Structure and connectivity of bluemouth, *Helicolenus dactylopterus*, populations in the NE Atlantic and Mediterranean

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Declarations of interest: none

17 Abstract

18 The bluemouth, *Helicolenus dactylopterus* (Delaroche 1809), is a marine demersal fish
19 that is widely distributed in the Atlantic and the Mediterranean. It is targeted by longline
20 fisheries around the Iberian Peninsula and it is a common by-catch associated with many
21 demersal fisheries. However, limited information exists about the population structure in
22 the NE Atlantic and Mediterranean. Thus, the bluemouth population structure in these
23 regions was studied using a landmark-based geometric morphometric analysis. The
24 purpose was to assess body shape variation among bluemouth and determine putative
25 populations and their relationships. Three populations were identified in the western
26 Mediterranean: two populations in the Alboran basin and another in the north-western
27 basin (Balearic Sea and Catalanian coast). The population from the central Mediterranean
28 did not seem to be effectively connected to the populations in the western Mediterranean.
29 In the NE Atlantic, our results showed that at least four different bluemouth populations
30 exist in the following areas: Galicia-Cantabrian Sea, Peniche (Portugal), the Gulf of Cadiz
31 and the Porcupine Bank. A connection between bluemouth from the NE Atlantic and
32 Mediterranean was observed, making unlikely that the Strait of Gibraltar acts as a barrier
33 isolating Atlantic and Mediterranean bluemouth populations. The results of this study
34 provide evidence that different bluemouth populations exist, which is relevant for stock
35 assessment and management of this species.

36 Keywords: *Helicolenus dactylopterus*, population structure, geometric morphometrics,
37 NE Atlantic, Mediterranean

1. Introduction

The bluemouth, *Helicolenus dactylopterus* (Delaroche 1809) is a marine demersal fish that is widely distributed in the Atlantic and the Mediterranean. This species is caught mainly as by-catch in bottom-trawl fisheries in the NE Atlantic, but in some regions such as the Azores and the Iberian Peninsula it is one of the species targeted in longline fisheries (Diogo et al., 2015, Sequeira et al., 2011, ICES, 2017). From the European countries that report significant landings of this species, Spain is by far the country with the highest bluemouth landings in the last decade (FAO, 2017). To the present, there are no specific management measures or landing regulations for the bluemouth in EC waters or in the NEAFC Regulatory Area (ICES, 2016).

The analysis of a species' population structure, including how the different populations are connected, is of primary importance in developing an optimal strategy for its efficient management (Coyle, 1998, Calò et al., 2013). At the present, the population structure of bluemouth in terms of age and size is relatively well studied in the Mediterranean (Massutí et al., 2000; 2001; Ribas et al., 2006; Consoli et al., 2010 and Pirrera et al., 2009), the Northeast Atlantic (Kelly et al., 1999; Heessen et al., 1996; Esteves et al., 1997; Abecasis et al., 2006; Sequeira et al., 2009) and the Northwest Atlantic (White et al., 1998). However, the population structure in terms of stock components (phenotypic and/or genotypic) of bluemouth has received less attention. Virtually all studies that have been focused on the identification of bluemouth populations have been carried out in Portuguese waters (i.e. Azores archipelago, Madeira and mainland Portugal). A variety of techniques, such as genetics (Aboim, 2005, Aboim et al., 2005), parasite tags (Sequeira et al., 2010), otolith composition (Neves et al., 2010), and morphometrics (Sequeira et al., 2011), have been used to identify bluemouth stocks in Portugal. In all of these studies, some degree of differentiation between the studied bluemouth populations has been

found, suggesting the existence of different populations in the Atlantic. Moreover, differences among growth patterns of bluemouth populations around the Iberian Peninsula have been detected (Rodríguez-Mendoza et al., 2011). These differences in growth may indicate that different populations exist in the NE Atlantic and Mediterranean.

Morphometric characters can be used as biological tags that allow the inclusion of an individual in a defined group that shares the same traits (Calò et al., 2013). In this sense, the study of body shape variation is a valuable tool for the identification of different populations and their connectivity patterns (Cadrin, 2005, Calò et al., 2013). To the present, morphometric techniques have been useful in determining the structure of populations of a variety of marine fish (e.g., Corti and Crosetti, 1996; O'Reilly and Horn, 2004; Silva, 2003; Murta et al., 2008).

Morphometric differences in fish populations can be caused by genetic or environmental factors, or both. (Swain et al. 2005). Thus, differences in salinity, temperature or food type and availability can promote morphological differentiation in fish populations. Overall environmental differences between the NE Atlantic and Mediterranean Sea have been well documented, for example, temperature is higher in the Mediterranean (12.8–13°C) and lower in the eastern Atlantic (10–4°C) (Ellett et al., 1986; Hopkins, 1985; Rice et al., 1991). Also, there are clear differences in the density, species richness and composition of demersal fauna in the Atlantic and the Mediterranean (Massutí et al., 2004), which affects the type and availability of food. Because of this, the Mediterranean Sea and the Northeast Atlantic are considered separate marine biogeographical regions and the Strait of Gibraltar has been proposed to be the division between them (Borsa et al., 1997). In some fish populations, the Strait of Gibraltar has been found to have a boundary effect with a reduction in gene flow between the two basins. However, a clear

phylogenetic break has never been observed, and some species show no differentiation at all between Atlantic and Mediterranean populations (Bargelloni et al., 2003). Other important biogeographical limits with boundary effects for fish populations have been identified between Galicia and the Cantabrian Sea: Cape Estaca de Bares and Cape Finisterre (Sánchez and Gil, 2000; Sánchez and Serrano, 2003; Serrano et al., 2008), and the Almeria-Oran front in the western Mediterranean (Tintore et al., 1988, Roldan et al., 1998, Naciri et al., 1999).

The purpose of this study was to assess body shape variation among bluemouth and determine putative populations around the Iberian Peninsula (NE Atlantic and Mediterranean) and their relationships. It was hypothesized that the structure of bluemouth populations is affected by: (1) boundary effects of biogeographical limits (e.g., the Strait of Gibraltar), (2) the species' sedentary behavior (Uiblein et al., 2003, Pakhorukov, 2008), (3) a limited larval dispersal in the pelagic stage (Aboim, 2005) and (4) the geographical distance existing between the different study areas.

2. Material and methods

To study the population structure of bluemouth around the Iberian Peninsula, a total of 1125 specimens were caught in 10 different locations in the NE Atlantic and the Mediterranean (Table 1 and Figures 1 and 2). The sampling locations were chosen taking into account their oceanographic characteristics and the available information regarding biogeographical limits with boundary effects in fish populations.

In the NE Atlantic, specimens were sampled from the Galician shelf (from the Miño River to Cape Estaca de Bares), the Cantabrian Sea (from Cape Estaca de Bares to the mouth of the Bidasoa River), Peniche (Portugal), the Gulf of Cadiz and the Porcupine Bank (Irish continental margin). Between Galicia and the Cantabrian Sea, two important biogeographical limits (with boundary effects for fish populations) have been identified: Cape Estaca de Bares and Cape Finisterrae (Figure 1) (Sánchez and Serrano, 2003; Serrano et al. 2008). In this study, the boundary between these sampling areas was considered to be Cape Estaca de Bares. However, the possibility of Cape Finisterrae being a boundary between bluemouth populations from Galicia and the Cantabrian Sea was assessed by analyzing the four adjacent subareas in these locations separately. Galicia was subdivided in two subareas, G1 (from the Miño River to Cape Finisterrae) and G2 (from Cape Finisterrae to Cape Estaca de Bares) and the Cantabrian Sea into subareas C1 (from Cape Estaca de Bares to Cape Peñas) and C2 (from Cape Peñas to the mouth of the Bidasoa River).

The Gulf of Cadiz is the first basin where the dense (i.e. salty and warm) Mediterranean outflow encounters the open ocean after crossing the Strait of Gibraltar (Ruiz and García-Lafuente, 2006; García-Lafuente et al., 2006). The Portuguese sampling location, Peniche, is situated on a region that is enclosed by several extensive submarine canyons (the Nazaré Canyon to the north and the Cascais and Setúbal-Lisbon canyons to the

south), which seem to affect the distribution and characteristics of demersal fish assemblages (Gomes et al., 2001; Sousa et al., 2005).

Specimens from the Mediterranean were caught in the Alboran Sea, close to the coast of Alicante (south-west of the Balearic Sea) and along the Catalan Sea. These locations were selected considering the work by Massutí et al. (2000) and Ribas et al. (2006), which indicate several well-defined areas that can be found in terms of oceanographic conditions. Bluemouth sampled in the Alboran Sea were divided into two subareas (called A1 and A2) based on the findings of Rodríguez-Mendoza et al. (2011), which showed two different growth trends in bluemouth from the Alboran basin. Thus, bluemouth caught along the Andalusian coastline and the western slopes of the Alboran Island were included in subarea A1. Specimens caught on the eastern slopes of the Alboran Island were included in subarea A2. The Alboran Island is located about 50 km north of the Moroccan coast and 90 km south of the province of Almería, Spain (Figure 1).

Two of the locations (the Porcupine Bank in the NE Atlantic and Sicily in the Central Mediterranean) were used as reference areas in order to understand the population structure of bluemouth at a larger scale and relativize the possible differences among bluemouth populations around the Iberian Peninsula.

Most of the samplings were carried out in bottom trawl research surveys in collaboration with the Spanish Oceanographic Institute (IEO). The depth range in which bluemouth specimens were captured in each survey is shown in Table 1. Only bluemouth from Peniche (Portugal) and from Sicily (Central Mediterranean) were obtained from regional commercial vessels.

2.1 Data acquisition

After collection, the fish were frozen at -20 °C in plastic bags (to avoid desiccation) and stored in a horizontal position (to avoid deformations) in the freezer until the morphometric analysis. To preserve shape, the evisceration to avoid the influence of the gonad size or stomach fullness on the shape of the fish, was done carefully. Around 90% of the bluemouth specimens that were caught in the 2007 DEMERSALES survey were eviscerated onboard. The rest of the bluemouth specimens from the 2007 DEMERSALES survey and those from the 2007 MEDITS, 2008 PORCUPINE and 2009 ARSA surveys and from commercial vessels were eviscerated later in the laboratory. Sex of the specimens was determined by macroscopic examination of the gonads.

Thirteen homologous landmarks were defined (Figure 3) to provide an adequate coverage of the body shape, based on a previous study by Sequeira et al. (2011). These landmarks were marked with black-headed entomological pins and then each specimen was placed on its left side on a white polystyrene board with a ruler with 1-cm gradations. A photograph was taken with a Nikon D1X digital camera (Nikon Corporation, Japan) using a focal length of 35 mm to avoid optic distortions of the images. The images were digitized using TpsDig software version 2.10 (Rohlf, 2006) to obtain the x,y coordinates of the landmarks, and, once digitized, each specimen was represented as a configuration of landmarks.

At the data acquisition step, 5 specimens were discarded: four specimens were the smallest individuals caught in all of the samplings (centroid size: 3.30–4.43 cm), and it was very difficult to locate and mark correctly the landmarks; and 1 specimen was damaged in the pectoral region in such way that landmark positions would not be reliable.

2.2 Morphometric and statistical analysis

2.2.1. Extraction of shape variables

Body shape was then analysed using landmark-based geometric morphometrics methods (Rohlf, 1990, Bookstein, 1991). To remove non-shape variation, a generalized Procrustes analysis (GPA) was carried out with the MorphoJ software (Klingenberg, 2011). This software uses a full Procrustes fit, as explained in Rohlf et al., (1990) and Zelditch et al., (2004). Then, data are projected to the tangent space by orthogonal projection (Dryden and Mardia, 1998).

2.2.2. Size-correction of shape variables

In this study, size composition (i.e. size structure or number of small, medium and large specimens) was different among the samples (Figure 2). Thus, there was a risk of confounding true differences between the groups with accidental differences in the size compositions of the samples due to the presence of allometric shape variation. Therefore, it was necessary to remove allometric shape variation prior to carrying out other statistical analyses. After the study carried out by Rodríguez-Mendoza (2013) it was concluded that the best choice for size-correction of the shape variables in the bluemouth dataset was a pooled within-group regression of the Procrustes coordinates on centroid size. The residuals of this regression could be then used as ‘size-free’ variables. Thus, these residuals were used as shape variables in subsequent statistical analyses. The R software (R Development Core Team, 2017) was used to carry out the size-correction of shape variables.

2.2.3. Sexual dimorphism

To the present, evidence that bluemouth exhibit sexual dimorphism is conflicting. According to Rodríguez-Mendoza (2013), bluemouth from a few locations show significant body shape differences by sex, while in other locations no shape differences

between sexes have been found. As it is not clear that bluemouth show sexual dimorphism, sexes were pooled for each area in the population structure analysis. In addition, sample sizes in many of the locations in our study would be significantly reduced if the samples were to be analyzed by sex.

2.2.4. Population structure analysis

First, Procrustes and Mahalanobis distances between mean shapes of the sampled bluemouth populations were calculated and the mean shape difference was evaluated using a permutation test with 10,000 runs. Then, a Canonical Variate Analysis (CVA) was used to provide a picture of the degree of separation and relationships among groups according to shape. For the purpose of group discrimination, the Mahalanobis distances can give a better picture of the groups' distinctness because they measure the differences between groups relative to the within-group variation and deal with the non-isotropic variation of the landmarks by transforming the multivariate space (Klingenberg and Monteiro, 2005). Therefore, the relationship among the bluemouth groups was also investigated using these distances in a cluster analysis. The results were depicted in a dendrogram using an average linkage method (i.e., UPGMA). Following the CVA, a jackknife cross-validation procedure was used to calculate an unbiased estimation of classification success. In this procedure, each individual was allocated to the group with the nearest centroid and the proportion of correctly allocated individuals calculated. The overall interpretation of results was based on the cluster analysis, the CV plot and the classification matrix validated by a jackknife procedure.

MorphoJ software (Klingenberg, 2011) and SPSS statistical software, release 19.0.0 (IBM Inc. 2010) were used to carry out the CVA and STATISTICA v. 6 was used to carry out the Cluster analysis.

3. Results

3.1. Overall population structure

Significant differences (all of p -values < 0.0001) were found among the mean shapes of bluemouth from the 10 areas (Table 2). The cluster analysis revealed three clusters (Figure 4). The first cluster shows that Alicante and Catalonia are the most similar groups and they cluster with subarea A1 in the Alboran Sea. The second cluster shows a similarity between Sicily and the Gulf of Cadiz, with the Porcupine Bank closely related to these two areas. Galicia and the Cantabrian Sea formed the third cluster which is well separated from the two previous clusters. Interestingly, subarea A2 in the Alboran Sea did not cluster closely with the other Mediterranean locations. Similarly, Portugal did not cluster together with other locations. In the next step of the analysis, the CVA showed overall significant differences among the groups (Wilks' Lambda: 0.0881, $F_{(198,9130)} = 15.53631$, $p < 0.05$). In general, the CVA plot (Figure 5) showed a similar picture as the one observed in the cluster analysis, although it was more difficult to see the degree of separation for some areas. The first CV separated Galicia and the Cantabrian Sea from most of locations while the second CV mainly showed the separation between three of the Mediterranean locations (Alicante, Catalonia and subarea A1) and two of the NE Atlantic locations (the Porcupine Bank and Portugal). Overall, the largest differences were observed between Galicia-Cantabrian Sea and the rest of locations, and the most similar locations were Cadiz and Alboran Sea A2.

The CVA yielded only a 57.6 % of correct classification (Table 3), despite the significant differences found in the CVA and in the comparison between the mean shapes based on Procrustes and Mahalanobis distances. This indicates that the obtained canonical variables were not able to separate bluemouth specimens effectively at least in some of

the areas. When analysing the classification matrix by areas, correct classification rates varied considerably (from 71.20 to 28.77 %).

The mean shapes did not show any specific trends of landmark displacements between Atlantic and Mediterranean locations (Figure 6). In other words, there were no specific shape changes related the Atlantic or Mediterranean origin of the samples. Instead, shape changes seem to be area-specific. These area-specific shape changes are described in detail in the next sections, where the Atlantic and Mediterranean locations are considered in detail.

3.2. Structure in the NE Atlantic locations

3.2.1. Cantabrian Sea and Galicia

A close morphological relationship was found between bluemouth from the Cantabrian Sea and the neighboring location to the Northwest, Galicia. These two groups had the most similar mean body shape (i.e., the lowest Procrustes distance, 0.1000) among the compared bluemouth samples and also the lowest Mahalanobis distance (1.9391) between any two NE Atlantic locations (Table 2). On the dendrogram (Figure 4), Galicia and the Cantabrian Sea clustered together and appeared separated from Portugal, the Gulf of Cadiz and the Porcupine Bank. In the CVA plot (Figure 5), Galicia and the Cantabrian Sea also appeared separated from the rest of locations by the first CV and the classification matrix confirmed these results: the greatest number of misclassified specimens from the Cantabrian Sea was allocated to Galicia (21.85 %; i.e., 26 out of 119 specimens), and the highest percentage of the bluemouth incorrectly classified from Galicia was assigned to the Cantabrian Sea (9.42 %, 18 out of 191 specimens) (Table 3). Shape differences were characterized by displacements of landmarks in the head region and the insertion of the ventral fin (Figure 6). Bluemouth from Galicia showed a smaller

and more contracted head while bluemouth from the Cantabrian Sea showed a deeper body.

The analysis using the four subareas (G1, G2, C1 and C2) showed that the morphological variation followed a gradient, with no clear differentiation between Galicia and the Cantabrian Sea (Figure 7 and Table 4). The misclassification rate between G1 and G2 was high (around 40 %). Likewise, subareas C1 and C2 from the Cantabrian Sea were confounded with subarea G2, with a misclassification rate of more than 20 % (Table 4).

3.2.2. Portugal

The greatest amount of morphological differentiation was found for bluemouth from Portugal according to both the Procrustes and Mahalanobis distances. The cluster analysis showed that Portugal did not group with neighboring locations to the north and south (Galicia and the Gulf of Cadiz) (Figure 4). On the CVA plot (Figure 5), Portugal was well separated from Galicia by the first and second CV's, and from the Gulf of Cadiz by the second and third CV's. In addition, Portugal was the second group with the highest classification success (70 %) in the classification matrix (Table 3). The misclassification rate of specimens from Portugal into other Atlantic locations was higher in the Porcupine Bank (6.67 %) than in both of the neighboring locations (3.33 %). The mean body shape of bluemouth from Portugal included relative displacements of landmarks in most of the body, especially at the insertion of the fins (Figure 6).

3.2.3. The Gulf of Cadiz

Moderate shape differences were found between the Gulf of Cadiz and the areas in the Mediterranean according to Procrustes distances. The lowest distance was found between this area and Alicante (0.0144). The relationship of the Gulf of Cadiz with other areas was not clear, because the dendrogram (Figure 4) showed a close relationship with Sicily,

but the CV plot (Figure 5) showed closeness to subarea A2 in the Alboran Sea. Additionally, the Gulf of Cadiz had a relatively low proportion of individuals correctly classified, with similar percentages of specimens incorrectly assigned to Atlantic locations (around 30%, especially to the Porcupine Bank) and to Mediterranean locations (around 22%, mostly to A1 and Alicante) (Table 3). In any case, the CVA showed a connection of the Gulf of Cadiz with the western Mediterranean.

3.2.4. The Porcupine Bank (Reference area in the NE Atlantic)

The Porcupine Bank did not cluster with the closest geographical locations in the NE Atlantic (i.e., Galicia, Portugal or Cantabrian Sea) (Figure 4). The CVA also showed that the Porcupine Bank was separated by the first CV from these locations. The percentage of specimens from the Porcupine Bank correctly classified reached almost 70% (Table 3), with a small number of specimens (7.14%) classified into Galicia, while the confusion with Portugal and the Cantabrian Sea was almost negligible (2.20% and 1.65% respectively). In the case of Galicia, a similar percentage of bluemouth specimens was misclassified into the Porcupine Bank (8.90%).

3.3. Structure in the Mediterranean

The results from the different analyses showed that bluemouth from the Alboran basin could be differentiated from bluemouth in the northeastern locations, Alicante and Catalonia, although a connection with these two locations seems to exist. In the CV plot (Figure 5), the locations in the Alboran basin were separated to a certain extent from Alicante and Catalonia by the first CV, while the second CV showed a moderate separation of subarea A2 and the rest of the western Mediterranean locations. The third CV separated subarea A1 from Alicante and Catalonia. In the dendrogram, subarea A1

joined Alicante and Catalonia, but at a greater distance; and subarea A2 did not join any of the Mediterranean locations.

The mean shape of bluemouth from subarea A1 (Figure 6) differed from that of Alicante mainly at the insertion point of the first dorsal fin (landmark 2), indicating differences in body depth. The mean shapes of bluemouth from Alicante and Catalonia showed similar body depths but differed mostly in the head shape and the pectoral and ventral regions. Shape changes were larger in the case of bluemouth from the Catalanian coast: there was an important upwards shift of the snout (landmark 1) and contraction of the area comprised by landmarks 9 (insertion of the pectoral fin), 11 (second preopercular spine) and 12 (tip of the upper mandible).

Interestingly, in the case of subarea A2, the morphological differentiation seems to be greater. The mean shape of bluemouth from subarea A2 showed that fish from this area presented slender bodies, indicated by a downwards shift of landmark 3 (insertion of the second dorsal fin) and an upwards shift of landmark 8 (insertion of the ventral fin) and a different head shape, with a pronounced downwards shift of landmark 1 (the tip of the snout), a rightwards displacement of landmark 12 (end of the upper mandible) and an upward displacement of landmarks 9, 10 and 11 (insertion of the pectoral fin, end of the opercle and second preopercular spine, respectively) (Figure 6).

3.3.1. The Alboran basin

The results showed morphological differentiation between bluemouth from the two examined subareas in the Alboran basin. The Procrustes distance was moderately high between subareas A1 and A2 (0.0264) and the dendrogram (Figure 4) did not show a close association between them. In the CV plot (Figure 5), the second CV also shows a separation between the two subareas. In the Mediterranean, subarea A1 in the Alboran

Sea had the highest correct classification rate (67.84 %), with a low number of specimens incorrectly classified into subarea A2 (5.85%) (Table 3). Subarea A2 had more than half of its individuals correctly classified (53.73%) but around 20% of the samples were incorrectly assigned to neighbouring locations.

3.3.2. Catalonia and Alicante

The analyses revealed a close morphological relationship between Catalonia and Alicante. The Procrustes distance was low between these two locations and the Mahalanobis distance was the lowest among Mediterranean locations. Accordingly, these two locations grouped together in the dendrogram (Figure 4), joined later by subarea A1. In the CV plot (Figure 5), Catalonia and Alicante also appear closely together in the three dimensions, and the results of the classification matrix showed a considerable confusion of specimens between Catalonia and Alicante (Table 3). The mean shapes of bluemouth from Catalonia and Alicante showed similar body depths but differed mostly in the head shape and the pectoral and ventral regions (Figure 6). Shape changes were larger in the case of bluemouth from the Catalanian coast: there was an important upwards shift of the snout (landmark 1) and contraction of the area comprised by landmarks 9 (insertion of the pectoral fin), 11 (second preopercular spine) and 12 (tip of the upper mandible).

3.3.3. Sicily (Reference area in the Mediterranean)

Sicily, located in the central Mediterranean, did not cluster with any of the western Mediterranean locations (Figure 4). On the other hand, the specimens from Sicily were largely confounded with Mediterranean locations, but also with Atlantic locations in a similar proportion (especially with the Gulf of Cadiz and the Porcupine Bank) (Table 3).

4. Discussion

This study shows morphological differences between bluemouth from some of the sampled areas in the NE Atlantic and the Mediterranean. As Thompson (1991) suggested, phenotypic differences between groups in the wild may reflect genetic differentiation, environmental differences, or a combination of the two. The genetic structure of bluemouth populations in the Atlantic has been studied mainly in Portuguese waters by Aboim et al., (2005), who concluded two important points regarding the genetic population differentiation of the bluemouth: (1) Isolation-by-distance did not seem to be the main cause of the differentiation found, and (2) the detection of relatively small-scale population units conforms to studies on bluemouth behavior, reproduction and tagging (internal fertilization and sedentary adults can promote differentiation between samples). Due to the lack of more information regarding the genetic population structure of bluemouth from the European continental slope, these two remarks should be taken into account in the interpretation of the morphometric differences between bluemouth populations observed here.

In addition to the genetic component, there are several environmental factors that can modify body shape in fishes (e.g., temperature, water velocity, quantity of food, type of food and feeding mode) because body shape is thought to reflect adaptation to specific ecological niches (Swain et al., 2005 and references therein). Therefore, because local environments vary, different characters that improve survival and reproduction are selected in different areas (Cadrin, 2005). For example, Lawton et al. (2010) observed plasticity in growth and morphology among adjacent populations of sea perch (*Helicolenus percooides*) in the Fiordland region in New Zealand, highlighting the importance of the local habitat quality and food resources for subpopulation structure.

398 In this study, bluemouth were sampled from several locations in the NE Atlantic and the
399 Mediterranean. Although the deep-sea (depth > 200 m) has been regarded as a relatively
400 uniform environment with respect to some variables such as temperature and salinity
401 (Hopkins, 1985; Merrett and Haedrich, 1997), the upper slope (200 – 500m), where the
402 bluemouth is abundant (Massutí et al., 2001; Sánchez et al., 2008; Romeo et al., 2009),
403 shows a variety of habitats, e.g. deep coral reefs, off-reefs habitats and transition habitats,
404 with different bottom types, e.g. rocks, sand, mud or a combination of these, that support
405 different fish and crustacean assemblages (Fariña et al., 1997, Demestre et al., 2000,
406 Roberts et al., 2008, Sánchez et al., 2009; Ross and Quattrini, 2007, 2009). In a recent
407 study, Ross and Quattrini (2007) observed that bluemouth was very abundant on the deep
408 coral reefs of the southeastern USA continental slope and the Rockall banks off Ireland,
409 but they also mention that bluemouth was regularly observed away from reef habitat,
410 associated to other structures (e.g. burrows or anemones). These observations indicate
411 that bluemouth indeed use a diversity of habitats with different characteristics. As many
412 demersal fish species, the bluemouth has a benthopelagic diet. It can be considered
413 specialized on medium-sized benthic decapods (Natantia, Brachyura and Macrura), but it
414 also feeds on demersal fish and sometimes pyrosomes, polychaetes and echinoderms
415 (Macpherson, 1979, 1985; Nouar and Maurin, 2000; Serrano et al., 2003). Thus, the
416 morphological differences detected in this study may be linked to differences in foraging
417 behavior, depending on the food type and availability in the studied areas. In this case,
418 morphometric differences would result from bone remodeling in response to differences
419 in loading regime induced by the diet or feeding mode in a particular area, a phenomenon
420 known as trophic morphological plasticity as described by Swain et al. (2005). However,
421 the same authors also point out that habitat and food similarity can also produce
422 morphological similarities among distant populations. This would explain the observed

similarities between the Gulf of Cadiz and Sicily samples, for example. In such cases, it is unlikely that fish from locations far apart from each other belong to the same stock unit, because stock units are normally distributed in space as gradients. Thus, body shape similarity would be caused by coincident environmental factors or even by similarities in the genetic pool that remained during the evolution, but not by a significant rate of interbreeding (Murta et al., 2008).

In the NE Atlantic, our results showed that at least four different bluemouth populations exist in the following areas: Galicia-Cantabrian Sea, Portugal, the Gulf of Cadiz and the Porcupine Bank. The results showed that the greatest morphological differentiation existed between bluemouth from Portugal and the neighboring locations: Galicia and the Cantabrian Sea to the north and the Gulf of Cadiz to the south. This indicates that Portugal can be considered as a separate population regarding body morphology. It has to be kept in mind that the sample from Portugal was obtained in waters in the vicinity of Peniche, which is enclosed by several extensive submarine canyons, as the Nazaré Canyon (De Stigter et al., 2007; Tyler et al., 2009). According to some studies, there seems to be a latitudinal boundary around this canyon that affects the distribution and characteristics of demersal fish assemblages (Gomes et al. 2001; Sousa et al. 2005). Thus, it is possible that the particular body shape of bluemouth from Portugal reflects the relative isolation of the region due to the canyons. In fact, a similar result was obtained in the morphometric study of bluemouth populations carried in Portuguese waters, which included Galicia as a reference area in the Iberian Peninsula (Sequeira et al. 2011). In a similar fashion, other morphometric studies for identification of stocks in other species have shown either a clear breakpoint between the northwestern Portuguese slope and Galicia (e.g., horse mackerel; Murta et al. 2008) or a high segregation of Portuguese samples (e.g., black anglerfish; Duarte et al. 2004).

In the case of Galicia and the Cantabrian Sea, a close morphological relationship was observed. The overall morphological variation along the Cantabrian Sea and Galicia seemed to follow a gradient, indicating that bluemouth in these areas are well connected. Thus, it seems that none of the existing biogeographical limits have a noticeable boundary effect on bluemouth populations. The sampling coverage in Galicia and the Cantabrian Sea was very good, without any significant gap between these regions, so if any clear division between the bluemouth populations existed; it is likely that it would have been identified in this study.

As expected, the Porcupine Bank was found to be separated from the other NE Atlantic locations. The offshore banks to the west of Ireland, such as the Rockall and Porcupine Banks, are important spawning and larval ground for many species, including the bluemouth. These banks are also known to be retention areas for eggs and larvae (Dransfeld et al. 2009), which could promote isolation of the bluemouth population from the Porcupine Bank, especially when its biology is also taken into account. Adult specimens of bluemouth are thought to be sedentary (Uiblein 2003, Pakhorukov, 2008; Aboim 2005 and references therein), its reproduction involves internal fertilization and zygoparity (Muñoz et al. 1999) and the larval dispersive phase is generally not sufficient to allow gene-flow between distant bluemouth populations (Aboim 2005). However, more samples from locations in between would be needed to see if bluemouth follow a gradient of morphological variation or if there is a clear boundary between these populations.

In this study, three populations were identified in the western Mediterranean: two populations in the Alboran basin (A1 and A2) and another in the north-western basin (Balearic Sea and Catalanian coast). It was interesting to find that bluemouth from subarea A2 presented important morphometric differences with respect to bluemouth

from the rest of the Alboran Basin (i.e., subarea A1), in accordance with the different growth trends detected by Rodríguez-Mendoza et al. 2011). Specimens belonging to subarea A2 were caught in a single haul to the east of the Alboran Island, while other bluemouth specimens were caught to the west of this island and the Andalusian coastline. The possibility of a methodological error was discarded after the photos of the 67 specimens from subarea A2 were re-examined and no irregularities were detected. In addition, all of these specimens were processed along with the specimens from other sectors of the Mediterranean by the same person and using the same equipment. Thus, several possibilities could explain the morphological differentiation of bluemouth from subarea A2, for example, that these fish have a different origin, e.g., from the African coasts delimiting the Alboran Sea to the south (which were not sampled in this study), or that particularly different environmental conditions exist at the local scale. However, there is no information that may support any of these hypotheses and further samplings within the Alboran Sea should be used to study the population structure of bluemouth in the region to determine if relatively small-scale population units exist, as in the case of the Azores archipelago (Aboim, 2005).

The bluemouth specimens from Alicante and Catalonia were found to belong to the same population, but different from the bluemouth populations in the Alboran Sea. Other studies have also reported that bluemouth populations are different in the two basins of the western Mediterranean and vary in their life history parameters and distribution (Massutí et al. 2000, Massutí et al. 2001, Ribas et al. 2006, Abad et al. 2007). In addition, the stock discrimination study based on otolith chemistry carried out by Swan et al. (2006), showed that the otolith elemental composition of adult *H. dactylopterus* from the Alboran Slope differed from those of the Catalan slope.

Nevertheless, the bluemouth populations in the Alboran basin and the population in the north-western basin Alicante-Catalonia seem to be connected, as shown by the cluster and CVA analyses. Ribas et al. (2006) suggested that dispersion of juvenile bluemouth from the Alboran Sea towards the Balearic Sea and Catalonia could be taking place, which may indicate that a considerable number of bluemouth in the Alicante sector originates in the Alboran Sea.

Contrary to what was expected, the results of this study did not support the hypothesis of the Strait of Gibraltar acting as a barrier between Atlantic and Mediterranean bluemouth populations. However, it is still possible that genetic differences exist between bluemouth populations on both sides of the Strait of Gibraltar, and that the morphological similarities that we observed here are merely related to similar environmental conditions due to water exchange between the Atlantic and Mediterranean. However, in recent years several studies have identified the Almeria-Oran Front (AOF) as a hydrographical and ecological boundary between Atlantic and Mediterranean populations of marine organisms (Quesada et al., 1995; Borsa et al., 1997; Pannacciulli et al., 1997; Naciri et al., 1999; Rios et al., 2002; Cimmaruta et al., 2005). For example, in the study by Cimmaruta et al., (2005), the allozyme data clearly indicated that hake stocks were separated by the Almeria- Oran front and not by the Strait of Gibraltar. In this context, the observed pattern of morphological variation bluemouth populations is compatible with the idea of the Almeria-Oran Front having some boundary effect for bluemouth (i.e., similarities between bluemouth from the Gulf of Cadiz and the Alboran Sea and a noticeable separation of bluemouth the Alboran Sea from Alicante and Catalonia). To our knowledge, the only study in which bluemouth populations in the NE Atlantic and Mediterranean have been compared is that carried out by Swan and collaborators in 2006, in which otolith analysis did not show clear differences between samples. Thus, it would

be interesting to compare gene frequencies between Atlantic and Mediterranean bluemouth populations in future studies to determine whether the AOF causes a reduction of gene flow between these populations or not. In this way, it would be easier to identify the underlying causes (genetic or phenotypic) of the morphometric variation between bluemouth populations in the NE Atlantic and Mediterranean.

In any case, the temporal and spatial stability of the bluemouth population structure determined in this study must be confirmed using a multidisciplinary (i.e. holistic) approach over a longer period of time. This is important because morphological variation is phenotypic and morphometric characters tend to have low to moderate heritability (Swain et al. 2005). Thus, it could be possible that the bluemouth populations studied in this study are affected by temporary differences in the environment and the picture of this stock structure is not definitive.

5. Conclusions

In the latest report, WGDEEP stated that no information is available regarding stock identity for bluemouth (ICES, 2017). From this perspective, the work carried out in this study is an important contribution for understanding the population structure of bluemouth in European waters. Even if the genetic structure for bluemouth is mostly unknown around Europe, the results of this study provide evidence that different phenotypic stocks exist, which can be used to model population dynamics for fishery stock assessment and management, regardless of genetic differences or similarities (Cadrin, 2000).

Moreover, the information provided about the population structure of bluemouth through this study can help to define meaningful areas for which data can be collected in order to carry out exploratory assessments of bluemouth stocks. For example, according to the results in this study, there is evidence that the bluemouth population that exists near Portugal is different from the one in Galicia and the one in the Gulf of Cadiz. This means that at least three different bluemouth populations coexist in ICES division 9.a, and if data were to be collected for formulating management advice, this structure would need to be considered. The opposite situation, when a homogeneous population is distributed in two different divisions, also needs to be taken into account. This could be the case for the bluemouth population in Galicia, which would be distributed in ICES divisions 8.c and 9.a.

Nevertheless, there are two key aspects that need to be addressed in the future. First, the temporal and spatial stability of the bluemouth population structure determined in this study needs to be studied. The second aspect is to contrast the results of this study with ecological and genetic markers, which can give other perspectives of the structure and dynamics of the bluemouth populations. In particular, the genetic approach will be very useful to complement the results of this study because it will allow a better understanding of the underlying causes of differences in morphology.

Acknowledgements

Many thanks to all the people who helped with the sampling of specimens. Samples from Sicily were obtained in collaboration with the Italian Institute of Coastal and Marine Environment (IAMC-CNR). Thanks to Dr. C.P. Klingenberg, Dr. A. Cardini and Dr. L. Outeiro. RRM's work was funded by a Marie Curie fellowship from the Early Stage Training site ECOSUMMER (MEST-CT-2005-020501) and a CONACyT Mexico grant.

568 This research was also supported by the project CTM2006-13964-C03-00 of the Spanish
569 Ministry of Science and Innovation. We would like to thank the anonymous reviewers
570 whose comments and suggestions helped to improve this manuscript.

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829

831 *Table 1. Total number of bluemouth specimens sampled in each location. Size is*
 832 *expressed as total length (TL) in cm. The column Source shows whether the samples were*
 833 *obtained from a research survey (indicating the name) or commercial vessels.*

834

	Study area	N	Size range (TL, cm)	Mean $\pm$ sd (TL, cm)	Dates	Depth range (m)	Source
NE Atlantic							
	Porcupine Bank	184	3.5 – 37.0	25.89 $\pm$ 6.73	Sept./Oct. 2008	225 – 739	Survey - Porcupine 2008
	Cantabrian Sea (2007)	119	10.6 – 39.7	20.12 $\pm$ 5.40	Sept./Oct. 2007	117 – 585	Survey - Demersales 2007
	Galicia (2007)	191	5.4 – 36.2	16.76 $\pm$ 4.68	Sept./Oct. 2007	112 – 696	Survey - Demersales 2007
	Portugal	60	21.6 – 28.5	23.89 $\pm$ 1.47	Feb. 2010	–	Commercial vessels
	Gulf of Cadiz	75	6.3 – 38.0	22.46 $\pm$ 6.49	March 2009	119 – 752	Survey - ARSA 2009
Mediterranean Sea							
	Alboran Sea						
	A1	172	7.4 – 30.2	19.76 $\pm$ 6.26	June 2007	150 – 744	Survey - Medits 2007
	A2	67	8.9 – 34.6	16.60 $\pm$ 5.11			
	Alicante	135	3.6 – 29.3	14.35 $\pm$ 4.41	June 2007	117 – 590	Survey - Medits 2007
	Catalonia	74	4.8 – 21.4	11.60 $\pm$ 3.33	June 2007	131 – 574	Survey - Medits 2007
	Sicily	48	16.1 – 262	19.81 $\pm$ 2.28	Oct. 2009	–	Commercial vessels

835

Table 2. (a) Procrustes distances and (b) Mahalanobis distances among the mean shapes of bluemouth of the studied areas. All of them are significant ($p < 0.05$).

(a)	Porc.	Cant.	Galicia	Portugal	Cadiz	A1	A2	Alicante	Catal.
NE Atlantic									
Porcupine Bank	0								
Cantabrian Sea	0.0213	0							
Galicia	0.0203	0.0100	0						
Portugal	0.0205	0.0306	0.0320	0					
Gulf of Cadiz	0.0218	0.0177	0.0226	0.0285	0				
Mediterranean Sea									
A1	0.0333	0.0256	0.0279	0.0442	0.0203	0			
A2	0.0172	0.0226	0.0239	0.0267	0.0195	0.0264	0		
Alicante	0.0238	0.0151	0.0178	0.0347	0.0144	0.0147	0.0186	0	
Catalonia	0.0369	0.0250	0.0264	0.0489	0.0256	0.0133	0.0325	0.0162	0
Sicily	0.0194	0.0254	0.0292	0.0172	0.0165	0.0332	0.0198	0.0256	0.0395

(b)	Porc.	Cant.	Galicia	Portugal	Cadiz	A1	A2	Alicante	Catal.
NE Atlantic									
Porcupine Bank	0								
Cantabrian Sea	2.9138	0							
Galicia	2.4804	1.9391	0						
Portugal	2.8826	3.5837	3.6958	0					
Gulf of Cadiz	2.0909	2.7339	3.1415	2.6236	0				
Mediterranean Sea									
A1	2.5458	2.9855	3.0032	3.2354	1.9418	0			
A2	2.5270	3.3179	3.2929	2.8011	2.5830	2.3708	0		
Alicante	2.4046	2.3526	2.6125	2.9572	2.0545	1.8687	2.2240	0	
Catalonia	2.7055	2.3926	2.6846	3.6185	2.4035	2.1138	3.0783	1.4782	0
Sicily	2.5626	3.3738	3.6621	2.0974	1.7739	2.5280	2.3283	2.4755	3.1079

Table 3. Jackknifed classification matrix from the CVA performed on the bluemouth samples from the 10 studied areas. Wilks' Lambda: 0.0881, $F(198,9130) = 15.5363$, $p < 0.05$. The overall correct classification rate was of 57.6%. Observed classifications are shown in rows; predicted classifications are shown in columns.

	Porcupine	Cantabr.	Galicia	Portugal	Cadiz	A1	A2	Alicante	Catalonia	Sicily
Porcupine Bank	68.13	1.65	7.14	2.20	4.40	7.14	2.20	4.40	1.65	1.10
Cantabrian Sea	5.04	53.78	21.85	1.68	4.20	1.68	0.84	4.20	5.88	0.84
Galicia	8.90	9.42	71.20	1.05	1.57	3.14	0	2.62	2.09	0
Portugal	6.67	0	3.33	70.00	3.33	1.67	1.67	6.67	0	6.67
Cadiz	20.00	5.33	4.00	2.67	45.33	9.33	2.67	9.33	1.33	0
A1	2.34	1.17	4.09	0.58	2.92	67.84	5.85	8.19	5.26	1.75
A2	11.94	2.99	1.49	2.99	1.49	13.43	53.73	7.46	1.49	2.99
Alicante	6.72	8.96	5.97	1.49	5.22	19.40	3.73	41.04	7.46	0
Catalonia	5.48	12.33	13.70	0	2.74	9.59	0	27.40	28.77	0
Sicily	10.42	0	0	6.25	18.75	10.42	4.17	14.58	0	35.42

Table 4. Jackknifed classification matrix from the CVA performed on the bluemouth samples from the four subareas in Galician waters and the Cantabrian Sea. Wilks' Lambda: 0.3164, $F_{(66,851)} = 6.0670$, $p < 0.05$. The overall correct classification rate was of 58.40 %. Observed classifications are shown in rows; predicted classifications are shown in columns. Subareas in Galicia are G1 (from the Miño River to Cape Finisterrae) and G2 (from Cape Finisterrae to Cape Estaca de Bares). Subareas in the Cantabrian Sea are C1 (from Cape Peñas to Cape Ajo) and C2 (from Cape Ajo to the Bidasoa River)

Subarea	G1	G2	C1	C2
G1	47.1	41.2	7.4	4.4
G2	17.1	69.9	9.8	3.3
C1	9.0	21.8	61.5	7.7
C2	2.4	26.8	34.1	36.6

861 *Figure 1. Map of the sampling locations around the Iberian Peninsula and Reference areas in the*
862 *NE Atlantic and Mediterranean.*

Figure 2. Size composition of the bluemouth samples in each location.

Figure 3. Scheme showing the location of the 13 landmarks used in the analysis.

867

868 *Figure 4. Cluster analysis linkage distance plot based on the Mahalanobis distances among the*

869 *10 groups of bluemouth considered in this study.*

870

871 *Figure 5. CVA plot showing the discrimination of the 10 bluemouth samples. The first three*
 872 *canonical variates shown in this graph accounted for 72.9 % of the among-group variation: CV1*
 873 *(37.7%), CV2 (21.6 %) and CV3 (13.6 %).*

874

NE Atlantic

Porcupine Bank

Cantabrian Sea

Galicia

Portugal

Gulf of Cadiz

Mediterranean Sea

Alboran Sea - A1

Alboran Sea - A2

Alicante

Catalonia

Sicily

875

876 *Figure 6. Mean shapes for bluemouth from the different locations. The figures represent the*

877 *transformation from the overall mean shape (light grey outline) to the mean shape for each*

878 *location (black outline). Shape changes have been exaggerated three-fold for better visualization.*

879 *The mean shapes were calculated using the size-corrected shape variables.*

880

881 *Figure 7. CVA plot showing the discrimination of the bluemouth samples from the four subareas*

882 *in Galicia and the Cantabrian Sea. The three canonical variates shown in this graph accounted*

883 *for 100.0 % of the among-group variation: CV1 (65.8%), CV2 (22.0%) and CV3 (12.2%).*