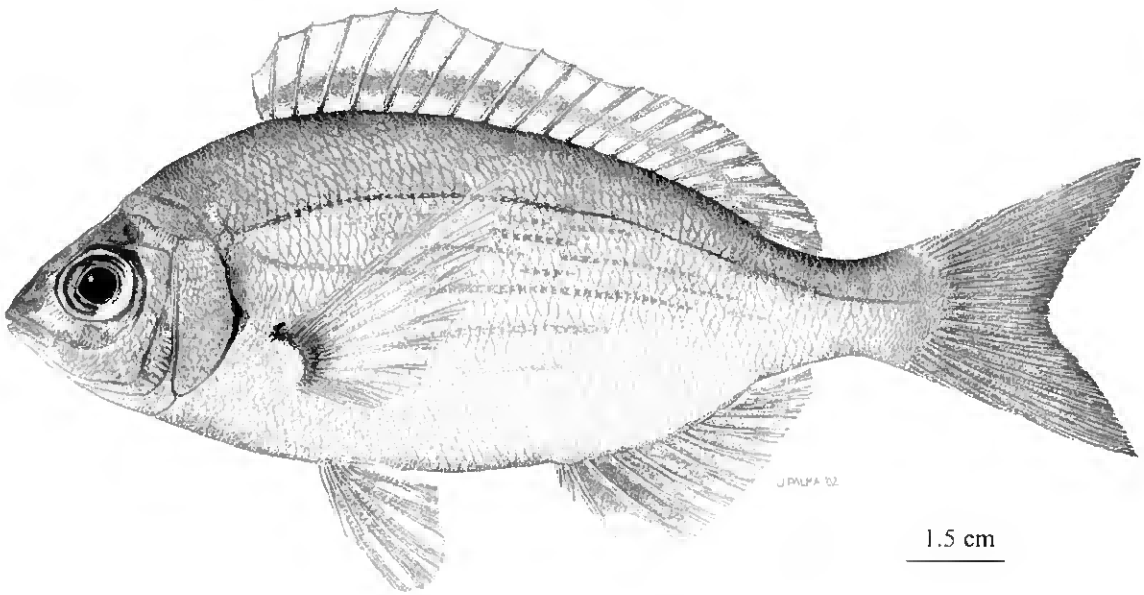


Figure 8 – Black seabream, *Spondyliosoma cantharus* (Linnaeus, 1758).

Table I - Descriptive statistics of the meristic traits recorded for each one of the reared samples of *Sparus aurata* (DF- dorsal fin, CF- caudal fin, AF- anal fin, RPF- right pectoral fin, LPF- left pectoral fin, URGR- upper right gill raker, ULGR- upper right gill raker, LRGR- lower right gill raker, LLGR- lower right gill raker)

**France**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 13 | 21 | 11 | 16 | 16 | 7 | 7 | 7 | 8 |
| Standard Error | 0 | 0.16 | 0 | 0.10 | 0.08 | 0.10 | 0.12 | 0.12 | 0.10 |
| Median | 13 | 21 | 11 | 16 | 16 | 7 | 7 | 7 | 8 |
| Mode | 13 | 21 | 11 | 16 | 16 | 7 | 7 | 7 | 8 |
| Standard Deviation | 0 | 0.78 | 0 | 0.49 | 0.37 | 0.49 | 0.58 | 0.59 | 0.50 |
| Range | 0 | 3 | 0 | 2 | 2 | 1 | 2 | 2 | 1 |
| Minimum | 13 | 20 | 11 | 15 | 15 | 6 | 5 | 7 | 7 |
| Maximum | 13 | 23 | 11 | 17 | 17 | 7 | 7 | 9 | 8 |
| Confidence Level (95.0%) | 0 | 0.34 | 0 | 0.21 | 0.16 | 0.21 | 0.25 | 0.26 | 0.22 |

**Portugal**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 13 | 21 | 11 | 14 | 14 | 6 | 6 | 8 | 8 |
| Standard Error | 0.09 | 0.12 | 0.07 | 0.09 | 0.09 | 0.09 | 0.09 | 0.10 | 0.09 |
| Median | 13 | 21 | 11 | 14 | 14 | 6 | 6 | 8 | 8 |
| Mode | 13 | 21 | 11 | 14 | 14 | 6 | 6 | 8 | 8 |
| Standard Deviation | 0.65 | 0.83 | 0.49 | 0.63 | 0.64 | 0.67 | 0.67 | 0.68 | 0.62 |
| Range | 3 | 6 | 2 | 2 | 3 | 3 | 3 | 3 | 2 |
| Minimum | 11 | 17 | 10 | 13 | 13 | 5 | 5 | 6 | 7 |
| Maximum | 14 | 23 | 12 | 15 | 16 | 8 | 8 | 9 | 9 |
| Confidence Level (95.0%) | 0.18 | 0.24 | 0.14 | 0.18 | 0.18 | 0.19 | 0.19 | 0.19 | 0.18 |

**Spain Atlantic**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 13 | 21 | 11 | 14 | 14 | 6 | 5 | 8 | 8 |
| Standard Error | 0.09 | 0.08 | 0.02 | 0.08 | 0.10 | 0.08 | 0.09 | 0.10 | 0.11 |
| Median | 13 | 21 | 11 | 14 | 15 | 6 | 5 | 7 | 7 |
| Mode | 13 | 21 | 11 | 14 | 15 | 6 | 5 | 7 | 7 |
| Standard Deviation | 0.65 | 0.58 | 0.14 | 0.59 | 0.71 | 0.54 | 0.67 | 0.70 | 0.81 |
| Range | 3 | 2 | 1 | 2 | 3 | 2 | 3 | 2 | 4 |
| Minimum | 12 | 20 | 11 | 13 | 13 | 4 | 3 | 7 | 6 |
| Maximum | 15 | 22 | 12 | 15 | 16 | 6 | 6 | 9 | 10 |
| Confidence Level (95.0%) | 0.18 | 0.17 | 0.04 | 0.17 | 0.20 | 0.15 | 0.19 | 0.20 | 0.23 |

**Spain Mediterranean**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 13.8 | 21.5 | 11.2 | 15.4 | 15.4 | 5.9 | 5.8 | 8.3 | 8.3 |
| Standard Error | 0.07 | 0.12 | 0.06 | 0.08 | 0.11 | 0.07 | 0.07 | 0.09 | 0.11 |
| Median | 14 | 21 | 11 | 15 | 15 | 6 | 6 | 8 | 8 |
| Mode | 14 | 21 | 11 | 15 | 15 | 6 | 6 | 8 | 8 |
| Standard Deviation | 0.48 | 0.86 | 0.45 | 0.54 | 0.76 | 0.50 | 0.52 | 0.61 | 0.75 |
| Range | 2 | 3 | 2 | 2 | 5 | 3 | 2 | 2 | 4 |
| Minimum | 13 | 20 | 10 | 15 | 12 | 4 | 5 | 7 | 6 |
| Maximum | 15 | 23 | 12 | 17 | 17 | 7 | 7 | 9 | 10 |
| Confidence Level (95.0%) | 0.14 | 0.25 | 0.13 | 0.15 | 0.21 | 0.14 | 0.15 | 0.17 | 0.21 |

**Italy**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 13 | 21 | 11 | 15 | 15 | 6 | 6 | 9 | 9 |
| Standard Error | 0.02 | 0.07 | 0.07 | 0.15 | 0.16 | 0.08 | 0.08 | 0.09 | 0.08 |
| Median | 13 | 21 | 11 | 15 | 15 | 6 | 6 | 9 | 9 |
| Mode | 13 | 21 | 11 | 15 | 15 | 6 | 6 | 9 | 9 |
| Standard Deviation | 0.14 | 0.52 | 0.51 | 1.05 | 1.10 | 0.55 | 0.59 | 0.61 | 0.55 |
| Range | 1 | 2 | 3 | 4 | 4 | 2 | 2 | 2 | 3 |
| Minimum | 12 | 20 | 10 | 12 | 12 | 5 | 5 | 7 | 7 |
| Maximum | 13 | 22 | 13 | 16 | 16 | 7 | 7 | 9 | 10 |
| Confidence Level (95.0%) | 0.04 | 0.15 | 0.14 | 0.30 | 0.31 | 0.16 | 0.17 | 0.17 | 0.16 |

**Greece**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 13 | 21 | 11 | 15 | 15 | - | - | - | - |
| Standard Error | 0.07 | 0.09 | 0.09 | 0.09 | 0.11 | - | - | - | - |
| Median | 13 | 21 | 11 | 15 | 15 | - | - | - | - |
| Mode | 13 | 21 | 11 | 15 | 15 | - | - | - | - |
| Standard Deviation | 0.49 | 0.64 | 0.64 | 0.68 | 0.84 | - | - | - | - |
| Range | 2 | 3 | 3 | 3 | 5 | - | - | - | - |
| Minimum | 12 | 20 | 10 | 14 | 13 | - | - | - | - |
| Maximum | 14 | 23 | 13 | 17 | 18 | - | - | - | - |
| Confidence Level (95.0%) | 0.13 | 0.17 | 0.17 | 0.19 | 0.23 | - | - | - | - |

Table II - Descriptive statistics of the meristic traits recorded for each one of the wild samples of *Sparus aurata* (DF- dorsal fin, CF- caudal fin, AF- anal fin, RPF- right pectoral fin, LPF- left pectoral fin, URGR- upper right gill raker, ULGR- upper right gill raker, LRGR- lower right gill raker, LLGR- lower right gill raker)

**Portugal**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 13 | 21 | 11 | 16 | 16 | 6 | 6 | 7 | 7 |
| Standard Error | 0.04 | 0.07 | 0.02 | 0.08 | 0.07 | 0.07 | 0.07 | 0.11 | 0.11 |
| Median | 13 | 21 | 11 | 16 | 16 | 6 | 6 | 7 | 7 |
| Mode | 13 | 21 | 11 | 16 | 16 | 6 | 6 | 7 | 7 |
| Standard Deviation | 0.31 | 0.50 | 0.14 | 0.55 | 0.52 | 0.51 | 0.53 | 0.75 | 0.78 |
| Range | 2 | 2 | 1 | 2 | 2 | 2 | 3 | 3 | 2 |
| Minimum | 12 | 20 | 11 | 15 | 15 | 5 | 4 | 6 | 6 |
| Maximum | 14 | 22 | 12 | 17 | 17 | 7 | 7 | 9 | 8 |
| Confidence Level (95.0%) | 0.09 | 0.14 | 0.04 | 0.16 | 0.15 | 0.14 | 0.15 | 0.21 | 0.22 |

**Spain Atlantic**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 13 | 21 | 11 | 16 | 16 | 5 | 5 | 8 | 8 |
| Standard Error | 0.05 | 0.10 | 0.02 | 0.07 | 0.07 | 0.12 | 0.11 | 0.08 | 0.10 |
| Median | 13 | 21 | 11 | 16 | 16 | 5 | 5 | 8 | 8 |
| Mode | 13 | 21 | 11 | 16 | 16 | 4 | 5 | 8 | 8 |
| Standard Deviation | 0.32 | 0.68 | 0.14 | 0.52 | 0.48 | 0.86 | 0.78 | 0.53 | 0.68 |
| Range | 2 | 3 | 1 | 2 | 1 | 2 | 3 | 2 | 3 |
| Minimum | 12 | 20 | 11 | 14 | 15 | 4 | 3 | 7 | 7 |
| Maximum | 14 | 23 | 12 | 16 | 16 | 6 | 6 | 9 | 10 |
| Confidence Level (95.0%) | 0.09 | 0.20 | 0.04 | 0.15 | 0.14 | 0.25 | 0.23 | 0.15 | 0.20 |

**Spain Mediterranean**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 13 | 21 | 11 | 16 | 16 | 6 | 6 | 8 | 8 |
| Standard Error | 0.05 | 0.06 | 0.02 | 0.05 | 0.05 | 0.07 | 0.06 | 0.08 | 0.08 |
| Median | 13 | 21 | 11 | 16 | 16 | 6 | 6 | 8 | 8 |
| Mode | 13 | 21 | 11 | 16 | 16 | 6 | 6 | 8 | 8 |
| Standard Deviation | 0.38 | 0.44 | 0.14 | 0.34 | 0.35 | 0.52 | 0.40 | 0.53 | 0.53 |
| Range | 2 | 1 | 1 | 2 | 1 | 2 | 2 | 2 | 2 |
| Minimum | 12 | 21 | 11 | 15 | 15 | 5 | 5 | 7 | 7 |
| Maximum | 14 | 22 | 12 | 17 | 16 | 7 | 7 | 9 | 9 |
| Confidence Level (95.0%) | 0.11 | 0.13 | 0.04 | 0.10 | 0.10 | 0.15 | 0.11 | 0.15 | 0.15 |

**Italy**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 13 | 21 | 11 | 16 | 16 | 7 | 6 | 7 | 7 |
| Standard Error | 0 | 0.07 | 0.05 | 0.06 | 0.11 | 0.08 | 0.08 | 0.09 | 0.07 |
| Median | 13 | 21 | 11 | 16 | 16 | 7 | 6 | 7 | 7 |
| Mode | 13 | 21 | 11 | 16 | 16 | 7 | 6 | 7 | 7 |
| Standard Deviation | 0 | 0.45 | 0.30 | 0.38 | 0.69 | 0.50 | 0.51 | 0.55 | 0.46 |
| Range | 0 | 1 | 1 | 2 | 4 | 1 | 1 | 2 | 2 |
| Minimum | 13 | 21 | 11 | 15 | 13 | 6 | 6 | 6 | 6 |
| Maximum | 13 | 22 | 12 | 17 | 17 | 7 | 7 | 8 | 8 |
| Confidence Level (95.0%) | 0 | 0.14 | 0.10 | 0.12 | 0.22 | 0.16 | 0.16 | 0.18 | 0.15 |

**Greece**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 13 | 21 | 11 | 16 | 16 | 5 | 5 | 8 | 8 |
| Standard Error | 0.03 | 0.10 | 0.10 | 0.06 | 0.09 | 0.04 | 0.03 | 0.07 | 0.08 |
| Median | 13 | 21 | 11 | 16 | 16 | 5 | 5 | 8 | 8 |
| Mode | 13 | 21 | 11 | 16 | 16 | 5 | 5 | 8 | 8 |
| Standard Deviation | 0.22 | 0.63 | 0.66 | 0.38 | 0.55 | 0.27 | 0.22 | 0.43 | 0.51 |
| Range | 1 | 2 | 3 | 2 | 3 | 1 | 1 | 2 | 2 |
| Minimum | 13 | 21 | 10 | 15 | 15 | 5 | 5 | 7 | 7 |
| Maximum | 14 | 23 | 13 | 17 | 18 | 6 | 6 | 9 | 9 |
| Confidence Level (95.0%) | 0.07 | 0.20 | 0.21 | 0.12 | 0.17 | 0.09 | 0.07 | 0.14 | 0.16 |

Table III - Descriptive statistics of the meristic traits recorded for each one of the samples of *Pagrus pagrus* (DF- dorsal fin, CF- caudal fin, AF- anal fin, RPF- right pectoral fin, LPF- left pectoral fin, URGR- upper right gill raker, ULGR- upper right gill raker, LRGR- lower right gill raker, LLGR- lower right gill raker)

**Portugal**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 10 | 22 | 8 | 15 | 15 | 7 | 7 | 9 | 9 |
| Standard Error | 0.03 | 0.11 | 0 | 0.05 | 0.04 | 0.06 | 0.06 | 0.05 | 0.05 |
| Median | 10 | 22 | 8 | 15 | 15 | 7 | 7 | 9 | 9 |
| Mode | 10 | 21 | 8 | 15 | 15 | 7 | 7 | 9 | 9 |
| Standard Deviation | 0.29 | 1.17 | 0 | 0.50 | 0.44 | 0.59 | 0.60 | 0.53 | 0.53 |
| Range | 2 | 4 | 0 | 2 | 2 | 3 | 3 | 2 | 3 |
| Minimum | 9 | 20 | 8 | 14 | 14 | 6 | 5 | 8 | 7 |
| Maximum | 11 | 24 | 8 | 16 | 16 | 9 | 8 | 10 | 10 |
| Confidence Level (95.0%) | 0.05 | 0.22 | 0 | 0.10 | 0.08 | 0.11 | 0.11 | 0.10 | 0.10 |

**Spain**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 10 | 22 | 8 | 15 | 15 | 7 | 7 | 10 | 10 |
| Standard Error | 0 | 0.15 | 0 | 0.10 | 0.08 | 0.11 | 0.14 | 0.10 | 0.12 |
| Median | 10 | 22 | 8 | 15 | 15 | 7 | 7 | 10 | 10 |
| Mode | 10 | 22 | 8 | 15 | 15 | 6 | 7 | 10 | 10 |
| Standard Deviation | 0 | 0.88 | 0 | 0.60 | 0.49 | 0.68 | 0.85 | 0.58 | 0.73 |
| Range | 0 | 3 | 0 | 3 | 2 | 2 | 4 | 2 | 3 |
| Minimum | 10 | 21 | 8 | 14 | 14 | 6 | 5 | 9 | 8 |
| Maximum | 10 | 24 | 8 | 17 | 16 | 8 | 9 | 11 | 11 |
| Confidence Level (95.0%) | 0 | 0.29 | 0 | 0.20 | 0.16 | 0.23 | 0.28 | 0.19 | 0.24 |

**Italy**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 10 | 22 | 8 | 15 | 15 | 6 | 6 | 10 | 10 |
| Standard Error | 0.03 | 0.12 | 0.03 | 0.07 | 0.08 | 0.10 | 0.10 | 0.10 | 0.11 |
| Median | 10 | 22 | 8 | 15 | 15 | 6 | 6 | 10 | 10 |
| Mode | 10 | 22 | 8 | 15 | 15 | 6 | 6 | 10 | 10 |
| Standard Deviation | 0.18 | 0.68 | 0.18 | 0.37 | 0.44 | 0.56 | 0.54 | 0.54 | 0.62 |
| Range | 1 | 3 | 1 | 2 | 2 | 2 | 2 | 2 | 2 |
| Minimum | 10 | 21 | 8 | 14 | 14 | 6 | 6 | 9 | 9 |
| Maximum | 11 | 24 | 9 | 16 | 16 | 8 | 8 | 11 | 11 |
| Confidence Level (95.0%) | 0.07 | 0.25 | 0.07 | 0.13 | 0.16 | 0.21 | 0.20 | 0.20 | 0.23 |

**Greece**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 10 | 22 | 8 | 15 | 15 | 6 | 6 | 9 | 9 |
| Standard Error | 0 | 0.11 | 0 | 0.05 | 0.05 | 0.10 | 0.08 | 0.07 | 0.07 |
| Median | 10 | 22.5 | 8 | 15 | 15 | 6 | 6 | 9 | 9 |
| Mode | 10 | 23 | 8 | 15 | 15 | 6 | 6 | 9 | 9 |
| Standard Deviation | 0 | 0.81 | 0 | 0.39 | 0.40 | 0.75 | 0.58 | 0.54 | 0.54 |
| Range | 0 | 4 | 0 | 2 | 2 | 4 | 2 | 2 | 3 |
| Minimum | 10 | 20 | 8 | 14 | 14 | 4 | 5 | 9 | 8 |
| Maximum | 10 | 24 | 8 | 16 | 16 | 8 | 7 | 11 | 11 |
| Confidence Level (95.0%) | 0 | 0.22 | 0 | 0.11 | 0.11 | 0.21 | 0.16 | 0.15 | 0.15 |

Table IV - Descriptive statistics of the meristic traits recorded for each one of the samples of *Diplodus sargus* (DF- dorsal fin, CF- caudal fin, AF- anal fin, RPF- right pectoral fin, LPF- left pectoral fin, URGR- upper right gill raker, ULGR- upper right gill raker, LRGR- lower right gill raker, LLGR- lower right gill raker)

**Portugal**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 14 | 23 | 13 | 16 | 16 | 7 | 8 | 9 | 9 |
| Standard Error | 0.09 | 0.11 | 0.06 | 0.05 | 0.05 | 0.06 | 0.07 | 0.07 | 0.07 |
| Median | 14 | 23 | 13 | 16 | 16 | 7 | 7 | 9 | 9 |
| Mode | 13 | 22 | 13 | 16 | 16 | 7 | 7 | 9 | 9 |
| Standard Deviation | 0.81 | 1.07 | 0.53 | 0.43 | 0.47 | 0.61 | 0.69 | 0.63 | 0.61 |
| Range | 3 | 4 | 3 | 2 | 3 | 3 | 4 | 3 | 2 |
| Minimum | 13 | 21 | 12 | 15 | 14 | 6 | 6 | 8 | 8 |
| Maximum | 16 | 25 | 15 | 17 | 17 | 9 | 10 | 11 | 10 |
| Confidence Level (95.0%) | 0.26 | 0.23 | 0.11 | 0.09 | 0.10 | 0.13 | 0.15 | 0.13 | 0.13 |

**Spain**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 13 | 23 | 13 | 16 | 16 | 8 | 8 | 10 | 10 |
| Standard Error | 0.06 | 0.19 | 0.10 | 0.10 | 0.10 | 0.10 | 0.12 | 0.10 | 0.13 |
| Median | 13 | 23 | 13 | 16 | 16 | 8 | 7 | 10 | 10 |
| Mode | 13 | 23 | 13 | 16 | 16 | 8 | 7 | 10 | 10 |
| Standard Deviation | 0.28 | 0.93 | 0.51 | 0.51 | 0.48 | 0.51 | 0.59 | 0.50 | 0.66 |
| Range | 1 | 4 | 2 | 2 | 2 | 1 | 2 | 2 | 2 |
| Minimum | 13 | 21 | 12 | 15 | 15 | 7 | 7 | 9 | 9 |
| Maximum | 14 | 25 | 14 | 17 | 17 | 8 | 9 | 11 | 11 |
| Confidence Level (95.0%) | 0.12 | 0.39 | 0.21 | 0.21 | 0.20 | 0.21 | 0.25 | 0.21 | 0.28 |

**Italy**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 14 | 23 | 13 | 16 | 16 | 9 | 9 | 9 | 9 |
| Standard Error | 0.09 | 0.12 | 0.08 | 0.17 | 0.07 | 0.13 | 0.10 | 0.08 | 0.09 |
| Median | 14 | 23 | 13 | 16 | 16 | 9 | 9 | 9 | 9 |
| Mode | 14 | 23 | 13 | 16 | 16 | 8 | 9 | 9 | 9 |
| Standard Deviation | 0.5 | 0.70 | 0.44 | 0.97 | 0.42 | 0.75 | 0.56 | 0.44 | 0.51 |
| Range | 2 | 3 | 2 | 6 | 1 | 3 | 2 | 2 | 2 |
| Minimum | 13 | 21 | 12 | 11 | 15 | 7 | 8 | 8 | 8 |
| Maximum | 15 | 24 | 14 | 17 | 16 | 10 | 10 | 10 | 10 |
| Confidence Level (95.0%) | 0.18 | 0.25 | 0.16 | 0.34 | 0.15 | 0.27 | 0.20 | 0.16 | 0.18 |

**Greece**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 14 | 25 | 13 | 16 | 16 | 8 | 7 | 10 | 10 |
| Standard Error | 0.12 | 0.2 | 0.07 | 0.07 | 0.11 | 0.11 | 0.13 | 0.1 | 0.11 |
| Median | 14 | 25 | 13 | 16 | 16 | 8 | 8 | 10 | 10 |
| Mode | 14 | 25 | 13 | 16 | 16 | 8 | 8 | 10 | 10 |
| Standard Deviation | 0.58 | 1 | 0.35 | 0.35 | 0.54 | 0.57 | 0.65 | 0.5 | 0.55 |
| Range | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Minimum | 13 | 23 | 12 | 15 | 15 | 7 | 6 | 9 | 9 |
| Maximum | 15 | 26 | 14 | 17 | 17 | 9 | 8 | 11 | 11 |
| Confidence Level (95.0%) | 0.24 | 0.41 | 0.14 | 0.14 | 0.22 | 0.23 | 0.27 | 0.21 | 0.23 |

Table V - Descriptive statistics of the meristic traits recorded for each one of the samples of *Diplodus puntazzo* (DF- dorsal fin, CF- caudal fin, AF- anal fin, RPF- right pectoral fin, LPF- left pectoral fin, URGR- upper right gill raker, ULGR- upper right gill raker, LRGR- lower right gill raker, LLGR- lower right gill raker)

**Portugal**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 13 | 23 | 12 | 16 | 16 | 6 | 6 | 9 | 9 |
| Standard Error | 0.05 | 0.17 | 0.03 | 0.06 | 0.07 | 0.12 | 0.10 | 0.09 | 0.09 |
| Median | 13 | 23 | 12 | 16 | 16 | 6 | 6 | 9 | 9 |
| Mode | 13 | 23 | 12 | 16 | 16 | 6 | 6 | 9 | 9 |
| Standard Deviation | 0.32 | 0.99 | 0.17 | 0.38 | 0.45 | 0.74 | 0.61 | 0.54 | 0.57 |
| Range | 1 | 3 | 1 | 1 | 2 | 3 | 3 | 2 | 2 |
| Minimum | 13 | 22 | 12 | 15 | 15 | 5 | 5 | 8 | 8 |
| Maximum | 14 | 25 | 13 | 16 | 17 | 8 | 8 | 10 | 10 |
| Confidence Level (95.0%) | 0.11 | 0.34 | 0.06 | 0.13 | 0.15 | 0.25 | 0.21 | 0.18 | 0.19 |

**Spain**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 13.05 | 23 | 11 | 14.88 | 14.85 | 5.60 | 5.73 | 9.98 | 9.73 |
| Standard Error | 0.07 | 0 | 0 | 0.11 | 0.11 | 0.09 | 0.11 | 0.10 | 0.10 |
| Median | 13 | 23 | 11 | 15 | 15 | 6 | 6 | 10 | 10 |
| Mode | 13 | 23 | 11 | 15 | 15 | 6 | 6 | 10 | 10 |
| Standard Deviation | 0.45 | 0 | 0 | 0.72 | 0.70 | 0.55 | 0.68 | 0.66 | 0.64 |
| Range | 2 | 0 | 0 | 4 | 4 | 2 | 3 | 2 | 2 |
| Minimum | 12 | 23 | 11 | 12 | 12 | 5 | 5 | 9 | 9 |
| Maximum | 14 | 23 | 11 | 16 | 16 | 7 | 8 | 11 | 11 |
| Confidence Level (95.0%) | 0.14 | 0 | 0 | 0.23 | 0.22 | 0.17 | 0.22 | 0.21 | 0.20 |

**Italy**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 13 | 23 | 12 | 15 | 15 | 6 | 6 | 9 | 9 |
| Standard Error | 0.07 | 0.11 | 0.08 | 0.06 | 0.03 | 0.08 | 0.09 | 0.08 | 0.07 |
| Median | 13 | 23 | 12 | 15 | 15 | 6 | 6 | 9 | 9 |
| Mode | 13 | 23 | 12 | 15 | 15 | 6 | 6 | 9 | 9 |
| Standard Deviation | 0.44 | 0.69 | 0.51 | 0.37 | 0.22 | 0.49 | 0.55 | 0.50 | 0.48 |
| Range | 2 | 3 | 2 | 2 | 1 | 1 | 2 | 2 | 2 |
| Minimum | 12 | 21 | 11 | 13 | 14 | 6 | 6 | 8 | 8 |
| Maximum | 14 | 24 | 13 | 15 | 15 | 7 | 8 | 10 | 10 |
| Confidence Level (95.0%) | 0.14 | 0.22 | 0.16 | 0.12 | 0.07 | 0.15 | 0.17 | 0.16 | 0.15 |

**Greece**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 13 | 25 | 12 | 15 | 15 | 5 | 5 | 10 | 10 |
| Standard Error | 0.07 | 0.12 | 0.07 | 0.06 | 0.06 | 0.11 | 0.11 | 0.07 | 0.07 |
| Median | 13 | 25 | 12 | 15 | 15 | 6 | 5 | 10 | 10 |
| Mode | 13 | 25 | 12 | 15 | 15 | 6 | 5 | 10 | 10 |
| Standard Deviation | 0.44 | 0.82 | 0.48 | 0.41 | 0.43 | 0.69 | 0.71 | 0.45 | 0.46 |
| Range | 2 | 3 | 1 | 2 | 2 | 2 | 3 | 1 | 1 |
| Minimum | 12 | 23 | 11 | 14 | 14 | 4 | 4 | 9 | 9 |
| Maximum | 14 | 26 | 12 | 16 | 16 | 6 | 7 | 10 | 10 |
| Confidence Level (95.0%) | 0.13 | 0.25 | 0.15 | 0.13 | 0.13 | 0.21 | 0.22 | 0.14 | 0.14 |

Table VI - Descriptive statistics of the meristic traits recorded for each one of the samples of *Lithognathus mormyrus* (DF- dorsal fin, CF- caudal fin, AF- anal fin, RPF- right pectoral fin, LPF- left pectoral fin, URGR- upper right gill raker, ULGR- upper right gill raker, LRGR- lower right gill raker, LLGR- lower right gill raker)

**Portugal**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 12 | 21 | 10 | 16 | 16 | 11 | 11 | 15 | 15 |
| Standard Error | 0.03 | 0.06 | 0.02 | 0.05 | 0.04 | 0.07 | 0.07 | 0.07 | 0.06 |
| Median | 12 | 21 | 10 | 16 | 16 | 11 | 11 | 15 | 15 |
| Mode | 12 | 21 | 10 | 16 | 16 | 11 | 11 | 15 | 15 |
| Standard Deviation | 0.30 | 0.69 | 0.18 | 0.49 | 0.48 | 0.77 | 0.78 | 0.81 | 0.68 |
| Range | 2 | 3 | 1 | 2 | 2 | 3 | 3 | 5 | 3 |
| Minimum | 11 | 19 | 10 | 15 | 15 | 9 | 9 | 12 | 13 |
| Maximum | 13 | 22 | 11 | 17 | 17 | 12 | 12 | 17 | 16 |
| Confidence Level (95.0%) | 0.05 | 0.13 | 0.03 | 0.09 | 0.09 | 0.14 | 0.14 | 0.15 | 0.12 |

**Spain**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 12 | 21 | 10 | 15 | 15 | 10 | 10 | 17 | 17 |
| Standard Error | 0.03 | 0.10 | 0 | 0.08 | 0.09 | 0.12 | 0.12 | 0.14 | 0.12 |
| Median | 12 | 21 | 10 | 15 | 15 | 10 | 10 | 17 | 17 |
| Mode | 12 | 21 | 10 | 15 | 15 | 10 | 10 | 17 | 17 |
| Standard Deviation | 0.18 | 0.54 | 0 | 0.46 | 0.53 | 0.67 | 0.65 | 0.77 | 0.68 |
| Range | 1 | 2 | 0 | 1 | 2 | 3 | 3 | 3 | 2 |
| Minimum | 11 | 20 | 10 | 15 | 14 | 9 | 9 | 16 | 16 |
| Maximum | 12 | 22 | 10 | 16 | 16 | 12 | 12 | 19 | 18 |
| Confidence Level (95.0%) | 0.07 | 0.20 | 0 | 0.17 | 0.19 | 0.25 | 0.24 | 0.28 | 0.25 |

**Italy**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 12 | 21 | 10 | 15 | 15 | 10 | 10 | 17 | 17 |
| Standard Error | 0.06 | 0.12 | 0.04 | 0.07 | 0.07 | 0.10 | 0.09 | 0.12 | 0.12 |
| Median | 12 | 21 | 10 | 15 | 15 | 10 | 10 | 17 | 17 |
| Mode | 12 | 21 | 10 | 15 | 15 | 10 | 10 | 17 | 17 |
| Standard Deviation | 0.29 | 0.58 | 0.2 | 0.35 | 0.35 | 0.49 | 0.45 | 0.58 | 0.6 |
| Range | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 |
| Minimum | 11 | 20 | 10 | 14 | 14 | 9 | 9 | 16 | 16 |
| Maximum | 13 | 22 | 11 | 16 | 16 | 11 | 11 | 18 | 18 |
| Confidence Level (95.0%) | 0.12 | 0.24 | 0.08 | 0.14 | 0.14 | 0.20 | 0.19 | 0.24 | 0.25 |

**Greece**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 12 | 22 | 10 | 15 | 15 | 9 | 10 | 17 | 17 |
| Standard Error | 0 | 0.04 | 0.06 | 0.08 | 0.08 | 0.21 | 0.17 | 0.13 | 0.12 |
| Median | 12 | 22 | 10 | 15 | 15 | 9.5 | 10 | 17 | 17 |
| Mode | 12 | 22 | 10 | 15 | 15 | 10 | 10 | 17 | 17 |
| Standard Deviation | 0 | 0.20 | 0.28 | 0.43 | 0.43 | 1.08 | 0.89 | 0.69 | 0.63 |
| Range | 0 | 1 | 2 | 1 | 1 | 4 | 3 | 3 | 2 |
| Minimum | 12 | 22 | 9 | 15 | 15 | 7 | 8 | 15 | 16 |
| Maximum | 12 | 23 | 11 | 16 | 16 | 11 | 11 | 18 | 18 |
| Confidence Level (95.0%) | 0 | 0.08 | 0.11 | 0.17 | 0.17 | 0.44 | 0.36 | 0.28 | 0.26 |

Table VII - Descriptive statistics of the meristic traits recorded for each one of the samples of *Pagellus bogaraveo* (DF- dorsal fin, CF- caudal fin, AF- anal fin, RPF- right pectoral fin, LPF- left pectoral fin, URGR- upper right gill raker, ULGR- upper right gill raker, LRGR- lower right gill raker, LLGR- lower right gill raker)

**Portugal**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 12 | 22 | 12 | 17 | 17 | 12 | 12 | 18 | 18 |
| Standard Error | 0.06 | 0.12 | 0.05 | 0.11 | 0.09 | 0.14 | 0.14 | 0.16 | 0.15 |
| Median | 12 | 22 | 12 | 17 | 17 | 12 | 12 | 18 | 18 |
| Mode | 12 | 22 | 12 | 17 | 17 | 12 | 12 | 18 | 18 |
| Standard Deviation | 0.31 | 0.66 | 0.25 | 0.61 | 0.48 | 0.79 | 0.76 | 0.88 | 0.81 |
| Range | 1 | 2 | 1 | 2 | 2 | 3 | 2 | 4 | 3 |
| Minimum | 11 | 21 | 11 | 16 | 16 | 10 | 11 | 16 | 16 |
| Maximum | 12 | 23 | 12 | 18 | 18 | 13 | 13 | 20 | 19 |
| Confidence Level (95.0%) | 0.11 | 0.25 | 0.09 | 0.23 | 0.18 | 0.30 | 0.28 | 0.33 | 0.30 |

**Spain**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 12 | 22 | 12 | 17 | 17 | 12 | 12 | 17 | 17 |
| Standard Error | 0.07 | 0.12 | 0 | 0.07 | 0.08 | 0.11 | 0.09 | 0.11 | 0.11 |
| Median | 12 | 21 | 12 | 17 | 17 | 12 | 12 | 17 | 17 |
| Mode | 12 | 21 | 12 | 17 | 17 | 12 | 12 | 17 | 17 |
| Standard Deviation | 0.46 | 0.82 | 0 | 0.47 | 0.53 | 0.79 | 0.63 | 0.77 | 0.80 |
| Range | 3 | 4 | 0 | 1 | 2 | 3 | 3 | 3 | 4 |
| Minimum | 10 | 20 | 12 | 16 | 15 | 11 | 11 | 16 | 15 |
| Maximum | 13 | 24 | 12 | 17 | 17 | 14 | 14 | 19 | 19 |
| Confidence Level (95.0%) | 0.13 | 0.23 | 0 | 0.14 | 0.15 | 0.23 | 0.18 | 0.22 | 0.23 |

**Italy**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 12 | 22 | 12 | 16 | 16 | 13 | 12 | 18 | 18 |
| Standard Error | 0.08 | 0.10 | 0.08 | 0.10 | 0.10 | 0.15 | 0.22 | 0.18 | 0.17 |
| Median | 12 | 22 | 12 | 16 | 16 | 13 | 13 | 18 | 18 |
| Mode | 12 | 22 | 12 | 16 | 16 | 13 | 13 | 18 | 18 |
| Standard Deviation | 0.43 | 0.50 | 0.4 | 0.49 | 0.49 | 0.74 | 1.14 | 0.89 | 0.86 |
| Range | 2 | 1 | 2 | 1 | 2 | 3 | 7 | 4 | 5 |
| Minimum | 10 | 22 | 11 | 16 | 15 | 12 | 8 | 16 | 15 |
| Maximum | 12 | 23 | 13 | 17 | 17 | 15 | 15 | 20 | 20 |
| Confidence Level (95.0%) | 0.17 | 0.20 | 0.16 | 0.20 | 0.20 | 0.30 | 0.46 | 0.36 | 0.35 |

**Greece**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 12 | 22 | 12 | 16 | 16 | 11 | 11 | 18 | 18 |
| Standard Error | 0.09 | 0.10 | 0 | 0.14 | 0.11 | 0.12 | 0.14 | 0.14 | 0.16 |
| Median | 12 | 22 | 12 | 17 | 16 | 11 | 11 | 18 | 18 |
| Mode | 12 | 22 | 12 | 17 | 16 | 11 | 11 | 18 | 18 |
| Standard Deviation | 0.46 | 0.54 | 0 | 0.74 | 0.57 | 0.65 | 0.77 | 0.78 | 0.87 |
| Range | 3 | 2 | 0 | 3 | 2 | 2 | 4 | 4 | 3 |
| Minimum | 10 | 21 | 12 | 15 | 15 | 10 | 9 | 16 | 17 |
| Maximum | 13 | 23 | 12 | 18 | 17 | 12 | 13 | 20 | 20 |
| Confidence Level (95.0%) | 0.18 | 0.21 | 0 | 0.28 | 0.22 | 0.25 | 0.29 | 0.30 | 0.33 |

Table VIII - Descriptive statistics of the meristic traits recorded for each one of the samples of *Dentex dentex* (DF- dorsal fin, CF- caudal fin, AF- anal fin, RPF- right pectoral fin, LPF- left pectoral fin, URGR- upper right gill raker, ULGR- upper right gill raker, LRGR- lower right gill raker, LLGR- lower right gill raker)

**Portugal**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 11 | 22 | 8 | 15 | 15 | 7 | 7 | 8 | 8 |
| Standard Error | 0.04 | 0.13 | 0.03 | 0.10 | 0.09 | 0.14 | 0.07 | 0.15 | 0.14 |
| Median | 11 | 22 | 8 | 15 | 15 | 7 | 7 | 8 | 8 |
| Mode | 11 | 23 | 8 | 15 | 15 | 7 | 7 | 8 | 8 |
| Standard Deviation | 0.23 | 0.79 | 0.16 | 0.61 | 0.54 | 0.85 | 0.46 | 0.92 | 0.89 |
| Range | 1 | 2 | 1 | 2 | 2 | 4 | 2 | 4 | 3 |
| Minimum | 10 | 21 | 7 | 14 | 14 | 5 | 6 | 7 | 8 |
| Maximum | 11 | 23 | 8 | 16 | 16 | 9 | 8 | 11 | 11 |
| Confidence Level (95.0%) | 0.07 | 0.26 | 0.05 | 0.20 | 0.18 | 0.28 | 0.15 | 0.30 | 0.29 |

**Spain**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 11 | 22 | 8 | 15 | 15 | 7 | 7 | 10 | 10 |
| Standard Error | 0.05 | 0.14 | 0 | 0.08 | 0.07 | 0.11 | 0.13 | 0.16 | 0.14 |
| Median | 11 | 22 | 8 | 15 | 15 | 7 | 7 | 10 | 10 |
| Mode | 11 | 21 | 8 | 15 | 15 | 7 | 7 | 10 | 10 |
| Standard Deviation | 0.26 | 0.74 | 0 | 0.44 | 0.37 | 0.60 | 0.72 | 0.86 | 0.75 |
| Range | 1 | 2 | 0 | 2 | 2 | 2 | 3 | 3 | 3 |
| Minimum | 10 | 21 | 8 | 14 | 14 | 6 | 6 | 9 | 9 |
| Maximum | 11 | 23 | 8 | 16 | 16 | 8 | 9 | 12 | 12 |
| Confidence Level (95.0%) | 0.10 | 0.28 | 0 | 0.17 | 0.14 | 0.23 | 0.28 | 0.33 | 0.29 |

**Italy**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 11 | 22 | 8 | 15 | 15 | 8 | 8 | 9 | 9 |
| Standard Error | 0.06 | 0.13 | 0 | 0.04 | 0.04 | 0.10 | 0.06 | 0.11 | 0.13 |
| Median | 11 | 22 | 8 | 15 | 15 | 8 | 8 | 9 | 9 |
| Mode | 11 | 22 | 8 | 15 | 15 | 8 | 8 | 9 | 9 |
| Standard Deviation | 0.30 | 0.62 | 0 | 0.21 | 0.21 | 0.46 | 0.29 | 0.54 | 0.60 |
| Range | 2 | 2 | 0 | 1 | 1 | 2 | 1 | 2 | 2 |
| Minimum | 10 | 21 | 8 | 15 | 15 | 7 | 7 | 8 | 8 |
| Maximum | 12 | 23 | 8 | 16 | 16 | 9 | 8 | 10 | 10 |
| Confidence Level (95.0%) | 0.13 | 0.27 | 0 | 0.09 | 0.09 | 0.20 | 0.12 | 0.23 | 0.26 |

**Greece**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 11 | 22 | 8 | 15 | 15 | 7 | 6 | 10 | 10 |
| Standard Error | 0.04 | 0.10 | 0 | 0.07 | 0.05 | 0.17 | 0.18 | 0.04 | 0.07 |
| Median | 11 | 22 | 8 | 15 | 15 | 7 | 7 | 10 | 10 |
| Mode | 11 | 22 | 8 | 15 | 15 | 7 | 7 | 10 | 10 |
| Standard Deviation | 0.25 | 0.69 | 0 | 0.50 | 0.37 | 1.14 | 1.19 | 0.30 | 0.46 |
| Range | 1 | 3 | 0 | 2 | 2 | 4 | 6 | 2 | 2 |
| Minimum | 10 | 20 | 8 | 14 | 14 | 4 | 2 | 9 | 9 |
| Maximum | 11 | 23 | 8 | 16 | 16 | 8 | 8 | 11 | 11 |
| Confidence Level (95.0%) | 0.08 | 0.21 | 0 | 0.15 | 0.11 | 0.34 | 0.36 | 0.09 | 0.14 |

Table IX - Descriptive statistics of the meristic traits recorded for each one of the samples of *Spondyliosoma cantharus* (DF- dorsal fin, CF- caudal fin, AF- anal fin, RPF- right pectoral fin, LPF- left pectoral fin, URGR- upper right gill raker, ULGR- upper right gill raker, LRGR- lower right gill raker, LLGR- lower right gill raker)

**Portugal**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 12 | 22 | 10 | 16 | 16 | 10 | 10 | 14 | 14 |
| Standard Error | 0.06 | 0.12 | 0.04 | 0.06 | 0.07 | 0.13 | 0.22 | 0.18 | 0.17 |
| Median | 12 | 21 | 10 | 16 | 16 | 10 | 10 | 14 | 14 |
| Mode | 12 | 21 | 10 | 16 | 16 | 9 | 9 | 14 | 14 |
| Standard Deviation | 0.38 | 0.75 | 0.28 | 0.39 | 0.43 | 0.81 | 1.40 | 1.13 | 1.09 |
| Range | 2 | 3 | 2 | 1 | 1 | 3 | 9 | 6 | 6 |
| Minimum | 10 | 21 | 9 | 15 | 15 | 8 | 8 | 10 | 10 |
| Maximum | 12 | 24 | 11 | 16 | 16 | 11 | 17 | 16 | 16 |
| Confidence Level (95.0%) | 0.12 | 0.24 | 0.09 | 0.13 | 0.14 | 0.26 | 0.46 | 0.37 | 0.35 |

**Spain**

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 12 | 22 | 10 | 15 | 15 | 9 | 9 | 16 | 16 |
| Standard Error | 0.04 | 0.12 | 0.10 | 0.07 | 0.07 | 0.11 | 0.12 | 0.12 | 0.18 |
| Median | 12 | 22 | 10 | 15 | 15 | 9 | 9 | 16 | 16 |
| Mode | 12 | 22 | 10 | 15 | 15 | 9 | 9 | 16 | 16 |
| Standard Deviation | 0.21 | 0.58 | 0.47 | 0.34 | 0.34 | 0.54 | 0.56 | 0.58 | 0.85 |
| Range | 1 | 2 | 3 | 1 | 1 | 2 | 2 | 2 | 4 |
| Minimum | 11 | 21 | 9 | 15 | 15 | 8 | 8 | 15 | 15 |
| Maximum | 12 | 23 | 12 | 16 | 16 | 10 | 10 | 17 | 19 |
| Confidence Level (95.0%) | 0.09 | 0.25 | 0.21 | 0.15 | 0.15 | 0.23 | 0.24 | 0.25 | 0.37 |

Table X - Analysis of variance of the meristic traits recorded for all the species (DF- dorsal fin, CF- caudal fin, AF- anal fin, RPF- right pectoral fin, LPF- left pectoral fin, URGR- upper right gill raker, ULGR- upper right gill raker, LRGR- lower right gill raker, LLGR- lower right gill raker)

| | | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|---|
| | France | 0 | 0.61 | 0 | 0.24 | 0.13 | 0.24 | 0.34 | 0.35 | 0.25 |
| | Portugal | 0.42 | 0.70 | 0.24 | 0.39 | 0.40 | 0.45 | 0.45 | 0.47 | 0.38 |
| *Sparus aurata* reared | Spain Atlantic | 0.42 | 0.34 | 0.02 | 0.34 | 0.50 | 0.29 | 0.44 | 0.49 | 0.65 |
| | Spain Mediterranean | 0.23 | 0.74 | 0.20 | 0.29 | 0.57 | 0.25 | 0.27 | 0.38 | 0.56 |
| | Italy | 0.02 | 0.27 | 0.26 | 1.09 | 1.22 | 0.31 | 0.35 | 0.38 | 0.30 |
| | Greece | 0.24 | 0.41 | 0.41 | 0.47 | 0.70 | - | - | - | - |
| | Portugal | 0.10 | 0.25 | 0.02 | 0.31 | 0.27 | 0.26 | 0.28 | 0.56 | 0.60 |
| | Spain Atlantic | 0.10 | 0.46 | 0.02 | 0.27 | 0.23 | 0.74 | 0.61 | 0.28 | 0.47 |
| *Sparus aurata* wild | Spain Mediterranean | 0.14 | 0.20 | 0.02 | 0.12 | 0.12 | 0.27 | 0.16 | 0.29 | 0.28 |
| | Italy | 0 | 0.20 | 0.09 | 0.14 | 0.47 | 0.25 | 0.26 | 0.30 | 0.21 |
| | Greece | 0.05 | 0.40 | 0.44 | 0.14 | 0.30 | 0.07 | 0.05 | 0.18 | 0.26 |
| | Portugal | 0.08 | 1.37 | 0 | 0.25 | 0.20 | 0.35 | 0.36 | 0.28 | 0.28 |
| *Pagrus pagrus* | Spain | 0 | 0.78 | 0 | 0.36 | 0.24 | 0.46 | 0.71 | 0.34 | 0.53 |
| | Italy | 0.03 | 0.46 | 0.03 | 0.13 | 0.20 | 0.32 | 0.29 | 0.29 | 0.38 |
| | Greece | 0 | 0.66 | 0 | 0.15 | 0.16 | 0.56 | 0.34 | 0.29 | 0.29 |
| | Portugal | 0.65 | 1.15 | 0.28 | 0.18 | 0.23 | 0.37 | 0.48 | 0.40 | 0.38 |
| *Diplodus sargus* | Spain | 0.08 | 0.86 | 0.26 | 0.26 | 0.23 | 0.26 | 0.35 | 0.25 | 0.43 |
| | Italy | 0.25 | 0.48 | 0.20 | 0.94 | 0.17 | 0.57 | 0.31 | 0.20 | 0.26 |
| | Greece | 0.34 | 1.00 | 0.12 | 0.12 | 0.29 | 0.32 | 0.43 | 0.25 | 0.31 |
| | Portugal | 0.10 | 0.99 | 0.03 | 0.14 | 0.20 | 0.54 | 0.37 | 0.29 | 0.32 |
| *Diplodus puntazzo* | Spain | 0.20 | 0 | 0 | 0.52 | 0.49 | 0.30 | 0.46 | 0.44 | 0.41 |
| | Italy | 0.20 | 0.48 | 0.26 | 0.14 | 0.05 | 0.24 | 0.30 | 0.25 | 0.23 |
| | Greece | 0.19 | 0.66 | 0.23 | 0.17 | 0.18 | 0.48 | 0.50 | 0.21 | 0.22 |
| | Portugal | 0.09 | 0.48 | 0.03 | 0.24 | 0.23 | 0.59 | 0.61 | 0.66 | 0.46 |
| *Lithognathus mormyrus* | Spain | 0.03 | 0.29 | 0 | 0.21 | 0.28 | 0.45 | 0.43 | 0.60 | 0.47 |
| | Italy | 0.08 | 0.33 | 0.04 | 0.12 | 0.12 | 0.24 | 0.21 | 0.33 | 0.36 |
| | Greece | 0 | 0.04 | 0.08 | 0.18 | 0.18 | 1.16 | 0.80 | 0.47 | 0.40 |
| | Portugal | 0.09 | 0.44 | 0.06 | 0.37 | 0.23 | 0.63 | 0.58 | 0.78 | 0.65 |
| *Pagellus bogaraveo* | Spain | 0.21 | 0.67 | 0 | 0.22 | 0.28 | 0.63 | 0.39 | 0.59 | 0.64 |
| | Italy | 0.19 | 0.25 | 0.16 | 0.24 | 0.24 | 0.55 | 1.30 | 0.80 | 0.73 |
| | Greece | 0.21 | 0.29 | 0 | 0.54 | 0.32 | 0.42 | 0.60 | 0.61 | 0.75 |
| | Portugal | 0.05 | 0.62 | 0.03 | 0.38 | 0.29 | 0.73 | 0.21 | 0.84 | 0.80 |
| *Dentex dentex* | Spain | 0.07 | 0.55 | 0 | 0.19 | 0.14 | 0.36 | 0.52 | 0.73 | 0.56 |
| | Italy | 0.09 | 0.38 | 0 | 0.04 | 0.04 | 0.21 | 0.08 | 0.29 | 0.36 |
| | Greece | 0.06 | 0.47 | 0 | 0.25 | 0.13 | 1.30 | 1.43 | 0.09 | 0.21 |
| *Spondyliosoma cantharus* | Portugal | 0.15 | 0.56 | 0.08 | 0.15 | 0.18 | 0.65 | 1.97 | 1.29 | 1.18 |
| | Spain | 0.04 | 0.34 | 0.23 | 0.12 | 0.12 | 0.29 | 0.31 | 0.33 | 0.72 |

Table XI - $p$ values, ($p<0.01$) of the Man-Whitney $U$ test performed for each one of the meristic characteristics between samples of wild *Sparus aurata* (Atl. - Atlantic, Med - Mediterranean).

**Dorsal fin**

| | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|
| Portugal | | 0.975 | 0.757 | 0.631 | 0.392 |
| Spain Atl. | 0.975 | | 0.738 | 0.615 | 0.383 |
| Spain Med. | 0.757 | 0.738 | | 0.871 | 0.592 |
| Italy | 0.631 | 0.615 | 0.871 | | 0.700 |
| Greece | 0.392 | 0.383 | 0.592 | 0.700 | |

**Caudal fin**

| | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|
| Portugal | | 0.130 | 0.447 | 0.406 | 0.178 |
| Spain Atl. | 0.130 | | 0.392 | 0.484 | 0.910 |
| Spain Med. | 0.447 | 0.392 | | 0.903 | 0.493 |
| Italy | 0.406 | 0.484 | 0.903 | | 0.587 |
| Greece | 0.178 | 0.910 | 0.493 | 0.587 | |

**Anal fin**

| | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|
| Portugal | | 0.992 | 0.997 | 0.512 | 0.379 |
| Spain Atl. | 0.992 | | 0.994 | 0.524 | 0.390 |
| Spain Med. | 0.997 | 0.994 | | 0.516 | 0.383 |
| Italy | 0.512 | 0.524 | 0.516 | | 0.773 |
| Greece | 0.379 | 0.390 | 0.383 | 0.773 | |

**Right pectoral fin**

| | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|
| Portugal | | 0.828 | 0.031 | 0.062 | 0.062 |
| Spain Atl. | 0.828 | | 0.052 | 0.095 | 0.095 |
| Spain Med. | 0.031 | 0.052 | | 0.871 | 0.871 |
| Italy | 0.062 | 0.095 | 0.871 | | 1 |
| Greece | 0.062 | 0.095 | 0.871 | 1 | |

**Left pectoral fin**

| | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|
| Portugal | | 0.823 | 0.062 | 0.113 | 0.082 |
| Spain Atl. | 0.823 | | 0.099 | 0.159 | 0.123 |
| Spain Med. | 0.062 | 0.099 | | 0.994 | 0.948 |
| Italy | 0.113 | 0.159 | 0.994 | | 0.939 |
| Greece | 0.082 | 0.123 | 0.948 | 0.939 | |

**Upper right gill raker**

| | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|
| Portugal | | 0.001 | 0.001 | 0.025 | 0.001 |
| Spain Atl. | 0.001 | | 0.001 | 0.001 | 0.386 |
| Spain Med. | 0.001 | 0.001 | | 0.001 | 0.001 |
| Italy | 0.025 | 0.001 | 0.001 | | 0.001 |
| Greece | 0.001 | 0.386 | 0.001 | 0.001 | |

**Upper left gill raker**

| | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|
| Portugal | | 0.001 | 0.006 | 0.041 | 0.001 |
| Spain Atl. | 0.001 | | 0.001 | 0.001 | 0.535 |
| Spain Med. | 0.006 | 0.001 | | 0.001 | 0.001 |
| Italy | 0.041 | 0.001 | 0.001 | | 0.001 |
| Greece | 0.001 | 0.535 | 0.001 | 0.001 | |

**Lower right gill raker**

| | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|
| Portugal | | 0.001 | 0.001 | 0.447 | 0.001 |
| Spain Atl. | 0.001 | | 0.879 | 0.001 | 0.232 |
| Spain Med. | 0.001 | 0.879 | | 0.001 | 0.295 |
| Italy | 0.447 | 0.001 | 0.001 | | 0.001 |
| Greece | 0.001 | 0.232 | 0.295 | 0.001 | |

**Lower left gill raker**

| | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|
| Portugal | | 0.001 | 0.001 | 0.777 | 0.001 |
| Spain Atl. | 0.001 | | 0.691 | 0.001 | 0.062 |
| Spain Med. | 0.001 | 0.691 | | 0.001 | 0.099 |
| Italy | 0.777 | 0.001 | 0.001 | | 0.001 |
| Greece | 0.001 | 0.062 | 0.099 | 0.001 | |

Table XII - $p$ values, ($p$ <0.01) of the Man-Whitney $U$ test performed for each one of the meristic characteristics between samples of reared *Sparus aurata* (Atl. - Atlantic, Med - Mediterranean).

**Dorsal fin**

| | France | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|---|
| France | | 0.006 | 0.056 | 0.001 | 0.891 | 0.522 |
| Portugal | 0.006 | | 0.001 | 0.001 | 0.001 | 0.011 |
| Spain Atl. | 0.056 | 0.001 | | 0.001 | 0.012 | 0.005 |
| Spain Med. | 0.001 | 0.001 | 0.001 | | 0.001 | 0.001 |
| Italy | 0.891 | 0.001 | 0.012 | 0.001 | | 0.515 |
| Greece | 0.522 | 0.011 | 0.005 | 0.001 | 0.515 | |

**Caudal fin**

| | France | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|---|
| France | | 0.004 | 0.009 | 0.803 | 0.330 | 0.676 |
| Portugal | 0.004 | | 0.535 | 0.000 | 0.003 | 0.000 |
| Spain Atl. | 0.009 | 0.535 | | 0.001 | 0.015 | 0.002 |
| Spain Med. | 0.803 | 0.000 | 0.001 | | 0.137 | 0.414 |
| Italy | 0.330 | 0.003 | 0.015 | 0.137 | | 0.462 |
| Greece | 0.676 | 0.001 | 0.002 | 0.414 | 0.462 | |

**Anal fin**

| | France | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|---|
| France | | 0.785 | 0.891 | 0.172 | 0.172 | 0.096 |
| Portugal | 0.785 | | 0.622 | 0.069 | 0.066 | 0.026 |
| Spain Atl. | 0.891 | 0.622 | | 0.120 | 0.119 | 0.051 |
| Spain Med. | 0.172 | 0.069 | 0.120 | | 0.970 | 0.623 |
| Italy | 0.172 | 0.066 | 0.119 | 0.970 | | 0.656 |
| Greece | 0.096 | 0.026 | 0.051 | 0.623 | 0.656 | |

**Right pectoral fin**

| | France | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|---|
| France | | 0.001 | 0.001 | 0.009 | 0.001 | 0.001 |
| Portugal | 0.001 | | 0.828 | 0.001 | 0.026 | 0.001 |
| Spain Atl. | 0.001 | 0.828 | | 0.001 | 0.018 | 0.001 |
| Spain Med. | 0.009 | 0.001 | 0.001 | | 0.002 | 0.066 |
| Italy | 0.001 | 0.026 | 0.018 | 0.002 | | 0.075 |
| Greece | 0.001 | 0.001 | 0.001 | 0.066 | 0.075 | |

**Left pectoral fin**

| | France | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|---|
| France | | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Portugal | 0.001 | | 0.444 | 0.001 | 0.067 | 0.001 |
| Spain Atl. | 0.001 | 0.444 | | 0.001 | 0.210 | 0.002 |
| Spain Med. | 0.001 | 0.001 | 0.001 | | 0.001 | 0.005 |
| Italy | 0.001 | 0.067 | 0.210 | 0.001 | | 0.225 |
| Greece | 0.001 | 0.001 | 0.002 | 0.005 | 0.225 | |

**Upper right gill raker**

| | France | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|---|
| France | | 0.001 | 0.001 | 0.001 | 0.001 | - |
| Portugal | 0.001 | | 0.333 | 0.145 | 0.021 | - |
| Spain Atl. | 0.001 | 0.333 | | 0.011 | 0.001 | - |
| Spain Med. | 0.001 | 0.145 | 0.011 | | 0.290 | - |
| Italy | 0.001 | 0.021 | 0.001 | 0.290 | | - |
| Greece | - | - | - | - | - | |

*Upper left gill raker*

| | France | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|---|
| France | | 0.001 | 0.001 | 0.001 | 0.001 | - |
| Portugal | 0.001 | | 0.058 | 0.321 | 0.193 | - |
| Spain Atl. | 0.001 | 0.058 | | 0.003 | 0.001 | - |
| Spain Med. | 0.001 | 0.321 | 0.003 | | 0.694 | - |
| Italy | 0.001 | 0.193 | 0.001 | 0.694 | | - |
| Greece | - | - | - | - | - | |

**Lower right gill raker**

| | France | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|---|
| France | | 0.002 | 0.601 | 0.001 | 0.001 | - |
| Portugal | 0.002 | | 0.002 | 0.076 | 0.001 | - |
| Spain Atl. | 0.601 | 0.002 | | 0.001 | 0.001 | - |
| Spain Med. | 0.001 | 0.076 | 0.001 | | 0.091 | - |
| Italy | 0.001 | 0.001 | 0.001 | 0.091 | | - |
| Greece | - | - | - | - | - | |

**Lower left gill raker**

| | France | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|---|
| France | | 0.013 | 0.726 | 0.001 | 0.001 | - |
| Portugal | 0.013 | | 0.003 | 0.059 | 0.001 | - |
| Spain Atl. | 0.726 | 0.003 | | 0.001 | 0.001 | - |
| Spain Med. | 0.001 | 0.059 | 0.001 | | 0.018 | - |
| Italy | 0.001 | 0.001 | 0.001 | 0.018 | | - |
| Greece | - | - | - | - | - | |

Table XIII - $p$ values, ($p<0.01$) of the Man-Whitney $U$ test performed for each one of the meristic characteristics between samples of *Pagrus pagrus*

**Dorsal fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.675 | 0.901 | 0.805 |
| Spain | 0.675 | | 0.820 | 1.000 |
| Italy | 0.901 | 0.820 | | 0.805 |
| Greece | 0.805 | 1.000 | 0.805 | |

**Caudal fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.305 | 0.111 | 0.071 |
| Espanha | 0.305 | | 0.442 | 0.431 |
| Itália | 0.111 | 0.442 | | 0.996 |
| Grécia | 0.071 | 0.431 | 0.996 | |

**Anal fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 1.000 | 0.062 | 1.000 |
| Espanha | 1.000 | | 0.275 | 1.000 |
| Itália | 0.062 | 0.275 | | 0.805 |
| Grécia | 1.000 | 1.000 | 0.805 | |

**Right pectoral fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.579 | 0.033 | 0.154 |
| Espanha | 0.579 | | 0.203 | 0.585 |
| Itália | 0.033 | 0.203 | | 0.354 |
| Grécia | 0.154 | 0.585 | 0.354 | |

**Left pectoral fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.501 | 0.430 | 0.465 |
| Espanha | 0.501 | | 0.245 | 0.245 |
| Itália | 0.430 | 0.245 | | 0.852 |
| Grécia | 0.465 | 0.245 | 0.852 | |

**Upper right gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.000 | 0.000 | 0.000 |
| Espanha | 0.000 | | 0.157 | 0.000 |
| Itália | 0.000 | 0.157 | | 0.013 |
| Grécia | 0.000 | 0.000 | 0.013 | |

**Upper left gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.009 | 0.000 | 0.000 |
| Espanha | 0.009 | | 0.043 | 0.002 |
| Itália | 0.000 | 0.043 | | 0.298 |
| Grécia | 0.000 | 0.002 | 0.298 | |

**Lower right gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.000 | 0.000 | 0.000 |
| Espanha | 0.000 | | 0.951 | 0.126 |
| Itália | 0.000 | 0.951 | | 0.121 |
| Grécia | 0.000 | 0.126 | 0.121 | |

**Lower left gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.000 | 0.000 | 0.003 |
| Espanha | 0.000 | | 0.190 | 0.001 |
| Itália | 0.000 | 0.190 | | 0.040 |
| Grécia | 0.003 | 0.001 | 0.040 | |

Table XIV - $p$ values, ($p<0.01$) of the Man-Whitney $U$ test performed for each one of the meristic characteristics between samples of *Diplodus sargus*

**Dorsal fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.001 | 0.097 | 0.301 |
| Spain | 0.001 | | 0.001 | 0.008 |
| Italy | 0.097 | 0.001 | | 0.012 |
| Greece | 0.301 | 0.008 | 0.012 | |

**Caudal fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.003 | 0.342 | 0.001 |
| Spain | 0.003 | | 0.004 | 0.001 |
| Italy | 0.342 | 0.004 | | 0.001 |
| Greece | 0.001 | 0.001 | 0.001 | |

**Anal fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.031 | 0.040 | 0.531 |
| Spain | 0.031 | | 0.716 | 0.168 |
| Italy | 0.040 | 0.716 | | 0.248 |
| Greece | 0.531 | 0.168 | 0.248 | |

**Right pectoral fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.201 | 0.015 | 0.907 |
| Spain | 0.201 | | 0.424 | 0.332 |
| Italy | 0.015 | 0.424 | | 0.066 |
| Greece | 0.907 | 0.332 | 0.066 | |

**Left pectoral fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.373 | 0.177 | 0.967 |
| Spain | 0.373 | | 0.815 | 0.516 |
| Italy | 0.177 | 0.815 | | 0.342 |
| Greece | 0.967 | 0.516 | 0.342 | |

**Upper right gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.584 | 0.001 | 0.266 |
| Spain | 0.584 | | 0.001 | 0.631 |
| Italy | 0.001 | 0.001 | | 0.001 |
| Greece | 0.266 | 0.631 | 0.001 | |

**Upper left gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.855 | 0.001 | 0.959 |
| Spain | 0.855 | | 0.001 | 0.834 |
| Italy | 0.001 | 0.001 | | 0.001 |
| Greece | 0.959 | 0.834 | 0.001 | |

**Lower right gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.001 | 0.001 | 0.002 |
| Spain | 0.001 | | 0.001 | 0.139 |
| Italy | 0.001 | 0.001 | | 0.001 |
| Greece | 0.002 | 0.139 | 0.001 | |

**Lower left gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.001 | 0.001 | 0.001 |
| Spain | 0.001 | | 0.001 | 0.447 |
| Italy | 0.001 | 0.001 | | 0.001 |
| Greece | 0.001 | 0.447 | 0.001 | |

Table XV - $p$ values, ($p<0.01$) of the Man-Whitney $U$ test performed for each one of the meristic characteristics between samples of *Diplodus puntazzo*

**Dorsal fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.693 | 0.683 | 0.443 |
| Spain | 0.693 | | 0.992 | 0.722 |
| Italy | 0.683 | 0.992 | | 0.727 |
| Greece | 0.443 | 0.722 | 0.727 | |

**Caudal fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.096 | 0.003 | 0.001 |
| Spain | 0.096 | | 0.038 | 0.001 |
| Italy | 0.003 | 0.038 | | 0.001 |
| Greece | 0.001 | 0.001 | 0.001 | |

**Anal fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.001 | 0.019 | 0.005 |
| Spain | 0.001 | | 0.001 | 0.001 |
| Italy | 0.019 | 0.001 | | 0.707 |
| Greece | 0.005 | 0.001 | 0.707 | |

**Right pectoral fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.001 | 0.001 | 0.001 |
| Spain | 0.001 | | 0.906 | 0.813 |
| Italy | 0.001 | 0.906 | | 0.710 |
| Greece | 0.001 | 0.813 | 0.710 | |

**Left pectoral fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.001 | 0.001 | 0.001 |
| Spain | 0.001 | | 0.802 | 0.953 |
| Italy | 0.001 | 0.802 | | 0.714 |
| Greece | 0.001 | 0.953 | 0.714 | |

**Upper right gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.044 | 0.016 | 0.005 |
| Spain | 0.044 | | 0.001 | 0.325 |
| Italy | 0.016 | 0.001 | | 0.001 |
| Greece | 0.005 | 0.325 | 0.001 | |

**Upper left gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.054 | 0.005 | 0.001 |
| Spain | 0.054 | | 0.001 | 0.024 |
| Italy | 0.005 | 0.001 | | 0.001 |
| Greece | 0.001 | 0.024 | 0.001 | |

**Lower right gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.001 | 0.706 | 0.001 |
| Spain | 0.001 | | 0.001 | 0.120 |
| Italy | 0.706 | 0.001 | | 0.001 |
| Greece | 0.001 | 0.120 | 0.001 | |

**Lower left gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.001 | 0.342 | 0.001 |
| Spain | 0.001 | | 0.001 | 0.982 |
| Italy | 0.342 | 0.001 | | 0.001 |
| Greece | 0.001 | 0.982 | 0.001 | |

Table XVI - $p$ values, ($p<0.01$) of the Man-Whitney $U$ test performed for each one of the meristic characteristics between samples of *Lithognathus mormyrus*

**Dorsal fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.928 | 0.752 | 0.738 |
| Spain | 0.928 | | 0.843 | 0.835 |
| Italy | 0.752 | 0.843 | | 1 |
| Greece | 0.738 | 0.835 | 1 | |

**Caudal fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.077 | 0.005 | 0.001 |
| Spain | 0.077 | | 0.229 | 0.001 |
| Italy | 0.005 | 0.229 | | 0.001 |
| Greece | 0.001 | 0.001 | 0.001 | |

**Anal fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.774 | 0.960 | 0.797 |
| Spain | 0.774 | | 0.798 | 1.000 |
| Italy | 0.960 | 0.798 | | 0.814 |
| Greece | 0.797 | 1.000 | 0.814 | |

**Right pectoral fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.001 | 0.001 | 0.001 |
| Spain | 0.001 | | 0.050 | 0.701 |
| Italy | 0.001 | 0.050 | | 0.122 |
| Greece | 0.001 | 0.701 | 0.122 | |

**Left pectoral fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.001 | 0.001 | 0.001 |
| Spain | 0.001 | | 0.125 | 0.665 |
| Italy | 0.001 | 0.125 | | 0.266 |
| Greece | 0.001 | 0.665 | 0.266 | |

**Upper right gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.001 | 0.001 | 0.001 |
| Spain | 0.001 | | 0.327 | 0.004 |
| Italy | 0.001 | 0.327 | | 0.031 |
| Greece | 0.001 | 0.004 | 0.031 | |

**Upper left gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.002 | 0.001 | 0.001 |
| Spain | 0.002 | | 0.249 | 0.037 |
| Italy | 0.001 | 0.249 | | 0.239 |
| Greece | 0.001 | 0.037 | 0.239 | |

**Lower right gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.001 | 0.001 | 0.001 |
| Spain | 0.001 | | 0.415 | 0.911 |
| Italy | 0.001 | 0.415 | | 0.451 |
| Greece | 0.001 | 0.911 | 0.451 | |

**Lower left gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.001 | 0.001 | 0.001 |
| Spain | 0.001 | | 0.553 | 1 |
| Italy | 0.001 | 0.553 | | 0.553 |
| Greece | 0.001 | 1 | 0.553 | |

Table XVII - $p$ values, ($p<0.01$) of the Man-Whitney $U$ test for each one of the meristic characteristics between samples performed of *Pagellus bogaraveo*

**Dorsal fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.844 | 0.902 | 0.413 |
| Spain | 0.844 | | 0.759 | 0.292 |
| Italy | 0.902 | 0.759 | | 0.505 |
| Greece | 0.413 | 0.292 | 0.505 | |

**Caudal fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.492 | 0.001 | 0.306 |
| Spain | 0.492 | | 0.001 | 0.080 |
| Italy | 0.001 | 0.001 | | 0.002 |
| Greece | 0.306 | 0.080 | 0.002 | |

**Anal fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.621 | 0.693 | 0.660 |
| Spain | 0.621 | | 1 | 1 |
| Italy | 0.693 | 1 | | 1 |
| Greece | 0.660 | 1 | 1 | |

**Right pectoral fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.174 | 0.003 | 0.037 |
| Spain | 0.174 | | 0.020 | 0.221 |
| Italy | 0.003 | 0.020 | | 0.463 |
| Greece | 0.037 | 0.221 | 0.463 | |

**Left pectoral fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.235 | 0.001 | 0.024 |
| Spain | 0.235 | | 0.005 | 0.172 |
| Italy | 0.001 | 0.005 | | 0.177 |
| Greece | 0.024 | 0.172 | 0.177 | |

**Upper right gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.160 | 0.001 | 0.013 |
| Spain | 0.160 | | 0.001 | 0.001 |
| Italy | 0.000 | 0.001 | | 0.001 |
| Greece | 0.013 | 0.001 | 0.001 | |

**Upper left gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.724 | 0.062 | 0.001 |
| Spain | 0.724 | | 0.007 | 0.001 |
| Italy | 0.062 | 0.007 | | 0.001 |
| Greece | 0.001 | 0.001 | 0.001 | |

**Lower right gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.002 | 0.657 | 0.722 |
| Spain | 0.002 | | 0.013 | 0.003 |
| Italy | 0.657 | 0.013 | | 0.879 |
| Greece | 0.722 | 0.003 | 0.879 | |

**Lower left gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.001 | 0.005 | 0.222 |
| Spain | 0.001 | | 0.070 | 0.002 |
| Italy | 0.005 | 0.070 | | 0.169 |
| Greece | 0.222 | 0.002 | 0.169 | |

Table XVIII - $p$ values, ($p<0.01$) of the Man-Whitney $U$ test performed for each one of the meristic characteristics between samples of *Dentex dentex*

**Dorsal fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.909 | 0.743 | 0.913 |
| Spain | 0.909 | | 0.685 | 0.987 |
| Italy | 0.743 | 0.685 | | 0.669 |
| Greece | 0.913 | 0.987 | 0.669 | |

**Caudal fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.054 | 0.050 | 0.004 |
| Spain | 0.054 | | 0.978 | 0.674 |
| Italy | 0.050 | 0.978 | | 0.688 |
| Greece | 0.004 | 0.674 | 0.688 | |

**Anal fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.854 | 0.864 | 0.837 |
| Spain | 0.854 | | 1 | 1 |
| Italy | 0.864 | 1 | | 1 |
| Greece | 0.837 | 1 | 1 | |

**Right pectoral fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.265 | 0.917 | 0.949 |
| Spain | 0.265 | | 0.285 | 0.191 |
| Italy | 0.917 | 0.285 | | 0.856 |
| Greece | 0.949 | 0.191 | 0.856 | |

**Left pectoral fin**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.356 | 0.795 | 0.780 |
| Spain | 0.356 | | 0.507 | 0.445 |
| Italy | 0.795 | 0.507 | | 0.984 |
| Greece | 0.780 | 0.445 | 0.984 | |

**Upper right gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.257 | 0.001 | 0.191 |
| Spain | 0.257 | | 0.001 | 0.021 |
| Italy | 0.001 | 0.001 | | 0.001 |
| Greece | 0.191 | 0.021 | 0.001 | |

**Upper left gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.459 | 0.001 | 0.060 |
| Spain | 0.459 | | 0.001 | 0.022 |
| Italy | 0.001 | 0.001 | | 0.001 |
| Greece | 0.060 | 0.022 | 0.001 | |

**Lower right gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.001 | 0.001 | 0.001 |
| Spain | 0.001 | | 0.001 | 0.089 |
| Italy | 0.001 | 0.001 | | 0.001 |
| Greece | 0.001 | 0.089 | 0.001 | |

**Lower left gill raker**

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Portugal | | 0.001 | 0.001 | 0.001 |
| Spain | 0.001 | | 0.001 | 0.034 |
| Italy | 0.001 | 0.001 | | 0.001 |
| Greece | 0.001 | 0.034 | 0.001 | |

Table XIX - *p* values, ($p<0.01$) of the Man-Whitney *U* test performed for each one of the meristic characteristics between samples of *Spondyliosoma cantharus* (DF- dorsal fin, CF- caudal fin, AF- anal fin, RPF- right pectoral fin, LPF- left pectoral fin, URGR- upper right gill raker, ULGR- upper right gill raker, LRGR- lower right gill raker, LLGR- lower right gill raker)

| | Portugal/Spain |
|---|---|
| DF | 0.821 |
| CF | 0.793 |
| AF | 0.884 |
| RPF | 0.001 |
| LPF | 0.001 |
| URGR | 0.001 |
| ULGR | 0.001 |
| LRGR | 0.001 |
| LLGR | 0.001 |

# ANNEX II

Table I - Descriptive statistics over the size (standard length) of the six reared samples of *S. aurata*.

| | France | Portugal | Sp. Atl. | Sp. Med. | Italy | Greece |
|---|---|---|---|---|---|---|
| Mean | 318.33 | 152.40 | 164.32 | 180.42 | 141.70 | 193.31 |
| Standard Error | 2.16 | 2.16 | 4.84 | 1.10 | 1.07 | 1.52 |
| Median | 315 | 153 | 151 | 180 | 142.5 | 194.5 |
| Mode | 312 | 145 | 143 | 175 | 149 | 201 |
| Standard Deviation | 13.65 | 15.25 | 34.24 | 7.75 | 7.56 | 10.93 |
| Sample Variance | 186.33 | 232.53 | 1172.30 | 60.00 | 57.19 | 119.55 |
| Range | 61 | 60 | 124 | 35 | 28 | 52 |
| Minimum | 289 | 119 | 109 | 160 | 127 | 162 |
| Maximum | 350 | 179 | 233 | 195 | 155 | 214 |

Table II - Descriptive statistics over the size (standard length) of the five wild samples of *S. aurata*.

| | Portugal | Sp. Atl. | Sp. Med. | Italy | Greece |
|---|---|---|---|---|---|
| Mean | 187.23 | 247.65 | 202.86 | 132.13 | 144.20 |
| Standard Error | 3.27 | 1.37 | 2.43 | 1.03 | 1.95 |
| Median | 186 | 247 | 206 | 132.5 | 142 |
| Mode | 189 | 245 | 220 | 128 | 151 |
| Standard Deviation | 28.66 | 9.51 | 17.36 | 6.50 | 12.32 |
| Sample Variance | 821.23 | 90.36 | 301.20 | 42.27 | 151.75 |
| Range | 180 | 42 | 76 | 26 | 48 |
| Minimum | 147 | 230 | 170 | 118 | 121 |
| Maximum | 327 | 272 | 246 | 144 | 169 |

Table III - Descriptive statistics over the size (standard length) of the four samples of *P. pagrus*.

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Mean | 185.36 | 208.95 | 169.35 | 121.85 |
| Standard Error | 3.12 | 3.90 | 1.73 | 4.30 |
| Median | 175 | 206 | 170 | 114 |
| Mode | 170 | 210 | 170 | 98 |
| Standard Deviation | 32.59 | 23.73 | 9.64 | 31.57 |
| Sample Variance | 1062.32 | 563.22 | 92.90 | 996.39 |
| Range | 147 | 110 | 40 | 103 |
| Minimum | 148 | 175 | 140 | 79 |
| Maximum | 295 | 285 | 180 | 182 |

Table IV - Descriptive statistics over the size (standard length) of the four samples of *D. sargus*.

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Mean | 209.66 | 200.75 | 160.00 | 181.68 |
| Standard Error | 4.66 | 3.40 | 1.69 | 4.38 |
| Median | 232 | 201.332 | 160 | 182.85 |
| Mode | 151 | 224 | 160 | 185 |
| Standard Deviation | 49.74 | 18.61 | 9.68 | 24.00 |
| Sample Variance | 2474.28 | 346.44 | 93.75 | 576.06 |
| Range | 147 | 75 | 40 | 100 |
| Minimum | 143 | 155 | 140 | 130 |
| Maximum | 290 | 230 | 180 | 230 |

Table V - Descriptive statistics over the size (standard length) of the four samples of *D. puntazzo*.

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Mean | 330.65 | 232.68 | 172.93 | 170.51 |
| Standard Error | 8.64 | 6.98 | 1.36 | 1.10 |
| Median | 352 | 234 | 170 | 171 |
| Mode | 364 | 210 | 170 | 175 |
| Standard Deviation | 51.12 | 44.67 | 8.73 | 7.23 |
| Sample Variance | 2613.11 | 1995.34 | 76.22 | 52.30 |
| Range | 191 | 182 | 40 | 35 |
| Minimum | 206 | 150 | 150 | 147 |
| Maximum | 397 | 332 | 190 | 182 |

Table VI - Descriptive statistics over the size (standard length) of the four samples of *L. mormyrus*.

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Mean | 223.96 | 218.36 | 180.96 | 184.60 |
| Standard Error | 4.24 | 4.38 | 1.68 | 2.81 |
| Median | 242 | 217.5 | 181.08 | 183.33 |
| Mode | 265 | 206 | 174 | 212 |
| Standard Deviation | 47.00 | 24.80 | 9.35 | 15.64 |
| Sample Variance | 2208.99 | 615.17 | 87.41 | 244.68 |
| Range | 135 | 135.4 | 37 | 62 |
| Minimum | 153 | 119.6 | 167 | 150 |
| Maximum | 288 | 255 | 204 | 212 |

Table VII - Descriptive statistics over the size (standard length) of the four samples of *P. bogaraveo*.

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Mean | 250.04 | 138.19 | 230.77 | 137.34 |
| Standard Error | 5.29 | 1.02 | 2.24 | 2.43 |
| Median | 255 | 139.19 | 231.39 | 134 |
| Mode | 290 | 140 | 240 | 126 |
| Standard Deviation | 45.22 | 7.12 | 12.70 | 13.73 |
| Sample Variance | 2044.87 | 50.76 | 161.16 | 188.41 |
| Range | 286 | 37 | 60 | 62 |
| Minimum | 24 | 123 | 190 | 119 |
| Maximum | 310 | 160 | 250 | 181 |

Table VIII - Descriptive statistics over the size (standard length) of the four samples of *D. dentex*.

| | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|
| Mean | 220.18 | 234.51 | 151.94 | 142.98 |
| Standard Error | 10.22 | 4.82 | 4.33 | 2.84 |
| Median | 181.5 | 230 | 150.40 | 142 |
| Mode | 180 | 212 | 150 | 160 |
| Standard Deviation | 62.98 | 26.85 | 23.72 | 19.02 |
| Sample Variance | 3966.53 | 720.99 | 562.82 | 361.79 |
| Range | 177 | 138 | 137 | 107 |
| Minimum | 162 | 162 | 129 | 93 |
| Maximum | 339 | 300 | 266 | 200 |

Table IX - Descriptive statistics over the size (standard length) of the two samples of *S.cantharus*.

| | Portugal | Spain |
|---|---|---|
| Mean | 199.97 | 244.20 |
| Standard Error | 3.98 | 9.64 |
| Median | 195 | 251 |
| Mode | 187 | 245 |
| Standard Deviation | 24.84 | 50.10 |
| Sample Variance | 616.97 | 2509.72 |
| Range | 144 | 211 |
| Minimum | 157 | 153 |
| Maximum | 301 | 364 |

Table X - Generalised Mahalanobis distances ($D^2$), for the linear discriminant functions based on the traditional measurements for the eight species in study. (Atl. - Atlantic; Med. - Mediterranean).

| | | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|---|
| | Portugal | - | | | | |
| *S. aurata* wild | Spain Atl. | 46.94 | - | | | |
| | Spain Med. | 8.34 | 40.73 | - | | |
| | Italy | 53.52 | 88.66 | 65.21 | - | |
| | Greece | 6.91 | 47.08 | 12.57 | 58.17 | - |

| | | France | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|---|---|
| | France | | | | | | |
| | Portugal | 1076.70 | - | | | | |
| *S. aurata* reared | Spain Atl. | 1067.13 | 58.80 | - | | | |
| | Spain Med. | 925.70 | 63.82 | 19.97 | - | | |
| | Italy | 1028.39 | 23.00 | 40.53 | 33.02 | - | |
| | Greece | 1116.39 | 38.20 | 72.40 | 49.63 | 40.56 | - |

| | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *P. pagrus* | Spain | 16.78 | - | | |
| | Italy | 30.28 | 19.32 | - | |
| | Greece | 11.31 | 15.29 | 23.58 | - |

| | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *D. sargus* | Spain | 5.46 | - | | |
| | Italy | 18.35 | 13.90 | - | |
| | Greece | 15.94 | 11.68 | 13.97 | - |

| | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *D. puntazzo* | Spain | 21.79 | - | | |
| | Italy | 25.43 | 15.47 | - | |
| | Greece | 30.35 | 12.49 | 6.41 | - |

| | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *L. mormyrus* | Spain | 16.96 | - | | |
| | Italy | 21.10 | 25.68 | - | |
| | Greece | 33.17 | 27.88 | 57.13 | - |

| | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *P. bogaraveo* | Spain | 46.01 | - | | |
| | Italy | 78.33 | 66.44 | - | |
| | Greece | 49.09 | 46.04 | 25.68 | - |

| | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *D. dentex* | Spain | 39.68 | - | | |
| | Italy | 28.15 | 18.89 | - | |
| | Greece | 31.46 | 11.19 | 5.47 | - |

Table XI - F-statistics for the linear discriminant functions based on the traditional measurements for the eight species in study. Atl. - Atlantic; Med. - Mediterranean; Asterisks denote **- P<0.001; ***- P<0.0001).

| df=12,24 | | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|---|
| | Portugal | - | | | | |
| | Spain Atl. | 108.62** | - | | | |
| *S. aurata* wild | Spain Med. | 20.04*** | 78.62** | - | | |
| | Italy | 109.91** | 150.58** | 113.85** | - | |
| | Greece | 14.18*** | 79.97** | 21.94** | 90.38** | - |

| df=12,28 | | France | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|---|---|
| | France | - | | | | | |
| | Portugal | 1874.05* | - | | | | |
| *S. aurata* reared | Spain Atl. | 1857.38* | 115.46* | - | | | |
| | Spain Med. | 1611.22* | 125.33* | 39.22* | - | | |
| | Italy | 1789.96* | 45.17* | 79.59* | 64.83* | - | |
| | Greece | 2010.36* | 77.95* | 147.75* | 101.29* | 82.78* | - |

| df=9.22 | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *P. pagrus* | Spain | 48.57** | - | | |
| | Italy | 76.22** | 33.89** | - | |
| | Greece | 43.1** | 35.15** | 48.42** | - |

| df=9.195 | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *D. sargus* | Spain | 13.44** | - | | |
| | Italy | 48.84** | 22.57*** | - | |
| | Greece | 39.26*** | 18.074*** | 22.7*** | - |

| df=10.147 | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *D. puntazzo* | Spain | 37.74** | - | | |
| | Italy | 44.04** | 24.11*** | - | |
| | Greece | 53.74** | 12.37*** | 29.15*** | - |

| df = 11,203 | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *L. mormyrus* | Spain | 36.33** | - | | |
| | Italy | 44.03** | 33.91** | - | |
| | Greece | 69.21** | 36.83** | 74.25** | - |

| df=9.174 | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *P. bogaraveo* | Spain | 140.75** | - | | |
| | Italy | 180.31** | 132.94** | - | |
| | Greece | 113** | 92.13** | 42.29** | - |

| df=8.133 | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *D. dentex* | Spain | 78.06 | - | | |
| | Italy | 54.35 | 33.08 | - | |
| | Greece | 75.10 | 23.71 | 11.35 | - |

Table XII - Percent classification success (PCS) for all traditional measurements for the eight species in study. Atl.- Atlantic, Med.- Mediterranean.

| | | PCS | Portugal | Sp. Atl. | Sp. Med. | Italy | Greece | N |
|---|---|---|---|---|---|---|---|---|
| | Portugal | 90.91 | 70 | 0 | 3 | 0 | 4 | 77 |
| | Sp. Atl. | 100 | 0 | 48 | 0 | 0 | 0 | 48 |
| *S. aurata* wild | Sp. Med. | 80.39 | 6 | 3 | 41 | 0 | 1 | 51 |
| | Italy | 100 | 0 | 0 | 0 | 40 | 0 | 40 |
| | Greece | 80 | 5 | 0 | 3 | 0 | 32 | 40 |
| | Total | 90.23 | | | | | | 256 |

| | | PCS | France | Portugal | Sp. Atl. | Sp. Med. | Italy | Greece | N |
|---|---|---|---|---|---|---|---|---|---|
| | France | 98 | 49 | 0 | 0 | 1 | 0 | 0 | 50 |
| | Portugal | 94 | 0 | 47 | 3 | 0 | 0 | 0 | 50 |
| *S. aurata* reared | Sp. Atl. | 98 | 0 | 1 | 49 | 0 | 0 | 0 | 50 |
| | Sp. Med. | 98 | 1 | 0 | 0 | 49 | 0 | 0 | 50 |
| | Italy | 100 | 0 | 0 | 0 | 0 | 54 | 0 | 54 |
| | Greece | 100 | 0 | 0 | 0 | 0 | 0 | 40 | 40 |
| | Total | 97.96 | | | | | | | 294 |

| | | PCS | Portugal | Spain | Italy | Greece | N |
|---|---|---|---|---|---|---|---|
| | Portugal | 94.50 | 103 | 3 | 0 | 3 | 109 |
| | Spain | 94.59 | 2 | 35 | 0 | 0 | 37 |
| *P. pagrus* | Italy | 100 | 0 | 0 | 31 | 0 | 31 |
| | Greece | 90.74 | 3 | 1 | 1 | 49 | 54 |
| | Total | 94.37 | | | | | 231 |

| | | PCS | Portugal | Spain | Italy | Greece | N |
|---|---|---|---|---|---|---|---|
| | Portugal | 93.86 | 107 | 5 | 1 | 1 | 114 |
| | Spain | 86.67 | 2 | 0 | 2 | 26 | 30 |
| *D. sargus* | Italy | 93.94 | 2 | 0 | 31 | 0 | 33 |
| | Greece | 70 | 6 | 21 | 1 | 2 | 30 |
| | Total | 89.37 | | | | | 207 |

| | | PCS | Portugal | Spain | Italy | Greece | N |
|---|---|---|---|---|---|---|---|
| | Portugal | 91.43 | 32 | 0 | 3 | 0 | 35 |
| | Spain | 95.35 | 0 | 0 | 2 | 41 | 43 |
| *D. puntazzo* | Italy | 82.93 | 0 | 0 | 34 | 7 | 41 |
| | Greece | 92.68 | 0 | 38 | 0 | 3 | 41 |
| | Total | 90.63 | | | | | 160 |

| | | PCS | Portugal | Spain | Italy | Greece | N |
|---|---|---|---|---|---|---|---|
| | Portugal | 97.56 | 120 | 2 | 1 | 0 | 123 |
| | Spain | 100 | 0 | 0 | 0 | 31 | 31 |
| *L. mormyrus* | Italy | 96.77 | 1 | 0 | 30 | 0 | 31 |
| | Greece | 87.5 | 4 | 28 | 0 | 0 | 32 |
| | Total | 96.31 | | | | | 217 |

| | | PCS | Portugal | Spain | Italy | Greece | N |
|---|---|---|---|---|---|---|---|
| | Portugal | 100 | 73 | 0 | 0 | 0 | 73 |
| | Spain | 100 | 0 | 49 | 0 | 0 | 49 |
| *P. bogaraveo* | Italy | 96.88 | 0 | 0 | 31 | 1 | 32 |
| | Greece | 96.88 | 0 | 1 | 0 | 31 | 32 |
| | Total | 98.92 | | | | | 186 |

| | | PCS | Portugal | Spain | Italy | Greece | N |
|---|---|---|---|---|---|---|---|
| | Portugal | 92.11 | 35 | 0 | 1 | 2 | 38 |
| | Spain | 82.22 | 0 | 4 | 4 | 37 | 45 |
| *D. dentex* | Italy | 93.33 | 0 | 0 | 28 | 2 | 30 |
| | Greece | 93.55 | 0 | 29 | 2 | 0 | 31 |
| | Total | 89.58 | | | | | 144 |

Table XIII - Generalised Mahalanobis distances ($D^2$) for the linear discriminant functions for all truss characters for all the eight species. (Atl.- Atlantic, Med.- Mediterranean)

| | | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|---|
| | Portugal | - | | | | |
| *S. aurata* wild | Spain Atl. | 43.5 | - | | | |
| | Spain Med. | 23 | 26 | - | | |
| | Italy | 50.5 | 65.7 | 53.4 | - | |
| | Greece | 25.5 | 33.4 | 8.1 | 59.1 | - |

| | | France | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|---|---|
| | France | - | | | | | |
| | Portugal | 535.4 | - | | | | |
| *S. aurata* reared | Spain Atl. | 545.8 | 40.5 | - | | | |
| | Spain Med. | 580.2 | 50.6 | 15.2 | - | | |
| | Italy | 708.9 | 129.5 | 73.4 | 62 | - | |
| | Greece | 520.8 | 65.7 | 27.1 | 15.6 | 86.8 | - |

| | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *P. pagrus* | Spain | 265.21 | - | | |
| | Italy | 232.78 | 20.66 | - | |
| | Greece | 542.52 | 398.89 | 378.26 | - |

| | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *D. sargus* | Spain | 15.08 | - | | |
| | Italy | 15.88 | 8.72 | - | |
| | Greece | 31.4 | 7.53 | 12.17 | - |

| | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *D. puntazzo* | Spain | 28.8 | - | | |
| | Italy | 34.01 | 15.92 | - | |
| | Greece | 41.27 | 23.02 | 7.18 | - |

| | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *L. mormyrus* | Spain | 17.78 | - | | |
| | Italy | 12.5 | 9.16 | - | |
| | Greece | 44.05 | 23.21 | 39.01 | - |

| | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *P. bogaraveo* | Spain | 86.42 | - | | |
| | Italy | 515.71 | 470.82 | - | |
| | Greece | 55.78 | 63.49 | 535.12 | - |

| | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *D. dentex* | Spain | 35.58 | - | | |
| | Italy | 42.59 | 25.37 | - | |
| | Greece | 53.88 | 31.81 | 19.24 | - |

Table XIV - F-statistics for the linear discriminant functions for all eight species in study (Atl.- Atlantic, Med.- Mediterranean). Asterisks denote **- p<0.001; ***- p<0.0001.

| df = 22.23 | | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|---|
| | Portugal | - | | | | |
| | Spain Atl. | 52.6** | - | | | |
| *S. aurata* wild | Spain Med. | 28.9** | 26.2** | - | | |
| | Italy | 54.2** | 58.4** | 48.7** | - | |
| | Greece | 27.3** | 29.6** | 7.4** | 48** | - |

| df = 26.263 | | France | Portugal | Spain Atl. | Spain Med. | Italy | Greece |
|---|---|---|---|---|---|---|---|
| | France | - | | | | | |
| | Portugal | 408** | - | | | | |
| *S. aurata* reared | Spain Atl. | 416.3** | 34.8** | - | | | |
| | Spain Med. | 442.6** | 43.6** | 13.1** | - | | |
| | Italy | 540.7** | 111.4** | 63.1** | 53.3** | - | |
| | Greece | 411** | 58.7** | 24.3** | 14** | 77.6** | - |

| df=18.21 | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *P. pagrus* | Spain | 368.02** | - | | |
| | Italy | 280.89** | 17.37** | - | |
| | Greece | 991.32** | 439.51** | 372.42** | - |

| df=9.195 | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *D. sargus* | Spain | 48.25*** | - | | |
| | Italy | 54.89*** | 18.39*** | - | |
| | Greece | 68.46*** | 15.15*** | 25.66*** | - |

| df=12.145 | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *D. puntazzo* | Spain | 55.8** | - | | |
| | Italy | 66.03** | 33.57*** | - | |
| | Greece | 81.75** | 49.72** | 15.5*** | - |

| df=10.204 | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *L. mormyrus* | Spain | 42.09** | - | | |
| | Italy | 28.84** | 13.37*** | - | |
| | Greece | 101.6** | 33.88** | 56.25** | - |

| df=14.169 | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *P. bogaraveo* | Spain | 165.08** | - | | |
| | Italy | 741.22** | 588.19** | - | |
| | Greece | 80.17** | 79.32** | 550.14** | - |

| df=14.127 | | Portugal | Spain | Italy | Greece |
|---|---|---|---|---|---|
| | Portugal | - | | | |
| *D. dentex* | Spain | 38.19** | - | | |
| | Italy | 44.86** | 36.77*** | - | |
| | Greece | 70.17** | 21.79** | 24.24*** | - |

Table XV - Percent classification success (PCS) for all eight species in study five sampling places. (Atl.- Atlantic, Med.- Mediterranean)

| | | PCS | Portugal | Sp. Atl. | Sp. Med. | Italy | Greece | N |
|---|---|---|---|---|---|---|---|---|
| | Portugal | 97.40 | 75 | 0 | 0 | 1 | 1 | 77 |
| | Spain Atl. | 100 | 0 | 48 | 0 | 0 | 0 | 48 |
| *S. aurata* wild | Spain Med. | 88.24 | 3 | 0 | 45 | 0 | 3 | 51 |
| | Italy | 100 | 0 | 0 | 0 | 40 | 0 | 40 |
| | Greece | 100 | 0 | 0 | 0 | 0 | 40 | 40 |
| | Mean | 96.88 | | | | | | 256 |

| | | PCS | France | Portugal | Sp. Atl. | Sp. Med. | Italy | Greece | N |
|---|---|---|---|---|---|---|---|---|---|
| | France | 100 | 40 | 0 | 0 | 0 | 0 | 0 | 40 |
| | Portugal | 98 | 0 | 49 | 0 | 1 | 0 | 0 | 50 |
| *S. aurata* reared | Spain Atl. | 92 | 0 | 0 | 46 | 4 | 0 | 0 | 50 |
| | Spain Med. | 90 | 0 | 0 | 1 | 45 | 0 | 4 | 50 |
| | Italy | 98 | 0 | 0 | 0 | 1 | 49 | 0 | 50 |
| | Greece | 96.30 | 0 | 0 | 0 | 2 | 0 | 52 | 54 |
| | Mean | 95.58 | | | | | | | 294 |

| | | PCS | Portugal | Spain | Italy | Greece | N |
|---|---|---|---|---|---|---|---|
| | Portugal | 100 | 109 | 0 | 0 | 0 | 109 |
| | Spain | 100 | 0 | 37 | 0 | 0 | 37 |
| *P. pagrus* | Italy | 96.77 | 0 | 1 | 30 | 0 | 31 |
| | Greece | 100 | 0 | 0 | 0 | 54 | 54 |
| | Mean | 99.57 | | | | | 231 |

| | | PCS | Portugal | Spain | Italy | Greece | N |
|---|---|---|---|---|---|---|---|
| | Portugal | 94.74 | 108 | 1 | 4 | 1 | 114 |
| | Spain | 90 | 0 | 27 | 1 | 2 | 30 |
| *D. sargus* | Italy | 90.91 | 1 | 2 | 30 | 0 | 33 |
| | Greece | 96.67 | 0 | 1 | 0 | 29 | 30 |
| | Mean | 93.72 | | | | | 207 |

| | | PCS | Portugal | Spain | Italy | Greece | N |
|---|---|---|---|---|---|---|---|
| | Portugal | 94.29 | 33 | 1 | 1 | 0 | 35 |
| | Spain | 85.37 | 0 | 35 | 6 | 0 | 41 |
| *D. puntazzo* | Italy | 85.37 | 0 | 3 | 35 | 3 | 41 |
| | Greece | 97.67 | 0 | 1 | 0 | 42 | 43 |
| | Mean | 90.63 | | | | | 160 |

| | | PCS | Portugal | Spain | Italy | Greece | N |
|---|---|---|---|---|---|---|---|
| | Portugal | 97.56 | 120 | 0 | 3 | 0 | 123 |
| | Spain | 93.75 | 1 | 30 | 1 | 0 | 32 |
| *L. mormyrus* | Italy | 96.77 | 1 | 0 | 30 | 0 | 31 |
| | Greece | 96.77 | 0 | 1 | 0 | 30 | 31 |
| | Mean | 96.77 | | | | | 217 |

| | | PCS | Portugal | Spain | Italy | Greece | N |
|---|---|---|---|---|---|---|---|
| | Portugal | 100 | 73 | 0 | 0 | 0 | 73 |
| | Spain | 100 | 0 | 49 | 0 | 0 | 49 |
| *P. bogaraveo* | Italy | 100 | 0 | 0 | 32 | 0 | 32 |
| | Greece | 100 | 0 | 0 | 0 | 32 | 32 |
| | Mean | 100 | | | | | 186 |

| | | PCS | Portugal | Spain | Italy | Greece | N |
|---|---|---|---|---|---|---|---|
| | Portugal | 100 | 38 | 0 | 0 | 0 | 38 |
| | Spain | 96.77 | 0 | 30 | 1 | 0 | 31 |
| *D. dentex* | Italy | 96.67 | 1 | 0 | 29 | 0 | 30 |
| | Greece | 97.78 | 0 | 0 | 1 | 44 | 45 |
| | Mean | 97.92 | | | | | 144 |

Table XVI - Stepwise Discriminant Analysis for the area comparison between *S. aurata* reared samples.
(Wilks' λ= 0.0065; F (30,1134)=95.284; $p$ <0.0001)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| A3 | 0,016 | 0,412 | 80,931 | 0,001 |
| A2 | 0,014 | 0,455 | 67,729 | 0,001 |
| A1 | 0,011 | 0,582 | 40,591 | 0,001 |
| A5 | 0,011 | 0,604 | 37,066 | 0,001 |
| A4 | 0,009 | 0,712 | 22,893 | 0,001 |
| A6 | 0,008 | 0,853 | 9,733 | 0,001 |

Table XVII - Stepwise Discriminant Analysis for the area comparison between *S. aurata* wild samples.
(Wilks' λ= 0.1076; F (24,859)=32.029; $p$ <0.0001)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| A2 | 0,168 | 0,642 | 34,365 | 0,001 |
| A6 | 0,183 | 0,589 | 42,969 | 0,001 |
| A4 | 0,174 | 0,617 | 38,186 | 0,001 |
| A3 | 0,163 | 0,659 | 31,796 | 0,001 |
| A1 | 0,128 | 0,842 | 11,522 | 0,001 |
| A5 | 0,125 | 0,864 | 9,698 | 0,001 |

Table XVIII - Stepwise Discriminant Analysis for the area comparison between *P. pagrus* samples.
(Wilks' λ= 0.246; F(15,616)=27.194 $p$ <0.0001)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| A5 | 0,437 | 0,563 | 57,651 | 0,0001 |
| A2 | 0,356 | 0,692 | 33,141 | 0,0001 |
| A4 | 0,343 | 0,718 | 29,199 | 0,0001 |
| A6 | 0,270 | 0,912 | 7,205 | 0,0001 |
| A1 | 0,265 | 0,928 | 5,733 | 0,0001 |

Table XIX - Stepwise Discriminant Analysis for the area comparison between *D. sagrus* samples.
(Wilks' λ= 0.19; F(18,560)=24.927; $p$ <0.0001)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| A6 | 0,320 | 0,592 | 45,558 | 0,001 |
| A3 | 0,274 | 0,691 | 29,488 | 0,001 |
| A2 | 0,243 | 0,781 | 18,455 | 0,001 |
| A1 | 0,242 | 0,783 | 18,323 | 0,001 |
| A4 | 0,221 | 0,859 | 10,856 | 0,001 |
| A5 | 0,216 | 0,875 | 9,388 | 0,001 |

Table XX - Stepwise Discriminant Analysis for area comparison between *D. puntazzo* samples.
(Wilks' λ= 0.103; F(12,405)=45.843; $p$ <0.0001)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| A3 | 0,134 | 0,772 | 15,060 | 0,001 |
| A1 | 0,199 | 0,518 | 47,361 | 0,001 |
| A5 | 0,152 | 0,681 | 23,844 | 0,001 |
| A2 | 0,111 | 0,932 | 3,720 | 0,013 |

Table XXI - Stepwise Discriminant Analysis for area comparison between *L. mormyrus* samples.
(Wilks' λ=0.262; F(18,588)=19.814; $p$ <0.0001)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| A4 | 0,396 | 0,661 | 35,479 | 0,000 |
| A1 | 0,455 | 0,576 | 51,052 | 0,000 |
| A2 | 0,336 | 0,779 | 19,719 | 0,000 |
| A5 | 0,300 | 0,873 | 10,102 | 0,000 |
| A3 | 0,287 | 0,912 | 6,717 | 0,000 |
| A6 | 0,282 | 0,928 | 5,355 | 0,001 |

Table XXII - Stepwise Discriminant Analysis for area comparison between *P. bogaraveo* samples.
(Wilks' λ=0.0005; F(18,501)=373.22; $p$ <0.0001)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| A4 | 0,001 | 0,853 | 10,196 | 0,000 |
| A6 | 0,002 | 0,265 | 163,486 | 0,000 |
| A3 | 0,001 | 0,461 | 69,111 | 0,000 |
| A2 | 0,001 | 0,678 | 28,043 | 0,000 |
| A1 | 0,001 | 0,733 | 21,483 | 0,000 |
| A5 | 0,001 | 0,867 | 9,076 | 0,000 |

Table XXIII - Stepwise Discriminant Analysis for area comparison between *D. dentex* samples.
(Wilks' λ=0.1009; F(18,382)=26.554; $p$ <0.0001)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| A2 | 0,125 | 0,810 | 10,552 | 0,000 |
| A1 | 0,227 | 0,444 | 56,330 | 0,000 |
| A6 | 0,151 | 0,667 | 22,424 | 0,000 |
| A3 | 0,137 | 0,738 | 15,944 | 0,000 |
| A5 | 0,128 | 0,786 | 12,268 | 0,000 |
| A4 | 0,113 | 0,889 | 5,625 | 0,001 |

Table XXIV - Stepwise Discriminant Analysis for area comparison between *S. cantharus* samples.
(Wilks' λ=0.6257; F(3,62)=12.364 $p$ < 0.0001)

| Descriptor | Wilks λ | Partial λ | F-remove | p-level |
|---|---|---|---|---|
| A3 | 0,698 | 0,896 | 7,160 | 0,010 |
| A4 | 0,669 | 0,936 | 4,244 | 0,044 |
| A6 | 0,647 | 0,967 | 2,122 | 0,150 |

# ANNEX III

Table I - Descriptive statistics of the meristic traits recorded for the hybrid *Sa* x *Pp* and the parental species (DF- dorsal fin, CF- caudal fin, AF- anal fin, RPF- right pectoral fin, LPF- left pectoral fin, URGR- upper right gill raker, ULGR- upper right gill raker, LRGR- lower right gill raker, LLGR- lower right gill raker)

*S.aurata*

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 13 | 21 | 11 | 15 | 15 | 6 | 6 | 8 | 8 |
| Standard Error | 0,03 | 0,05 | 0,03 | 0,05 | 0,06 | 0,04 | 0,05 | 0,05 | 0,05 |
| Median | 13 | 21 | 11 | 15 | 15 | 6 | 6 | 8 | 8 |
| Mode | 13 | 21 | 11 | 15 | 15 | 6 | 6 | 8 | 8 |
| Standard Deviation | 0,58 | 0,75 | 0,48 | 0,86 | 0,93 | 0,64 | 0,69 | 0,75 | 0,78 |
| Range | 2 | 6 | 3 | 5 | 6 | 4 | 5 | 3 | 4 |
| Minimum | 12 | 17 | 10 | 12 | 12 | 4 | 3 | 6 | 6 |
| Maximum | 14 | 23 | 13 | 17 | 18 | 8 | 8 | 9 | 10 |
| Confidence Level (95.0%) | 0,07 | 0,09 | 0,06 | 0,10 | 0,11 | 0,08 | 0,09 | 0,10 | 0,10 |

Hybrid *Sa* x *Pp*

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 11 | 22 | 10 | 15 | 15 | 7 | 7 | 9 | 9 |
| Standard Error | 0,07 | 0,11 | 0,08 | 0,07 | 0,06 | 0,04 | 0,11 | 0,06 | 0,05 |
| Median | 11 | 22 | 10 | 15 | 15 | 7 | 7 | 9 | 9 |
| Mode | 11 | 22 | 10 | 15 | 15 | 7 | 7 | 9 | 9 |
| Standard Deviation | 0,64 | 0,95 | 0,73 | 0,61 | 0,57 | 0,40 | 0,95 | 0,51 | 0,49 |
| Range | 2 | 3 | 3 | 4 | 2 | 2 | 5 | 3 | 3 |
| Minimum | 10 | 20 | 8 | 12 | 13 | 6 | 3 | 7 | 7 |
| Maximum | 12 | 23 | 11 | 16 | 15 | 8 | 8 | 10 | 10 |
| Confidence Level (95.0%) | 0,14 | 0,21 | 0,16 | 0,14 | 0,13 | 0,09 | 0,21 | 0,11 | 0,11 |

*P.pagrus*

| | DF | CF | AF | RPF | LPF | URGR | ULGR | LRGR | LLGR |
|---|---|---|---|---|---|---|---|---|---|
| Mean | 10 | 22 | 8 | 15 | 15 | 7 | 7 | 9 | 9 |
| Standard Error | 0,01 | 0,07 | 0,00 | 0,03 | 0,03 | 0,05 | 0,05 | 0,04 | 0,04 |
| Median | 10 | 22 | 8 | 15 | 15 | 7 | 7 | 9 | 9 |
| Mode | 10 | 22 | 8 | 15 | 15 | 7 | 7 | 9 | 9 |
| Standard Deviation | 0,21 | 1,00 | 0,07 | 0,49 | 0,44 | 0,80 | 0,75 | 0,62 | 0,67 |
| Range | 2 | 4 | 1 | 3 | 2 | 5 | 4 | 3 | 4 |
| Minimum | 9 | 20 | 8 | 14 | 14 | 4 | 5 | 8 | 7 |
| Maximum | 11 | 24 | 9 | 17 | 16 | 9 | 9 | 11 | 11 |
| Confidence Level (95.0%) | 0,03 | 0,13 | 0,01 | 0,06 | 0,06 | 0,10 | 0,10 | 0,08 | 0,09 |

Table II - Descriptive statistics of the tooth counts recorded on the *S. aurata* sample. (BCR - big canine right, BCL - big canine left, SCR - small canine right, SCL - small canine left, ITR - intermediate tooth right, ITL - intermediate tooth left, RM1R - right molars on first row, RM2R - right molars on second row, RM3R - right molars on third row, RM4R - right molars on fourth row, RM5R - right molars on fifth row, LM1R - left molars on first row, LM2R - left molars on second row, LM3R - left molars on third row, LM4R - left molars on fourth row, LM5R - left molars on fifth row).

Upper jaw

| | BCR | BCL | SCR | SCL | ITR | ITL | RM1R | RM2R | RM3R | RM4R | RM5R | LM1R | LM2R | LM3R | LM4R | LM5R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean | 3 | 3 | 2 | 2 | 3 | 3 | 4 | 5 | 5 | 4 | 1 | 4 | 5 | 4 | 4 | 1 |
| Standard Error | 0,07 | 0,06 | 0,08 | 0,08 | 0,07 | 0,09 | 0,12 | 0,18 | 0,14 | 0,13 | 0,12 | 0,13 | 0,19 | 0,18 | 0,17 | 0,17 |
| Median | 3 | 3 | 2 | 2 | 3 | 3 | 4 | 5 | 5 | 4 | 0 | 4 | 5 | 5 | 4 | 0 |
| Mode | 3 | 3 | 2 | 2 | 3 | 3 | 3 | 5 | 4 | 4 | 0 | 4 | 6 | 5 | 5 | 0 |
| Standard Deviation | 0,63 | 0,62 | 0,74 | 0,79 | 0,70 | 0,83 | 1,16 | 1,71 | 1,32 | 1,29 | 1,16 | 1,25 | 1,82 | 1,72 | 1,66 | 1,66 |
| Sample Variance | 0,40 | 0,38 | 0,55 | 0,62 | 0,49 | 0,68 | 1,34 | 2,91 | 1,75 | 1,66 | 1,34 | 1,56 | 3,32 | 2,94 | 2,77 | 2,74 |
| Range | 3 | 3 | 4 | 4 | 4 | 5 | 6 | 7 | 7 | 7 | 4 | 7 | 8 | 7 | 7 | 8 |
| Minimum | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Maximum | 3 | 3 | 4 | 4 | 5 | 6 | 6 | 8 | 8 | 7 | 4 | 7 | 9 | 7 | 7 | 8 |
| Confidence Level (95.0%) | 0,13 | 0,13 | 0,15 | 0,16 | 0,14 | 0,17 | 0,24 | 0,35 | 0,27 | 0,27 | 0,24 | 0,26 | 0,38 | 0,36 | 0,34 | 0,34 |

Lower jaw

| | BCR | BCL | SCR | SCL | ITR | ITL | RM1R | RM2R | RM3R | LM1R | LM2R | LM3R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean | 3 | 3 | 1 | 1 | 2 | 2 | 6 | 5 | 3 | 6 | 5 | 3 |
| Standard Error | 0,08 | 0,07 | 0,12 | 0,14 | 0,07 | 0,07 | 0,15 | 0,12 | 0,17 | 0,18 | 0,14 | 0,18 |
| Median | 3 | 3 | 1 | 1 | 2 | 2 | 6 | 5 | 3 | 6 | 5 | 3 |
| Mode | 3 | 3 | 1 | 1 | 2 | 2 | 5 | 5 | 3 | 6 | 6 | 3 |
| Standard Deviation | 0,76 | 0,65 | 0,64 | 0,74 | 0,68 | 0,69 | 1,45 | 1,19 | 1,62 | 1,77 | 1,31 | 1,72 |
| Sample Variance | 0,57 | 0,43 | 0,41 | 0,55 | 0,47 | 0,48 | 2,11 | 1,42 | 2,61 | 3,13 | 1,72 | 2,95 |
| Range | 3 | 3 | 2 | 3 | 3 | 3 | 6 | 5 | 6 | 9 | 6 | 7 |
| Minimum | 1 | 1 | 0 | 0 | 1 | 1 | 3 | 3 | 0 | 2 | 2 | 0 |
| Maximum | 4 | 4 | 2 | 3 | 4 | 4 | 9 | 8 | 6 | 11 | 8 | 7 |
| Confidence Level (95.0%) | 0,16 | 0,14 | 0,25 | 0,29 | 0,14 | 0,14 | 0,30 | 0,25 | 0,33 | 0,37 | 0,27 | 0,36 |

Table III - Descriptive statistics of the tooth counts recorded on the *Sa*x*Pp* sample. (BCR - big caninee right, BCL - big canine left, SCR - small canine right, SCL - small canine left, ITR - intermediate tooth right, ITL - intermediate tooth left, RM1R - right molars on first row, RM2R - right molars on second row, RM3R - right molars on third row, RM4R - right molars on fourth row, LM1R - left molars on first row, LM2R - left molars on second row, LM3R - left molars on third row, LM4R - left molars on fourth row).

Upper jaw

| | BCR | BCL | SCR | SCL | ITR | ITL | RM1R | RM2R | RM3R | RM4R | LM1R | LM2R | LM3R | LM4R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean | 3 | 3 | 2 | 2 | 4 | 4 | 4 | 6 | 5 | 3 | 4 | 7 | 5 | 3 |
| Standard Error | 0,03 | 0,03 | 0,04 | 0,04 | 0,05 | 0,05 | 0,07 | 0,08 | 0,09 | 0,15 | 0,08 | 0,08 | 0,10 | 0,14 |
| Median | 3 | 3 | 2 | 2 | 4 | 4 | 4 | 6,5 | 5 | 3 | 4 | 7 | 5 | 3 |
| Mode | 3 | 3 | 2 | 2 | 4 | 4 | 4 | 7 | 5 | 0 | 4 | 7 | 6 | 0 |
| Standard Deviation | 0,56 | 0,51 | 0,75 | 0,74 | 0,95 | 0,96 | 1,36 | 1,45 | 1,74 | 2,75 | 1,48 | 1,51 | 1,86 | 2,61 |
| Sample Variance | 0,31 | 0,26 | 0,57 | 0,55 | 0,91 | 0,92 | 1,86 | 2,11 | 3,04 | 7,55 | 2,18 | 2,27 | 3,46 | 6,83 |
| Range | 4 | 3 | 4 | 5 | 6 | 5 | 7 | 8 | 10 | 11 | 7 | 8 | 11 | 10 |
| Minimum | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 |
| Maximum | 5 | 4 | 4 | 5 | 7 | 6 | 7 | 11 | 10 | 11 | 7 | 11 | 11 | 10 |
| Confidence Level (95.0%) | 0,06 | 0,05 | 0,08 | 0,08 | 0,10 | 0,10 | 0,14 | 0,15 | 0,18 | 0,29 | 0,15 | 0,16 | 0,19 | 0,27 |

Lower jaw

| | RC | LC | ITR | ITL | RM1R | RM2R | RM3R | LM1R | LM2R | LM3R |
|---|---|---|---|---|---|---|---|---|---|---|
| Mean | 3 | 3 | 4 | 4 | 4 | 10 | 1 | 4 | 10 | 1 |
| Standard Error | 0,02 | 0,02 | 0,06 | 0,06 | 0,09 | 0,14 | 0,09 | 0,08 | 0,13 | 0,07 |
| Median | 3 | 3 | 4 | 4 | 3 | 10 | 0 | 3 | 10 | 0 |
| Mode | 3 | 3 | 4 | 4 | 3 | 10 | 0 | 3 | 11 | 0 |
| Standard Deviation | 0,47 | 0,46 | 1,06 | 1,04 | 1,63 | 2,56 | 1,61 | 1,53 | 2,50 | 1,39 |
| Sample Variance | 0,22 | 0,21 | 1,11 | 1,07 | 2,67 | 6,58 | 2,58 | 2,34 | 6,25 | 1,92 |
| Range | 3 | 3 | 6 | 7 | 9 | 11 | 7 | 10 | 11 | 6 |
| Minimum | 1 | 1 | 0 | 0 | 0 | 4 | 0 | 0 | 3 | 0 |
| Maximum | 4 | 4 | 6 | 7 | 9 | 15 | 7 | 10 | 14 | 6 |
| Confidence Level (95.0%) | 0,05 | 0,05 | 0,11 | 0,11 | 0,17 | 0,27 | 0,17 | 0,16 | 0,26 | 0,15 |

Table IV - Descriptive statistics of the tooth counts recorded on the *P. pagrus* sample. (BCR - big caninee right, BCL - big canine left, SCR - small canine right, SCL - small canine left, ITR - intermediate tooth right, ITL - intermediate tooth left, RM1R - right molars on first row, RM2R - right molars on second row, LM1R - left molars on first row, L M2R - left molars on second row).

Upper jaw

| | BCR | BCL | SCR | SCL | ITR | ITL | RM1R | RM2R | LM1R | LM2R |
|---|---|---|---|---|---|---|---|---|---|---|
| Mean | 2 | 2 | 2 | 2 | 2 | 2 | 7 | 4 | 7 | 4 |
| Standard Error | 0,04 | 0,03 | 0,08 | 0,29 | 0,07 | 0,08 | 0,13 | 0,11 | 0,13 | 0,11 |
| Median | 2 | 2 | 2 | 2 | 2 | 2 | 7 | 4 | 7 | 4 |
| Mode | 2 | 2 | 2 | 2 | 2 | 2 | 7 | 4 | 7 | 4 |
| Standard Deviation | 0,40 | 0,30 | 0,83 | 3,01 | 0,73 | 0,81 | 1,32 | 1,15 | 1,29 | 1,16 |
| Sample Variance | 0,16 | 0,09 | 0,69 | 9,05 | 0,54 | 0,66 | 1,73 | 1,32 | 1,67 | 1,36 |
| Range | 3 | 2 | 5 | 32 | 3 | 4 | 7 | 8 | 6 | 6 |
| Minimum | 1 | 1 | 0 | 0 | 1 | 1 | 3 | 1 | 3 | 1 |
| Maximum | 4 | 3 | 5 | 32 | 4 | 5 | 10 | 9 | 9 | 7 |
| Confidence Level (95.0%) | 0,08 | 0,06 | 0,16 | 0,58 | 0,14 | 0,16 | 0,25 | 0,22 | 0,25 | 0,23 |

Lower jaw

| | BCR | BCL | SCR | SCL | ITR | ITL | RM1R | RM2R | LM1R | LM2R |
|---|---|---|---|---|---|---|---|---|---|---|
| Mean | 3 | 3 | 2 | 2 | 3 | 3 | 7 | 5 | 7 | 5 |
| Standard Error | 0,05 | 0,06 | 0,07 | 0,07 | 0,08 | 0,07 | 0,13 | 0,13 | 0,13 | 0,14 |
| Median | 3 | 3 | 2 | 1,5 | 3 | 3 | 7 | 5 | 7 | 5 |
| Mode | 3 | 3 | 2 | 1 | 3 | 3 | 7 | 5 | 7 | 4 |
| Standard Deviation | 0,47 | 0,58 | 0,67 | 0,69 | 0,78 | 0,73 | 1,37 | 1,33 | 1,32 | 1,46 |
| Sample Variance | 0,22 | 0,33 | 0,45 | 0,48 | 0,61 | 0,53 | 1,89 | 1,77 | 1,73 | 2,12 |
| Range | 1 | 3 | 5 | 5 | 4 | 3 | 9 | 8 | 8 | 9 |
| Minimum | 2 | 1 | 0 | 0 | 1 | 1 | 2 | 2 | 2 | 1 |
| Maximum | 3 | 4 | 5 | 5 | 5 | 4 | 11 | 10 | 10 | 10 |
| Confidence Level (95.0%) | 0,09 | 0,11 | 0,13 | 0,13 | 0,15 | 0,14 | 0,26 | 0,26 | 0,25 | 0,28 |

Table V - Sample variance of the different tooth types in the upper jaw of the hybrid *Sa* x *Pp* and the parental species (BCR- big canin right, BCL- big canin left, SCR- small canin right, SCL- small canin left, ITR- intermediate tooth right, ITL- intermediate tooth left, RM#R- right molars in each one of the rows, LM#R- right molars in each one of the rows)

| | BCR | BCL | SCR | SCL | ITR | ITL | RM1R | RM2R | RM3R | RM4R | RM5R | LM1R | LM2R | LM3R | LM4R | LM5R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Sparus aurata* | 0,40 | 0,38 | 0,55 | 0,62 | 0,49 | 0,68 | 1,34 | 2,91 | 1,75 | 1,66 | 1,34 | 1,56 | 3,32 | 2,94 | 2,77 | 2,74 |
| Hybrid *Sa* x *Pp* | 0,31 | 0,26 | 0,57 | 0,55 | 0,91 | 0,92 | 1,86 | 2,11 | 3,04 | 7,55 | - | 2,18 | 2,27 | 3,46 | 6,83 | - |
| *Pagrus pagrus* | 0,16 | 0,09 | 0,69 | 0,56 | 0,54 | 0,66 | 1,73 | 1,32 | - | - | - | 1,67 | 1,36 | - | - | - |

Table VI - Sample variance of the different tooth types in the lower jaw of the hybrid *Sa* x *Pp* and the parental species (BCR- big canin right, BCL- big canin left, SCR- small canin right, SCL- small canin left, ITR- intermediate tooth right, ITL- intermediate tooth left, RM#R- right molars in each one of the rows, LM#R- right molars in each one of the rows)

| | BCR | BCL | SCR | SCL | ITR | ITL | RM1R | RM2R | RM3R | LM1R | LM2R | LM3R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Sparus aurata* | 0,57 | 0,43 | 0,41 | 0,55 | 0,47 | 0,48 | 2,11 | 1,42 | 2,61 | 3,13 | 1,72 | 2,95 |
| Hybrid *Sa* x *Pp* | 0.22* | | 0.21* | | 1,11 | 1,07 | 2,67 | 6,58 | 2,58 | 2,34 | 6,25 | 1,92 |
| *Pagrus pagrus* | 0,22 | 0,33 | 0,45 | 0,48 | 0,61 | 0,53 | 1,89 | 1,77 | - | 1,73 | 2,12 | - |

* - only one type of canin was identified

Table VII - Tested relationships between hybrids and parental species in the upper jaw (Tukey HSD test).

Big left canins

| | *S. aurata* | *Sa* x *Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | | 0,999 | 0,001 |
| *Sa* x *Pp* | 0,999 | | 0,001 |
| *P. pagrus* | 0,001 | 0,001 | |

Small left canins

| | *S. aurata* | *Sa* x *Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | | 0,003 | 0,061 |
| *Sa* x *Pp* | 0,003 | | 0,852 |
| *P. pagrus* | 0,061 | 0,852 | |

Intermediate left tooth

| | *S. aurata* | *Sa* x *Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | | 0,001 | 0,001 |
| *Sa* x *Pp* | 0,001 | | 0,001 |
| *P. pagrus* | 0,001 | 0,001 | |

Molars, left first row

| | *S. aurata* | *Sa* x *Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | | 0,763 | 0,001 |
| *Sa* x *Pp* | 0,763 | | 0,001 |
| *P. pagrus* | 0,001 | 0,001 | |

Molars, left second row

| | *S. aurata* | *Sa* x *Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | | 0,001 | 0,04 |
| *Sa* x *Pp* | 0,001 | | 0,001 |
| *P. pagrus* | 0,04 | 0,001 | |

Big right canins

| | *S. aurata* | *Sa* x *Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | | 0,998 | 0,001 |
| *Sa* x *Pp* | 0,998 | | 0,001 |
| *P. pagrus* | 0,001 | 0,001 | |

Small right canins

| | *S. aurata* | *Sa* x *Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | | 0,020 | 0,007 |
| *Sa* x *Pp* | 0,020 | | 0,532 |
| *P. pagrus* | 0,007 | 0,532 | |

Intermediate right tooth

| | *S. aurata* | *Sa* x *Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | | 0,001 | 0,01 |
| *Sa* x *Pp* | 0,001 | | 0,01 |
| *P. pagrus* | 0,001 | 0,001 | |

Molars, right first row

| | *S. aurata* | *Sa* x *Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | | 0,977 | 0,001 |
| *Sa* x *Pp* | 0,977 | | 0,001 |
| *P. pagrus* | 0,001 | 0,001 | |

Molars, right second row

| | *S. aurata* | *Sa* x *Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | | 0,001 | 0,031 |
| *Sa* x *Pp* | 0,001 | | 0,001 |
| *P. pagrus* | 0,031 | 0,001 | |

Table VIII - Tested relationships between hybrids and parental species in the lower (Tukey HSD test).

Big left canins

| | *S. aurata* | *Sa* x*Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | | 0,058 | 0,030 |
| *Sa* x*Pp* | 0,058 | | 0,001 |
| *P. pagrus* | 0,030 | 0,001 | |

Intermediate left tooth

| | *S. aurata* | *Sa* x*Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | | 0,001 | 0,001 |
| *Sa* x*Pp* | 0,001 | | 0,001 |
| *P. pagrus* | 0,001 | 0,001 | |

Molars, left first row

| | *S. aurata* | *Sa* x*Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | | 0,001 | 0,001 |
| *Sa* x*Pp* | 0,001 | | 0,001 |
| *P. pagrus* | 0,001 | 0,001 | |

Molars, left second row

| | *S. aurata* | *Sa* x*Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | | 0,001 | 0,001 |
| *Sa* x*Pp* | 0,001 | | 0,442 |
| *P. pagrus* | 0,001 | 0,442 | |

Big right canins

| | *S. aurata* | *Sa* x*Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | | 0,595 | 0,029 |
| *Sa* x*Pp* | 0,595 | | 0,015 |
| *P. pagrus* | 0,029 | 0,015 | |

Intermediate right tooth

| | *S. aurata* | *Sa* x*Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | | 0,001 | 0,001 |
| *Sa* x*Pp* | 0,001 | | 0,001 |
| *P. pagrus* | 0,001 | 0,001 | |

Molars, right first row

| | *S. aurata* | *Sa* x*Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | | 0,001 | 0,001 |
| *Sa* x*Pp* | 0,001 | | 0,001 |
| *P. pagrus* | 0,001 | 0,001 | |

Molars, right second row

| | *S. aurata* | *Sa* x*Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | | 0,001 | 0,001 |
| *Sa* x*Pp* | 0,001 | | 0,961 |
| *P. pagrus* | 0,001 | 0,961 | |

Table IX - Percent classification success (PCS), F-statistics and $D^2$ for the tooth counts for the hybrids and parental species.

Upper jaw

| | PCS | *S. aurata* | *Sa*x*Pp* | *P. pagrus* | N |
|---|---|---|---|---|---|
| *S. aurata* | 45,65 | 49 | 42 | 1 | 92 |
| *Sa*x*Pp* | 91,81 | 325 | 22 | 7 | 354 |
| *P. pagrus* | 95,28 | 5 | 0 | 101 | 106 |
| Total | 84,78 | | | | 552 |

Lower jaw

| | PCS | *S. aurata* | *Sa*x*Pp* | *P. pagrus* | N |
|---|---|---|---|---|---|
| *S. aurata* | 69,57 | 64 | 6 | 22 | 92 |
| *Sa*x*Pp* | 89,27 | 13 | 316 | 25 | 354 |
| *P. pagrus* | 71,70 | 22 | 8 | 76 | 106 |
| Total | 82,61 | | | | 552 |

Upper jaw (df = 10,511)

| F-values | *S. aurata* | *Sa*x*Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | - | | |
| *Sa*x*Pp* | 21,24 | - | |
| *P. pagrus* | 33,40 | 86,58 | - |

Lower jaw (df = 7,534)

| F-values | *S. aurata* | *Sa*x*Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | - | | |
| *Sa*x*Pp* | 99,89 | - | |
| *P. pagrus* | 12,85 | 97,16 | - |

Upper jaw

| $D^2$ | *S. aurata* | *Sa*x*Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | - | | |
| *Sa*x*Pp* | 3,22 | - | |
| *P. pagrus* | 7,41 | 11,35 | - |

Lower jaw

| $D^2$ | *S. aurata* | *Sa*x*Pp* | *P. pagrus* |
|---|---|---|---|
| *S. aurata* | - | | |
| *Sa*x*Pp* | 9,83 | - | |
| *P. pagrus* | 1,87 | 8,55 | - |

Table X - Descriptive statistics of the recorded characteristics through the traditional morphometry for the samples of *S. aurata*, hybrids *Sa x Pp* and *P. pagrus*.

*Sparus aurata*

| | SL | MFH | POL | OOL | PSL | HL | HD | UJL | LJL | CPL | DFL | AFL | LPA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean | 161.51 | 64.89 | 20.61 | 12.86 | 20.16 | 46.66 | 38.41 | 17.28 | 17.65 | 26.21 | 88.84 | 46.82 | 33.71 |
| Standard Error | 2.56 | 1.04 | 0.35 | 0.17 | 0.33 | 0.69 | 0.69 | 0.27 | 0.29 | 0.49 | 1.48 | 0.77 | 0.56 |
| Median | 155.59 | 64.06 | 19.83 | 12.23 | 18.98 | 46.00 | 35.20 | 16.62 | 16.59 | 24.88 | 84.21 | 45.72 | 31.98 |
| Mode | 223.47 | 90.36 | 28.49 | 11.21 | 28.18 | 63.13 | 55.49 | 22.56 | 23.67 | 37.10 | 124.18 | 64.29 | 47.13 |
| Standard Deviation | 40.98 | 16.68 | 5.57 | 2.73 | 5.22 | 11.00 | 11.04 | 4.32 | 4.57 | 7.87 | 23.65 | 12.30 | 9.03 |
| Sample Variance | 1679.69 | 278.08 | 30.99 | 7.46 | 27.24 | 121.04 | 121.85 | 18.69 | 20.91 | 61.89 | 559.49 | 151.29 | 81.60 |
| Range | 172.73 | 65.67 | 24.18 | 10.35 | 21.79 | 46.16 | 52.78 | 21.39 | 19.21 | 41.94 | 93.47 | 47.19 | 35.3 |
| Minimum | 95.2 | 35.96 | 11.78 | 8.57 | 12.12 | 28.61 | 23.71 | 10.92 | 10.71 | 12.48 | 52.48 | 28.03 | 20.59 |
| Maximum | 267.93 | 101.63 | 35.96 | 18.92 | 33.91 | 74.77 | 76.49 | 32.31 | 29.92 | 54.42 | 145.95 | 75.22 | 55.89 |
| Confidence Level (95.0%) | 5.04 | 2.05 | 0.69 | 0.34 | 0.64 | 1.35 | 1.36 | 0.53 | 0.56 | 0.97 | 2.91 | 1.51 | 1.11 |

Hybrid SaxPp

| | SL | MFH | POL | OOL | PSL | HL | HD | UJL | LJL | CPL | DFL | AFL | LPA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean | 145.44 | 57.53 | 16.30 | 11.72 | 18.37 | 42.58 | 35.41 | 15.59 | 11.59 | 29.83 | 74.99 | 44.52 | 28.89 |
| Standard Error | 2.41 | 0.92 | 0.29 | 0.13 | 0.30 | 0.63 | 0.56 | 0.29 | 0.20 | 0.54 | 1.26 | 0.82 | 0.65 |
| Median | 149 | 59 | 16.48 | 11.84 | 18.78 | 43.14 | 36.14 | 15.96 | 11.9 | 30.07 | 75.93 | 44.6 | 27.96 |
| Mode | 136 | 60 | 18.59 | 11.44 | 16.28 | 43.73 | 37.04 | 17.4 | 12.78 | 32.54 | 81.25 | 44.63 | 26.43 |
| Standard Deviation | 21.68 | 8.29 | 2.63 | 1.20 | 2.71 | 5.70 | 5.01 | 2.63 | 1.76 | 4.89 | 11.38 | 7.40 | 3.60 |
| Sample Variance | 470.20 | 68.70 | 6.93 | 1.45 | 7.37 | 32.45 | 25.10 | 6.89 | 3.11 | 23.87 | 129.42 | 54.74 | 12.92 |
| Range | 113 | 42 | 14.71 | 5.39 | 13.52 | 27.87 | 26.1 | 13.05 | 9.4 | 24.6 | 60.22 | 39.05 | 14.99 |
| Minimum | 89 | 37 | 9.99 | 8.73 | 11.38 | 27.89 | 22.48 | 9.32 | 6.25 | 17.66 | 44.95 | 26.18 | 24.56 |
| Maximum | 202 | 79 | 24.7 | 14.12 | 24.9 | 55.76 | 48.58 | 22.37 | 15.65 | 42.26 | 105.17 | 65.23 | 39.55 |
| Confidence Level (95.0%) | 4.79 | 1.83 | 0.58 | 0.27 | 0.60 | 1.26 | 1.11 | 0.58 | 0.39 | 1.08 | 2.52 | 1.64 | 1.32 |

*Pagrus pagrus*

| | SL | MFH | POL | OOL | PSL | HL | HD | UJL | LJL | CPL | DFL | AFL | LPA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean | 145.93 | 57.87 | 17.78 | 14.88 | 18.13 | 45.48 | 34.16 | 14.53 | 14.82 | 25.03 | 73.80 | 41.98 | 26.27 |
| Standard Error | 2.40 | 1.06 | 0.33 | 0.22 | 0.29 | 0.70 | 0.57 | 0.25 | 0.26 | 0.45 | 1.32 | 0.82 | 0.46 |
| Median | 144.82 | 57.65 | 17.39 | 14.5 | 18.08 | 44.81 | 35.64 | 14.66 | 15.26 | 25.37 | 73.65 | 41.89 | 26.11 |
| Mode | 81.01 | 56.85 | 16.4 | 12.57 | 17.74 | 47.17 | 37.69 | 16.43 | 13.9 | 30.89 | 74.27 | 41.89 | 13.26 |
| Standard Deviation | 36.44 | 16.11 | 5.03 | 3.28 | 4.44 | 10.67 | 8.69 | 3.83 | 3.98 | 6.77 | 20.06 | 12.41 | 6.95 |
| Sample Variance | 1327.59 | 259.45 | 25.34 | 10.74 | 19.71 | 113.75 | 75.50 | 14.70 | 15.80 | 45.81 | 402.46 | 154.08 | 48.35 |
| Range | 166.71 | 73.68 | 25.57 | 15.82 | 23.09 | 49.74 | 56.18 | 20.69 | 19.48 | 36.07 | 104.12 | 58.09 | 32.82 |
| Minimum | 69.43 | 21.65 | 7.52 | 7.45 | 7.68 | 22.58 | 15.42 | 5.96 | 5.89 | 10.26 | 21.95 | 13.12 | 11.23 |
| Maximum | 236.14 | 95.33 | 33.09 | 23.27 | 30.77 | 72.32 | 71.6 | 26.65 | 25.367 | 46.33 | 126.07 | 71.21 | 44.05 |
| Confidence Level (95.0%) | 4.72 | 2.09 | 0.65 | 0.42 | 0.58 | 1.38 | 1.13 | 0.50 | 0.52 | 0.88 | 2.60 | 1.61 | 0.90 |