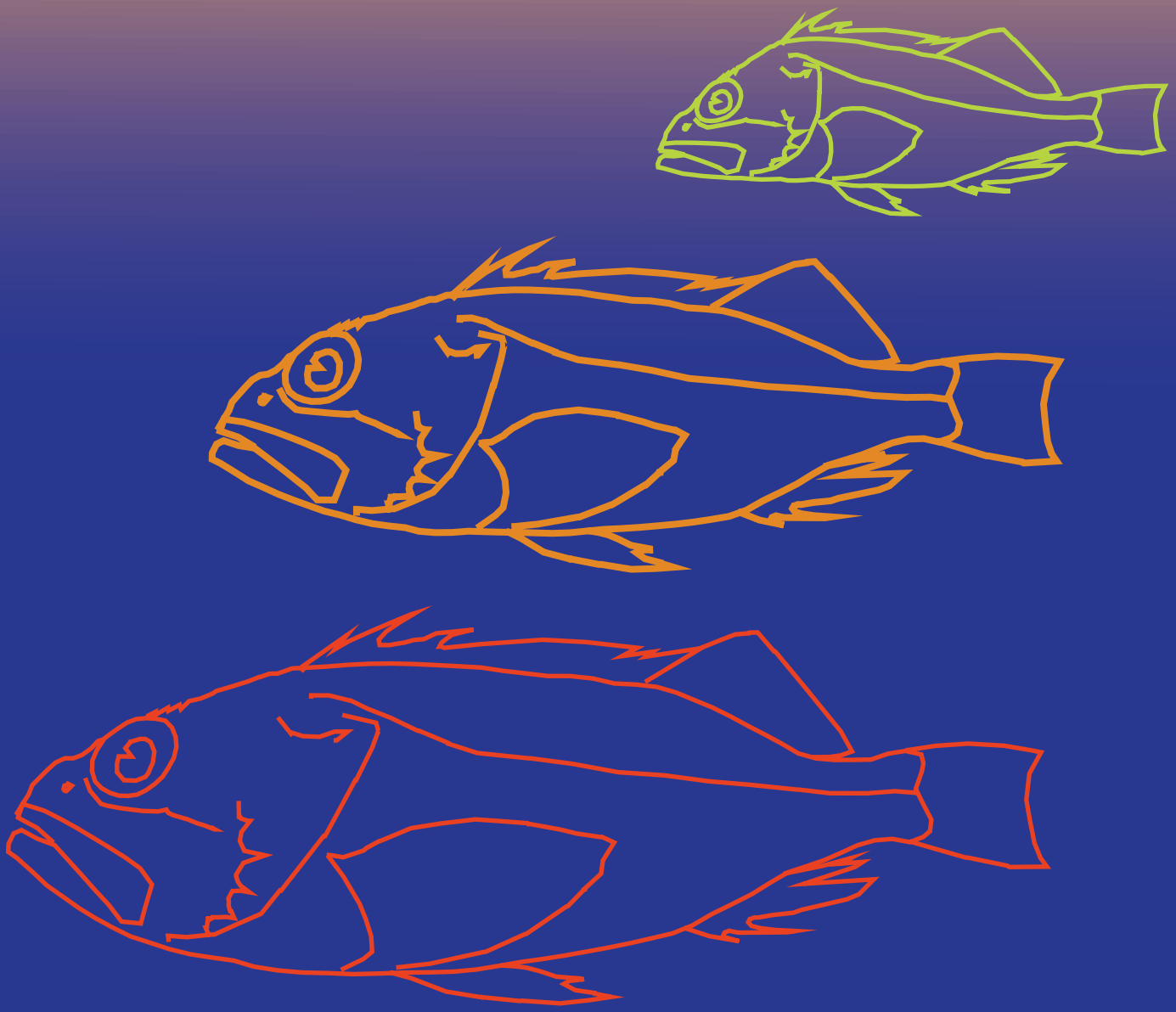
Population structure of the bluemouth, *Helicolenus dactylopterus* (Teleostei: Sebastidae), in the Northeast Atlantic and Mediterranean using geometric morphometric techniques



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PhD Thesis

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Universidade Vigo

Depto. de Ecología y Biología Animal

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Population structure of the bluemouth, *Helicolenus dactylopterus* (Teleostei: Sebastidae), in the Northeast Atlantic and Mediterranean using geometric morphometric techniques

Memoria de Tesis Doctoral
para optar al grado de Doctor
por la Universidad de Vigo

Presentada por:
Rebeca Paola Rodríguez Mendoza

Vigo, 2013

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Autorizan la presentación de la memoria adjunta, titulada **"Population structure of the bluemouth, *Helicolenus dactylopterus* (Teleostei: Sebastidae), in the Northeast Atlantic and Mediterranean using geometric morphometric techniques"**, realizada por Rebeca Paola Rodríguez Mendoza bajo su dirección, para optar al grado de Doctor por la Universidad de Vigo.

Y para que así conste, se expide el presente certificado en Vigo, a 23 de abril de 2013.

Fdo. Juan Francisco Saborido Rey

Fdo. Graham J. Pierce

Fdo. Bernardino González Castro

A mis padres,
a mis hermanos,
a Alejandro,
a toda mi familia,
y a mis amigos…

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Summary

The bluemouth, *Helicolenus dactylopterus* (Delaroche 1809) is a marine demersal fish that is widely distributed in the Atlantic and the Mediterranean. The bluemouth is mostly considered a deep-sea species, but it has a wide bathymetric range of distribution (from 62 m to 1135 m depth). Precisely because of this distribution, it is caught in many fisheries that exploit marine organisms on the continental shelf and the deep-sea. Since the late nineties, the biology of the bluemouth has been studied in the NE and NW Atlantic, the Mediterranean and the North Sea, focusing mainly in the distribution, age, growth and reproduction of the species. However, as with other deep-sea species, there are still many gaps in what regards the biology, ecology and population dynamics of this species.

As it is now widely recognized, information on a species' population structure is of primary importance in developing an optimal strategy for its efficient management (Coyle, 1998). Thus, the overall goal of this study was to provide baseline information on the population structure of bluemouth, *Helicolenus dactylopterus*, around the Iberian Peninsula. Moreover, this thesis provides the first comparative study of bluemouth populations in the Northeast Atlantic and the Mediterranean.

To characterize the population structure of bluemouth in terms of growth and stock components, a morphological approach was followed, because the analysis of morphological characters (i. e., meristic and morphometric characters) has proved useful for characterizing populations of a variety of marine fish (Swain et al., 2005).

To achieve this, bluemouth from 9 areas were sampled. In the NE Atlantic, specimens were caught off Galicia, the Cantabrian Sea, the Gulf of Cadiz, Portugal and the Porcupine Bank (Irish continental margin). Specimens from the Mediterranean were sampled in the Alboran Sea, off-shore Alicante (south-west of the Balearic Sea), the Catalonian coast and in Italian waters (Sicily). The samples from the Porcupine Bank and Sicily 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.

The first specific objective of this study was to study the ontogenetic allometry of the bluemouth in the Northeast Atlantic and Mediterranean using geometric morphometrics. In this part of the study, the shape changes that occur during the growth of bluemouth were characterized to better understand its biology and ecology. The

general pattern of ontogenetic changes observed in this study seemed to be related to the changing ecology of the species (i.e. ontogenetic diet and habitat adaptations) and consisted of a relative expansion of the area between the second preopercular spine and the pectoral fin, a relative deepening and shortening of the body and an upward shift of the snout as the head becomes more compact in relation to the body. However, some specific growth patterns were detected in the different areas, indicating that growth trajectories were not homogeneous among bluemouth populations. The study of allometry was also used to determine the best method to correct for allometry for the bluemouth population structure dataset. For this purpose, a pooled within-group regression yielded the best results.

The second specific objective was to identify bluemouth phenotypic stocks in the Northeast Atlantic and western Mediterranean based on geometric morphometrics and meristics.

The greatest morphological differentiation was found 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 bluemouth from Portugal can be considered as a separate phenotypic stock. The overall morphological variation along the Cantabrian Sea and Galicia seemed to follow a gradient, with no clear breakpoints that could indicate the isolation between bluemouth from these regions. Thus, bluemouth from these two areas seem to constitute a single phenotypic stock.

In the western Mediterranean, there was evidence that at least two bluemouth phenotypic stocks exist: one in the south-western basin (Alboran Sea) and another in the north-western basin (Balearic Sea and Catalonian coast) that extends to the transition zone in the Alicante region. A third stock in the western Mediterranean (or a sub-population) might be present, because bluemouth from subarea A2 in the Alboran Sea presented important morphometric differences with respect to bluemouth from the neighboring areas, but his needs to be further investigated.

In general, meristic variables were stable among bluemouth from the different locations, with the exception of the counts of gill rakers (GRV and GRH), where some variability was observed. Thus, the usefulness of meristic variables to identify bluemouth phenotypic stocks is questionable. It appears that in *Helicolenus*, the study of meristic characters may be more useful at the interspecific level rather than at the intraspecific level.

The work carried out in this thesis is a major contribution for understanding the population structure of bluemouth in European waters, providing evidence that different phenotypic stocks exist. These stocks can be used as a first approach to model population dynamics for fishery stock assessment and management. However, more work needs to be done to understand the bluemouth stock structure in the NE Atlantic and Mediterranean. A multidisciplinary study, covering both basins, is necessary to identify correctly the stock components and understand the dynamics of bluemouth populations.

Chapter 1
General Introduction

1.1 Overview

The bluemouth, *Helicolenus dactylopterus* (Delaroche 1809) is a marine demersal fish that is widely distributed in the Atlantic and the Mediterranean. The bluemouth is mostly considered a deep-sea species, but it has a wide bathymetric range of distribution (from 62 m to 1135 m depth). Precisely because of this distribution, it is caught in many fisheries that exploit marine organisms on the continental shelf and the deep-sea. Since the late nineties, the biology of the bluemouth has been studied in the NE and NW Atlantic, the Mediterranean and the North Sea, focusing mainly in the distribution, age, growth and reproduction of the species (Heessen et al., 1996; Esteves, 1997; White et al., 1998; Kelly et al., 1999; Massutí et al., 2000a and b, 2001; Abecasis et al., 2006; Ribas et al., 2006; Mamie et al., 2007; Muñoz et al., 1999, 2000; Allain, 2001; Sequeira et al., 2003; Mendonça et al., 2006 and Vila, et al., 2007). However, as with other deep-sea species, there are still many gaps in what regards the biology, ecology and population dynamics of this species.

In this section, an overview of the current knowledge about the biology and ecology of the bluemouth is presented. Then, the bluemouth fishery is described and finally, the objectives and the organization of this thesis are outlined.

1.2. The bluemouth

1.2.1 Taxonomy

The bluemouth is currently classified in the family Sebastidae within the Order Scorpaeniformes (Figure 1-1) (Eschmeyer & Fricke, 1998; 2010). Some authors, however, have included it in the family Scorpaenidae (Eschmeyer, 1969; Nelson, 1984; Hureau & Litvinenko, 1986) and it seems that this classification is still the commonly used (e.g., Massutí et al., 2000a; Massutí et al., 2001; Pirrera et al., 2009; Romeo et al., 2009; Smith et al., 2009). In both classifications, the genus *Helicolenus* has been included in the subfamily Sebastinae.

The subfamily Sebastinae was defined by Matsubara (1943). Two of the genera in this subfamily, *Sebastes* (including *Sebastodes*) and *Sebasticus*, have an incomplete suborbital stay which does not attach to the operculum. In contrast, the genera

Helicolenus and *Hozukius* have a complete suborbital stay. Species of *Helicolenus* are characterized by the lack of an airbladder and by having 25 vertebrae (Abe & Eschmeyer, 1972). According to WoRMS (2012), the genus *Helicolenus* comprises 10 species (Table 1-1). However, the status of *Helicolenus lahillei* is not clear as it is considered as a subspecies of *Helicolenus dactylopterus* by some authors. In fact, Eschmeyer (1969) considered that *H. dactylopterus lahillei* is one of the two Atlantic subspecies of *H. dacylopterus* (the other is *Helicolenus dactylopterus dactylopterus*). He described that *H. d. dactylopterus* was composed of four separate populations (the north-east Atlantic and the Mediterranean, the Gulf of Guinea, South Africa and the north-west Atlantic), while *H. d. lahillei* was only found off the coasts of Uruguay and Argentina.

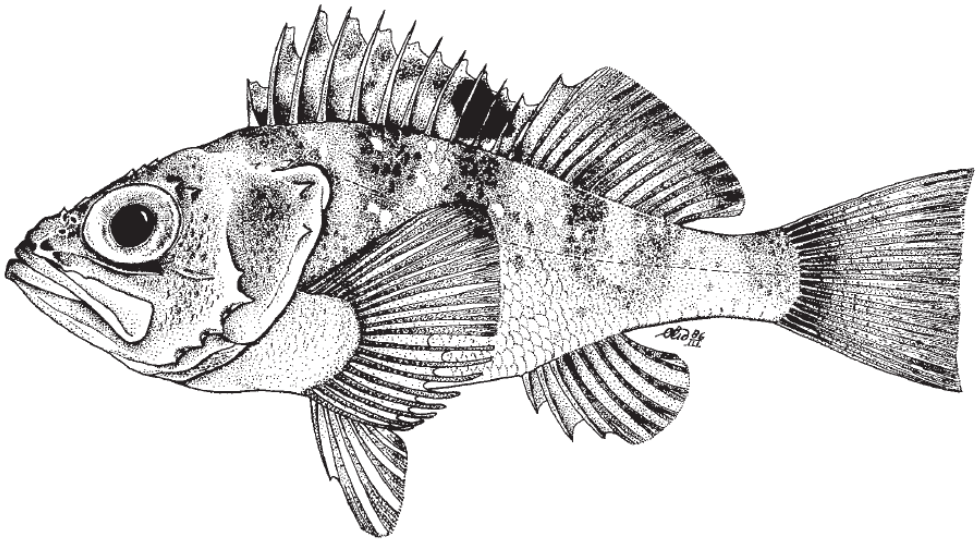


Figure 1-1. The bluemouth, Helicolenus dactylotpterus (Delaroche, 1809). Source: FAO, 2002. The Living Marine Resources of the western Central Atlantic. K. E. Carpenter (Ed.). FAO Rome.

Table 1-1. Species within the genus Helicolenus.

Genus ***Helicolenus***	**Species**
1	*Helicolenus alporti* (Castelnau 1873)
2	*Helicolenus avius* (Abe & Eschmeyer 1972)
3	*Helicolenus barathri* (Hector 1875)
4	*Helicolenus dactylopterus* (Delaroche 1809)
5	*Helicolenus fedorovi* (Barsukov 1973)
6	*Helicolenus hilgendorfii* (Döderlein 1884)
7	*Helicolenus lahillei* (Norman 1937)
8	*Helicolenus lengerichi* (Norman 1937)
9	*Helicolenus mouchezi* (Sauvage 1875)
10	*Helicolenus percoides* (Richardson & Solander 1842)

1.2.2 Morphology

Bluemouth adults have a robust but flexible muscular body, typical of benthic sit-and-wait predators (Webb, 1984; Uiblein et al., 2003). Fish from the genus *Helicolenus* can reach sizes of about 50 cm (Paul & Horn, 2009). The largest specimen of *H. dactylopterus*, as recorded in scientific literature, was of 47 cm (Abecasis et al., 2006). The head is of moderate size, without cirri or tabs; the snout is short and the eyes are large. Small teeth occur in its jaws, palatine and vomer. The preorbital bone has rounded lobes over maxilla. The suborbital ridge either lacks spines or has a single spine and the nasal, preocular, supraocular, postocular and pterotic spines are poorly developed. The preopercle has 5 spines, with the second being the longest (McEachran & Fechhelm, 1998). The color of their body is variable, from pale orange to bright red with vertical bands of darker pigmentation on the flanks. The belly is white or with a pinkish coloration. The mouth and tongue are white, but the throat and the peritoneum are dark grey or black. Young specimens have a dark area at base of dorsal fin. The body is covered with ctenoid scales. Bluemouth have 25 vertebrae and the gasbladder is absent (Abe & Eschmeyer, 1972; Moser et al. 1977). The first gill arch has 7 or 8 gill rakers on the vertical branch and 16 to 18 on the horizontal branch. The number of rays of the pectoral fin is 17 – 19. The dorsal fin has 12 spines and 11 to 13 soft rays. The anal fin has 3 spines and 5 soft rays. The caudal fin is very slightly emarginated (McEachran & Fechhelm, 1998).

1.2.3 Distribution and habitat

The bluemouth is widely distributed in the Eastern Atlantic, from the Norwegian coasts to the south-west coast of Africa, around the Macaronesian archipelagos (Azores, Madeira, Canaries and Cape Verde) and in the Mediterranean except in the Black Sea (Hureau & Litvinenko, 1986). On the western coast of the Atlantic, it is found from Canada to Venezuela (Quéro & Vayne, 1997 in Abecasis, et al., 2006). Bluemouth are also common on seamounts of the Mid-Atlantic Ridge (Hureau & Litvinenko, 1986) and non-axial seamounts such as the Josephine Bank (Maul, 1976).

Recent records from North Sea ground fish surveys have confirmed the presence of *H. dactylopterus* in the North Sea since 1991. The stock is dominated by a single year

class (1990) that has been spreading south and east of Shetland since 1991 (Heessen, 1994).

Bluemouth have been recorded at a wide range of depths, from 62 m to 1135 m depth (Table 2-2). According to its distribution, the bluemouth is primarily considered a deep-sea species. The deep sea is normally characterized by depths of >800m where the biotic and abiotic factors seem to vary very little. The light is scarce or inexistent, the pressure is very high, temperature low (-1ºC to +4ºC), the salinity constant, oxygen concentration near saturation and the food input very small (Gage & Tyler, 1991). However, most scientists consider the beginning of the deep sea habitat to be around 200m on the transition from the continental shelves to the continental slope, which determine the threshold between shallow-water fauna and deep-sea fauna (Tyler, 2003). This is the definition that will be used throughout this study. It is considered here that the deep-sea starts on the "shelf break" at 200m deep, and spreads through the continental slope (200-2000m), the oceanic rise (2000-4000m) and the abyssal plains (4000-6000m) reaching depths of >11,000 m in trenches of the Pacific Ocean (Hopper, 1995).

Literature indicates that *H. dactylopterus* uses a wide range of habitats and that it is strongly associated with the sea bottom (Uiblein et al., 2003; Ross & Quattrini, 2007). However, it seems that deep-water coral banks are especially important for this species. The coral banks consist of a complex three-dimensional structure providing several ecological niches for a large diversity of associated species (Rogers, 1999). At the same time, they represent a refuge for prey as well as a spawning and nursery area for many species (Mastrototaro, 2010). Ross and Quattrini (2007) reported that bluemouth is the most common scorpaenid on the deep-water coral reefs banks off the southeastern United States. These authors reported that adults and juveniles were observed perched under coral bushes, perched on top of coral (live or dead), or sitting on the substrate near corals. When observed away from reef habitat, it was nearly always closely associated with whatever structure was available (e. g., burrows, anemones,etc.). Uiblein and colleagues (2003) reported that *Helicolenus dactylopterus* was the most abundant species on the upper slope (around 500 m), where the bottom type was highly structured and showed a mixture of soft and hard substrates. This species is also common on coral habitat at Rockall Banks off Ireland where it displays the same behavior as noted above (SWR, pers. obs. in Ross & Quattrini, 2007). The biodiversity of the Santa Maria di Leuca (SML) coral bank in the Ionian Sea has been recently studied by means of underwater

video systems, benthic samplers and fishing gears. Mastrototaro and colleagues (2010) reported a high occurrence of *Helicolenus dactylopterus* in this coral habitat and they suggest this type of habitat could be related to their spawning requirements. Moreover, D'Onghia et al. (2010) has reported a noteworthy number of large specimens of bluemouth, both maturing and mature, captured in the Santa Maria di Leuca coral banks. D'Onghia and colleagues (2010) have suggested that the remarkable density of young-of-the-year indicates that SML coral banks act also as a nursery area for bluemouth and other deep-water species, which find suitable environmental conditions and refuge from fishing. In the western Mediterranean, high abundances of bluemouth have been observed at the small seamount Seco de los Olivos in the Alboran Sea. This seamount hosts a great variety of corals and gorgonias, and it is characterized by rocky bottoms that are interpolated with (trawlable) sandy bottoms (Abad et al., 2007). This species is also widely known from trawl samples over supposedly soft substrata (e. g., Haedrich & Merrett, 1988; Gordon et al., 1996; Table 1), however, trawls generally obscure habitat data (Ross & Quattrini, 2007).

Table 2-2. Records of the bathymetric distribution for the Bluemouth found in scientific literature.

Study	Area	Depth range (m)	Observations
Merret & Marshall (1981)	Off the coast of North Africa (Central Eastern Atlantic)	279 – 500	
Cardador & Pestana (1995)[a]	Off the Portuguese south-west coast (NE Atlantic)	200 – 500	
Kelly et al. (1999)	Rockall Trough (NE Atlantic)	285 – 1135	Peak abundance on the upper slope (500 – 800 m)
Sánchez & Serrano (2003)	Cantabrian Sea (NE Atlantic)	120 – 500	Typical distribution according to the authors
Massutí et al. (2004)	Rockall Trough (NE Atlantic)	? – 880	From trawl surveys
Menezes et al. (2006)	Off the Azores (Central Eastern Atlantic)	125 – 925	From trawl surveys
Pakhorukov (2008)	Seamounts of the southern Azores complex (NE Atlantic)	180 – 760	From submersible observations
Serrano et al. (2008)	Galician continental shelf (NE Atlantic)	200 – 500	Typical distribution according to the authors
Katsanevakis & Maravelias (2009)	Aegean and Ionian Seas (Mediterranean)	62 – 877	Depth of peak density for bluemouth was estimated to be 627 m
Pirrera et al. (2009)	Off the Italian coasts (Mediterranean)	150 – 1000	Juveniles are mainly located around 150 - 300 m depth; adult specimens are spread over a wider depth range from 200 m to as deep as 1000 m
Ross& Quattrini (2009)	Western North Atlantic Ocean	366 – 603	From submersible observations

[a] Not seen; cited from Kelly et al. (1999).

1.2.4 Behaviour

Bluemouth adults are thought to lead a very sedentary life style according to seabed observations and tagging experiments. Between 1982 and 1986, a series of visual observations and trawls performed by R/V *Ikhtiandr* were carried out to study the ichthyofauna on seamounts of the Southern Azores complex (Pakhorukov, 2008). According to the results, most of the bluemouth that were observed were lying motionless on the bottom, resting on their pectoral fins, with the head slightly raised. They, as a rule, were situated near stones, pits, crevices, and under benches. They did not react to the underwater inhabited device (UID), and when the device passed above them at a distance of 0.5–1.0 m, they remained on their site without changing their posture. When UID passed very near (0.2–0.3 m) from the ground, they ascended over the bottom, slowly swam aside, and again descended to the bottom 1.5 m away from it; in this case, some individuals turned their head towards the device, assuming a threatening posture, raising their head high, and widely drawing apart their gill covers. More recently, Uiblein and colleagues (2003) performed underwater observations with a submersible at depths between 400 and 2000 m in the Bay of Biscay, NE Atlantic. The authors describe that *H. dactyloperus* was encountered attached to the bottom and inactive, without showing any locomotion or response to the submersible. Thus, based on their observations, they characterized this species as a typical sit-and-wait predator that may attack its prey at rather short distances. Ross & Quattrini (2007) also reported that bluemouth appeared to be inactive most of the time (i. e., perched on corals or sitting on the substrate near corals). Tagging experiments around the Azores archipelago also indicate that bluemouth is rather sedentary. In these experiments, most tagged specimens have been recaptured, after more than one year, exactly in the same places as they were originally caught and tagged (Menezes unpubl. data in Aboim, 2005).

1.2.5 Age and Growth

The bluemouth has been characterized as a slow growing and long-lived species that can live more than 30 years (Massutí et al., 2000a; Abecasis et al. 2006; Sequeira et al., 2009). In fact, the *Helicolenus* species found in New Zealand (*H. percoide*s), has been reported to live much longer than that. The oldest specimen recorded was a 59-year old

male of 50 cm in length that was caught in the Chatham Rise area in New Zealand (Paul & Horn, 2009).

The age composition and growth patterns of *H. dactylopterus* have been studied by means of otolith reading and by fitting the von Bertalanffy growth equation to length-at-age data. A summary of the studies that have addressed age and growth of bluemouth is available in Tables 1-3 and 1-4. A large variation of results can be observed in the von Bertalanffy growth parameters for bluemouth obtained by different authors in Table 4. Massutí et al. (2000a) and Sequeira et al. (2009) explain that the observed variation may be a consequence of several factors: the method used (otolith reading or length-frequency analysis), the heterogeneity of the sample studied (fish of different size distribution caught with different types of sampling gear), different environmental conditions, different latitudes, and different fishing pressures. Moreover, Sequeira et al. (2009) suggested that since bluemouth dwell mostly around submarine mountains in the neighbourhood of deep canyons and lead a rather sedentary existence the difference in growth parameters among bluemouth from different areas could indicate that local populations exist.

Growth of male and female bluemouth has been studied systematically in most works concerning age and growth, but the results among the different works are not consistent. In the western Mediterranean, Massutí et al. (2000a) and Ribas et al. (2006) observed that males grew faster than females. A study in the Azores was not conclusive about possible differences in growth rates between sexes, as growth curves estimated using whole otolith readings showed that males grew faster but growth curves estimated by sliced otoliths failed to show differences between sexes (Abecasis et al., 2006). Moreover, a very recent study by Sequeira et al. (2009) of bluemouth in the Portuguese continental slope found no significant differences between sexes when comparing female and male growth curves. Growth studies for *H. percoides* in two adjacent areas off southeastern New Zealand showed clear between-sex differences in growth and a strong indication of between-area differences as well.

Table 1-3 Maximum otolith ages obtained in age and growth studies of species of Helicolenus.

Species	Area	Method	Maximum age	Length range (cm)	Reference
H. dactylopterus	Ligurian Sea	–	9	–	Peirano & Tunesi (1986)a
H. dactylopterus	Azores	Whole	16	16–41	Isidro (1987)
H. dactylopterus	Tyrrhenian Sea	Whole	9	<5 to >28	Ragonese (1989)
H. dactylopterus	Ionian Sea	–	7	–	D'Onghia et al. (1992)a
H. dactylopterus	Strait of Sicily	Whole	10	4–34	Ragonese & Reale (1995)
H. dactylopterus	Azores	Whole	14	14–47	Esteves et al. (1997)
H. dactylopterus	Adriatic Sea	Whole	25	–	Romanelli et al. (1997)
H. dactylopterus	Carolinas, USA	Thin section	30	17–42	White et al. (1998)
H. dactylopterus	Rockall Trough	Thin section	43	10–38	Kelly et al. (1999)
H. dactylopterus	NE Atlantic	Thin section	43	6–32	Allain & Lorance (2000)
H. dactylopterus	Alboran Sea	Whole	30	3–36	Massutí et al. (2000a)
H. dactylopterus	Balearic Sea	Whole	24	2–30	Massutí et al. (2000b)
H. dactylopterus	Azores	Thin section	32	12–47	Abecasis et al. (2006)
H. percoides	SE Australia	Thin section	42	6–45	Withell & Wankowski (1988)
H. percoides	ECSI (New Zealand)	Thin section	35	5 - 48	Paul & Horn (2009)
H. percoides	Chatham Rise (New Zealand)	Thin section	59	13 – 53	Paul & Horn (2009)

(–) Information not available.

a Not seen; cited in Morales-Nin (2001).

Table 1-4. The estimated von Bertalanffy growth parameters and growth performance indices for Helicolenus dactylopterus *obtained in various studies in the Atlantic and Mediterranean.*

Study/ Area	Sex	Method	L_∞ (cm)	k (year $^{-1}$)	t_0	n	Age range	TL range	Φ
Sequeira et al., 2009/ Portuguese slope	F	Whole otoliths	45.29	0.05	-4.17	450	0 – 27	6 – 37	1.98
	M	Whole otoliths	43.30	0.05	-3.68	483	0 – 26	6 – 35	2.01
	F and M	Whole otoliths	45.50	0.05	-4.01	901	0 – 26	5 – 36	2.01
Isidro (1987)/ Azores	F	Whole otoliths	38.69	0.18	0.42	287	3 – 16	16 – 38	2.43
	* M	Whole otoliths	44.80	0.11	1.83	273	3 – 12	16 – 41	2.36
Ragonese & Reale (1995)/ Strait of Sicily	F and M	Whole otoliths	39.20	0.13	1.46	1125	0 – 10	8 – 33	2.29
Romanelli et al. (1997)/ Adriatic sea	F and M	Whole otoliths	–	–	–	434	–	13 – 41	–
Esteves et al. (1997)/ Azores	F	Whole otoliths	54.70	0.10	-1.16	173	3 – 12	14 – 46	2.48
	* M	Whole otoliths	50.20	0.16	0.05	228	3 – 14	15 – 47	2.61
	* F	Back-calculation	52.60	0.11	-0.24	173	1 – 9	–	2.48
	M	Back-calculation	57.40	0.11	-0.32	228	1 – 11	–	2.56
	F	LFA	56.00	0.15	1.08	173	4 – 9	–	2.67
	M	LFA	65.30	0.13	0.71	228	4 – 9	–	2.74
Krug et al. (1998)/ Azores	F and M	Whole otoliths	50.50	0.14	-1.23	1745	1 – 16	14 – 47	2.55
		MULTIFAN	50.50	0.16	-0.46	6630	1 – 16	14 – 47	2.62
White et al. (1998)/ Carolinas (USA)	F and M	Sliced otoliths	–	–	–	1134	7 – 30	6 – 41	–
Kelly et al. (1999)/ Rockall Trough	F	Whole otoliths and sliced	31.00	0.09	-3.00	–	1 – 37	10 – 38	1.94
	M	Whole otoliths and sliced	37.20	0.06	-4.00	–	1 – 43	10 – 38	1.92

Table 1-4. (continued) The estimated von Bertalanffy growth parameters and growth performance indices for Helicolenus dactylopterus *obtained in various studies in the Atlantic and Mediterranean.*

Study/ Area	Sex	Method	L_∞ (cm)	k (year $^{-1}$)	t_0	n	Age range	TL range	Φ
Allain and Lorance (2000) NE Atlantic	F and M	Whole otoliths		–		1210	1 – 17		
		Sliced otoliths	29.00	0.10	-2.79	1179	1 – 43	6 – 32	
Massutí et al. (2000b)/ Iberian Mediterranean coast	F and M	Whole otoliths	25.50	0.25	-0.53	787	0 – 19	3 – 36	2.21
Massutí et al. (2000a) Alboran Sea	F	Whole otoliths	27.10	0.12	-2.65	561	0 – 26	28	1.95
	M	Whole otoliths	32.30	0.09	-3.31	575	0 – 30	29	1.97
	F and M	Whole otoliths	30.00	0.10	-2.86	1455	–	–	1.95
Balearic Sea	F	Whole otoliths	27.00	0.16	-1.62	178	1 – 22	7 – 28	2.07
	M	Whole otoliths	32.50	0.10	-2.62	198	1 – 21	8 – 30	2.02
	F and M	Whole otoliths	29.90	0.13	-1.75	938	–	–	2.07
Massutí et al. (2001) Alboran Sea	F and M	Whole otoliths	–	–	–	1739	0 – 30	3 – 36	–
Eivissa Channel	F and M	Whole otoliths	–	–	–	652	0 – 10	2 – 23	–
Balearic Sea	F and M	Whole otoliths	–	–	–	928	0 – 22	3 – 30	–
Abecasis et al. (2006) Azores	F	Whole otoliths	56.52	0.06	-1.13	230	2 – 15	13 – 41	2.28
	M	Whole otolith	59.06	0.07	-0.21	330	3 – 16	14 – 46	2.39
	F	Sliced otolith	57.08	0.05	-2.28	92	2 – 28	14 – 42	2.21
	M	Sliced otoliths	54.81	0.06	-2.29	138	2 – 32	12 – 47	2.26
Mamie et al. (2007) North Sea	F and M	LFA	28.20	0.12	2.10	–	–	5 – 26	1.98

– , data not available. LFA: Length frequency analysis

[2]

1.2.6 Diet

In general, the diet of bluemouth consists of benthic decapod crustaceans (Natantia, Brachyura and Macrura), demersal fish and sometimes pyrosomes, polychaetes and echinoderms (Macpherson, 1979, 1985; Nouar & Maurin, 2000; Serrano et al., 2003). However, the proportions of these preys in the diet vary according to the size of the fish. For example, Macpherson (1979) reported that the diet of small bluemouth individuals from 4 to 9 cm in the Mediterranean consisted mainly of fish (51.9%) such as silvery pout (*Gadiculus argenteus argenteus*) and gobies (*Deltentosteus quadrimaculatus* and *Lesueurigobius friesii*) and decapods like *Alpheus glaber* (20.9%), *Calocaris macandreae* (5.9%) and *Goneplax rhomboides* (4.2%). In contrast, the main prey of adult specimens (20 – 29 cm in length) was the decapod crustacean *Goneplax rhomboides* (49.4%), followed by other decapods such as *Calocaris macandreae* (17.6%) and *Alpheus glaber* (14.1%) and a small percentage of pyrosomes (9.4%) and fish (8.2%). A more recent study by Consoli et al. (2010) showed a shift in feeding habits between small (4.0–6.3 cm TL) and larger fishes. In particular, small fishes feed mainly on mysids with a preference for *Lophogaster typicus* whereas adults are feeders of reptantian decapods (mostly *G. rhomboides*).

1.2.7 Reproduction

Species within the subfamily Sebastinae are mostly viviparous (Wourms, 1991). Within the genus *Helicolenus* the reproductive strategy ranges from a zygoparous form of oviparity (early developmental embryos released into the environment), characteristic of *H. dactylopterus* (Muñoz & Casadevall, 2002; Sequeira et al., 2003), to viviparity (release of full-term embryos) in *H. percoides* (Wourms, 1991). In the genus *Helicolenus* fertilization occurs internally and in *H. dactylopterus*, sperm can be stored for up to 10 months inside the female ovaries (Muñoz et al., 1999; 2000). In this way, sperm cells are maintained in a viable state and protected from the female immune system until oocytes mature (Muñoz et al., 2002; Vila et al., 2007).

Females of the genera *Helicolenus* present the cystovarian type II-3 ovary that seems to be an important structure in the formation of egg masses that are included in a gelatinous matrix (Koya & Muñoz, 2007). In this type of ovary, the lobes are paired

structures that fuse at the caudal end and have a muscular connective stroma crossing them longitudinally from which ovigerous lamellae are suspended within the ovarian cavity by highly irrigated fibromuscular peduncles. The oocytes are located on the surface of these peduncles and its development stage increases from the central stroma to the ovary's periphery. The ovarian cavity is located between the surrounding ovarian wall and the central ovarian stroma (Koya & Muñoz, 2007). The two testicles of male bluemouth are flat, elongated structures of approximately the same size, situated in the dorsal region of the abdominal cavity. They are of the lobular type and spermatogonia can be found all along the length of the whole testicular lobule (Muñoz et al., 1999). The sperm cells have an elongated head and middle piece, necessary for internal fertilization and to penetrate the gelatinous matrix in which eggs are suspended (Muñoz et al., 2002)
In spawning females, embryos are released in the initial development stages enclosed in a gelatinous matrix. According to Sequeira et al. (2011b) the gelatinous matrix is produced by the ovarian wall and peduncular epithelia and it undergoes consistency changes until embryo emission occurs. The embryos are embedded in the gelatinous matrix within the ovaries for a presumed period of 12-18 h, which corresponded to the late blastula stage. The matrix is constituted mainly by water and proteins, which is indicative that in bluemouth, a support role of the gelatinous matrix is more probable than a nourishing function (Sequeira et al., 2011b).

The spawning period in the western Mediterranean was from the end of December to May (Muñoz et al., 2010), which was a little longer than the period defined for Greek waters (Terrats & Petrakis, 2001) or around the Azores in the north-east Atlantic Ocean, which is from January to March (Mendonça et al., 2006). In the Celtic Sea, the spawning period was ascertained to be in November and December by Quéro & Vayne (1997), and Allain (2001) determined that spawning off the British Isles occurs from March to June. In the Portuguese continental slope, spawning occurs between January and March, as recorded for the Azores (Sequeira et al., 2011b).

The strategy of releasing eggs over a several months can be advantageous because it increases the probability of offspring survival, but it can also be understood as a necessity in highly fecund species, where a physical limitation occurs (Muñoz et al., 2010). This second explanation is further enhanced in the genus *Helicolenus*, since the increase in the volume of the ovaries is caused not only by the hydration of the oocytes but also by the accumulation of the gelatinous matrix that encloses the eggs, and the

embryonic development and subsequent increase in size of the fertilized eggs (Muñoz et al., 2010).

The characteristics of the reproductive biology that may affect the reproductive potential of the species (i. e., fecundity, atresia and spawning frequency), and that are critical to determine the population dynamics of this deep-sea species have been less studied in bluemouth populations. The most recent information comes from a study of the reproductive biology of bluemouth in the western Mediterranean (Muñoz et al., 2010). Here, the authors found that bluemouth is a highly fecund species, when taking into account its other reproductive characteristics (i. e., internal fertilization and zygoparity). This fecundity is comparable to other related species that fertilize externally and reproduce by the simpler oviparous mode. They also observed that the potential fecundity of *H. dactylopterus* is a function of the total length and the total mass of the fish. Their study also provided the first insights into the regulatory mechanisms of potential fecundity of bluemouth. In this species, potential fecundity is regulated through the mobilization of oocytes in cortical alveoli or early vitellogenesis towards oocyte final maturation in a rapid process dependent on surplus energy, and hence there is less need for follicular atresia to act as a regulatory mechanism. In many females however, the spawning season ends before all oocytes are developed, so there is a need to eliminate the underdeveloped non-ovulated oocytes, which is done through atresia, as shown by the sudden and marked increase in the relative intensity of atresia (up to 50%) detected in the ovaries at the end of the spawning season. Finally, they determined that development of oocytes in the ovary is asynchronous, because oocytes of all stages of development are present in the ovary and the oocyte size-frequency distribution is continuous. Only when hydration occurs, a clearly size differentiated stock of oocytes becomes evident. This oocyte size-frequency distribution confirms *H. dactylopterus* as a batch-spawner, whose eggs are recruited and ovulated from the population of yolked oocytes in several batches over a protracted period during the annual spawning season.

Thus, although recent research shows that *H. dactylopterus* is a highly fecund species with a relatively long spawning period, two factors may underpin the reproductive potential of this species when fisheries are taken into account: (1) the complex reproductive strategy of this species (zygoparous species with internal fertilization and asynchronous reproductive cycles of males and females) and (2) the relationship observed between potential fecundity and the size of individuals (Muñoz et al., 2010).

1.3 Deep-sea fisheries

1.3.1 Overview of deep-sea fisheries

Generally, the deep-sea is defined as the ocean's water body beyond 200 m depth, which is the point where the continental slope begins (Merret & Haedrich, 1997). Deep-sea fisheries, however, are considered those fisheries that exploit marine organisms at depths greater than 400 - 500 m (Gordon et al., 2003, Koslow et al., 2000). Yet, the bathymetric distribution of many deep-sea species extends onto the continental shelf and large quantities of these species are caught by a number of fleets and a variety of gears. This applies especially to the juveniles of some of the species, which are distributed in relatively shallow waters and so are caught and discarded by other fisheries (ICES, 2011). The most important species targeted by deep-sea fisheries in the NE Atlantic are the ling (*Molva molva*), the blue ling (*Molva dypterygia*), the tusk (*Brosme brosme*), the greater silver smelt (*Argentina silus*), the orange roughy (*Hoplostethus atlanticus*), the roundnose grenadier (*Coryphaenoides rupestris*), the black scabbardfish (*Aphanopus carbo*), the greater forkbeard (*Phycis blennoides*), the red (black spot) sea bream (*Pagellus bogaraveo*), the Alfonsinos (*Beryx spp*.), the Greenland halibut (*Reinhardtius hippoglossoides*), the beaked redfish (*Sebastes mentella)*, the blue whiting *(Micromesistius poutassou)* and sharks like *Centroseymnus coelolepis* and *Centrophorus squamosus* (Gordon 2003; Gordon et al., 2003; ICES, 2011; FAO, 2011; ICES, 2012). Other deep-sea species of lower commercial interest are the roughhead grenadier (*Macrourus berglax*), the common Mora (*Mora moro*), the rabbit fish (*Chimaera monstrosa*), Baird's and Risso's smoothhead (*Alepocephalus bairdii* and *A. rostratus*), the wreckfish (*Polyprion americanus*), the bluemouth (*Helicolenus dactylopterus*), the silver scabbard fish (*Lepidopus* caudatus), the deep-water cardinal fish (*Epigonus telescopus*) and the deep-water red crab (*Chaceon affinis*).

Most deep-sea fisheries in the Northeast Atlantic originated as artisanal fisheries, particularly in southern Europe where the continental shelf is narrow and deep water is close to land, (e.g. in the Azores, mainland Portugal and southern Spain). In these southern regions, deep-water fishes are still landed primarily by small vessels using traditional gear (handlines or longlines). However, most deep-sea catches in the Northeast

Atlantic today stem from highly mechanized longline and trawl fisheries. The major expansion and industrialization of these fisheries started after World War II, and has subsequently been accompanied by a steady improvement in vessels and gear technology and by dedicated exploration of new fishing grounds (often subsidized by national governments) (Gordon et al., 2003, Koslow et al., 2000).

1.3.2 Impacts of deep-sea fishing

The negative impacts of deep-sea fisheries are undeniable. Although some deep-sea species are highly productive (e.g., blue whiting, *Micromesistius poutassou*), many of the exploited deep-water species exhibit clear ‘‘K-selected’’ life history characteristics: they are slow-growing, with a relatively high age of first maturity (e.g., orange roughy and the roundnose grenadier) and may not spawn every year and thus have intermittent recruitment. As a consequence, these species can be notably unproductive, highly vulnerable to overfishing, and have potentially little resilience to overexploitation (Koslow et al., 2000; FAO, 2011).

In many deep-sea trawling fisheries, a large proportion of catches (more than 50%) can consist of unpalatable species and numerous small species, including juveniles of the target species, which are usually discarded (Allain et al., 2003). The survival of these discards is unknown, but considered to be virtually zero because of fragility of these species and the effects of pressure changes during retrieval (Gordon, 2001). Therefore such fisheries tend to deplete the whole fish community biomass, which can in turn induce major changes to fish communities through removal of key predatory or forage species (e. g., Basson et al., 2001) (Koslow et al., 2000). The effects of fishing on the benthic habitat relate to the physical disturbance by the gear used. This includes the removal of physical features, reduction in complexity of habitat structure and resuspension of sediment (ICES, 2011). More attention has been paid to biogenic habitat that occurs along the slope, mainly the cold-water corals, which, in the Northeast Atlantic include the corals *Lophelia pertusa*, *Madrepora oculata*, *Solenosmilia variabilis*, *Desmophyllum cristagalli,* and *Enallopsammia rostrata*. A dense and diverse range of megafauna are associated with *Lophelia* reefs, inncluding fixed (anthipatarians, gorgonians, sponges) and mobile invertebrates (echinoderms, crustaceans). Several species of deep-water fish occur associated with corals, some in more abundance than in

surrounding non-coral areas, but the functional links between fish and coral are still to be fully elucidated. However, it is accepted that structurally complex habitats such as corals, offer a greater diversity of food and physical shelter to fish and other macrofauna. Any long-lived sessile organisms that stand proud of the seabed will be highly vulnerable to destruction by towed demersal fishing gear. There are a number of documented reports of damage to *Lophelia* reefs in various parts of the Northeast Atlantic by trawl gear where trawl scars and coral rubble have been observed (e.g. Hall-Spencer et al., 2002). Damage can also be caused on a smaller scale by static gears such as gillnets and longlines (Grehan et al., 2005). The recovery rates for damaged coral are likely to be extremely slow (Risk et al., 2002).

1.3.3 Assessment and management of deep-sea fisheries

Although some information on the fish populations and their biology pre-dates the fisheries (from as early as the 1860s and 1870s according to Gordon (2003), it was not until the late 1980s that the rapid increase in commercial exploitation started raising concerns about the sustainability of these fisheries. In the early 90s, the International Council for the Exploration of the Seas (ICES) recognized the growing importance of these new fisheries and in 1994, it created the ICES Study Group on the Biology and Assessment of Deep-Sea Fisheries Resources (SGDEEP), which is currently the Working Group on the Biology and Assessment of Deep-Sea Fisheries Resources (WGDEEP). Since its creation, this group provides annual information regarding many deep-water fisheries within the ICES area (e. g., biological and landings data of deep-sea species, assessments and management recommendations for deep-sea stocks, etc.). Other ICES groups, i.e., the North-Western Working Group (NWWG) and the Arctic Fisheries Working Group (AFWG) also provide advice for commercially important deep-sea species, such as the Greenland halibut (*Reinhardtius hippoglossoides*) and redfish (*Sebastes* spp.). In the Mediterranean Sea, assessments and management advice are provided by the Scientific Advisory Committee (SAC) of the General Fisheries Commission for the Mediterranean (GFCM). To the present, SAC provides only in a few deep-sea species (e. g., blackspot seabream – *Pagellus bogaraveo* and red shrimp – *Aristeus antennatus*).

Since then, the quality and quantity of fisheries and biological data available for assessments have improved. However, knowledge on most deepwater species still lags considerably behind that of the commercially exploited shelf-based species. Areas where our current knowledge is particularly poor are recruitment processes and their variation, stock identity, fish migration, and fish behaviour (Hammer & Zimmermann, 2005).

In the NE Atlantic (except the Baltic Sea), the North East Atlantic Fisheries Commission (NEAFC) is the competent organization for recommending measures to promote the rational exploitation of fisheries in the NEAFC area, beyond areas under national fisheries jurisdiction. The contracting parties are of NEAFC are Denmark (in respect of the Faroe Islands and Greenland), the European Union, Iceland, Norway and the Russian Federation. Besides the areas under the fisheries jurisdiction of NEAFC's Contracting Parties (i.e., national waters), three large areas of international waters exist and constitute the NEAFC Regulatory Area. Some of the recommendations for conservation and management of resources in the NEAFC regulatory area include limiting effort put into the directed fishing for deep-sea species and other measures for the protection of deep-sea species such as blue whiting, blue ling (e.g., seasonal prohibition of bottom contacting gear), orange roughy (e.g., fishing restrictions in many areas), etc. (NEAFC, 2013).

Within the European Union, deep-sea fisheries are mainly regulated according to EC regulation 2347/2002, which establishes specific access requirements (i. e. fishing permits) and associated conditions applicable to fishing for deep-sea stocks. Landings of the main deep-water species caught in ICES Subareas VI and VII are managed by EU TACs since 2003 for black scabbardfish, argentine, tusk, blue ling, ling, roundnose grenadier, orange roughy and blackspot sea bream. TACs have been also introduced for deep-water sharks and greater forkbeard. TACs are revised every second year. They were reduced at each revision (for 2005/2006, 2007/2008 and 2009/2010). Other measures have been adopted to protect deep-sea stocks, for example, reductions of fishing effort levels (e. g., Council Regulation (EC) No 27/2005) or regulations on the use of certain gears (e. g., Council Regulation (EC) No 51/2006 which banned the use of gillnets by Community vessels at depths greater than 200 m in ICES Divisions VIa,b and VIIb,c,j,k.).

The Joint Norwegian-Russian Fisheries Commission (JNRFC) provides joint management of the most important fish stocks of Norway and the Russian Federation (e.g. Greenland halibut), in the Barents Sea and the Norwegian Sea. This Commission has

dealt with issues such as the stipulation of quotas and minimum sizes for jointly managed live marine resources, regulation of mesh width in nets, use of fish sorting grids in trawlers and the introduction of satellite monitoring of fishing and transport vessels in addition to a number of other issues related to strengthening control of catches of live marine resources (JNRFC, 2013).

1.3.4 Current situation of the bluemouth in the fisheries

In the NE Atlantic, bluemouth is taken as by-catch in: a) hake fisheries by Spanish trawlers in ICES subareas VI and VII, (i. e., on the Porcupine, Rockall and Great Sole Banks) (ICES, 2011; Vázquez-Rowe et al., 2011); b) Spanish trawl fishery targets species such as hake, megrim, anglerfish, and *Nephrops* in ICES subarea VIII; c) bottom-trawl fishery at the southern part of the Portuguese continental coastal, targeting crustaceans, some on deeper grounds such as *Nephrops norvegicus* and *Aristeus antennatus* and d) the French trawl fishery, exploiting the slope of the northeast Atlantic (Gordon & Hunter, 1994, Allain et al., 2003).

In the western Mediterranean, bluemouth is a by-catch in deep-water shrimp fisheries (Sardà et al., 2004). In the Central Mediterranean, the bluemouth is caught by bottom set-nets and trawl fisheries; the former uses long-lines, trammel net and traps, and exploits mainly adult specimens (between 300 and 400mm in total length), whereas the latter catches mainly smaller specimens (between 120 and 150 mm) (Romeo et al., 2009). Whether bluemouth is retained or discarded depends on several factors. The most important are the size of the captured specimens, the commercial value of bluemouth in the market where the fleet lands the capture and factors like processing facilities on board and duration of the trips (Piñeiro et al., 2001). Significant quantities of bluemouth (mostly juveniles) are discarded from the trawl fishery around Irish waters (i. e. ICES Divisions VIb Rockall Bank, VIIc Porcupine Bank, VIIb West of Achill) (Borges et al., 2005; ICES, 2011). In Scotland, for example, bluemouth are not commercially important and they are discarded (Kelly et al., 1999), however, it is possible that the species could become more important to the Scottish fishing economy as its distributional range advances further north (Mamie et al., 2007). In ICES Subareas with longline fisheries directed at ling and tusk (ICES Subareas II, IV, V, VI, VII and XIV), bluemouth is generally discarded (Kelly et al., 1999).

According to FAO (2011), bluemouth caught in the NE Atlantic and Mediterranean are landed mainly as by-catch in four countries, Spain, Portugal, France and the UK (Figure 1-2 and 1-3). From these, Spain is by far the country with the highest bluemouth landings. From Spanish landings, the largest proportion of bluemouth is landed in four ports located in Galicia: Burela, Celeiro, A Coruña and Vigo (Xunta de Galicia, 2011). The landings in this area are shown in Figure 1-4. In other regions in Spain, this species is also appreciated. For example, in Catalonia, bluemouth is the most commercial scorpionfish species with an important economic value, as it is shown by the fact that in 2003 a total of 48,119 kg were landed and sold in Catalonian fish markets (Ribas et al., 2006)

To the present, there are no specific management measures for the bluemouth. According to ICES (2011), no quotas are set for bluemouth in EC waters or in the NEAFC Regulatory Area. Moreover, it is not included in Appendix I of Council Regulation (EC) No 2347/2002, meaning that vessels are not required to hold a Deep-water Fishing Permit in order to land bluemouth; they are therefore not necessarily affected by EC regulations governing deep-water fishing effort. However, within the EU, data on length structure of bluemouth landings and discards must be collected according to the Data Collection Framework (DCF) that was established in 2008 for the collection, management and use of data in the fisheries sector and support for scientific advice regarding the Common Fisheries Policy (Council Regulation (EC) No 199/2008 and EC 2010/93/EU). In these regulations, the bluemouth is included in Group 2, which designates “Other internationally regulated species and major non-internationally regulated by-catch species”.

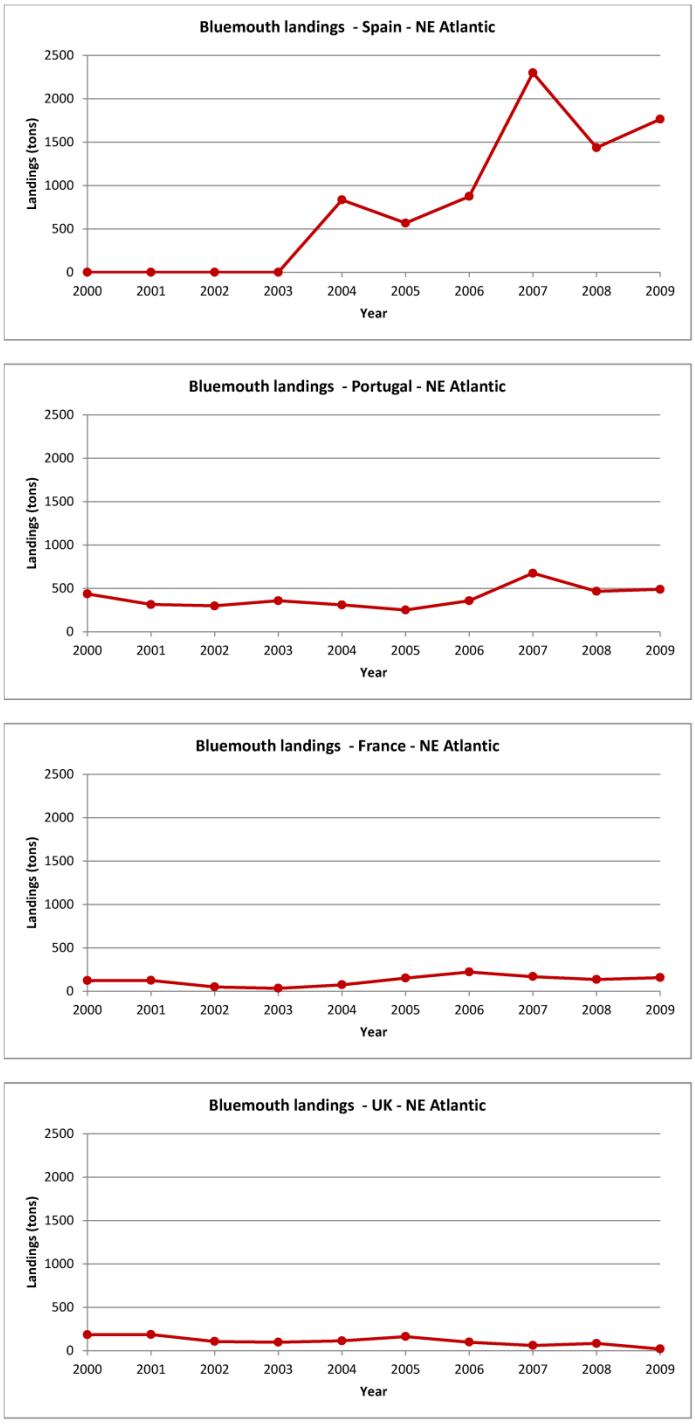


Figure 1-2. Bluemouth landings from NE Atlantic fisheries (Source of the data: FAO (2011). Data for 2010 were not available at the time of consultation.

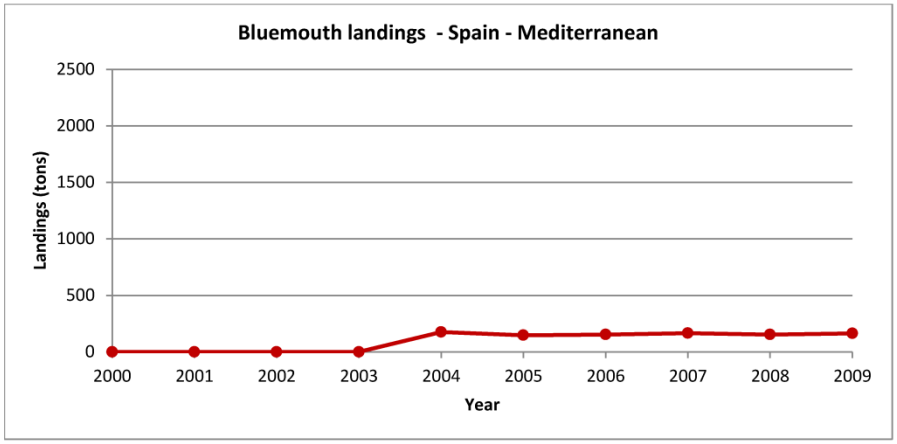


Figure 1-3. Bluemouth landings from Mediterranean fisheries (Source of the data: FAO (2011). Data for 2010 were not available at the time of consultation.

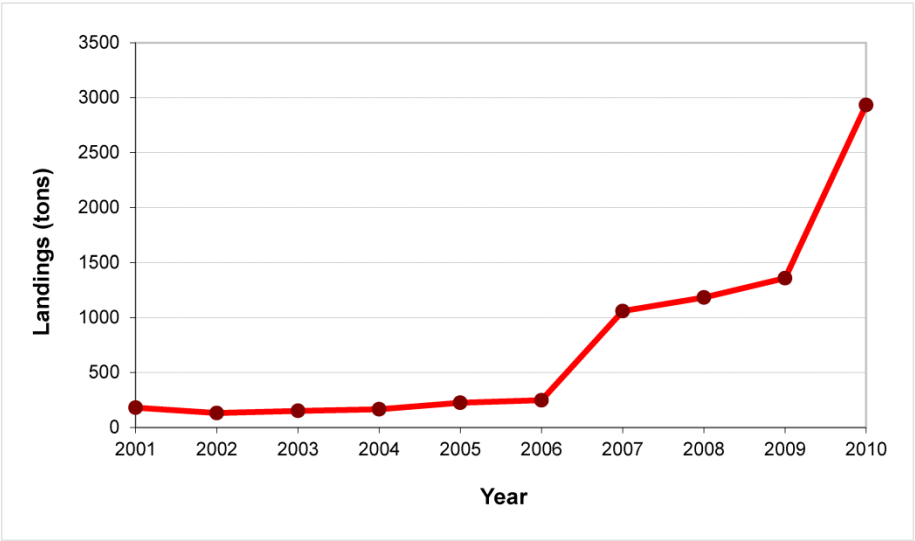


Figure 1-4. Bluemouth landings in Galicia. Source: www.pescadegalicia.com – Plataforma tecnolóxica da pesca - Xunta de Galicia - Consellería do Mar.

1. 4 Population structure of marine fish and management

The analysis of a species' population structure is of primary importance in developing an optimal strategy for its efficient management. In this way, the systematic organization of populations is taken into account; their historically formed hereditary heterogeneity is preserved; and the autoregulation mechanisms are maintained to enable efficient adaptation of populations to varying environmental conditions (Coyle, 1998).

Many organizations take a categorical species approach to management which fails to recognize variation within a species. If managers are to include intraspecific variation in their decision-making considerations, they will need information on the biological differences between discrete local groups of species and they will need to understand the genetic and ecological processes that influence discreteness (Maclean & Evans, 1981 in Coyle, 1998). In fisheries, each of these groups is usually called a stock or population. There are several definitions for the term stock, but one of the most accepted is the one by Ihssen et al. (1981), who defined a stock as an intraspecific group of randomly mating individuals (fish) with temporal and spatial stability (Waldman, 2005).

1.4.1 Stock identification

Stock identification is an interdisciplinary field that involves the recognition of self-sustaining components within natural fish populations (i. e., stocks) (Cadrin et al., 2005). Most commonly, a stock is considered equivalent to a population, at least partly reproductively isolated from other populations, and genetically different from them as a result of adaptation to its local environment (Swain et al., 2005).

Identification of stocks that are isolated from other stocks and maintain homogeneous vital rates is essential for management of living marine resources, whether for conservation biology or fishery management (Eagle et al., 2008). Although these two fields have different objectives, they both involve inferences about how populations respond to perturbations or restoration efforts (Cadrin, 2010). Fishery management is usually focused on maintaining maximum sustainable yield (MSY) and its determination is most appropriate for demographically independent units that are essentially isolated from other groups on ecological time scales (i.e., years to decades). Conservation biology

considers both long-term and short-term dynamics to determine the appropriate stock units to manage. Evaluating the risk of extinction for a species involves the recognition of "evolutionarily significant units" that are reproductively isolated over geologic time scales (i.e., millennia) and have developed unique adaptations (Cadrin, 2010).

The process to stock identification involves three basic steps: (1) a hypothesis is mounted based on whatever evidence is available of the existence of putative stocks (versus the null hypothesis of a single stock), (2) a well-conceived survey is undertaken to obtain representative samples and (3) an efficient discrimination technique is applied to the collections (Kutkuhn 1981; Waldman, 2005).

To demarcate the spatial distribution of a stock unit, it is assumed that fish from two sampling locations belong to the same stock unit if there was not a location in between that seemed to belong to a different stock unit (Murta et al., 2008). In this case, if similarity between individuals from distant locations exists, it 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.

The methods used to identify fish stock use characters or traits that can be purely genetic, purely environmental or a combination of both (Swain et al., 2005). These methods include the use of meristic and morphometric characters (e. g., Cadrin, 2005 and Waldman, 2005), life-history parameters (Begg, 2005), genetics (e. g., Waldman, 2005), or environmental markers such as otolith microchemistry (Swan et al., 2006), parasite tags (e. g., MacKenzie & Abaunza, 2005) or fatty acid profiles (Grahl-Nielsen, 2005). However, the strongest inferences on stock structure are drawn from a suite of complementary techniques that cover multiple aspects of the biology of a fish species (i.e., a holistic approach) (Begg & Waldman 1999). Thus, by using this approach, we can maximize the likelihood of correctly defining stocks (Hohn, 1997). The holistic approach can be applied in three different ways: a) by collating all available stock identification information previously known into a single review to infer stock structure; b) by using two or more different stock identification techniques in a single study on a range of samples; or ideally, c) by using a wide range of stock identification techniques on the same samples for more direct comparisons.

Genotypic approaches have been regarded as the avatar of stock identification, and they do offer many advantages to alternative techniques, including permanence across an

individual's life cycle, freedom from environmental modulation, often large substrates of variation and nonfatal sampling (Waldman, 2005 and references therein).

Phenotypic stocks are groups of fish characterized by phenotypic differences that may be entirely environmentally induced (Booke, 1981). The phenotypic stock definition is less conservative than the genetic stock definition because it allows for some mixing among stocks, but partial isolation is enough that geographic differences persist (Cadrin, 2005). Moreover, phenotypic differences between groups in the wild may reflect genetic differentiation, environmental differences, or a combination of the two (Thompson, 1991 in Swain et al, 2005).

The importance of delineating "phenotypic" stocks is being increasingly emphasized (Swain et al., 2005), because any detectable differences among stocks are both valid and useful (Waldman, 2005), and meristic, morphometric and life history characters are clearly appropriate for delineating phenotypic stocks (Swain et al., 2005). Meristic characters are the numbers of discrete, serially repeated, countable characters such as vertebrae, gill rakers and fin rays; morphometric characters describe aspects of body shape such as its length, width, etc.; and life history traits describe population parameters such as abundance, growth, reproduction and mortality (Begg, 2005; Swain et al., 2005). The main advantage in using these traits in studies of population structure is that they are often related to fitness and respond to selection, and thus may reveal genetic differentiation not evident in neutral genetic traits. Their main disadvantage results from phenotypic plasticity, i.e., the ability of a genotype to produce different phenotypes across an environmental gradient (Swain et al. 2005 and references therein).

There have been only few studies on the stock structure of deepwater fish species in the ICES area, and for assessment purposes stock units have been defined on the basis of current knowledge of species distribution and similarity of observed catch-rate trends between ICES areas (ICES, 1998, 2000). Thus, stock units are currently individual or groups of ICES subareas or occasionally ICES divisions. This is not ideal because the ICES statistical areas are devised for the fishery on the continental shelf and are, in many instances, inappropriate for deepwater fisheries (Hammer & Zimmerman, 2005).

In 2007, in addition to updating fishery information, the ICES Working Group on the Biology and Assessment of Deep-sea Fisheries Resources (WGDEEP) was required to hold a three day workshop on stock discrimination. The group evaluated techniques that could be used for stock discrimination in deep-water species and examined the

available information to identify stock units in the ICES area. Information for most species was not sufficient to discriminate stocks and the WG recommended that there was no reason to change from the current practice in ICES. However, for tusk there was genetic evidence available that allowed five separate stock units to be identified. WGDEEP recommended that these be adopted for future assessments.

1.4.2 Geometric morphometrics and the study of marine populations

Morphometrics is a subfield of statistics that deals with the quantitative analysis of shape. The term shape is used to denote the geometric properties of an object that are independent of the object's overall size, position, and orientation (Mitteroecker & Gunz, 2009).

At the present, there are two main approaches to analyze the morphological variation of organisms (Adams & Rohlf, 2004; Mitteroecker & Gunz, 2009; Strauss, 2010). The first one, referred to as traditional morphometrics, consists of applying multivariate statistical analyses to sets of morphological variables such as linear distance measurements, ratios and angles. The second and more recent approach is known as geometric morphometrics. In geometric morphometrics, shape information is characterized thorough a series of landmarks (or outlines) in such a way that the geometry of the morphological structures of interest is preserved throughout the analyses (Adams & Rohlf, 2004).

Geometric morphometrics is based directly on the digitized x,y coordinate positions of landmarks, points representing the spatial positions of putatively homologous structures in two or three dimensions (Bookstein, 1991; Dryden & Mardia, 1998; Rohlf, 1993; Strauss, 2010). Once landmark coordinates have been obtained for a set of forms, they must be standardized to be directly comparable. For this purpose, the Procrustes method (Bookstein, 1996; Small, 1996; Dryden & Mardia, 1998) is the most widespread and best understood in its mathematical and statistical properties (Mitteroecker & Gunz, 2009). The geometric morphometrics methodology is explained in detail in Chapter 2: Material and Methods.

Morphometric analysis is a valuable tool for scientists involved in studying marine populations, because it helps to identify intraspecific groups of animals that can be effectively monitored and conserved. Among a wide variety of methodological approaches, geographic patterns in morphology provide a unique perspective on spatial population structure (Cadrin, 2010). Moreover, the recent development of image processing techniques has improved traditional methods of morphometric stock identification by facilitating better data collection, more effective descriptions of shape, and new analytical tools (Cadrin & Friedland, 1999). Along with that, conventions for sampling, analysis and interpretation have been developed to promote representative and meaningful conclusions, and many case studies have demonstrated the value of morphometric analysis for stock identification and resource conservation (Cadrin, 2010).

Morphological variation is phenotypic (i.e., it is influenced by both genetic composition and environmental factors), and heritability of morphometric characters is generally low to moderate (Swain et al., 2005). Therefore, temporal stability in geographic variation of morphology is essential for stock identification applications, so that stocks are not determined on the basis of ephemeral differences in the environment. Despite the influence of environmental factors on morphological variation, patterns of variation can indicate groups that are isolated enough to maintain phenotypic differences. Morphometric patterns are also often associated with geographic differences in growth, maturity, or mortality which are critical to population dynamics. Adaptive significance of morphological features can add powerful interpretability to patterns of variation. For example, body form, fin size and fin location are adaptive for movement and maneuverability of fishes (Webb, 1984) and cetaceans (Fish, 1998); position of the mouth and head shape are associated with trophic ecology (e.g., Costa & Cataudella, 2007); abdomen size reflects energetic investment, feeding and spawning condition (e.g., Armstrong & Cadrin, 2001); body armor (e.g., scutes, spines) can indicate different predatory environments (e.g., Walker, 1996); and secondary sex characters (e.g., dorsal humps and fin size of fishes; chelipeds of crustaceans) can indicate behavioral differences and size at maturity (Cadrin, 2000).

Morphometric analysis offers a unique perspective to the investigation of population structure (Cadrin, 2010). Patterns in morphology have been used to identify and discriminate stocks for nearly a century (Teissier, 1936). More recently, morphometric patterns are considered in the context of information on genetic variation

and movement patterns for an interdisciplinary analysis of population structure. Moreover, landmark methods have been applied to finfish to study ontogenetic changes (seabass *Dicentrarchus labrax*, Perciformes, Loy et al., 1996; sea bream *Diplodus vulgaris*, Sparidae, Loy et al., 1998; and catfish *Callichthys* spp., Callichthyidae, Reis et al., 1998) and geographic variation in populations of grey mullet - *Mugil cephalus*, (Corti & Crossetti, 1996); sticklebacks - *Gasterosteus aculeatus*, Gasterosteidae; (Walker, 1996,1997), the silverside - *Atherinops affinis* (O'Reilly & Horn, 2004), sardine (Silva, 2003), horse mackerel (Murta et al., 2008) and scorpaenids like *Sebastes* spp. (Valentin et al., 2002).

1.5 Aims and objectives

As it is now widely recognized, information on a species' population structure is of primary importance in developing an optimal strategy for its efficient management (Coyle, 1998). Thus, the overall goal of this study was to provide baseline information on the population structure of bluemouth, *Helicolenus dactylopterus*, around the Iberian Peninsula. Moreover, this thesis provides the first comparative study of bluemouth populations in the Northeast Atlantic and the Mediterranean.

To characterize the population structure of bluemouth in terms of growth and stock components, a morphological approach was followed, because the analysis of morphological characters (i. e., meristic and morphometric characters) has proved useful for characterizing populations of a variety of marine fish (Swain et al., 2005).

The **first specific objective** of this study was to study the ontogenetic allometry of the bluemouth in the Northeast Atlantic and Mediterranean using geometric morphometrics. In this part of the study, the shape changes that occur during the growth of bluemouth were characterized to better understand its biology and ecology. The study of allometry was also used to determine the best method to correct for allometry for the bluemouth population structure dataset.

The **second objective** was to identify bluemouth phenotypic stocks in the Northeast Atlantic and western Mediterranean based on geometric morphometrics and

meristics. To achieve this, bluemouth from 9 areas were compared. In the NE Atlantic, specimens were sampled from the coasts off Galicia, the Cantabrian Sea, the Gulf of Cadiz, Portugal and the Porcupine Bank (Irish continental margin). Specimens from the Mediterranean were caught in the Alboran Sea, off-shore Alicante (south-west of the Balearic Sea), the Catalonian coast and in Italian waters (Sicily). The samples from the Porcupine Bank and Sicily 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.

In this **Chapter 1** an overview of the current knowledge about the biology and ecology of the bluemouth was provided. Also, the fisheries where this species is caught were described.

Chapter 2 describes the materials and methods used in this thesis. This includes a description of the sampling locations and the bluemouth samples used to study the population structure and growth of this species. Also, the protocol for the acquisition of morphometric and meristic data and the statistical analysis that were carried out are described in detail.

Chapter 3 refers to the study of ontogenetic allometry of bluemouth populations. Here, the allometric shape trajectories for bluemouth from the different study areas in the NE Atlantic and western Mediterranean were determined and the variation of growth patterns in the different environments of the study areas was examined. Growth patterns of males and females were also analyzed to determine if sexual dimorphism existed. Finally, in order to determine the best way to remove the effects of allometric size on the shape variables, a comparison of the results yielded by several methods commonly used for size-correction was done.

In **Chapter 4** deals with the identification of phenotypic stocks of *H. dactylopterus* around the Iberian Peninsula, using landmark-based geometric morphometric techniques to assess body shape variation. Body shape differences between males and females within each study area were also analyzed to determine if sexual

dimorphism existed. In addition, meristic characters were analyzed to complement the information provided by the morphometric analysis.

In **Chapter 5**, a general discussion of the results is presented. Methodological caveats and advantages are discussed and recommendations regarding the use of geometric morphometrics for studying fish populations are given. The population structure of bluemouth, in the context of stock identification, is defined and the implication for fisheries management and conservation is discussed.

Chapter 2
Material and Methods

2 Material and methods

In this chapter, the bluemouth samples and locations used to study the population structure and growth of this species are described first. Due to the different nature of morphometric and meristic data, the protocol for the acquisition of each type of data and the statistical analysis performed on them are separated into two different sections: *Morphometrics* and *Meristics*.

2.1 Specimens and sampling locations

To study the population structure of bluemouth around the Iberian Peninsula, a total of 1294 specimens were caught in the NE Atlantic and the western Mediterranean (Table 2-1 and Figures 2-1 and 2-2). Two reference areas were included 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: the Porcupine Bank in the NE Atlantic and Sicily (Italy) in the Central Mediterranean. 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 Finisterre), 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). Galicia and the Cantabrian Sea ecosystems are considered to be divided by Cape Estaca de Bares, which is described as a biogeographic limit and a larval retention area of mesoscale hydrographic anomalies, i.e. anticyclonic eddies (Sánchez & Gil, 2000). The other locations in the NE Atlantic are also interesting in terms of their oceanographic characteristics. 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 and the water mass circulation in its continental shelf provides warm and biologically productive waters that are markedly suitable for the reproduction of many fish species (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. The Nazaré Canyon (latitude 39° 35' N, longitude 9°

25' W) cuts the full width of the shelf and slope, extending from shallow water less than 1 km off the coastline to a depth of 5000 m at 210 km offshore (De Stigter et al., 2007; Tyler et al., 2009). Moreover, some studies have suggested a latitudinal boundary around this canyon that affects the distribution and characteristics of demersal fish assemblages (Gomes et al. 2001; Sousa et al. 2005). The Porcupine Bank is a submarine shelf break bank that is partly attached to the Irish continental shelf and it has a high productivity due to closed circulation patterns around the bank that promote the retention of organic matter over it. It is also worth noting that it hosts an important number of deep cold-water ecosystems (White 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 Catalonian coast (Figures 2-1 and 2-2). 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 in the western Mediterranean: 1) the south-western basin (Alboran Sea), 2) the north-western basin (Catalonian coast) and 3) the transition zone, which goes from Cape Palos to Sagunto (Alicante sector). Bluemouth samples from Sicily (Central Mediterranean) were obtained from commercial trawlers near Mazara del Vallo, in collaboration with the Italian Institute of Coastal and Marine Environment (IAMC-CNR). The Strait of Sicily divides the Mediterranean into western and eastern sub-basins. As described by Gasparini et al. (2005), the Strait of Sicily is a topographically complex region comprising two sill systems separated by an internal deep basin: the eastern sill with a maximum depth of about 540 m (which connects the Strait with the Ionian Basin), the central basin with deep trenches more than 1700 m deep, and the western sill, composed of two narrow passages, which have a maximum depth of 530 m. The width of the strait, wide at the surface, reduces with depth, becoming very narrow at the sills. Dynamically, the strait is a two layer system: the upper layer (about 200 m thick) is composed of AW and flows eastward; the deep layer, composed of Levantine Intermediate Water (LIW), flows in the opposite direction, from east to west (Gasparini *op. cit.*).

Most of the samplings were carried out in bottom trawl research surveys in collaboration with the Instituto Español de Oceanografía (IEO). In these surveys, the samplings were not specifically designed for the capture of bluemouth, but were aimed at estimating the abundance of demersal resources in the study area on sea bottoms ranging

from 100 to 600 m. Fishing surveys were uniformly performed on five depth ranges (100– 200; 201–300; 301–400; 401–500; 501–600 m). The depth range in which bluemouth specimens were captured in each survey is shown in Table 2-1.

Samples from Galicia and the Cantabrian Sea were obtained during the 2006 and 2007 DEMERSALES surveys. Samples from the Porcupine Bank were obtained in the 2008 PORCUPINE survey and samples from the Gulf of Cadiz in the 2009 ARSA survey. Bluemouth samples from the Mediterranean were obtained in the 2007 MEDITS survey. Only bluemouth from Peniche (Portugal) and from Sicily (Central Mediterranean) were obtained from regional commercial vessels (Table 2-1).

In our sampling design, we set 60 specimens per area as the minimum number necessary to carry out the morphological analysis. However, specimens from Sicily were not easy to obtain and the sample size was lower (48 specimens).

In most of the areas, a wide size range of bluemouth specimens was obtained, from juveniles to adults. The exceptions are Portugal and Sicily, where the sample consisted of adult specimens only. The size and sex distribution of each of the samples is detailed in Table 2-2 and Figure 2-3.

The protocol used to obtain adequate samples for the morphometric study was adapted from that developed by Garabana (2005) for the morphometric study of redfish (*Sebastes* spp.). A description of the sampling process is presented in the next sections.

Table 2-1. Total number of bluemouth specimens sampled in each location. Size is expressed as total length (TL) in cm.

	Study area	N	Size range (TL, cm)	Mean ± sd (TL, cm)	Dates	Depth range (m)	Source
NE Atlantic	Porcupine Bank	184	3.5 – 37.0	25.89±6.73	Sept./Oct. 2008	225 – 739	Porcupine 2008
	Cantabrian Sea (2007)	119	10.6 – 39.7	20.12±5.40	Sept./ Oct. 2007	117 –585	Demersales 2007
	Cantabrian Sea (2006)	50	9.6 – 33.9	18.73±6.69	Sept./ Oct. 2006	120 – 500	Demersales 2006
	Galicia (2007)	191	5.4 – 36.2	16.76±4.68	Sept./ Oct. 2007	112 – 696	Demersales 2007
	Galicia (2006)	119	4.8 – 40.5	18.96±7.68	Sept./ Oct. 2006	120 – 650	Demersales 2006
	Portugal	60	21.6 – 28.5	23.89±1.47	Feb. 2010	–	Commercial vessels
	Gulf of Cadiz	75	6.3 – 38.0	22.46±6.49	March 2009	119 – 752	ARSA 2009
Mediterranean Sea							
	Alboran Sea	239	7.4 – 34.6	18.84±6.13	June 2007	150 – 744	Medits 2007
	Alicante	135	3.6 – 29.3	14.35±4.41	June 2007	117 – 590	Medits 2007
	Catalonia	74	4.8 – 21.4	11.60±3.33	June 2007	131 – 574	Medits 2007
	Sicily	48	16.1 – 262	19.81±2.28	Oct. 2009	–	Commercial vessels

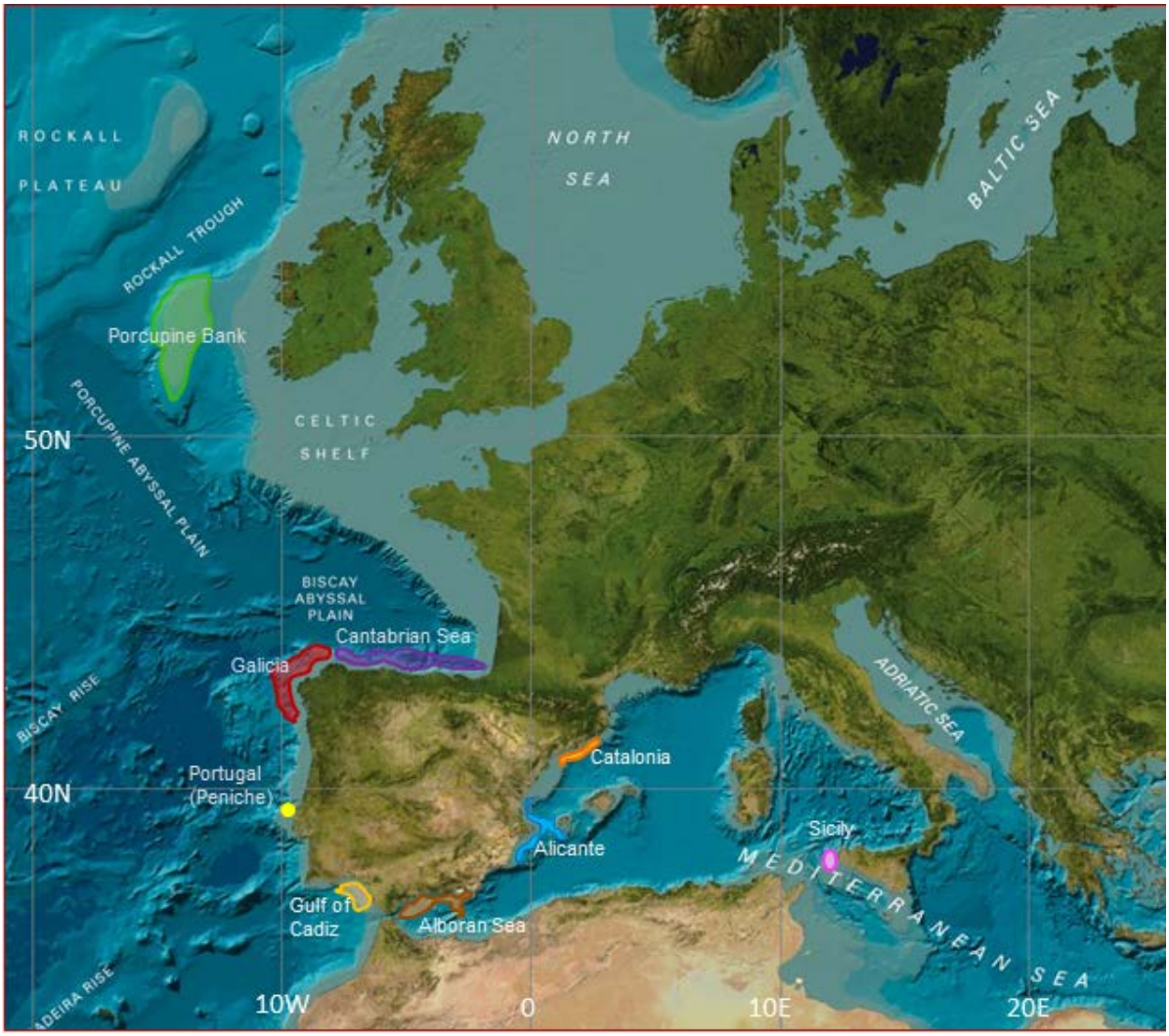


Fig. 2-1. Map of the study area with the sampling areas (shown in yellow) in the Northeast Atlantic and Mediterranean.

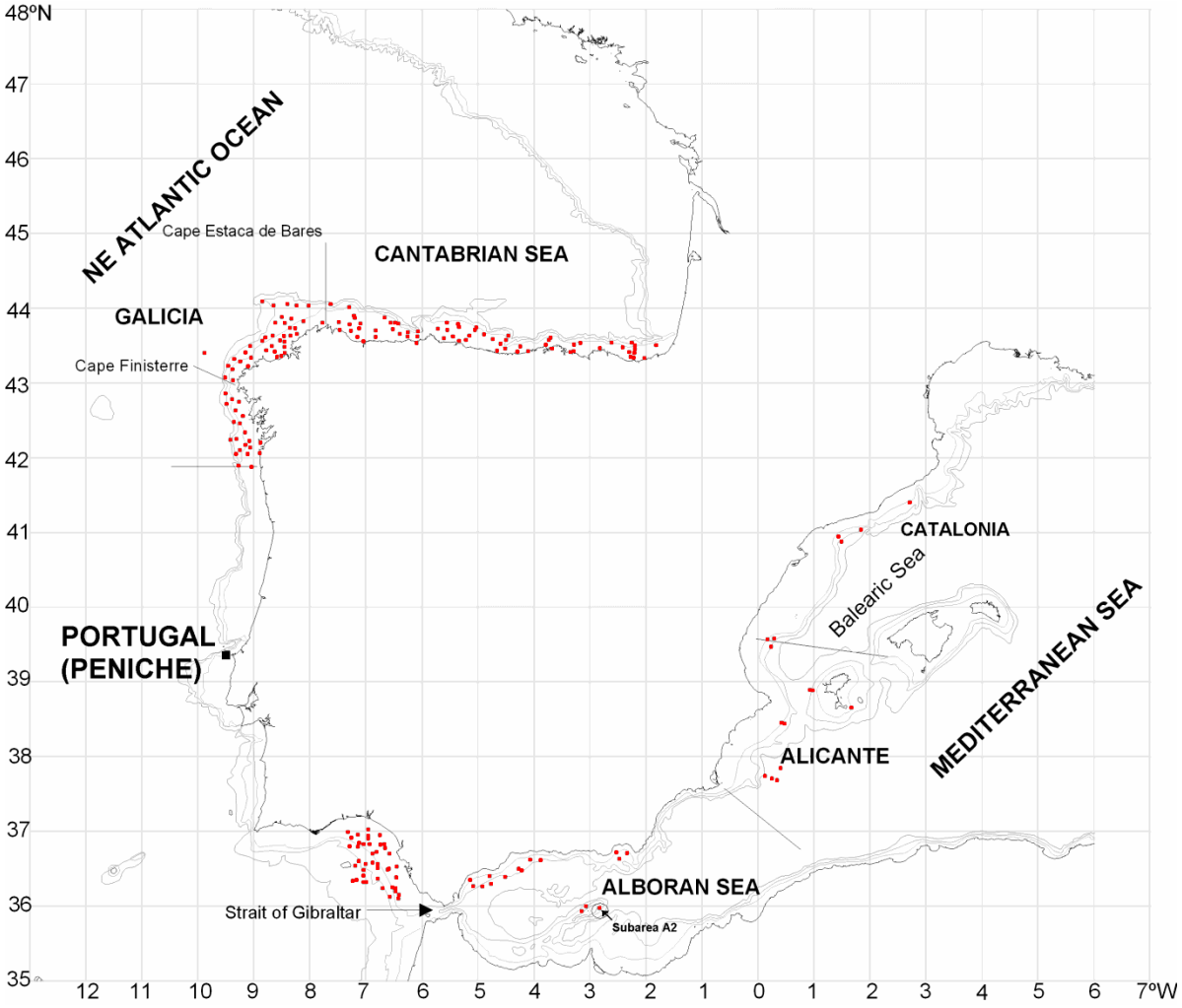


Fig. 2-2. Detailed map of the sampling locations around the Iberian Peninsula. Red small squares indicate hauls were bluemouth specimens were caught during research surveys.

Table 2-2. Number of bluemouth specimens by sex and length class caught in each of the (a) NE Atlantic and (b) Mediterranean locations. Size is expressed as total length (TL) in cm.

(a)							
NE Atlantic	**Porcupine**	**Cant. 07**	**Cant. 06**	**Gal. 07**	**Gal. 06**	**Portugal**	**Cadiz**
Sex							
Unsexed/ undifferentiated							
0 – 5	**2**	0	**0**	0	**1**	0	0
5 – 10	**1**	0	**1**	**15**	**2**	0	**3**
10 – 15	**6**	0	**15**	**12**	**43**	0	**2**
15 – 20	0	**7**	**17**	**2**	**28**	0	**1**
20 – 25	0	0	**5**	0	**19**	0	**2**
25 – 30	0	0	**9**	0	**12**	0	**1**
30 – 35	0	0	**3**	0	**9**	0	0
35 – 40	0	0	0	0	**4**	0	0
> 40	0	0	0	0	**1**	0	0
N	**9**	**7**	**50**	**29**	**119**	**0**	**9**
Males							
0 – 5	0	0	0	0	0	0	0
5 – 10	0	**0**	0	0	0	0	0
10 – 15	**4**	**7**	0	**13**	0	0	0
15 – 20	**8**	**18**	0	**32**	0	**0**	**4**
20 – 25	**12**	**15**	0	**26**	0	**18**	**9**
25 – 30	**20**	**5**	0	**3**	0	**8**	**11**
30 – 35	**58**	**2**	0	0	0	0	**7**
35 – 40	**6**	**1**	0	**1**	0	0	0
> 40	0	0	0	0	0	0	0
N	**108**	**48**	**0**	**75**	**0**	**26**	**31**
Females							
0 – 5	0	0	0	0	0	0	0
5 – 10	0	0	0	**0**	0	0	**1**
10 – 15	**2**	**9**	0	**22**	0	0	**4**
15 – 20	**14**	**32**	0	**48**	0	0	**6**
20 – 25	**25**	**14**	0	**15**	0	**29**	**15**
25 – 30	**15**	**5**	0	**2**	0	**5**	**7**
30 – 35	**8**	**2**	0	0	0	0	**1**
35 – 40	**3**	**2**	0	0	0	0	**1**
> 40	0	0	0	0	0	0	0
N	**67**	**64**	**0**	**87**	**0**	**34**	**35**
Total N	**184**	**119**	**50**	**191**	**119**	**60**	**75**

Table 2- 2 (continued) Number of bluemouth specimens by sex and length class caught in each of the (a) NE Atlantic and (b) Mediterranean locations. Size is expressed as total length (TL)in cm.

b) **Mediterranean Sea**	**Alboran Sea**	**Alicante**	**Catalonia**	**Sicily**
Sex				
Unsexed/ Undifferentiated				
0 – 5	0	**1**	**1**	0
5 – 10	**24**	**19**	**35**	0
10 – 15	**28**	**52**	**23**	0
15 – 20	**18**	**1**	0	0
20 – 25	**2**	0	0	0
25 – 30	0	0	0	0
30 – 35	0	0	0	0
35 – 40	0	0	0	0
>40	0	0	0	0
N	**69**	**35**	**59**	0
Males				
0 – 5	0	0	0	0
5 – 10	0	0	0	0
10 – 15	**6**	**3**	**1**	0
15 – 20	**20**	**15**	**3**	**14**
20 – 25	**29**	**6**	**2**	**14**
25 – 30	**32**	**3**	0	**1**
30 – 35	**2**	0	0	0
35 – 40	0	0	0	0
>40	0	0	0	0
N	**89**	**73**	**6**	**29**
Females				
0 – 5	0	0	0	0
5 – 10	0	0	0	0
10 – 15	0	**9**	**3**	0
15 – 20	**7**	**20**	**6**	**14**
20 – 25	**20**	**5**	0	**5**
25 – 30	**44**	**1**	0	0
30 – 35	**7**	0	0	0
35 – 40	0	0	0	0
>40	0	0	0	0
N	**78**	**35**	**9**	**19**
Total N	**239**	**35**	**74**	**48**

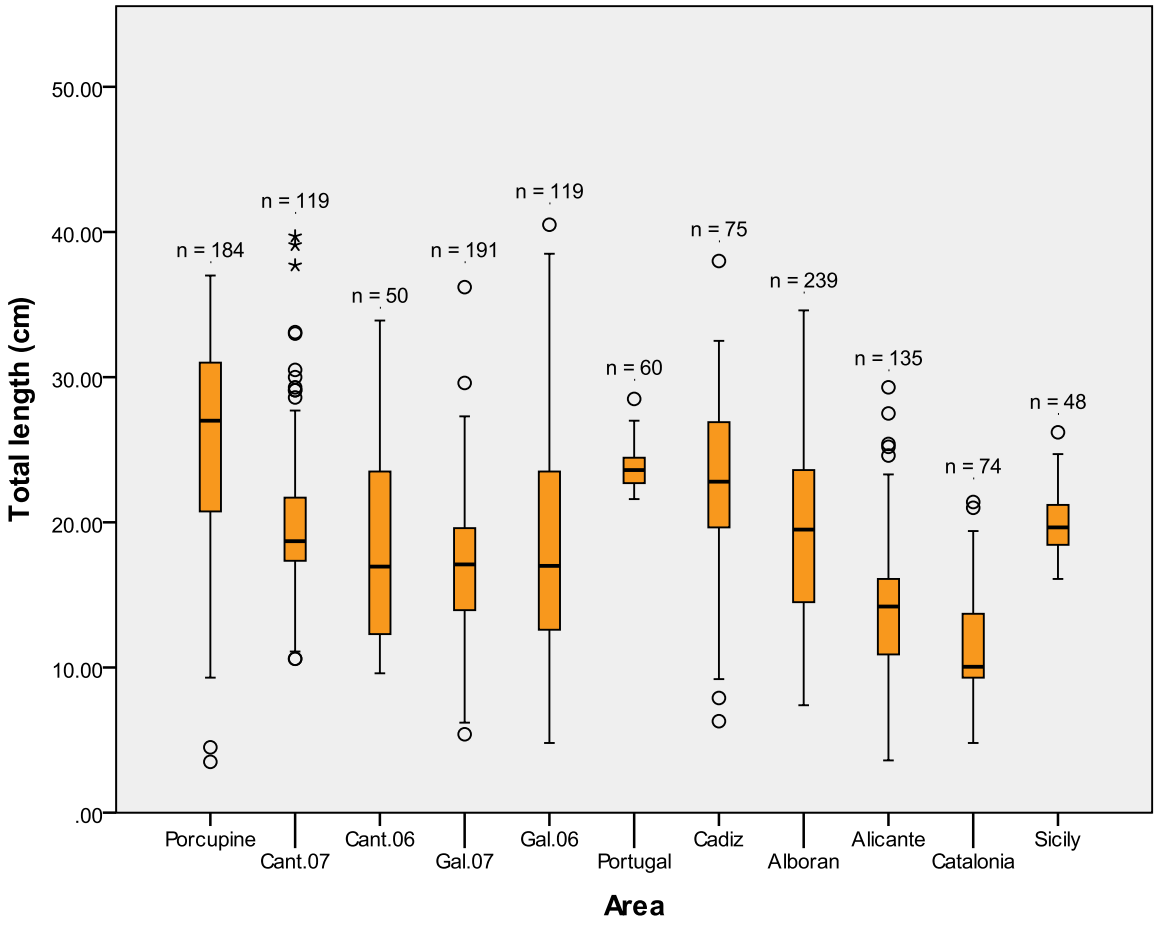


Figure 2-3 Size composition of the bluemouth samples in each location.

2.2 Selection and processing of samples

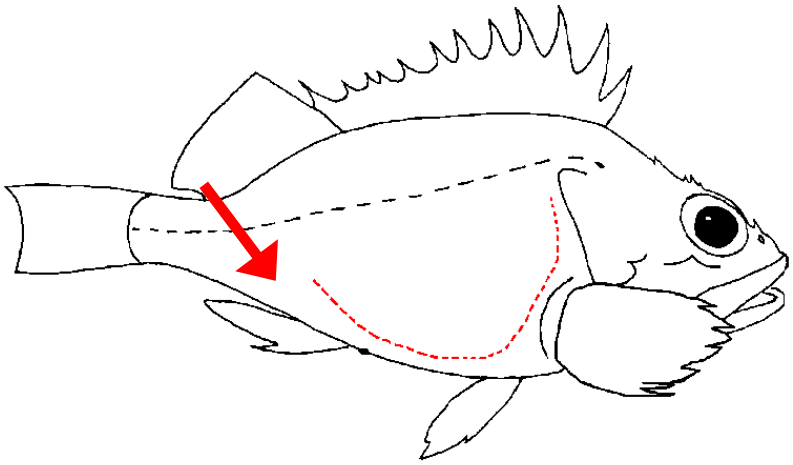
The morphometric and meristic study required that bluemouth specimens met two basic criteria: the fish had to be intact (no damage to the body and fins) and with no apparent deformations. In addition, it was established in the protocol that specimens that were to be processed on-board (i.e., sexed and/or sampled for feeding studies) should be eviscerated through an opening on the right side of the fish, so the left side remained intact for the images (Fig. 2-4a). The first samples obtained for this study were bluemouth caught in the 2006 DEMERSALES survey. However, in 2006, the sampling protocol was still being developed. Thus, around 55% of the specimens in this survey were eviscerated with a cut on the left side of the fish, instead of the right side that was later established in

the morphometric protocol. This issue is further discussed in the section *Bilateral symmetry* below.

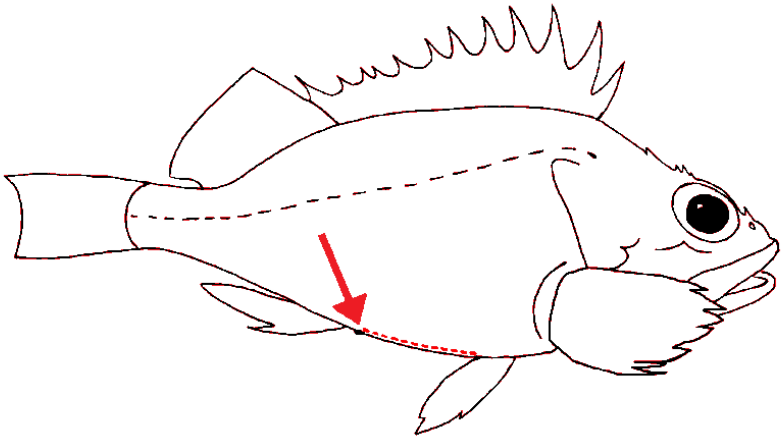
Around 90% of the bluemouth specimens that were caught in the 2007 DEMERSALES survey were processed onboard and were eviscerated through an opening on the right side of the fish (as in Fig. 2-4a). The bluemouth specimens from the 2007 MEDITS were cut by doing a small incision in the abdominal region to observe the gonads and determine the sex of the fish (Fig. 2-4b). These specimens were eviscerated later at the laboratory. The rest of the bluemouth specimens caught during the 2007 DEMERSALES survey and those from the 2008 PORCUPINE, 2009 ARSA surveys and from commercial surveys were stored with the body intact and were eviscerated later in the laboratory using the method established in the morphometric protocol (Fig. 2-4a). All specimens were immediately placed in plastic bags (to avoid desiccation) and stored in a horizontal position (to avoid deformations) in the freezer of the vessel. The samples were transported to the facilities at the IIM-CSIC and stored in the freezer at -20ºC until the time of the analysis.

At the laboratory, total length (TL) and weight were recorded and each fish was labeled with a specific identification code. Once the fish were thawed, specimens that had not been eviscerated on-board were eviscerated (as described in the previous section). This allowed avoiding the influence of the gonad size or stomach fullness on the shape of the fish.

The sex of the specimens was determined by macroscopic examination of the gonads. For the specimens in this study, fish were classified as male, female or indeterminate. This last class included the sexually undifferentiated specimens as well as a small number individuals which could not be sexed for various reasons (e. g., the gonads were damaged). In the case of the specimens from the Cantabrian Sea and Galicia 2006, no records regarding the sex of the specimens were available because the majority of individuals were eviscerated on-board for feeding studies not related to this project.
Then, pictures of each specimen were obtained for the morphometric analysis (see section *Morphometrics* below). Meristic characters were recorded after the photograph of the fish was taken (see section *Meristics*).



a)

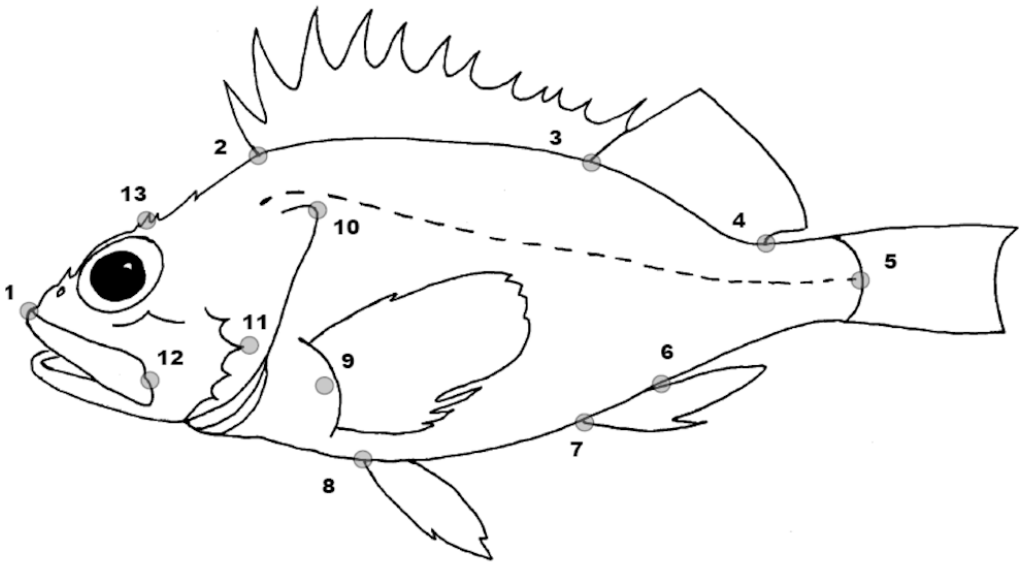


b)

Figure 2-4. Scheme showing the procedure to eviscerate bluemouth specimens. A) Fish were eviscerated through a small opening on the right side of the fish, so the left side remained intact for the images. B) Fish from the 2007 MEDITS survey were eviscerated through a small incision from the anus to the ventral fin.

2.3 Morphometrics

To describe body shape of bluemouth specimens, 13 landmarks were defined (Figure 2-5) based on previous work by Garabana (2005) on similar species (*Sebastes* spp.). Biologically, landmarks are defined as discrete, homologous anatomical loci; mathematically, landmarks are points of correspondence, matching within and between populations (Zelditch et al. 2004). To ensure an accurate localization of the selected points, black-headed entomological pins were placed on each landmark (Figure 2-6). Once the landmarks were located, each fish was placed on its left side on a white polystyrene board with a ruler with 1-cm gradations. A photograph was taken with a digital camera Nikon D1X using a focal length of 35 mm to avoid optic distortions of the images (see Garabana, 2005 for the details). The *x*,*y* coordinates of the landmarks were recorded using the TpsDig software version 2.10 (Rohlf, 2006) on the digital images.



Landmark	Description
1	Marks the snout, i. e., the tip (most distal part) of the upper jaw
2	Base of the first dorsal spine
3	Point between the first and second dorsal fins
4	Marks the end of the second dorsal fin, at the base of the last soft ray
5	End of the hypurals, mid-point
6	Posterior end of the anal fin
7	Base of the first spine of the anal fin
8	Base of the first ray of the ventral fin
9	Mid-point of the insertion of the pectoral fin
10	Posterior limit of the operculum
11	Tip of the second preopercular spine
12	Mid-point of the posterior end of the upper jaw
13	Second supraocular spine

Figure 2-5. Scheme showing the location of the 13 landmarks used in the analysis.

Figure 2-6. Image of a bluemouth specimen with the 13 landmarks marked with black-headed entomological pins, ready to be digitized.

2.3.1 Statistical analysis of morphometric data

Once digitized, each specimen was represented as a configuration of landmarks, called a configuration matrix. A configuration matrix is a $K \times M$ matrix of Cartesian coordinates that describes a particular set of K landmarks in M dimensions (Dryden & Mardia, 1998, Zelditch et al. 2004). In our case M equals 2.

Size

To quantify the size of a specimen, centroid size (CS) was computed from the raw coordinates of the landmarks (Dryden & Mardia, 1998) using the MorphoJ software package (Klingenberg, 2011). Centroid size is the most commonly used size measure in geometrical shape analysis, because it is uncorrelated with the entire space of shape variation. As Bookstein (1991) indicated, in the absence of allometry, centroid size "explains" nothing about shape. CS is calculated as the square root of the summed squared distances of each landmark from the centroid of the landmark configuration. The centroid is the *M*-dimensional vector (two in the case of the two-dimensional landmarks for bluemouth specimens) whose components are the averages of the X and Y coordinates of the landmarks. Thus, the centroid size of a configuration (X) is:

$$CS(\mathbf{X}) = \sqrt{\sum_{i=1}^{K}\sum_{j=1}^{M}(\mathbf{X}_{ij} - C_j)^2}$$

Where the sum is over the rows i and columns j of the matrix $\mathbf{X}$. $\mathbf{X}_{ij}$ is a standard notation from linear algebra specifying the value located on the ith row and the jth column of the matrix $\mathbf{X}$, and in this case C_j stands for the location of the jth component of the centroid

In the present study, CS was highly correlated with total length of the specimens ($r^2 = 0.9935$, $p < 0.01$) (Fig. 6).

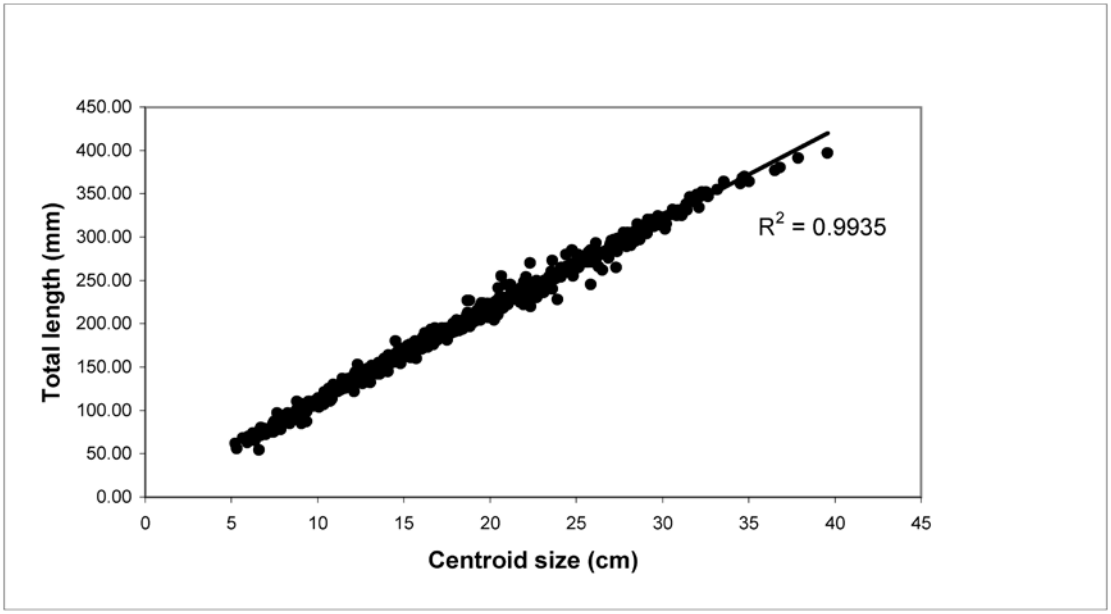


Figure 2-7. Relationship between centroid size (CS) and total length (TL) for all of the specimens in this study (r^2 = 0.9935, $p < 0.01$). The relationship between centroid size (CS) and total length (TL) is given in order to allow for comparisons of our results with the results of other studies.

Shape

Body shape was analyzed using landmark-based geometric morphometrics methods (Rohlf, 1990a and b; 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 and projects the data to the tangent space by orthogonal projection (Dryden & Mardia, 1998).

The necessary calculations to perform a Procrustes fit are described in Rohlf et al. (1990b) and Zelditch et al. (2004), but the procedure can be summarized as follows:
Following digitization, each is individual in the sample is represented as a landmark configuration, which represents the positions of the landmarks describing that individual. The first step of the Procrustes fit is to superimpose the landmark configurations to have a common centroid. The superimposition is done by centering each configuration of landmarks at the origin by subtracting the coordinates of its centroid from the corresponding (x or y) coordinates of each landmark. Then, the landmark configurations are rescaled to unit centroid size by dividing each coordinate of each landmark by the centroid size of that particular configuration. When only two configurations are involved (i. e., two individuals) the analysis is called ordinary Procrustes fit. In this analysis, one of the configuration is chosen to be the reference, and the second configuration is rotated to minimize the summed squared distances between homologous landmarks (over all landmarks) between the forms. In other words, the second configuration is rotated to minimize the partial Procrustes distance.

The Procrustes distance is the basic metric in shape theory. It is defined as the square root of the sum of the squared differences between the coordinates of corresponding landmarks in two optimally (by least-squares) superimposed configurations at centroid size, and it is a measure of the absolute magnitude of the shape deviation (Slice et al., 1998).

When there are more than two configurations (i. e., generalized Procrustes fit), the superimposition procedure is iterative. First, all configurations are rotated to optimal alignment on the first configuration; the mean shape is then calculated and all are rotated to optimal alignment on the mean shape, which is the new reference. At this point, the mean shape is recalculated. If it differs from the previous reference, the rotations are recalculated using this newest reference. When the newest reference is the same as the

previous, the iterations stop. The final reference is the one that minimizes the average distances of shapes from the reference (Zelditch et al. 2004).

At this stage, the superimposed landmark configurations are said to be in partial Procrustes superimposition on the "reference" or "consensus" configuration (i. e., the mean configuration of landmarks in a sample of configurations). A slightly closer fit of the landmark configurations on the reference can be achieved when the scale for individual configurations is allowed to vary, while holding the centroid size of the reference constant at 1.0. This procedure is called the full Procrustes fit (Dryden & Mardia, 1998; Klingenberg, 2007). In this kind of superimposition, the full Procrustes distance is minimized, which is defined as the distance between two landmark configurations in the linear space tangent to Kendall's shape space (i. e., the tangent space) when centroid size is allowed to vary to minimize the distance between shapes rather than fixing it to unit size.

Finally, the specimens are projected to a linear shape tangent space. Here, the "reference" or "consensus" is the configuration of landmarks that corresponds to the point of tangency between the exact curved shape space and the approximating tangent space in which the linear multivariate statistical analyses are performed (Rohlf, 1999; Rohlf & Slice, 1990; Slice, 2001). The coordinates of the aligned specimens are the Procrustes coordinates and they were used as shape variables in the statistical analyses in this study.

2. 3. 2 Detection of outliers, quantification of measurement error and evaluation of bilateral symmetry

Quantification of error

Three common sources of undesirable shape variability in morphometric datasets are the error produced when **locating landmarks** on the specimens, the **orientation error** (error derived from the positioning of the specimen when the photograph is taken) and the **digitization error** (error during the digitization of landmarks on the image). It is important to know how large these errors are with respect to the natural variability

between specimens, because the lower the error, the more discriminative power the set of landmarks will have during further analysis (Adriaens, 2007).

The quantification of the error was based in the protocol for error testing in landmark based geometric morphometrics developed by D. Adriaens in 2007 (http://www.fun-morph.ugent.be/Miscel/Methodology/Morphometrics.pdf), using the TpsDig and TpsUtil software (Rohlf, 2008):

To quantify the total error (due to landmark location, orientation and digitization), 10 specimens were selected considering different study areas, sizes and sexes in order to represent as best as possible the range of natural variation in the dataset. The landmarks were located on the body of each fish using entomological pins, the fish was positioned on the Styrofoam board and a photograph was taken. This procedure was repeated 8 times for each of the 10 specimens, resulting in 80 images that were used as a test dataset for error qunatification. All images were digitized in a random order using the TpsDig software (Rohlf, 2008). Then, the Procrustes distance was calculated as a measure of the shape variability a) between all of the specimens to estimate the overall shape variation in the test dataset and b) between all of the replicas of each specimen to estimate the shape variability caused by the processes of locating the landmarks each time, placing the specimen (orientation error) and digitizing the photograph (digitization error). The amount of error with respect to the total variability was expressed as a percentage, by calculating the ratio of the mean Procrustes distance for each set of replicas and the mean Procrustes distance for the whole dataset.

For our test dataset, the total error accounted for 33.65% of the total shape variability. From our perspective, this seems as a high amount of error, since it leaves only a 66.35 % of shape variability as the “natural” variability in the dataset in which we are interested and it could lead to an important reduction of discriminative power. However, this result does not necessarily mean that the measurement error in the whole population structure dataset reaches this magnitude, but it is difficult to say to what extent the results of the test dataset may be extrapolated to the whole dataset, which is much larger in number of specimens and probably more variable. In this case, a significantly larger sample of specimens from the population structure dataset would be needed to yield a better estimate of the total shape variability and the amount of error present, but unfortunately, in the present study this was not possible due to time and economic

constraints. Nevertheless, this information was taken into account when interpreting the results of the analysis of the bluemouth population structure dataset.

Analysis of the arching effect

Another important source of measurement error is the distortion associated with the specimen's posture when the photograph is taken, because it can strongly affect the configuration of individual landmarks (Arnqvist & Mårtensson, 1998; Valentin et al., 2008).

In organisms like fish, which have flexible bodies, the arching effect (Valentin et al., 2008) refers to an upward or downward arching of the body. The problem with the arching effect is that it produces undesirable shape variability in the dataset that can obscure true shape variation related to biological or ecological factors, or introduces bias if the variation caused by arching is unevenly distributed in the samples.

Thus, arching effect in the population structure dataset used in this study was investigated using a PCA-model of the arching coupled with Burnaby's orthogonal projection, according to Valentin et al. (2008). The detailed methodology and results of this analysis are presented in Appendix I. In the analysis, the presence of an arching effect in the original dataset was detected, but when the results of the canonical variate analysis performed on the original data set and the dataset corrected for the arching effect were compared, there was no improvement in group discrimination in terms of Wilk's lambda values and correct classification rate, that is, both analyses yielded very similar results. Thus, we decided to use the original dataset to determine the bluemouth population structure.

Detection of Outliers

Before any statistical analyses were carried out, landmark configurations were inspected to find possible outliers using MorphoJ v. 1.03c (Klingenberg, 2011). This software uses a diagram with the cumulative distribution of the distances of individual specimens from the average shape of the entire sample and it compares it to a curve expected for a multivariate normal distribution fitted to the data. This diagram is an approximate guide to the quality of the data, but it is useful for detecting patterns where

the curve representing the data is stretched out to the right at the top of the diagram, indicating that there are one or a few specimens that deviate very strongly from the others. Depending on the relationship between the dimensionality of the data and the number of specimens in the dataset, either the Procrustes distance or the squared Mahalanobis distance is used (e.g. Klingenberg & Monteiro, 2005). Procrustes distance is a measure of the absolute magnitude of the shape deviation, whereas Mahalanobis distance provides an indication of how unusual an individual is relative to the others in the sample (in larger samples). According to this, individuals in our dataset that strongly deviated from the overall mean shape (using Mahalanobis distances due to the large sample size) were considered outliers. In total, 5 specimens were discarded; two from the Porcupine Bank, one from Alicante, one from the Catalonian coast and one from the Alboran Sea. The first four specimens were the smallest individuals caught in all of the samplings (CS 3.30 – 4.43 cm), and it was very difficult to locate and mark correctly the landmarks. Thus, it was not clear if the large Mahalanobis distances of these specimens from the mean shape were caused by errors in the position of the landmarks or by true differences in shape due to allometry. In the last specimen, the pectoral region was slightly damaged and this could have affected the relative positions of the landmarks.

Bilateral symmetry

The first samples obtained for this study were bluemouth caught in the DEMERSALES 2006 survey, when the sampling protocol was still being developed. During the sampling onboard, more than half of the bluemouth specimens were eviscerated with a cut on the left side of the fish, instead of the right side that was established in the morphometric protocol used in this study. This means that the photograph was taken using the right side of the fish (which was intact), and to be able to compare these specimens to the rest of samples (where the photograph was taken using the left side of the fish) the photographs needed to be reflected. However, to do this, we needed to know if the left and right sides of the fish were symmetric or if systematic differences between the sides existed. To test this, 60 intact specimens were photographed using both of their sides, resulting in 120 photographs. These images were divided in two groups (left side and right side) and their mean shapes were compared using the Procrustes and Mahalanobis distances and a permutation test with 10,000 runs was used to test the null hypothesis of no mean difference between sides. This analysis was done

through a Discriminant Function Analysis in MorphoJ v. 1.03c software package (Klingenberg, 2011).

No significant body shape differences between the left and right sides of uncut fish were observed. The Procrustes and Mahalanobis distances between the mean shapes of the left and right side were 0.0080 and 1.2049 respectively but none of them were significant at the 5 % level. Thus, we included the photographs from the bluemouth specimens caught in the DEMERSALES 2006 survey that were taken using the right side of the fish in the population structure dataset.

2. 3. 3 Statistical analyses

The data used in morphometric analyses are inherently multivariate, because many variables are needed to characterize body shape. This is even more evident in the case of geometric morphometrics, where the geometry of the specimens is preserved throughout the analysis, implying that many shape variables must be analyzed at the same time. In the next sections, the multivariate techniques used in this study to analyze the shape variables are described. The first set of methods, *Methods for size correction*, is aimed at disentangling the influence of size on shape and characterizing allometry, prior to the comparison of bluemouth populations. The second set of methods, *Methods used to determine the bluemouth population structure*, was used to determine the population structure of bluemouth by characterizing the patterns of shape variation and separating the populations according to their shape characteristics.

MorphoJ software (Klingenberg, 2011), SPSS statistical software, release 19.0.0 (IBM Corp., 2010), and R software (R Development Core Team, 2011) were used to carry out the analyses.

Methods for size correction

While fishes grow, body proportions change as the larvae, juvenile and adult fish adapt to habitat and diet transitions. The change in proportions related to variation in size (i.e. growth) is called ontogenetic allometry and it has been studied in fishes for a long time (e.g. Barlow, 1961; Strauss & Fuiman, 1985 and Klingenberg & Froese, 1991). However, the presence of allometry poses a problem if one wants to compare the morphology of a group of fish populations, because there is the risk of confounding real differences between populations with accidental differences in size composition of the samples. Thus, it has long been a goal for allometric analyses to provide methods that can be used to correct the data for the effects of size variation. These methods use allometric approaches to construct variables that are unaffected by size variation (Klingeberg, 1996).

In this study, we used five of the most commonly used methods to characterize allometry in our dataset:

a) Principal components analysis (PCA)
b) Burnaby's method
c) Size-and-shape PCA
d) Overall multivariate regression
e) Pooled within-group multivariate regression

Principal component analysis (PCA)

This multivariate statistical technique has been traditionally used to reduce the dimensionality of a dataset consisting of a large number of interrelated variables, while retaining as much as possible of the variation present in the dataset. This is achieved by transforming to a new set of variables, the principal components (PCs), which are linear combinations of the original variables in the dataset. The PCs are uncorrelated (orthogonal) to each other and they are ordered so that the first few retain most of the variation present in all of the original variables (Jolliffe, 2002; McGarigal et al., 2000).
In the context of allometry, the utility of PCA is a result of the empirical observation that most variation in many samples is attributable to variation in the size of the specimens in the sample. In that case, the first PC would capture the relationship between shape and size, which might be allometry (Jolicoeur, 1963; Slice & Stiztel, 2004).

The procedure to calculate the PCs was summarized strightforwardly by Klingenberg, (1996) as follows: PCA decomposes a covariance matrix **S** into eigenvectors and eigenvalues, so that **S** = **BLB'**. The matrix of **B** eigenvectors is used to transform the original data **X** into a set of new variables **Y** = **XB**, the principal components (PCs). The matrix **L** is the covariance matrix of the PCs, and as the PCs are uncorrelated among each other, all off-diagonal elements of **L** are zero. The diagonal elements of **L**, the eigenvalues, are the variances for which the associated eigenvectors account. These eigenvalues are difficult to interpret by themselves, because they depend on the measurement units used in the data, however, the proportion of the total variance for which the PC1 accounts is important to assess how well the model of simple allometry fits the data.

Burnaby's method

The procedure proposed by Burnaby (1966) eliminates the effects of growth from multivariate data by projecting data points onto a subspace that is orthogonal to the growth vector. This growth-invariant subspace has one dimension fewer than the original space (Klingenberg, 1996). It is a simple method of sweeping the effect of one of more extraneous variables from the data and then carrying out the statistical analyses on the adjusted data matrix. Usually, PC1 of the pooled within-groups covariance matrix has been used as the growth vector in Burnaby's procedure (Klingenberg, 1996). The growth-adjusted data matrix, *X*', as calculated by Burnaby (1966) and Rohlf and Bookstein (1987) is the following:

$$X' = X(I_p - F(F^tF)^{-1}F^t)$$

where X is the $n \times p$ original data matrix (n is the number of cases and p the number of variables), F is the growth vector of p values that one wishes to correct for, and I_p is the $p \times p$ identity matrix.

The steps are mathematically equivalent to projecting the *n* specimens onto the within-group eigenvectors, replacing the values for the projections onto the first axis with zeros, and then rotating the *n* specimens back into the original space. They will now have different values since the effects of differences in within-group size have been completely removed and the data points now all lie on a hyperplane within the original space. The matrix $\mathbf{X}'$ may be then used in place of **X** in further statistical analysis (Rohlf & Bookstein, 1987).

Size-and-shape PCA

This method for determining allometry is as variant of the PCA method mentioned above and it was proposed by Mitteroecker et al. (2004): In this method, a PCA is carried out on the data matrix (a matrix containing the shape variables) augmented by one single additional column for the logarithm of Centroid Size (CS). Typically, log CS will have by far the largest variance of any column of this matrix and thus the PC1 of this size-and-shape will be closely aligned with size.

Multivariate regression

This method models explicitly the relationship between size (or other variables) and shape, and because of this, it is the method of choice to assess the influence of a single factor such as size, age or any other environmental variable on shape. In several studies, the regression of shape on the logarithm of CS has been identified as the optimal measure of allometry (Monteiro, 1999; Mitteroecker et al., 2004; Mitteroecker & Gunz, 2009; Slice & Stitzel, 2004). Moreover, multivariate regression is not sensitive to the number of dependent shape variables or to their covariance structure and the resulting vector of regression coefficients (quantifying the average effect on shape) can be visualized as shape deformation (Mitteroecker & Gunz, 2009)

The key idea is that regression separates the component of variation in the dependent variables that is predicted by the independent variable from the residual component of variation, which is uncorrelated with the independent variables. The predicted component can be computed from the slope of the regression line and the deviation of the data point from the mean in the direction of size. The residual is the difference of the total shape deviation of the data point from the mean and the predicted component (Klingenberg, 2011 - from MorphoJ's help files). Using the residuals from a regression of shape on size for further analyses is therefore a method of size correction for the shape data.

The calculation of a multivariate regression for shape data is described in Slice and Stitzel (2004):

Let $\mathbf{Y}$ be an $n \times pk$ matrix of the Procrustes coordinates (pk columns for p points in k dimensions) for all of our n specimens (rows) and $\mathbf{X}$ be an $n \times 2$ matrix with an initial column of ones and a second column of centroid sizes (log-transformed to help to normalize and linearize the relationship). Then we solve the linear model:

$\mathbf{Y} = \mathbf{XB} + \mathbf{E}$ for the $2 \times pk$ matrix, $\mathbf{B}$, the coefficients for the grand mean and the regression of size onto each of the coordinate values. $\mathbf{E}$ represents the error term. The least-squares estimates of these parameters are simply: $\hat{\mathbf{B}} = (\mathbf{X}^t\mathbf{X})^{-1}\mathbf{X}^t\mathbf{Y}$.

Pooled within-group regression

If the dataset contains multiple groups, the question arises whether this group structure should be considered for size correction. In other words, the question is whether

the correction should be specifically for the within-group allometric relationship or for the total allometry. This question does matter because the within- and between-group allometries cannot be expected to coincide in general (Klingenberg, 2011 - from MorphoJ's help files).

Thus, if we are considering more than one group at a time and if the regression slopes in the groups are the same, we can perform a *pooled within-group regression*. This method uses the regression slopes within samples to separate the predicted and residual components of variation in the dependent variables. Note that there is not just a separation of predicted and residual deviations for individual data points, but now also for the means of the subsamples corresponding to the different groups. This results in a reduction of shape variation within groups, which sometimes can lead to a substantial increase in the separation of groups.

The calculation of the predicted and residual components of a pooled within-group regression of Procrustes coordinates (Y) on log centroid size (X) is the following:
First we calculate the vector of pooled within-group coefficients $\mathbf{b}_w$:

$$\mathbf{b}_w = \mathbf{x}_1^{\mathbf{t}}\mathbf{Y}_1 \cdot (\mathbf{x}_1^{\mathbf{t}}\mathbf{x}_1)^{-1}$$

where $\mathbf{x}_1$ is a vector of the group mean–centered covariate values (the appropriate group mean is subtracted from each value of the covariate) and $\mathbf{Y}_1$ is a matrix of group mean–centered Procrustes coordinates, of $n \times pk$ dimensions (pk columns for p landmarks in k dimensions) for all of our n specimens (rows).

The predicted values are calculated as:

$$\mathbf{Y}_{pred} = \mathbf{1} \cdot \bar{\mathbf{Y}} + \mathbf{b}_w{}^t(\mathbf{X}_{obs} - \bar{\mathbf{X}})$$

where **1** is a matrix of ones of dimensions $n \times 1$, $\bar{\mathbf{Y}}$ is a row vector $(1 \times pk)$containing the grand mean of each of the Procrustes coordinates, $\mathbf{b}_w$ is the (row) vector containing the pooled within-group coefficients, $(\mathbf{X}_{obs} - \bar{\mathbf{X}})$ is a $n \times 1$ vector of centered covariate values (subtracting the grand mean of the covariate).

And the residuals:

$$\mathbf{Y}_{res} = \mathbf{Y}_{obs} - \mathbf{b}_w{}^t(\mathbf{X}_{obs} - \bar{\mathbf{X}})$$

Where $\mathbf{Y}_{obs}$ is the $n \times pk$ matrix of the Procrustes coordinates (pk columns for p landmarks in k dimensions) for all of our n specimens (rows), $\mathbf{b}_w$ is the (row) vector

containing the pooled within-group coefficients, $(\mathbf{x}_{obs} - \bar{\mathbf{X}})$ is a $n \times 1$ vector of centered covariate values (subtracting the grand mean of the covariate).

Characterization of growth trajectories using multivariate regressions

In chapter 3, a multivariate regression of the Procrustes coordinates on the logarithm of centroid size was used to determine growth trajectories and characterize morphological changes in response to size. This method was chosen because it models explicitly the relationship between size (or other variables) and shape. The amount of shape variation for which each regression accounted was expressed as a percentage of the total variation around sample means. A permutation test using 10,000 runs (Good, 1994) was used to test the null hypothesis of independence between shape and size.

To visualize the strength of the association between size and shape, we calculated shape scores according to Drake & Klingenberg (2008) and plotted them against log centroid size. A shape score is defined by projecting the shape data onto a line in the direction of the regression vector for the independent variable (centroid size). If the regression model is written as $\boldsymbol{y} = \boldsymbol{\beta}x + \boldsymbol{\varepsilon}$ (where $\boldsymbol{y}$ is the row vector of shape variables; $\boldsymbol{\beta}$ is the regression vector; x is the independent variable; and $\boldsymbol{\varepsilon}$ is the row vector of error terms), the shape score s can be computed as $s = \boldsymbol{y}\boldsymbol{\beta}'(\boldsymbol{\beta}\boldsymbol{\beta}')^{-0.5}$. This shape score is the shape variable associated with the shape changes predicted by the regression model, but also includes the residual variation in that direction in shape space (Drake & Klingenberg, 2008). These analyses were carried out with the MorphoJ software package (Klingenberg, 2011). The similarity of growth trajectories between sexes and among areas was evaluated following the approach explained in Zelditch et al. (2003a and 2003b), with the program VecCompare - IMP software (Sheets, 2000). To compare each pair of regression vectors, this program first calculates the angle between these vectors (i.e. between-group angle). That angle is obtained as the arccosine of the signed inner products between normalized regression vectors. Then, the between-group angle is compared with the upper 95 confidence interval of within-group angle ranges assessed by a bootstrapping approach with 900 runs. The null hypothesis is that the observed angle could have been produced by two independent samplings of a single group (i.e. area or sex). If the between group angle exceeds the 95 confidence interval of the two within-group angles, the difference is judged statistically significant at the 5 level.

Visualization of ontogenetic shape changes

To visualize the shape changes associated to the growth of bluemouth specimens, **warped outline drawings** were done using the thin-plate spline interpolation function (Bookstein, 1989). Visualizations were done in the MorphoJ software package (Klingenberg, 2011).

Sexual dimorphism

The presence of sexual dimorphism of body shape between males and females within each area was investigated prior to the population structure analysis. To find out if shape differences existed between sexes, a parametric Hotelling's T^2 test was done. This test is the multivariate equivalent of the *t*-test and tests whether two vectors of means for the two groups are sampled from the same sampling distribution.

Comparisons between the mean shapes of males and females within each area were also carried out based on Procrustes and Mahalanobis distances with a permutation test with 10,000 runs. For all the comparisons between male and female mean shapes, size-corrected variables (i.e., regression residuals from a multivariate regression) were used in the cases whenever significant allometry was present (Chapter 3 – Table 3-2). These areas were: the Porcupine Bank, the Cantabrian Sea, Galicia, the Gulf of Cadiz, subareas A1 and A2 of Alboran Sea and Alicante. The original variables were used in cases where no significant allometry was detected (Table 3-2), namely, Portugal and Sicily. The shape comparison for Catalonia was not performed due to insufficient sexed specimens in the area. These analyses were done in MorphoJ software (Klingenberg, 2011) and SPSS statistical software, release 19.0.0 (IBM Corp., 2010).

Methods used to determine the bluemouth population structure

Discriminant analysis has been a widely used technique in geometric morphometrics and continues to be one of the most useful techniques for separating populations of organisms according to shape. This technique, together with the cluster analysis was used to provide a picture of the degree of separation and relationships among groups according to shape. MorphoJ software (Klingenberg, 2011) and SPSS statistical

software (IBM Corp., 2010) were used to carry out the DFA/CVA and STATISTICA 6.0 (StatSoft Inc., 2001) was used to carry out the Cluster Analysis.

Discriminant Function Analysis/ Canonical variate analysis

Discriminant function analysis (DFA) is a multivariate technique, introduced by Fisher (1936). He developed the technique to create a linear discriminant function to establish maximum separation among three species of iris flowers based upon four measurements. This is a key method in morphometric studies, where groups of specimens are compared to see if they can be distinguished morphologically. In addition, this technique can be used to obtain an overview of the structure of variation among a number of groups, such as samples from different geographical locations, different species in a clade or different genotypes (Klingenberg unpublished course material). Excellent references regarding the use of this technique in geometric morphometrics are Klingenberg and Monteiro (2005), Zelditch et al. (2004) and Mitteroecker and Bookstein (2011).

Since the terminology of discriminant analysis can be somewhat confusing (e. g. McGarigal et al., 2000; Zelditch et al., 2004; Klingenberg & Monteiro, 2005; Strauss, 2010), we decided to adopt the terminology used in Klingenberg and Monteiro, 2005, where the analysis is referred to as Discriminant function analysis (DFA) when only two groups are involved in the analysis and Canonical Variate Analysis (CVA) when more than two groups are analyzed at the same time.

Like PCA (See *Methods for size correction – PCA*), DFA is a form of eigenanalysis, except that in this case the axes are eigenvectors of the among-group covariance matrix rather than the total covariance matrix. Thus, in contrast to PCA, discriminant analysis is explicitly a multigroup procedure, and assumes that groups are known a priori on the basis of extrinsic criteria and that all individuals are members of one (and only one) of the known groups. DFA optimizes discrimination between groups by one or more axes, the discriminant functions (or canonical variates). These are mathematical functions in the sense that the projection scores of data points on the axes are linear combinations of the variables, as in PCA. For k groups, DFA finds the k-1 discriminant axes that maximally separate the k groups. Like PCs, DFs have corresponding eigenvalues that specify the

amount of among-group variance (rather than total variance) accounted by the scores of each DF (Strauss, 2010).

The procedure used to calculate the discriminant functions and canonical variates is described in Klingenberg and Monteiro (2005) as follows:

The discriminant function between two groups is computed as $\mathbf{W}^{-1}\mathbf{d}$, where $\mathbf{d}$ is the difference vector between the two group means and $\mathbf{W}^{-1}$ is the inverse of the within-group covariance matrix $\mathbf{W}$. The discriminant functions therefore correspond to lines connecting pairs of group means, the discriminant scores are computed by orthogonal projection of the data points onto those lines. Similarly, canonical variate analysis is based on a transformation of the among-group covariance matrix $\mathbf{B}$ by premultiplying with $\mathbf{W}^{-1}$, followed by a principal component analysis of the matrix $\mathbf{W}^{-1}\mathbf{B}$. The resulting canonical variates are those variables that account for the maximum amount of among-groups difference relative to the within-group variation.

However, the optimal discriminant functions derived from a dataset need not be effective discriminators (discriminant functions are calculated regardless of whether the differences between groups are statistically significant) (Zelditch et al., 2004). To determine if a discriminant function is an effective discriminator, the Wilk's lambda statistic (λ) is used (as in a single-factor MANOVA). Wilk's lambda is ratio of the determinant of the within-groups sum of squares (**W**) and the determinant of the total sum of squares (**B**) (Zelditch et. al 2004):

$$\lambda = \frac{\det(\mathbf{W})}{\det(\mathbf{T})} = \frac{\det(\mathbf{W})}{\det(\mathbf{W}+\mathbf{B})}$$

Conveniently, λ can be calculated as the product of the eigenvalues of $\mathbf{W}(\mathbf{W}+\mathbf{B})^{-1}$. The sampling distribution of the Wilk's lambda statistic is not well understood, and to test for statistical significance lambda is usually converted to an approximate *F*-ratio statistic (Quinn & Keough, 2002 and references therein).

Due to the transformation by $\mathbf{W}^{-1}$, the resulting canonical or discriminant space is different from the space of the original variables to the degree that $\mathbf{W}$ differs from being proportional to an identity matrix. The distances in the transformed space are known in multivariate statistics as the *Mahalanobis distances*, and they measure the differences between groups relative to the within-group variation. Mahalanobis distances can be used to evaluate the utility of the discriminant functions (or canonical variates) for discriminating among groups, by computing the distance between specimens from the

group mean. The means are computed using the a priori group assignments. As described in Zelditch et al. (2004), the Mahalanobis distance between a specimen **X** and the mean **M** of a group is given by:

$$\mathbf{D} = \sqrt{(\mathbf{X} - \mathbf{M})^{t}\mathbf{S}^{-1}(\mathbf{X} - \mathbf{M})}$$

where $\mathbf{S}^{-1}$ is the inverse of the variance-covariance matrix of the CV scores of the specimens. The predicted group membership of each specimen based on the scores is determined by assigning each specimen to the group whose mean is closest (under the Mahalanobis distance) to the specimen. The results of the assignment of the specimens to the groups can be presented in a *classification matrix*. This is a table were *a priori* group assignments is compared to the classification that results from using the Mahalanobis distances of the specimens to the group means. As a direct measure of predictive accuracy, the correct classification rate (i. e., the percentage of samples classified correctly) is the most intuitive measure of discrimination. This percentage can also be used as an indirect measure of the amount of canonical discrimination contained in the variables. The higher the correct classification rate, the greater the degree of group discrimination achieved by the discriminant functions (McGarigal et al., 2000).

However, the discriminant functions tend to over-estimate the separation between groups, particularly if the sample size is small relative to the number of dimensions (i. e. many landmarks in the analysis). A good separation of the groups on its own does therefore not mean that observations can be reliably classified (Klingenberg, 2011 - MorphoJ help files). The most common validation procedure, when the sample size is large enough, is to randomly divide the total sample of specimens into two groups (McGarigal et al., 2000). In this method, one subset referred to as the training sample is used to derive the discriminant functions, and the other, referred to as the test sample, is used to test the efficiency of the functions. In this study, the sample was not large enough to use this method, so we recurred to another method that uses a resampling procedure, the leave-one-out cross-validation or jackknife validation (e.g. Lachenbruch, 1967). The jackknife validation proceeds as follows (McGarigal et al., 2000): (1) a single specimen is omitted from the dataset; (2) the discriminant functions are derived; (3) the omitted specimen is classified by assigning that specimen to the group whose mean is closest (under the Mahalanobis distance) to the specimen (4) the process is repeated sequentially for each specimen; and (5) the resulting jackknife correct classification rate is calculated to judge the reliability and robustness of the canonical functions. If the jackknife

classification rate is much lower than the rate from the full dataset, then we must suspect that the estimation of means and dispersions is not reliable, resulting in unstable functions.

Cluster analysis

Cluster analysis refers to a large family of techniques, each of which attempts to organize objects (i. e., sampling units or entities) into discrete classes or groups such that within-group similarity is maximized and among-group similarity is minimized according to some objective criteria. This is in contrast to ordination, which attempts to organize samples along a continuum. In addition, in contrast to discriminant analysis, cluster analysis operates on datasets for which prespecified well-defined groups do not exist, but are suspected (McGarigal et al., 2000).

There are several methods to achieve clustering, but the most used ones are of the kind known as agglomerative hierarchical clustering. Agglomerative methods start with individual objects and join objects and then objects and groups until all of the objects are in one big group. Most algorithms for agglomerative cluster analysis start with a matrix of pairwise similarities or dissimilarities between the objects and the steps are as follows (Quinn & Keough, 2002):

1) Calculate a matrix of dissimilarities between all pairs of objects.
2) The first cluster is formed between the two objects with the smallest dissimilarity.
3) The dissimilarities between this cluster and the remaining objects are then recalculated.
4) A second cluster is formed between cluster 1 and the object most similar to cluster 1.
5) The procedure continues until all objects are linked in clusters.

The graphical representation of the cluster analysis is a dendrogram (i. e. a tree-like plot) showing the links between groups of objects with the lengths of the lines representing dissimilarity.

The major difference between the variety of available hierarchical agglomerative clustering methods is how the dissimilarities between clusters and between clusters and objects (step 3) are recalculated. These are termed linkage methods. In this study, we used an average linkage method, known as unweighted pair-group method using the arithmetic

mean (UPGMA). This method designates distance values between groups to be the mean dissimilarity between clusters (McGarigal et al., 2000).

Also, in this study, the initial dissimilarity matrix consisted of Mahalanobis distances between bluemouth samples from different locations.

2.4 Meristics

Meristic characters are the numbers of discrete, serially repeated, countable characters such as vertebrae, gill rakers, and fin rays (Swain et al., 2005). The meristic variables used in this study were: the number of spines of the first dorsal fin (SDF1), the number of rays of the second dorsal fin (RDF2), the number of rays of the pectoral (RPF), ventral (RVF) and anal (RAF) fins, and gill rakers of the horizontal (GRH) and vertical (GRV) segment of the gill.

Meristic characters were recorded after the photograph of the fish was taken (for the morphometric analysis). In some cases, the meristic characters were recorded immediately after the photograph was taken, but since the whole procedure for acquiring morphometric and meristic data is time consuming, there were occasions were the fish were stored and frozen again after the photograph was taken and the meristic variables were recorded at later stage. In general, the protocol for the acquisition of meristic variables was the following: First, the bluemouth specimens were thawed and identified according to their code. Then, each fish was placed with its head facing to the left and the most exterior gill arch was extirpated with scissors and forceps. The gill arch was rinsed with water to eliminate all tissue remains. The number of gill rakers was counted separately for the vertical (GRV) and horizontal (GRH) segments of the gill arch (Fig. 2-8). Then, the number of spines of the first dorsal fin (SDF1), the number of soft rays of the second dorsal fin (RDF2, Fig. 2-9), the number of spines and rays of the anal fin (RAF, Fig. 2-10), the number of spines and rays of the ventral fin (RVF) and the number of spines and rays of the pectoral fin (RPF, Fig. 2-11) were counted.

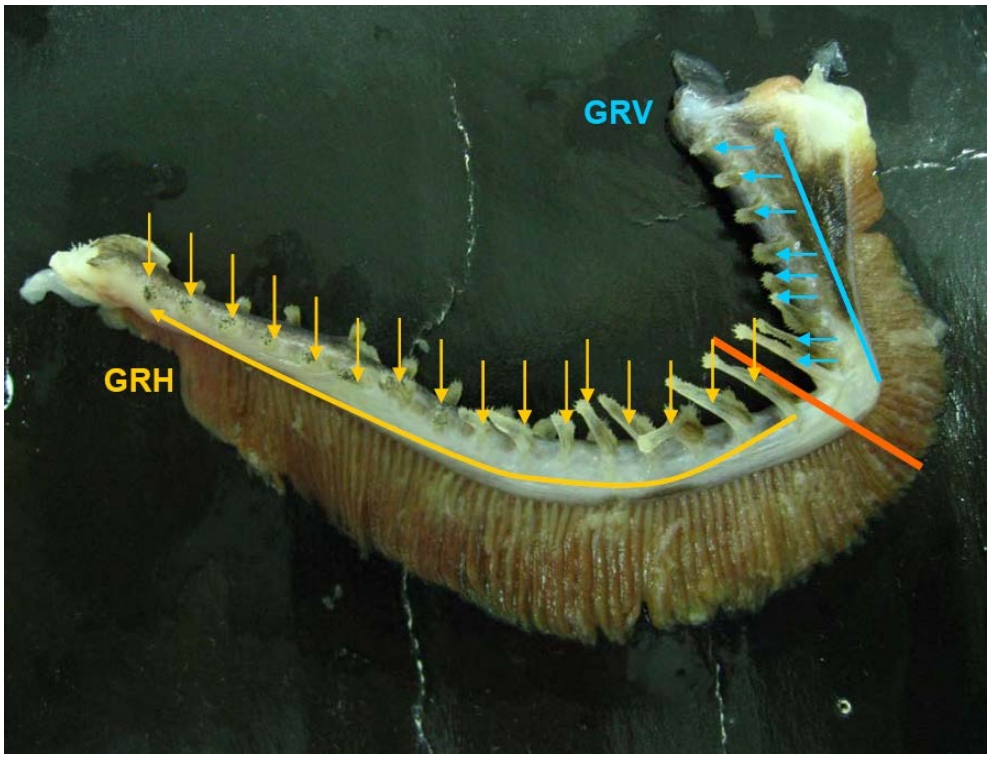


Figure 2-8. Gill rakers of the vertical (GRV, blue arrows) and horizontal (GRH, yellow arrows) segments of the most exterior gill arch.

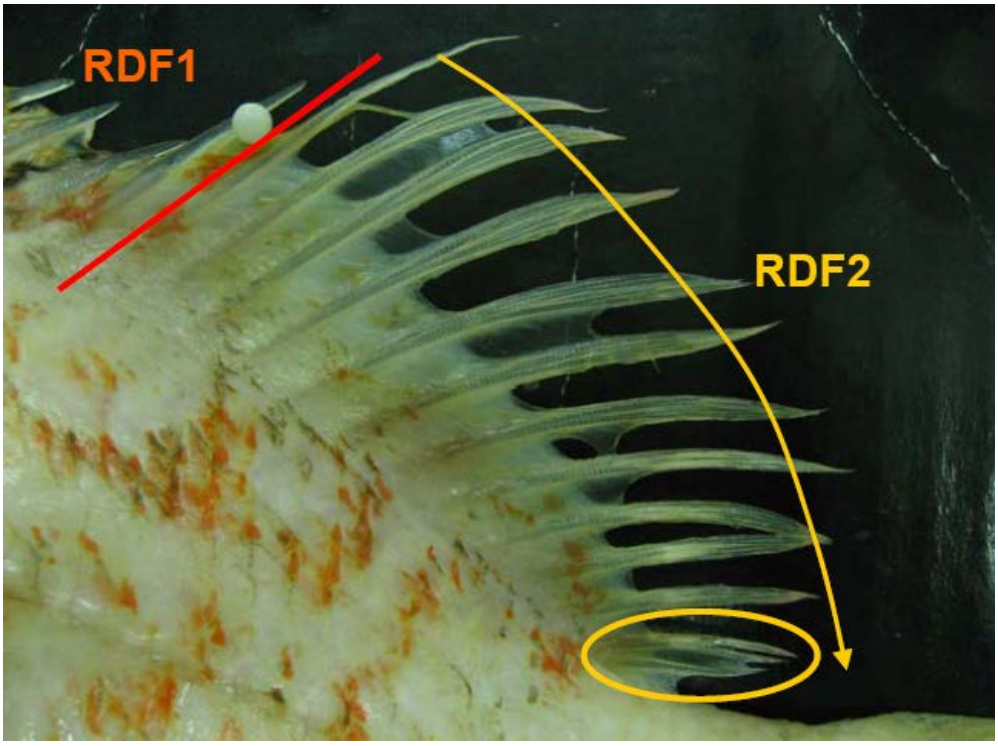


Figure 2-9. Rays of the second dorsal fin. Note: The last two rays (in the oval) are counted separately. Thus, on this image we can count 13 soft rays on the second dorsal fin.

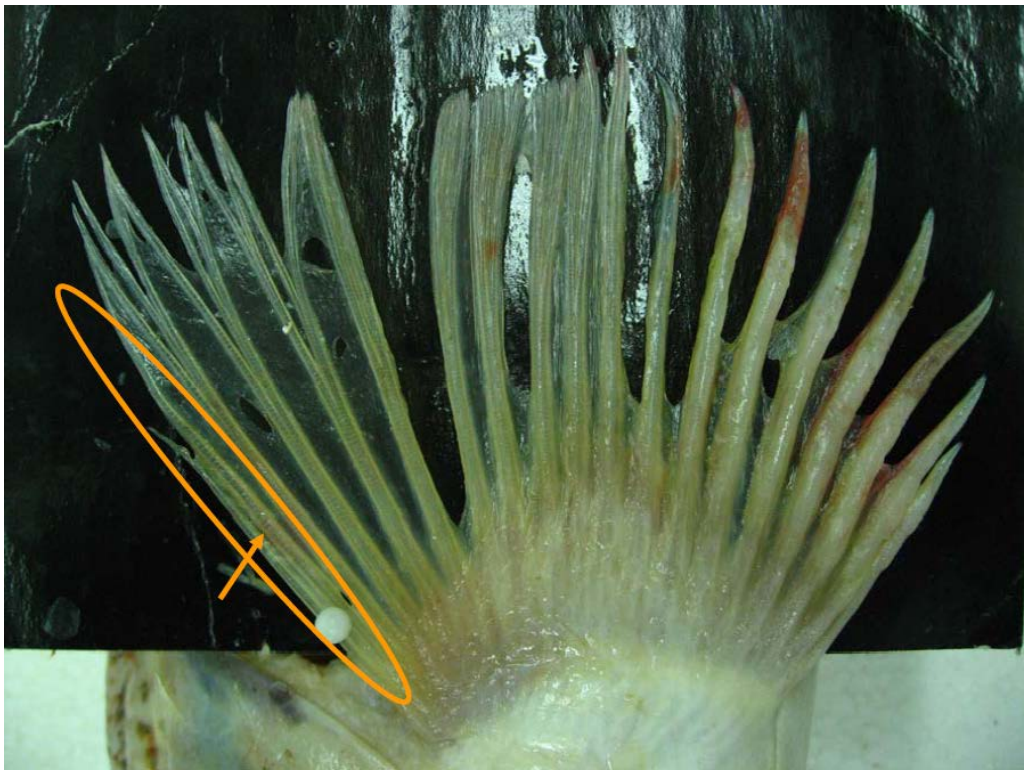


Figure 2-10. Rays of the pectoral fin. Note: The two rays indicated with the arrow were actually counted as one, because these rays are usually together and very difficult to separate in small fish. In this image, we can count 18 rays on the pectoral fin.

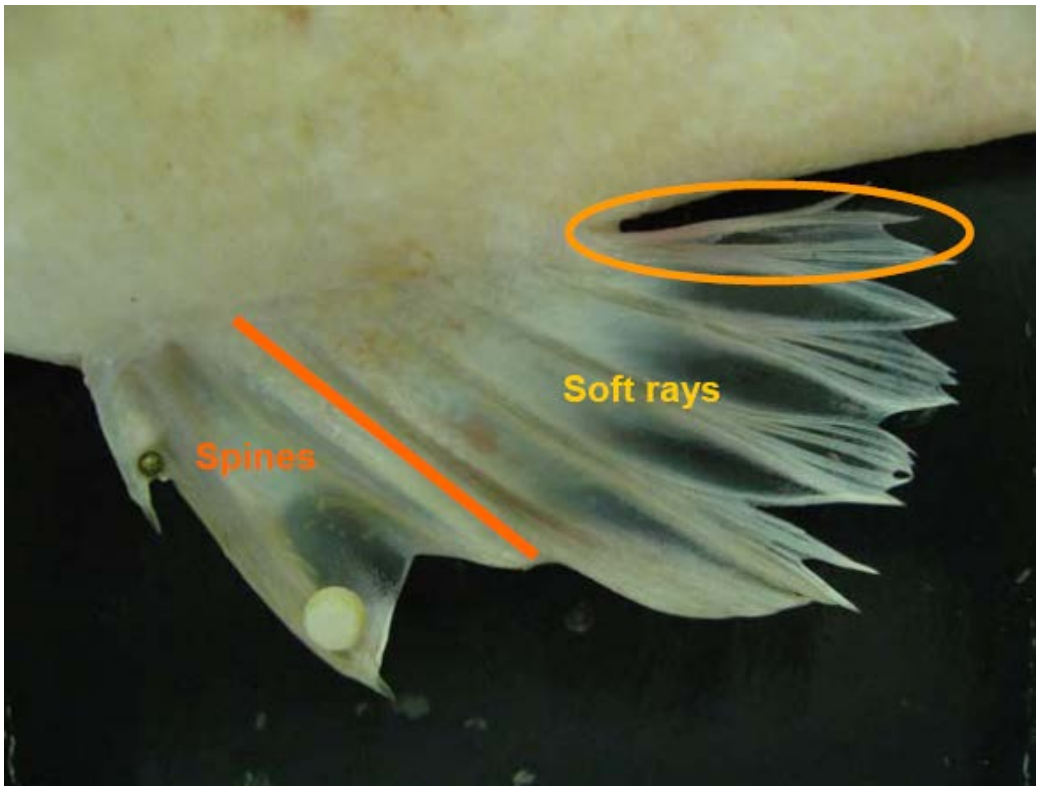


Figure 2-11. Rays of the anal fin. Note: In this image, we can see three spines and five soft rays on the anal fin. The last ray (in the oval) is bifurcated only at its tip, and it is counted as a single ray

2.4.1 Statistical analysis of meristic variables

Meristic characters are enumerable morphological features of fishes (Waldman, 2005), which means that the data obtained for meristic analysis are discrete, not continuous as in the case of data obtained to study body morphometrics. All parametric tests (e. g., t-test, ANOVA, etc.) require that the data are continuous and the populations are distributed normally. When these two assumptions are not met, as it is the case of meristic variables, non-parametric statistical methods are appropriate. The non-parametric tests that have been used in this work are rank-based non-parametric methods, where the ranks of the measurements are employed in the procedure instead of the actual measurements. A good review of the different non-parametric methods can be found in Zar (1984), Siegel and Castellan (1988), Quinn and Keough (2002) and Field (2005).

Before the meristic variables were compared among bluemouth from different areas, the effect of sex and size of the fish on these variables was investigated within each area. To determine if the counts of meristic variables differed between males and females, a Mann – Whitney test was carried out. The relationship between size and meristic counts was assessed using the Spearman's correlation coefficient (r_s). In the case of meristic variables, which are fixed early in the ontogeny of fish (Barlow, 1961; Waldman, 2005), when a significant relationship between the size of the fish and meristic variables is found it usually indicates the presence of different year classes in the samples (e.g., Garabana, 2005), and not a change in meristic counts as the result of fish growth. The size of the fish was expressed as Centroid size (see section *Morphometric and Statistical Analysis- Size* of this chapter). The analysis of meristic variables was carried out using SPSS statistical software, release 19.0.0 (SPSS Inc. 2010).

The Mann-Whitney *U* test for two groups

The Mann-Whitney *U* test is the non-parametric analogue of the t-test. The null hypothesis being tested is that the two samples (groups) come from populations with identical distributions against the alternative hypothesis that states that the samples come from different populations which differ only in location (mean or median) (Quinn & Keough, 2002). The procedure is as follows:

First, all the observations are ranked, ignoring the groups. Tied observations get the average of their ranks. Second, the sum of the ranks for both samples is calculated (if the null hypothesis is true, we would expect a similar mixture of ranks in both samples). The *U* statistic is then calculated for each group as follows:

$$U = N_1 N_2 + \frac{N_1(N_1+1)}{2} - R_g$$

Where N_1 is the number of observations in the first group, N_2 is the number of observations in the second group and R_g is the sum of ranks of the group for which the U statistic is being calculated (i. e., first or second group).

The probability distribution this statistic approximates a normal distribution and the *z* statistic can be used to test for significance.

Spearman's rank correlation coefficient

Spearman's correlation coefficient (r_s) is simply the Pearson correlation coefficient after the two variables have been separately transformed to ranks but the (y_{i1}, y_{i2}) pairing is retained after ranking. The null hypothesis being tested is that there is no monotonic relationship between Y_1 and Y_2 in the population. (Note: A monotonic relationship is a relationship that does one of the following: (a) as the value of one variable increases so does the value of the other variable or (b) as the value of one variable increases the other variable value decreases). The equation for Pearson's correlation coefficient between two variables, *x* and *y* that is applied to the data after ranking is:

$$r = \frac{\sum (x_i - \overline{x})(y_i - \overline{y})}{(N-1)s_x s_y}$$

Where x_i is the *i*th observation of variable *x*, $\overline{x}$ is the mean of variable *x*, y_i is the *i*th observation of variable *y*, $\overline{y}$ is the mean of variable y, N is the total number of

observations, s_x is the standard deviation of variable *x* and s_y is the standard deviation of variable *y*.

The Kruskal-Wallis H test

The Kruskal-Wallis technique tests the null hypothesis that the *k* samples come from the same population or from identical populations with the same median. Then, the alternative hypothesis is that at least one of the pairs of groups has different medians. The procedure is described in Siegel and Castellan (1988) as follows:

First, each of the *N* observations is replaced by ranks. That is, all of the scores from all of the *k* samples are combined and ranked into a single series. The smallest score is replaced by rank 1, the next smaller score is replaced by rank 2, and the largest score is replaced by rank *N*, where *N* is the total number of independent observations in the *k* samples. When this is done, the sum of ranks in each sample is computed. From these sums, the average rank for each sample or group is calculated. In this way, if the samples come from the same or identical populations, the average ranks should be about the same, whereas if the samples were from different populations with different medians, the average ranks should differ.

The equation to calculate the Kruskal-Wallis statistic (H or *KW*):

$$KW = \frac{12}{N(N+1)} \sum_{j=1}^{k} n_j \left(\bar{R}_j - \bar{R} \right)^2$$

where *k* = number of samples or groups
n_j = number of cases in the *j*th sample
N = number of cases in the combined sample (the sum of n_j's)
$\bar{R}_j$ = average of the ranks in the *j*th sample or group
$\bar{R}$ = (*N* + 1)/2 = the average of the ranks in the combined sample (the grand mean) and the summation is across the *k* samples.

If the *k* samples actually are drawn from the same population or from identical populations, that is, if the null hypothesis is true, then the sampling distribution of the statistic *KW* can be calculated and the probability of observing different values of *KW* can be tabled. However, when there are more than *k* = 3 groups, and when the number of observations in each group exceeds five, the sampling distribution of *KW* is well

approximated by the χ^2 distribution with $df = k - 1$, so this test statistic (χ^2) can be used to test for significance.

Post-hoc multiple comparisons

When the obtained value of *KW* is significant, it indicates that at least one of the groups is different from at least one of the others, however, it does not tell us which one(s) is/are different. What is needed is a procedure which will enable us to determine which groups are different. The null hypothesis is that there is no difference between the mean ranks of two particular groups. In this study, the procedure explained in Siegel and Castellan (1988) was used for determining which pairs of groups are different:

First, we obtain the differences between the mean ranks between all pairs of groups $\left|\overline{R}_u - \overline{R}_v\right|$. When the sample size is large, these differences are approximately normally distributed. However, there are a large number of differences and because the differences are not independent, the comparison procedure must be adjusted appropriately. For that, the value of α is divided by the number of possible comparisons (for k groups, the number of possible comparisons is $k(k-1)/2$).

Then we can test the significance of individual pairs of differences by using the following inequality. If

$$\left|\overline{R}_u - \overline{R}_v\right| \geq Z_{\alpha/k(k-1)}\sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_u}+\frac{1}{n_v}\right)}$$

then we may reject the hypothesis that there is no difference between the mean ranks of two particular groups (u and v). The value obtained by calculating the part to the right of the inequality is called the critical difference or critical value. In this inequality, the value of $Z_{\alpha/k(k-1)}$ is the abscissa value from the unit normal distribution above which lies $\alpha/k(k-1)$ percent of the distribution.

It has to be noted that the value of the critical difference depends on the sample size, so if the sample sizes between groups are unequal, each of the observed differences have to be compared against different critical differences.

Chapter 3

Ontogenetic Allometry and Size Correction

3.1 Introduction

As described in the general introduction (Chapter 1), bluemouth has been characterized as a slow growing and long-lived species. The majority of the ontogenetic studies on bluemouth has focused on analyzing the adult growth (size at age), and particularly on the comparison between male and female growth producing contradictory results (see *Age and Growth* in Chapter 1). However, there are no studies exploring the morphological changes that take place as fish increase in size.

Body form in fishes is a product of ontogeny (Cadrin, 2005). It is affected by the genetic makeup of an individual, but it also reflects adaptation to environmental factors such as temperature, food availability, feeding mode, swimming behavior or habitat use (Barlow, 1961; Wimberger, 1992; Swain et al., 2005). During the growth of fishes, body proportions change as the larvae and juvenile fish adapt to habitat and diet transitions until they reach adulthood. This change in proportions, related to variation in size (i.e. growth), is termed ontogenetic allometry.

The study of allometry is important to understand the relationships between size, shape and function (i.e., respiration, locomotion and feeding) of organisms (e. g., Kováč, 2002; Frédérich et al., 2008). For example, considerations of function suggest that, generally, teleosts share the positive allometry of the head and caudal body during larval growth, with postlarval growth being characterized by positive allometry of the region between (Zelditch et al., 2004).

Allometry also offers insights into growth and development, because these processes cause the changes in shape and size. For example, in studies of skeletal form, allometric coefficients provide information of the spatial distribution of relative growth rates (Zelditch et al., 2004). Moreover, allometry has been one of the main frameworks for studying ontogeny in the context of evolutionary biology (Klingenberg, 1998). This is because evolutionary changes in the spatiotemporal dynamics of growth can be discovered by comparative studies of ontogenetic allometry (Zelditch et al., 2004). Thus, by comparing how allometric growth differs between species, it is possible to reveal differences in their pathways of development that promote the morphological differentiation of species (Weston, 2003; Zelditch et al., 2004). For example, a study showed that evolution from anadromous stickleback (*Gasterosteus aculeatus*) into lacustrine forms involved prominent reshaping of the opercular bone (Kimmel et al.,

2008). In that study, the covariance between size and shape was distinctive for the two forms, suggesting that evolution modified the ancestral trajectory of allometric growth. Other examples of these applications in fish species can be found in Parsons et al. (2011) and Corse et al. (2012).

Traditionally, changes in proportion are represented as growth trajectories that describe the growth of an organism from its inception to its mature form (Alberch et al., 1979) and more recently, with the tools of geometric morphometrics, we can visualize shape changes to identify what happens during the growth organisms (e.g., Loy et al., 1996; Loy et al., 1998; Frost et al., 2003; Mitteroecker et al., 2004; Kouttouki et al., 2006; Drake & Klingenberg, 2008). In fishes, allometric trajectories have been studied for some time using traditional morphometric characters (e. g., Strauss & Fuiman, 1985; Meyer, 1990; Klingenberg & Froese, 1991). Since the late 1990's, the newer landmark-based geometric morphometrics have overtaken traditional methods in studies involving comparison and visualization of allometric trajectories. For example, Loy et al. (1998) used geometric morphometric to analyze the allometric shape changes that occur during the transition from pelagic to benthic stages of the two-banded sea bream (*Diplodus vulgaris*) and Frédérich et al. (2008) determined allometric trajectories and defined the developmental changes that lead to morphological differences between two species of damselfishes using geometric morphometrics.

The study of allometry has also an important application for size-correction of morphological variables when comparisons of multiple groups of specimens are carried out. The importance of size-correction in these cases derives from the risk of confounding true differences between the groups with accidental differences in the size compositions of the samples when allometric variation is not removed. Thus, the development of methods for size-correction has been active for a long time (e. g. Burnaby, 1966; Mosimann, 1970; Humphries et al., 1981; Thorpe, 1983; Claytor & MacCrimmon, 1987; Klingenberg & Froese, 1991; Klingenberg, 1996; Lleonart et al., 2000; Mitteroecker et al., 2004).

The methodological problem for size-correction has generally been the separation of empirical morphological differences among groups of specimens in two components: a) the morphological differences that are a consequence of size variation according to an allometric model, and b) the morphological differences derived from changes in shape that are inherent in the different groups under study. In population structure studies, this

inherent shape variation can be analyzed once the size-related variation has been removed.

There is a variety of methods that are used to characterize allometry and to remove size-related variation from the shape variables. In multivariate allometry, the first principal component (PC1) of the pooled within-group covariance matrix has been usually used to characterize the allometric trajectory, e.g., in multigroup PCA, Burnaby's procedure and in the shearing procedure (Jolicoeur, 1963; Humphries et al., 1981; Rohlf & Bookstein, 1987; Klingenberg, 1996). Burnaby's method (Burnaby, 1966) is one of the most important methods used to separate size-related and size-unrelated variation and it has long been used with distance data in traditional morphometrics. Although this approach is older than geometric morphometrics, it can be used in this new context as well. The procedure proposed by Burnaby (1966) eliminates the effects of growth from multivariate data by projecting points onto a subspace that is orthogonal to the growth vector. This growth-invariant subspace has one dimension fewer than the original space (Klingenberg, 1996).

However, approaches that characterize allometry using PC1 may not work well for geometric morphometric data, because during the Generalized Procrustes analysis (see Material and Methods), isometric size is factored out from the samples in the rescaling step. In this way, only in cases where allometric growth is substantially present will the first Principal component be associated to size (Slice & Stitzel, 2004). For example, in a study of different kangaroo and wallaby species, the PC2 rather than the PC1 was found to be associated with size (Milne & O'Higgins, 2002). In cases like this, no single PC may have a clear association with size, with allometric effects distributed over several PC's. Therefore, considerable care is needed in this kind of analysis, and the decision of which PC should count as allometric component may be difficult (Klingenberg, 2007).

A fundamentally different approach using a principal component analysis (PCA) has been proposed by Mitteroecker et al. (2004), in which a size variable (log Centroid size) is first added to the shape data (the Procrustes coordinates) to produce a size-and-shape space. Allometry is then characterized by a PCA of the covariance matrix of the data in this size-and-shape space. The amount of size variation usually far exceeds the amount of shape variation (as measured by Procrustes distance), thus, the size measure will dominate the PC1 in this analysis. As a result, the PC1 will represent the variation of size itself and

those shape features that are linearly associated with it, and can be interpreted as an allometric vector.

However, in geometric morphometrics, the preferred method for size-correction of the variables is to use the residuals of a pooled-within group regression as 'size-free' variables (Klingenberg, 2008), because in this way, only shape variation that is due to size variation is eliminated, contrary to what happens with methods that use Principal components, where an entire dimension is removed from the analysis.

All these methods for the analysis of allometry are means to characterize morphological change in response to size change. If there is a very strong allometric effect, that is, size alone accounts for the most of the variation, then different methods will produce similar results, which differ primarily in the form of presentation (Klingenberg, 2007).

3.2 Objectives

Thus, this chapter has two main goals: 1) to characterize the shape changes that occur during the growth of bluemouth to better understand its biology and ecology and 2) to determine the best method to correct for allometry for the bluemouth population structure dataset. To achieve the first goal, the allometric shape trajectories for bluemouth from the different study areas in the NE Atlantic and western Mediterranean were determined (for each area separately), using a PCA approach and a multivariate regression. Once this was done, the variation of growth patterns in the different environments of the study areas was examined. Growth patterns of males and females were also analyzed to determine if sexual dimorphism existed.

Then, to determine the best way to remove the effects of allometric size on the shape variables, a comparison of the results yielded by several methods commonly used for size-correction was done. For each method, both the amount of shape variation explained by the allometric vector and the results of a discriminant function analysis (DFA) using the data from two different bluemouth populations after size-correction were considered, specifically the Wilk's lambda value and the percentage of classification success from the jackknifed classification matrix. The methods used for size-correction were:

f) Principal components analysis (PCA)
g) Burnaby's method
h) Size-and-shape PCA
i) Overall multivariate regression
j) Pooled within-group multivariate regression

3.3 Results

3.3.1 Characterization of allometric shape trajectories

In this section, allometric trajectories for bluemouth from each area and between sexes (within the areas) were determined. Two methods were used as possible options to characterize these trajectories, namely, a principal components analysis (PCA) and a multivariate regression within each group. From this analysis, the multivariate regression provided the best characterization of the allometric trajectories in the form of regression vectors. The regression vectors were then used to: a) compare growth patterns among areas and sexes and b) visualize the patterns of shape changes that occur during growth of bluemouth.

Principal components analysis (PCA)

Shape variation related to size was found to be spread over several principal components (Table 3-1) and the distribution of this variation was considerably heterogeneous among the bluemouth samples from the different areas. Thus the size-related shape variation was concentrated mostly on the first PC in Cantabrian Sea and Gulf of Cádiz, on the second PC's in Porcupine Bank, Galicia, Alboran Sea and Alicante; and finally, in Portugal, Catalonia and Sicily it was related mostly to PC3. Significant relationships between the PC and centroid size were obtained in more than one PC in all areas except in Portugal. Therefore, it was not possible to characterize the allometric trajectory for bluemouth from each area using the PCA method. Thus, allometry was further analyzed using the regression vectors from a multivariate regression for specimens within each of the study areas.

Multivariate regression

The allometric trajectories for bluemouth from each area and between sexes were determined using a multivariate regression and the results are presented below. The trajectories for each area are represented individually using scatterplots of shape scores as a function of log centroid size. These shape scores were defined by projecting the shape data onto a line in the direction of the regression vector for log centroid size. Then, differences between the growth trajectories (between areas and between sexes) were determined by comparing the angles between the regression vectors in each case. Finally, visualizations of the shape changes that take place during growth are presented using warped outline drawings.

Growth trajectories by area

Ontogenetic allometry was present in the majority of studied areas, as shown by the statistically significant multivariate regressions (Table 3-2), and the scatter plots between shape scores and centroid size used to visualize growth trajectories (Fig. 3-1 and 3-2). The only cases where significant allometry was not detected was for bluemouth from Portugal and Sicily. In both cases, samples were obtained from commercial vessels, where the size range of the bluemouth specimens that are caught is limited and largely determined by the fishing method and the characteristics of the fishing area itself. Therefore, the allometric trajectories for these areas were not defined accurately because allometric trajectories are best defined when very small and very large specimens are included in the sampling design (Klingenberg, 2007).

However, the amount of shape variation accounted by the significant regressions differed considerably among the studied areas, ranging from 4.57 for Catalonia to 24.13 for the Gulf of Cadiz. For Catalonia, the growth trajectory might not be accurately represented despite the significant relationship between shape and size, because the sample for this area consisted mainly of small specimens with a mean size of 10.71 cm CS. Thus, the results for this area should be interpreted with some caution. Also, some areas from the Iberian Peninsula showed a considerable amount of dispersion around the growth trajectory (e.g. the Cantabrian Sea, the Alboran Sea and Alicante). We examined more closely these locations to see if there was any pattern indicating a possible substructure of the bluemouth sample within these areas that could explain the observed

dispersion and thus be considered in the study. It seems that only in the case of the Alboran Sea, it appears that there are two different growth trends (Fig. 3-3), one shown by bluemouth specimens caught mainly along the coastline (subarea A1, N = 171) and another one presented by specimens caught off the coast, on the slopes of the Alboran Island at the position 35º58.44´N, 2º49.53´W (subarea A2, N = 67). Thus, we carried out separate regressions of shape on size for each of the subareas in the Alboran Sea (A1 and A2) and both were statistically significant ($p < 0.01$), however, the amount of shape variation accounted by the regressions was noticeably different (7.77% for subarea A1 and 22.82% for subarea A2).

Table 3-1. Size-related variation (allometry) associated to each of the first five PC's (expressed in percentage of the shape variation accounted by each PC).

Area	N	PC1	PC2	PC3	PC4	PC5
NE Atlantic						
Porcupine Bank	182	17.42**	30.59**	4.51**	6.94**	7.92**
Cantabrian Sea	119	28.58**	1.79	0.12	5.52**	3.19
Galicia	191	5.32**	60.38**	0.42	0.20	2.88**
Portugal	60	0.50	0.27	6.66**	1.02	0.00
Gulf of Cadiz	75	55.54**	2.65	3.43	21.26**	3.21
Mediterranean Sea						
Alboran Sea	238	8.38**	35.48**	13.87**	6.23**	1.24
Alicante	134	14.24**	36.26**	12.65**	2.53	0.21
Catalonia	73	0.24	0.00	45.90**	6.15**	1.31
Sicily	48	2.82	4.67	16.44**	1.72**	0.01

** Significant regression ($p < 0.01$) between the PC and log Centroid size.

Table 3-2. Results of the multivariate regression of shape on size for bluemouth specimens within the studied locations.

Area	N	% of predicted shape variation related to size
NE Atlantic		
Porcupine Bank	182	12.0634**
Cantabrian Sea	119	16.2398**
Galicia	191	8.9842**
Portugal	60	1.4792 $^{n.s.}$
Gulf of Cadiz	75	24.1378**
Mediterranean Sea		
Alboran Sea	238	9.0962**
Subarea A1	171	7.7699**
Subarea A2	67	22.8231**
Alicante	134	11.2923**
Catalonia	73	4.5719*
Sicily	48	4.0089 $^{n.s.}$

** Significant at the 1% level ($p < 0.01$)
* Significant at the 5% level ($p < 0.05$)
$^{n.s.}$ Not significant at the 5% level ($p > 0.05$)

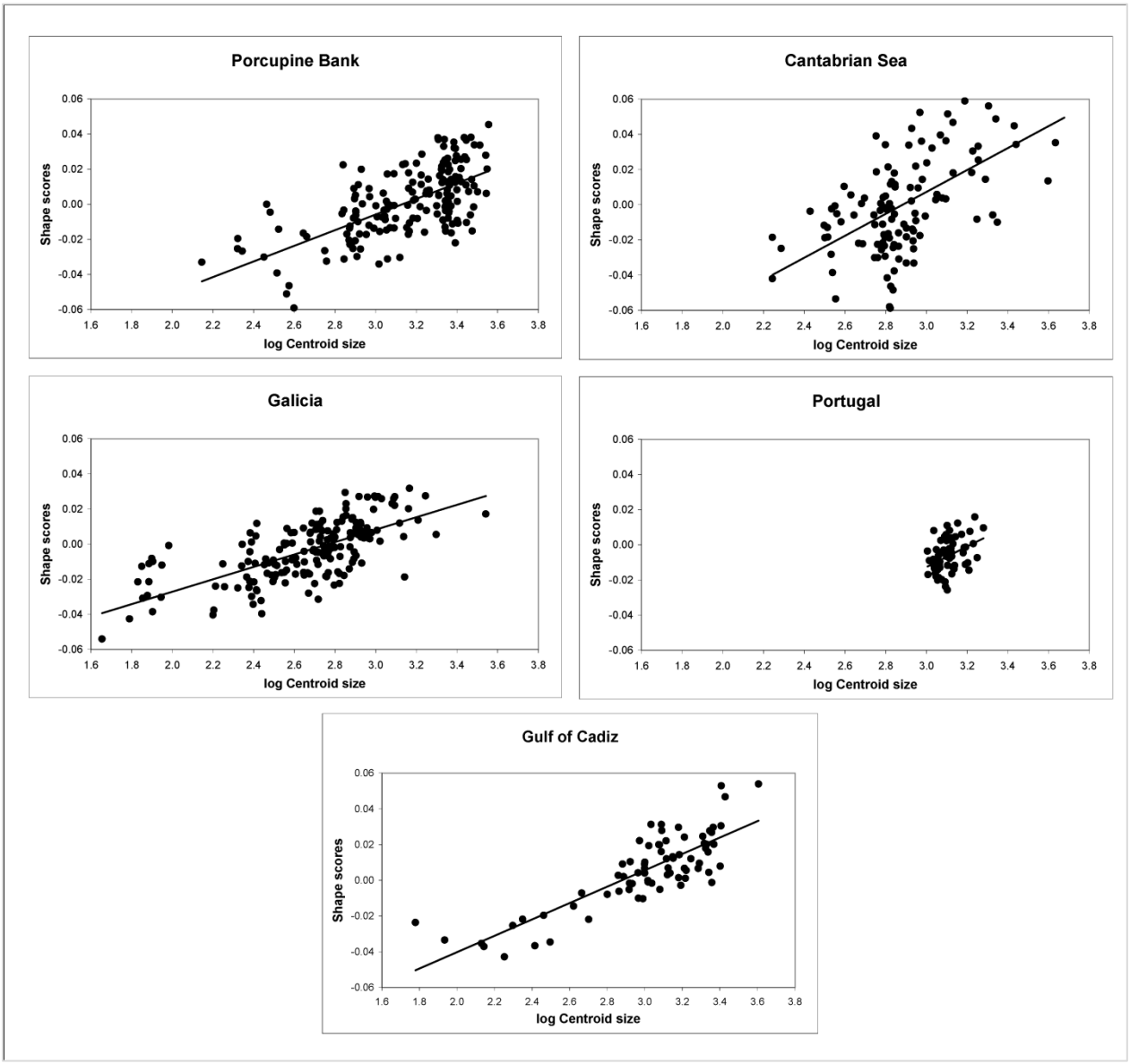


Figure. 3-1 Ontogenetic allometry of bluemouth from the studied areas in the Northeast Atlantic. The growth trajectories are represented with shape scores as a function of log (Centroid size).

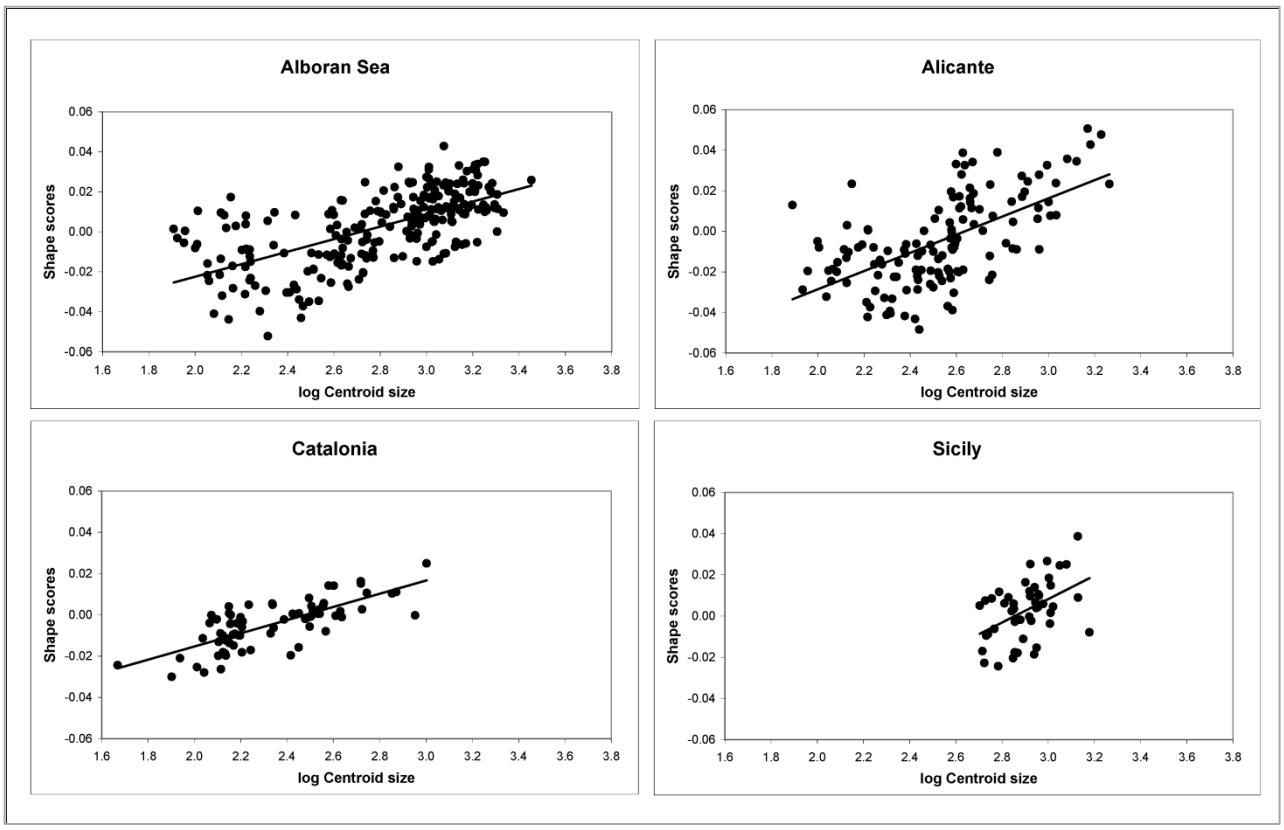


Figure. 3-2 Ontogenetic allometry of bluemouth from the studied areas in the Mediterranean Sea. The growth trajectories are represented with shape scores as a function of log (Centroid size).

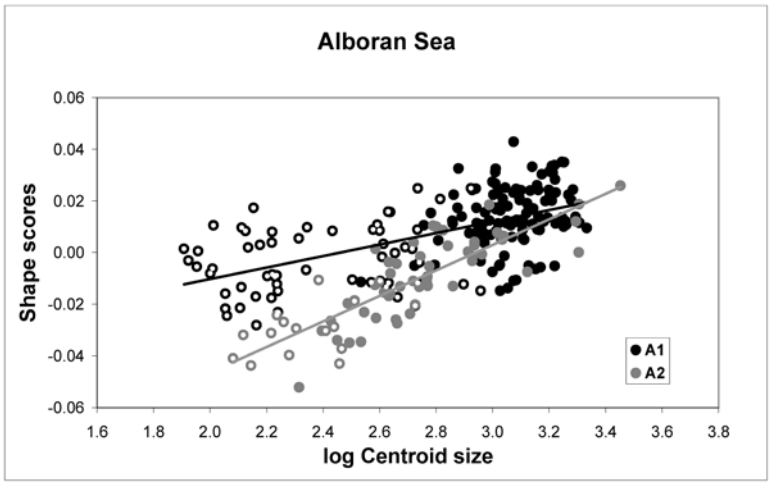


Figure. 3-3 Growth trajectories for bluemouth from the two subareas in the Alboran Sea (A1 and A2). The trajectories are represented with shape scores as a function of log (centroid size). Filled circles indicate sexed specimens (males and females) and open circles indicate unsexed specimens.

Growth trajectories by sex.

Multivariate regressions of shape on size were also done for males and females separately within each of the study areas in which significant allometric shape variation was detected (Table 3-3). Therefore, the samples from Portugal and Sicily were excluded from this analysis. Ontogenetic allometry was detected for both sexes from all the NE Atlantic samples, since the relationship between shape and size was statistically significant ($p < 0.01$) but no statistical differences between growth trajectories of males and females within these locations were found at the 5% level (Table 3-4). In the Mediterranean locations, the analysis could not be done for the sample from Catalonia because the number of specimens where sex was recorded was too low (6 males and 9 females only). For the Alboran Sea, the regressions for both sexes from subarea A2 were significant ($p < 0.01$), but those for subarea A1 were not ($p > 0.05$) for both males and females). In the case of subarea A1, the growth trajectories were probably not well defined (and therefore not significant), because the size range of the sexed specimens was very limited. From the 114 males and females in the sample, there was only one specimen smaller than 15 cm CS or 2.7 log centroid size (Fig. 3-3). Thus, we did not compare the growth vectors for males and females from subarea A1 and we decided to use sexed and unsexed specimens together (N = 171) to determine the growth trajectory for comparison with other areas. For subarea A2, the angle between ontogenetic vectors of males and females was of 44.8º and the 95th percentile of the ranges of the within-sex angles were 40.9º for females and 44.6º for males. Although the inter-sex angle was significant at the 5% level, its value was very close to the 95th percentile of the range of angles for the males and this result should also be interpreted with caution. As with subarea A1, we also used all the available specimens from subarea A2 (N = 67) to determine the growth trajectory for comparison with other areas. For Alicante, the regressions of shape on size for males ($p < 0.01$) and females ($p < 0.05$) were significant (Table 3-3) and the growth trajectories were similar for males and females in this area (Table 3-4).

Table 3-3. Results of the multivariate regression for males and females within the studied locations. The shape variation predicted by each regression is expressed as a percentage of the total shape variation. The regressions for Catalonia were not done due to insufficient sexed specimens in the area. The samples from Portugal and Sicily were excluded from this analysis because no significant allometric shape variation was detected (Table 3-2).

Area	Sex	N	Predicted shape variation (%)
NE Atlantic			
Porcupine Bank	Females	67	9.95**
	Males	108	15.26**
Cantabrian Sea	Females	64	21.18**
	Males	48	15.64**
Galicia	Females	87	9.69**
	Males	75	11.26**
Gulf of Cadiz	Females	35	30.38**
	Males	31	11.33**
Mediterranean Sea			
Alboran Sea	Females	78	20.77**
	Males	89	12.35**
Subarea A1	Females	54	3.17 n.s.
	Males	60	1.99 n.s.
Subarea A2	Females	24	23.71**
	Males	29	20.96**
Alicante	Females	35	9.48*
	Males	27	11.94**
Catalonia	Females	9	-
	Males	6	-

** Significant at the 1% level ($p < 0.01$)
* Significant at the 5% level ($p < 0.05$)
n.s. Not significant at the 5% level ($p > 0.05$)

Table 3-4. Angle between growth trajectories of males and females by area. The comparison between growth trajectories of males and females from subarea A1 were not done because the regressions for males and females were not significant at the 5% level. The regressions for Catalonia were not done due to insufficient sexed specimens in the area. The samples from Portugal and Sicily were excluded from this analysis because no significant allometric shape variation was detected (Table 3-2).

Area	Angle
NE Atlantic	
Porcupine Bank	32.8 $^{n.s.}$
Cantabrian Sea	18.3 $^{n.s.}$
Galicia	32.4 $^{n.s.}$
Gulf of Cadiz	34.1 $^{n.s.}$
Mediterranean Sea	
Alboran Sea - Subarea A2	44.8*
Alicante	28.8 $^{n.s.}$
Catalonia	-

* Significant at the 5% level ($p < 0.05$)
$^{n.s.}$ Not significant at the 5% level ($p > 0.05$)

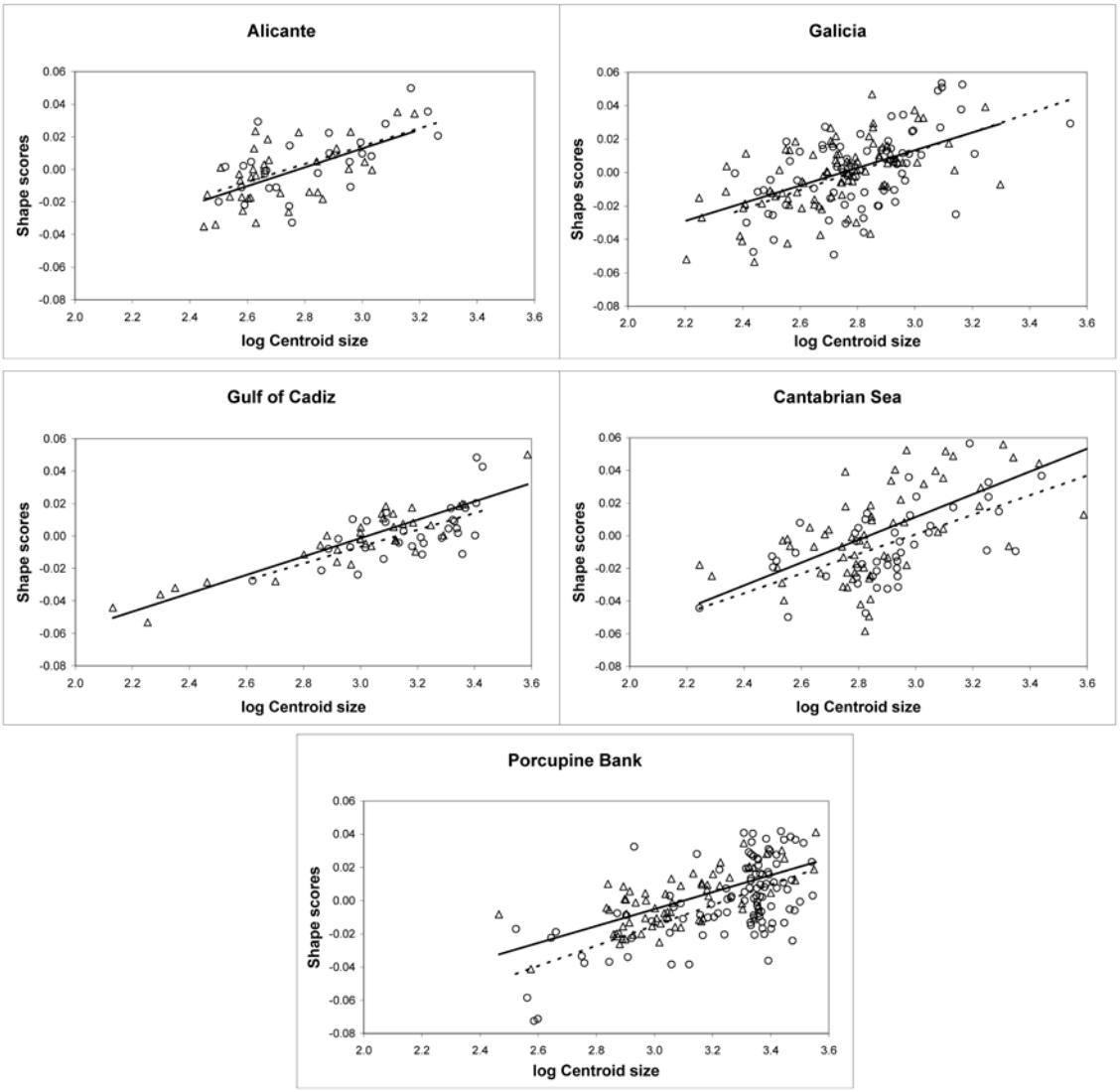


Figure. 3-4 Growth trajectories for bluemouth males (open black circles and dashed line) and females (open black triangles and solid line) for some of the studied areas in the Northeast Atlantic and Mediterranean. The growth trajectory for the Alboran Sea is shown separately in Fig. 3-2 (see Results- Growth trajectories for males and females section). . The regressions for Catalonia were not done due to insufficient sexed specimens in the area. The samples from Portugal and Sicily were excluded from this analysis because no significant allometric shape variation was detected (Table 3-2).

Comparison of growth trajectories between areas

Growth trajectories were compared pairwise by calculating the angle between the regression vectors of the studied areas (Table 3-5). We did not find any clear pattern of geographical variation for the differences between growth trajectories. Bluemouth from the Gulf of Cadiz showed similar ontogenetic shape changes to those from Galicia, subarea A2 in the Alboran Sea, Alicante and Catalonia, indicating that there are no growth patterns specific to the NE Atlantic or the Mediterranean Sea only. Interestingly, the growth trajectories for the two subareas within the Alboran Sea differed considerably. Only the growth trajectory for bluemouth from the Cantabrian Sea was different to all others.

Shape changes during growth

The patterns of shape changes during growth of bluemouth are shown in Fig. 3-5 for the Mediterranean locations and in Fig. 3-6 for the NE Atlantic locations. In general, the shape changes associated with increases in size in bluemouth specimens consisted of: a) a relative expansion of the area comprised by landmark 9 (midpoint of the insertion of the pectoral fin), landmark 11 (tip of the second preopercular spine) and landmark 12 (midpoint of the jaw end), b) a contraction of the head area in relation to body size accompanied in most cases by an upward shift of the tip of the snout and c) a dorsoventral expansion together with a relative shortening of the body. Thus, as expected, we observed a trend towards a more robust body as the fish become larger. Still, some specific shape changes were identified in bluemouth specimens from the Cantabrian Sea. The estimated shape for large specimens showed a considerable up-rightward displacement of landmark 10 (end of the operculum), an up-leftward displacement of landmark 5 (insertion of the hypural plate) and a larger downward displacement of landmarks 8 (insertion of the ventral fin) and 9 (insertion of the pectoral fin).

Table 3-5. Results for the pairwise comparisons of the growth vectors for bluemouth from the studied areas.

Area	**NE Atlantic**				**Mediterranean Sea**			
	Porcupine Bank	Cantabrian	Galicia	Cadiz	Alboran (A1)	Alboran (A2)	Alicante	Catalonia
NE Atlantic								
Porcupine Bank	0							
Cantabrian Sea	41.8*	0						
Galicia	38.8*	49.6*	0					
Gulf of Cadiz	20.5	29.3*	34.7	0				
Mediterranean Sea								
Alboran Sea (A1)	51.8*	76.1*	40.2*	57.4*	0			
Alboran Sea (A2)	30.9*	26.3*	38.8*	20.5	62.5*	0		
Alicante	31.9*	29.7*	35.7*	20.9	57.9*	27.4	0	
Catalonia	60.9*	70.1*	45.1	57.0	54.5	66.4*	51.1	0

* Growth trajectories are significantly different at the 5% level ($p < 0.05$).

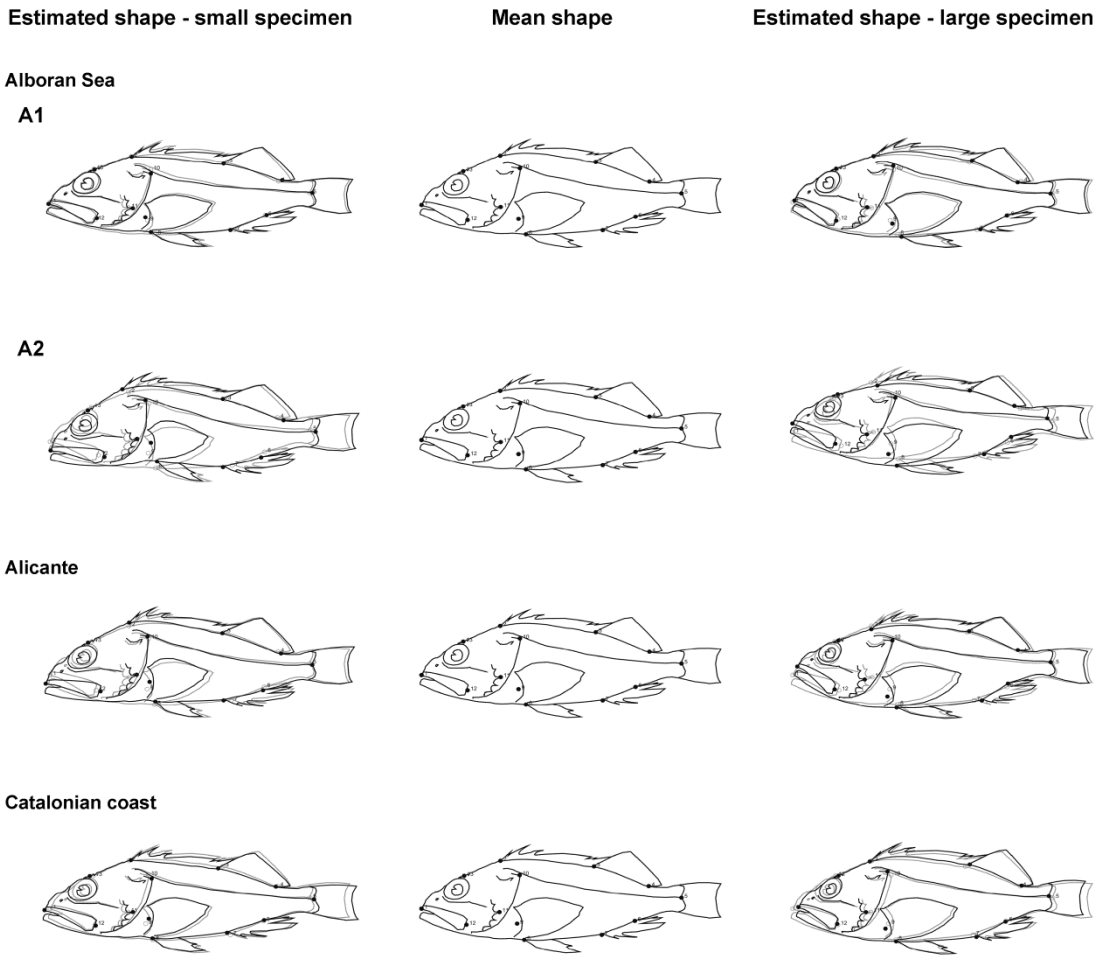


Figure 3-5 Visualization of shape changes associated to growth for bluemouth from the western Mediterranean. The mean shape is shown in the center and is also represented as light grey outline drawings in the figures in the left and right columns. Left column: the black outline shows the shape change for a decrease in log Centroid size by 1.5 units, representing the estimated shape for a small specimen. Right column: the black outline shows the shape change for an increase in log Centroid size by 1.5 units, representing the estimated shape for a large specimen.

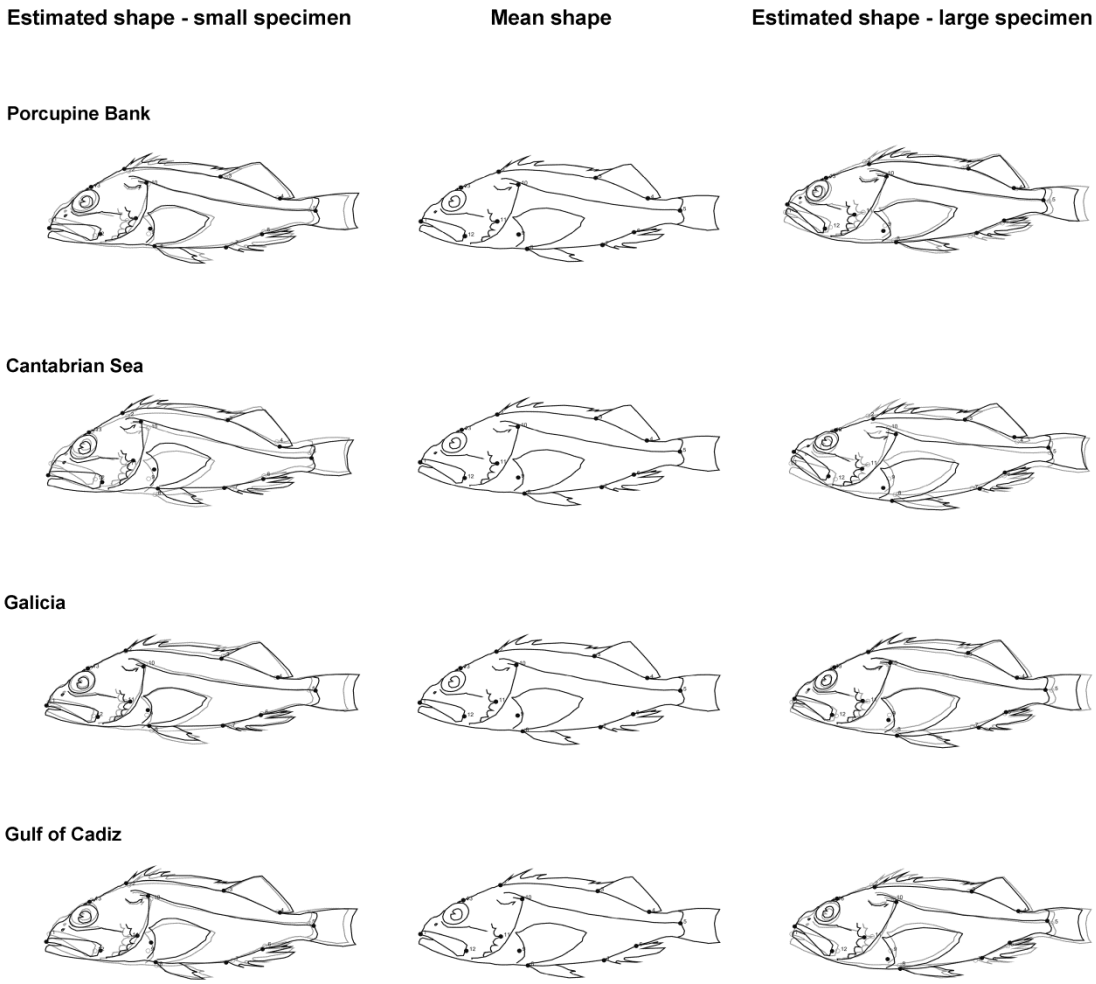


Figure 3-6 Visualization of shape changes associated to growth for bluemouth from the NE Atlantic. The mean shape is shown in the center and is also represented as light grey outline drawings in the figures in the left and right columns. Left column: the black outline shows the shape change for a decrease in log Centroid size by 1.5 units, representing the estimated shape for a small specimen. Right column: the black outline shows the shape change for an increase in log Centroid size by 1.5 units, representing the estimated shape for a large specimen.

3.3.2 Comparison of the size-correction methods

One of the main objectives of this chapter was to determine the best method to remove the effects of allometric size on the shape variables. For that purpose, a comparison of the results from five methods commonly used for size-correction was done. The methods considered in this section are: a) a principal components analysis (PCA), b) Burnaby's method, c) a size-and-shape PCA, d) an overall multivariate regression and d) a pooled within-group multivariate regression (see chapter 2 - Material and Methods). For each method, the characteristics of the allometric vector and the results of a discriminant function analysis (DFA) using size-corrected variables were considered. Two bluemouth populations, assumed to be different, were selected to carry out the comparison. In this way, the method that yielded a good characterization of the allometry present in the two-population dataset and the lowest Wilk's lambda value and the highest correct classification rate in the discriminant analysis was considered the best method to eliminate allometric variation.

Originally, the reference areas (i. e., the Porcupine Bank in the NE Atlantic and Sicily in the central Mediterranean) were chosen to compare the effectiveness of different size correction methods, because these populations were assumed to belong to different stocks based mainly on the geographical distance that separates the sampling locations. However, no significant allometry was detected in the Sicilian sample (see Table 3-2). For that reason, one of the populations from the western Mediterranean was used. The sample from Alicante was chosen because a significant allometry was detected and considerable number of samples was available.

Thus, the bluemouth populations from the Porcupine Bank (n = 182) and Alicante (n = 134) were used to compare the success of the five methods in eliminating the size effect on shape variables. These populations were assumed to belong to different stocks based on a) the considerable geographical distance that separates the sampling locations, b) characteristics of the bluemouth such as adult sedentarism (Uiblein et al., 2003; Pakhorukov, 2008) and c) the environment in these locations, for example, the closed circulation patterns in the Porcupine Bank (White et al., 2005) and existence of possible oceanographic barriers such as the Strait of Gibraltar or the Almería- Oran Front (Tintoré et al., 1988).

Before the comparison, it was determined that the two populations differed in size composition ($t_{(311.698)} = 19.709$; $p<0.01$). The mean centroid size of bluemouth from the Porcupine Bank was 24.25 ± 6.03 cm while the mean size in the Alicante sample was 13.09 ± 4.06 cm. Allometry was present in both of the samples, as indicated by a significant relationship between size and shape according to the within-group multivariate regressions (Table 3-2). In addition, growth trajectories between the Porcupine Bank and Alicante were found to be significantly different at the 5% level (Table 5).

The results of each method and the comparison of the five methods are presented below.

PCA and Burnaby's method

Both methods use a principal component analysis (PCA) of the pooled within-group covariance matrix to characterize the allometric trajectory, always using a single PC, usually PC1, to extract the size-related shape variation. To find out which PC characterized the best the allometric trajectory, a PCA was first carried out using the two selected bluemouth samples, from the Porcupine Bank and Alicante (N = 316). Then, the amount of size-related shape variation in each PC was calculated by performing a regression of each PC on log Centroid size (Table 3-6).This analysis showed that PC1, PC2, PC3 and PC6 contained significant size-related variation ($p<0.01$) according to a permutation test using 10000 replicates. The percentage of size-related variation in PC1, PC2, PC3 and PC6 was 3.57%, 60.33%, 3.08% and 2.45% respectively. This result shows that PC2 contains most of the size-related variation, therefore, it was decided to use this PC as the allometric vector needed for size-correction in the PCA and Burnaby's method. Nevertheless, when PC2 is used as the allometric vector, a small amount of size-related variation (i. e., that contained in PC1, PC3 and PC6) will remain present in the dataset (Table 6). Therefore, the effect of size will not be removed entirely and this will need to be considered when interpreting the results of these methods. Moreover, an entire dimension will be removed in both methods and it is possible that meaningful shape information (not related to size) will be lost.

Table3-6. PCA of the dataset Porcupine-Alicante and results of the regression of the first 10 PCs on log CS to determine the amount of size-related variation.

	Distribution of the variation in the dataset by PC (%)	Cumulative distribution of the variation in the dataset by PC (%)	Size-related variation in the PC (%)	Size-related variation expressed as percentage of the total variation in the dataset (%)
PC1	**41.896**	**41.896**	**3.5688** *	**1.4951**
PC2	**11.611**	**53.507**	**60.3299** *	**7.0049**
PC3	**7.517**	**61.025**	**3.0757** *	**0.2312**
PC4	6.969	67.994	0.8567	0.0597
PC5	4.174	72.168	0.1153	0.0048
PC6	**3.759**	**75.926**	**2.4491***	**0.0920**
PC7	3.433	79.360	0.0941	0.0032
PC8	3.078	82.437	0.3741	0.0115
PC9	2.726	85.163	0.0209	0.0005
PC10	2.168	87.331	0.1992	0.0043

*****Significant according to permutation test .using 10.000 replicates, p-value: < 0.01.

In the PCA method, PC2 was removed from the dataset and the remaining 21 PCs were used as shape variables in a discriminant function analysis (DFA). Significant differences between bluemouth from the two geographical areas were found (Wilks' λ = 0.7845, $F_{(21,\ 294)}$ = 3.6571, p< 0.01). The overall correct classification rate (from the Jackknifed classification matrix) was of 63.8%.

The adjusted shape variables obtained from Burnaby's correction method were used in a DFA. As with the PCA method, significant shape differences between the two bluemouth samples were found (Wilks' λ = 0.5211, $F_{(21,\ 294)}$ = 12.8659, p < 0.01). The overall correct classification rate was of 81.3%. The results of the DFA are summarized also in Table 3-7 below.

Size-and-shape PCA

The first PC of the PCA done on the covariance matrix of Procrustes coordinates with the log Centroid size added accounted for 99.24% of the total size-and-shape

variation. This PC was removed and the remaining 22 PC'S were used as shape variables in a DFA to evaluate the results of the size-correction. According to the DFA on the size-corrected shape variables, significant differences among bluemouth from the two geographical areas were found (Wilks' $\lambda = 0.7845$, $F_{(22, 293)} = 3.6571$, $p < 0.01$) (Table 3-7). The correct classification rate from the Jackknifed classification matrix was of 63.8%. In the case of size-and-shape PCA, it is important to point out that the PC1 extracted from the size-and-shape PCA represents the common allometry shared by all the specimens and that group structure is not considered.

Overall multivariate regression

The overall multivariate regression was significant ($p<0.01$) and it accounted for 9.0057% of the total shape variation. As in the previous method, the estimated allometric vector also represents the common allometry shared by all specimens with no consideration of the group structure in the sample. Then, the residuals of this regression were used as size-corrected shape variables in a DFA. In the DFA conducted on the size-corrected shape variables, significant overall differences between bluemouth samples from the Porcupine Bannk and Alicante were detected (Wilks' $\lambda = 0.7848$, $F_{(22, 293)} = 73.423$, $p < 0.01$) (Table 3-7). The correct classification rate from the Jackknifed classification matrix was of 64.2%.

Pooled within-group multivariate regression

Here, the pooled within-group multivariate regression was used to estimate an allometric vector. From all the methods that were compared, this is the only one that considers the group structure in the dataset. The regression was significant ($p<0.01$) and it accounted for 18.4232% of the total shape variation. In the DFA using the regression residuals as size-corrected shape variables, significant differences between bluemouth from the two geographical areas were also found (Wilks' $\lambda = 0.3534$, $F_{(22, 293)} = 315.138$, $p < 0.01$) and the classification success from the Jackknifed classification matrix was of 87.7% (Table 3-7).

Comparison of the five size-correction methods

The allometric vector was defined either as a principal component in the case of the PCA, Burnaby's and size-and-shape PCA methods or as a regression vector in the overall and pooled within-group multivariate regressions. The characterization of an allometric vector in the PCA and Burnaby's method was not straightforward, because several PCs contained significant shape variation. Nevertheless, size-related variation was concentrated in PC2, which represented about 7% of the total variation in the dataset. Size-related variation in the allometric vector obtained using an overall multivariate regression represented about 9% of the total variation in the dataset. When the group structure was taken into account using the pooled-within group regression, a higher amount (about 18%) of size-related variation was captured in the allometric vector.

In the case of the size-and-shape PCA, the first PC accounted for 99.24% of the variation in the dataset. This was expected because the amount of size variation (as measured by log CS) far exceeds the amount of shape variation (as measured by Procrustes distance). Consequently, the PC1 of the size-and-shape PCA represents the variation of size itself and those shape features that are linearly associated with it. As a result, this method did not provide directly an estimate of the amount of allometric shape variation alone and could not be compared to the estimates provided by the other methods. In addition, the first PC in this method only represents the overall allometric trajectory (that is shared by all of the groups), and within-group size-related shape differences that might be present in the remaining PC's are ignored.

From these results, the allometric vector obtained using a pooled-within group regression captured the highest amount of size-related shape variation.

The five DFA analyses indicated significant differences between the two bluemouth samples, however the values of Wilks' lambda were variable, ranging from 0.7848 to 0.3534 (Table 3-7). Since Wilk's lambda approaches zero if the groups are well separated, the best group separation was obtained with the size-corrected variables from the pooled within-group regression (Wilk's lambda = 0.3534). The classification success, ranged from 63.8 % to 87.7% (Table 3-7), and the highest rate was achieved with the pooled within-group regression method. Thus, results from the DFA performed using the pooled within-group regression showed both the lowest Wilks' lambda value and the highest percentage of classification success, indicating the best discrimination of the two

bluemouth samples. Overall, the pooled within-group regression yielded the best results for size-correction of the dataset.

Table3-7. Comparison of the results of a discriminant analysis using size-corrected variables obtained from different size-correction methods

	Size-and-shape PCA	**PCA**	**Burnaby's method**	**Overall regresion**	**Pooled within-group regression**
Wilk's lambda	0.7845	0.7535	0.5211	0.7848	0.3534
F statistic	3.6571[a]	85.880 [b]	197.822 [b]	3.6518[a]	24.3640[a]
P-value	<0.01	<0.01	<0.01	<0.01	<0.01
Classification success[c] (%)	63.8	70.3	81.3	64.2	87.7

[a] $F_{(22,293)}$
[b] $F_{(21,294)}$
[c] from Jackknifed classification matrix

3.4 Discussion

Body form in fishes is a product of their ontogeny (Cadrin, 2005). It is affected by the genetic makeup of an individual but it also reflects adaptation to environmental factors such as temperature, food availability, feeding mode, swimming behavior and habitat use (Barlow, 1961; Wimberger, 1992; Swain et al., 2005). During the growth of fishes, body proportions change as the larvae and juvenile fish adapt to habitat and diet transitions until they reach adulthood. According to our results, bluemouth specimens from both NE Atlantic and Mediterranean locations seem to follow a pattern of ontogenetic shape changes that is probably related to the changing ecology of the species over the course of its life history: bluemouth juveniles have a streamlined body shape during their pelagic stage (Furlani, 1997 and references therein) while adults have robust but flexible muscular bodies typical of benthic sit-and-wait predators (Webb, 1984; Uiblein et al., 2003).

For most of the studied areas, ontogenetic shape changes were most evident in the head and pectoral area, affecting the position of the snout, preopercular spines and pectoral fins, but changes in body depth and length were also important.(Figs. 3-5 and 3-6). Changes in body depth and length are mostly related to swimming capacity and locomotor adaptations to food capture and escape from predators (Webb, 1984). Functionally, mouth shape changes have also many repercussions in the life of fish. This is because mouth morphology plays an essential role in determining the type of prey consumed and morphological variations can lead to changes in foraging/ predation ability and subsequently differential exploitation of food resources (Karpouzi & Stergiou, 2003).

In this sense, the observed changes in mouth shape and position are very likely to be related to ontogenetic changes in the diet of bluemouth. In general, their diet consists of benthic decapod crustaceans (Natantia, Brachyura and Macrura), demersal fish and sometimes pyrosomes, polychaetes and echinoderms (Macpherson, 1979, 1985; Nouar & Maurin, 2000; Serrano et al., 2003), but the proportions of these prey types in their diet vary according to the size of the fish. For example, Macpherson (1979) reported that the diet of small bluemouth individuals from 4 to 9 cm in the Mediterranean consisted mainly of fish (51.9%) such as silvery pout (*Gadiculus argenteus argenteus*) and gobies (*Deltentosteus quadrimaculatus* and *Lesueurigobius friesii*) and decapods like *Alpheus glaber* (20.9%), *Calocaris macandreae* (5.9%) and *Goneplax rhomboides* (4.2%). In

contrast, the main prey of adult specimens (20 - 29 cm in length) was the decapod crustacean *Goneplax rhomboides* (49.4%), followed by other decapods such as *Calocaris macandreae* (17.6%) and *Alpheus glaber* (14.1%) and a small percentage of pyrosomes (9.4%) and fish (8.2%). A more recent study by Consoli et al. (2010) showed a shift on feeding habits between small (4.0–6.3 cm TL) and larger bluemouth. In particular, small fishes feed mainly on mysids with a preference for *Lophogaster typicus* whereas adults are feeders of reptantian decapods (mostly *G. rhomboides*). In that study, the ontogenetic shift toward bigger prey was related to a size increase in the mouth gape of adult fishes and the contribution of prey types appeared to be depth related, maybe because of the different composition of the macrobenthonic communities along the bathymetric gradient. In the case of *Helicolenus percoides*, ontogenetic diet changes have been also observed, as the proportions of Crustacean and fish are inversely related as length increases. For this species, Brachyura were the single most important prey in fish of less than 20 cm, but they were replaced by *Pyrosoma atlanticum* and teleosts in larger size classes (Blaber & Bulman, 1987).

However, the degree to which the above described ontogenetic shape changes were present in bluemouth from each of the studied areas was different, reflecting the differences in growth trajectories that we found in this study. The factors that cause these growth differences are likely to be complex. Phenotypic variation can result from either genetic differentiation or phenotypic plasticity. Genetic information on bluemouth populations is still scarce. To our knowledge, only one study has focused on the genetic population structure of the bluemouth in the North Atlantic (the Azores, Madeira and Cape Verde, the coast of Portugal (Peniche) and the Northwest Atlantic (off the coast of South Carolina, USA) (Aboim et al., 2005). In that study, using mitochondrial DNA, some genetic differentiation was detected between populations within the NE Atlantic region (Azores, Peniche and Madeira) but limited sample sizes and the poor resolution of phylogenetic analyses limited the interpretation of the data. A later study using microsatellites, however, revealed genetic isolation of the Peniche population and some differentiation at the local scale within the Azores archipelago (Aboim, 2005).

Phenotypic plasticity is the ability of a genotype to produce different phenotypes in response to different environmental stimuli (Wimberger, 1992). In fishes, as with most indeterminately growing organisms, the influence of the environment on life history traits is realized primarily through factors that affect body size and the rate at which body size

changes throughout an individual's life (Swain et al., 2005). Therefore, fish growth and survival depend on many components of the habitat in which fish live i.e., prey resources, predation risk, temperature, sediment type, water depth, etc. (Hayes et al., 1996). In addition to environmental factors, growth in fish can be affected by population density and fishing mortality (Rochet, 1998; Law, 2000; Sánchez Lizaso et al., 2000). In general, size structure of deep-sea fishes has been shown to be different between the NE Atlantic and the Mediterranean (Tortonese, 1960; Stefanescu et al., 1992), resulting therefore in differences in growth as well. More recently, Massutí et al. (2004) compared the deep-sea fish assemblages between these areas and they also found evidence that for almost all species, those in the Mediterranean tended to grow to a smaller adult size. As a consequence, these fish will have smaller mouths and will therefore use a different component of the available food resources (Massutí et al., *op. cit*.). The authors of that study suggested that the primary cause of the differences observed in size structure is a result of adaptations at both the species and ecosystem level to different trophic relationships between these two areas. However, they also indicated that a high temperature in the Mediterranean (~13ºC compared to ~10ºC in the eastern Atlantic areas) could also play an important part in explaining size structure differences. In the same study, size differences between NE Atlantic and Mediterranean bluemouth were found, as the minimum size of captured specimens for locations in the NE Atlantic was at least double than that found in the Mediterranean, and the maximum size was found in the Porcupine Seabight (west off Ireland, NE Atlantic).

In our study, bluemouth from the NE Atlantic generally attained larger sizes but we did not find that NE Atlantic growth patterns were clearly differentiated from those presented by bluemouth from Mediterranean locations. For example, bluemouth specimens from the Gulf of Cadiz, which is located next to the Strait of Gibraltar, exhibited similar ontogenetic shape changes to bluemouth from Galicia and the Porcupine Bank (NE Atlantic) but also to bluemouth from subarea A2 in the Alboran Sea and Alicante (western Mediterranean). In our study, bluemouth from the Cantabrian Sea presented a unique growth pattern, probably caused by a combination of factors (i.e., food availability along with a low fishing mortality and singular environmental conditions). The Cantabrian Sea is a well delimited area in the Bay of Biscay with particular characteristics that differentiate it from the rest of the Atlantic (Sánchez, 1993) and it also supports an important demersal ecosystem (Le Danois Bank) where no regular fishery

operates, allowing for a well-preserved bluemouth spawning stock (Sánchez et al., 2008). In the Cantabrian Sea at the summit of Le Danois Bank where bluemouth are more abundant (400 – 550 m depth), some of the decapods that are considered to be the main preys of adult bluemouth (i.e. the crab *Goneplax rhomboides*, and the shrimps *Calocaris macandreae* and *Alpheus glaber*) are scarce or even absent due to the low proportion of mud in the sediments, which is required by these burrowing species (Cartes et al., 2007). Therefore, morphological adaptations of the snout in bluemouth from the Cantabrian Sea could arise as the fish use other food resources in this area. In contrast, these decapods are very abundant in other areas considered in this study, such as the southern part of the Galician shelf and the upper slope, where there are fine sediments due to outwelling from the Rías Baixas (Fariña et al., 1997).

On the Mediterranean coasts of the Iberian Peninsula, the abundance of *Goneplax rhomboides*, *Calocaris macandreae*, and *Alpheus glaber* also varies in the different geographical sectors, being most abundant in the Alboran Sea and the northern Catalonia (Abelló et al., 2002). In general, the Alboran Sea has been described as an area with particular hydrographical characteristics due to the influence of Atlantic waters and with a high productivity within the general oligotrophic context of the Mediterranean (Massutí et al., 2001; Abad et al., 2007). Interestingly, the growth trend presented by bluemouth from subarea A1 in the Alboran Sea was different from the one exhibited by bluemouth from adjacent areas, including subarea A2 also in the Alboran Sea. It has been suggested the existence of a well-developed bluemouth spawning stock in the Alboran basin, contrary to what it was found in the areas with a high fishing pressure north of the Alboran Sea where older fish are poorly represented (Massutí et al., 2001). In a more recent study, Abad et al. (2007) also found a high abundance of bluemouth on the small seamount Seco de los Olivos in the eastern Alboran Sea, which is an area where trawled sandy bottoms are interspersed with rocky bottoms, and food is highly available due to strong localized currents and upwelling. Thus, food availability on the continental slope of the Alboran Sea in combination with a lower fishing mortality and the oceanographic conditions in the area are likely to produce a different growth pattern that the ones observed in adjacent areas.

Bluemouth caught in subarea A2 in the Alboran basin showed a similar growth pattern to the ones observed in contiguous areas, both in the Atlantic and in the Mediterranean, i.e. Gulf of Cadiz and Alicante, respectively. There is a possibility that a

group of individuals from these areas migrated to subarea A2, because occasional migrations of adult specimens may occur (Aboim, 2005), or that very particular environmental conditions exist in that location that affect the growth of these individuals. In any case, further study is needed to determine the factors that cause different growth patterns within the Alboran basin and the temporal and spatial stability of the observed patterns has to be confirmed.

In the present study, we also compared growth trajectories between males and females. Information about sexual dimorphism is required for understanding the ecology, behavior, and life history of a fish species (Kitano et al., 2007), and allometry has been suggested to be a main component of sexual shape dimorphism because it accounts for size dimorphism (Gidaszewski et al., 2009). Up to the present, only differences in sexual size dimorphism and growth rates between sexes have been studied for the bluemouth in the NE Atlantic and Mediterranean (White et al., 1998; Kelly et al., 1999; Massutí et al., 2000a; Abecasis et al., 2006; Ribas et al., 2006; Sequeira et al., 2009). However, both of these topics are still being studied for the bluemouth, as some of these authors have found that females grow faster and achieve a larger asymptotic length, while others have found the opposite trend and in some studies no differences in growth rates were detected at all. However, these discrepancies could be related to differences in the length ranges sampled in the various studies (Sequeira et al., 2009). For other species of the same genus, such as *Helicolenus percoides* in the South-eastern Australian waters, growth rates of the sexes seemed to be comparable but females attained a larger size (Withell & Wankowski, 1988), although a recent study found that males grew slightly faster than females (Paul & Horn, 2009). In the case of *Helicolenus lengerichi*, no differences in growth rates were observed between males and females (Petrova & Chekunova, 1979, as cited in Withell & Wankowski, 1988).

Regarding ontogenetic shape changes, no differences in the growth patterns of males and females was observed within any of the NE Atlantic locations. However, in the Mediterranean, we could only compare growth trajectories for males and females from two of the five areas included in this study: Alicante and subarea A2 in the Alboran Sea. More specifically: a) the analysis could not be done for the sample from Catalonia because the number of specimens where sex was recorded was too low; b) The comparison between growth trajectories of males and females from subarea A1 was not done because the regressions for males and females were not significant; and c) the

sample from Sicily was excluded from this analysis because no significant allometric shape variation was detected (see Table 3-2).

For Alicante, we did not find differences in allometric growth between sexes. However, our study was inconclusive about possible differences between sexes for subarea A2 due to a relatively low sample size and because the angle between the regression vectors was marginally significant. Perhaps in future studies, a combination of the study of growth rates and allometric shape changes between sexes can be used to better understand sexual dimorphism in bluemouth populations.

In fisheries, differences in life history parameters between groups of fish are assumed to be evidence that populations are geographically and/or reproductively isolated and can be considered discrete stock units for management purposes (Ihssen et al. 1981; Begg, 2005). In this sense, the information provided in the present study can be used to complement further studies regarding stock identification of bluemouth around the Iberian Peninsula (which is the matter of the next chapter). Moreover, in the context of stock identification, morphological discrimination among groups of fish is often difficult because samples may differ in size composition and because allometric growth is taking place. This situation implies the risk of confounding real differences between fish populations with accidental differences in size composition of the samples. Thus, in morphometric studies, it is necessary to eliminate shape variation associated with size before we can compare multiple groups (Burnaby, 1966; Mosimann, 1970; Humphries et al., 1981; Thorpe 1976, 1983; Rohlf & Bookstein, 1987; Klingenberg, 1996). According to our results, bluemouth from around the Iberian Peninsula and the Porcupine Bank exhibit allometric growth. Therefore, this fact has to be taken into account if morphological comparisons of bluemouth from different areas are to be made for the purpose of stock identification in Iberian waters.

In this study, five methods commonly used for size-correction of shape variables were tested to determine the best method to eliminate allometry of a test dataset that included two bluemouth samples (Porcupine Bank and Alicante). These methods were: a PCA, Burnaby's method, a size-and-shape PCA and the total and pooled within-group regression. However, in the PCA method and Burnaby's method, the characterization of a single allometric vector was not straightforward, because significant size-related shape variation was distributed along several PCs.

In fact, PCA may not work well for geometric morphometric data for two reasons: first, because no single PC may have a clear association with size, with allometric effects distributed over several PC's (Klingenberg unpublished course material); and second, because during the Generalized Procrustes analysis (see chapter 2 - *Materials and Methods*), isometric size is factored out from the samples in the rescaling step. In this way, only in cases where allometric growth is substantially present will the first principal component be associated with size (Slice & Stitzel, 2004). Another disadvantage of these methods is that an entire dimension is removed from the analysis, i.e., all the variation in the direction of the allometric axis, and in this way other information with biological significance may be removed as well.

In contrast, the regression approaches do not present this drawback, because only the part of shape variation that is predicted by size variation is removed (Klingenberg, 2008). In this study, the size-and-shape PCA was found to be equivalent to the method using the overall multivariate regression for the size-correction. The similarity of the results of the size-and-shape PCA and the total multivariate regression is due to the fact that both methods use the common (overall) allometric trajectory to estimate allometry, that is, none of these methods takes into account the group structure in the dataset (e. g., Cardini & Elton, 2008). On the contrary, the pooled within-group regression does consider the group structure and it actually enhances group separation by eliminating the within-group allometric variation. The improvement of group separation in the DFA performed with the residuals of the pooled within-group regression was reflected in a lower Wilks' lambda value and a higher correct classification rate. Also, the pooled within-group regression also explained a higher amount of shape variation (18.42%) than the overall multivariate regression (9.00%), which is a fact that must be also considered in the choice of the size-correction method for the population structure analysis. Taking into account the above mentioned, the best choice for size-correction of the shape variables in the population structure dataset would be to use the residuals of the pooled within-group regression.

However, the central assumption of all the size-correction methods is that the groups in the analysis must share the same allometric trajectories (Klingenberg, 1996), and in this study we found evidence that the growth trajectories for bluemouth between the samples from the Porcupine Bank and Alicante were not homogeneous (i. e., parallel),

representing a problem for size correction of the shape variables that should be addressed prior to morphometric analysis.

Nevertheless, Klingenberg (2009) indicated that in practice, if the allometric regressions in the different groups are not drastically different from each other, the pooled within-group regression can still provide a 'compromise' estimate of allometry that can be used for size correction. In our case, because some differences were found among allometric trajectories, the residuals from the regression on size will be slightly correlated with size within groups. However, because the variation within groups was reduced substantially when the pooled within-group regression was used, the group discrimination was enhanced. Thus, to remove allometry and the differences in size composition of the bluemouth samples in the studied areas, the size-correction of the shape variables using the residuals of a pooled-within area regression is an adequate method for the morphometric comparison performed in the next chapter.

Another way to avoid ‘size-effects’ would be to compare samples with similar size compositions or to only use fish of the same size (selective sampling). However, for demersal species like the bluemouth, it is not easy to obtain homogeneous samples from all of the study areas, because most of the time samplings depend on fisheries that target other species (e.g. European hake, *Merluccius merluccius*, or Blue and red shrimp, *Aristeus antennatus*) and the size range of the captured specimens in each area can be affected by factors such as depth and the type of bottom of the fishing area and fishing gears used, i.e. trawling nets, long-lines or gill-nets (Demestre et al., 2000; Massutí et al., 2001; Santos et al., 2002). In addition, bluemouth samples from trawling research surveys also vary in size composition, as in this study. In the case of size selective sampling, the shape variation outside the chosen size range is ignored, the covariance is reduced and the ability to distinguish groups is therefore weakened (Cadrin, 2000). Another potential drawback of size selective sampling is that if the growth rate is very different among putative populations and it is uncoupled from shape changes, we could be comparing specimens of very different ages and thus resulting in confounding effects.

3.5 Concluding remarks

In this study, geometric morphometric techniques allowed us to determine and visualize ontogenetic shape trajectories for bluemouth specimens from several areas in the NE Atlantic and Mediterranean, mostly around the Iberian Peninsula. The general pattern of ontogenetic changes seemed to be related to the changing ecology of the species (i.e., ontogenetic diet and habitat adaptations) and consisted of a relative expansion of the area between the second preopercular spine and the pectoral fin, a relative deepening and shortening of the body and an upward shift of the snout as the head becomes more compact in relation to the body. However, the degree to which the above described ontogenetic shape changes were present in bluemouth from each of the studied areas was different, indicating that the growth trajectories are not homogeneous. The factors that cause these growth differences are likely to be complex, but a combination of factors such as food availability along with a low fishing mortality and unique environmental conditions is likely to produce distinctive growth patterns such as the ones that we found in areas like the Cantabrian Sea and the Alboran Sea. For the purpose of fisheries management, these observed differences in the way that bluemouth grow could be an indicator that different populations exist and should be further studied.

However, if morphological comparisons are to be used as a tool to identify phenotypic stocks, the fact that growth differences exist should be considered because most size-correction methods assume equal or parallel growth trajectories to remove the effect of size from shape variables. In this study, from the five methods for size-correction of the shape variables that were tested, the best results were obtained with the pooled within-group regression method. Although growth differences were detected among bluemouth from the different study areas, this method provides a 'compromise' estimate of allometry that can be used for size-correction of the bluemouth population structure.

Finally, this kind of shape information could be also used to complement traditional growth curves, showing what shape changes occur and when they take place during growth.

Chapter 4

Population Structure Based on Geometric Morphometrics and Meristics

4.1 Introduction

As described in the general introduction in Chapter 1, the analysis of a species' population structure is of primary importance in developing an optimal strategy for its efficient management (Coyle, 1998). Moreover, information on the biological differences between discrete groups within a species is necessary to understand the genetic and ecological processes that influence structuring of populations (Maclean & Evans 1981 in Coyle, 1998). In fisheries, self-sustaining components within natural fish populations (i.e., stocks) are usually identified based on genotypic and/or phenotypic features (Cadrin et al., 2005).

Phenotypic stocks are groups of fish characterized by phenotypic differences such as meristic, morphometric and life history characters. A powerful tool for the identification of phenotypic stocks based on morphometric characters is the application of geometric morphometric techniques (Cadrin, 2005). These techniques have been useful in separating populations of a variety of marine fish (e.g., Corti & Crosetti, 1996; O'Reilly & Horn, 2004; Silva, 2003; Murta et al. 2008).

4.1.1 Population structuring of marine fish in the NE Atlantic and Mediterranean

In general, three hypotheses may explain population structuring in marine pelagic and demersal fish species (Palumbi, 1994; McLean et al., 1999; Avise, 2000; Bahri-Sfar et al., 2000): (1) Environmental factors, including past sea level changes, and present or past physical barriers such as ocean currents, may disrupt fish populations from different geographic locations; (2) increasing geographical distance is expected to enhance isolation among populations; (3) life history traits, including potential for dispersal, homing to spawning zones, and larval retention, may play also an important role in population structuring (Zardoya et al., 2004)

The Strait of Gibraltar has been proposed to be the division between two important marine biogeographical regions, the Mediterranean Sea and the Northeast Atlantic (Borsa et al., 1997). The western Mediterranean is a subtropical, semi-enclosed area separated from the Atlantic by a sill in the Strait of Gibraltar, with a high degree of

environmental stability for both temperature (12.8 – 13 ºC) and salinity (38 – 38.6 ‰) below a depth of 200 m (Hopkins, 1985; Massutí et al., 2004). Several environmental factors could cause overall morphological differences between fish populations from the NE Atlantic and the Mediterranean. For example, food availability and the partitioning of the main trophic resources, i.e. among mostly fish in the Atlantic and between decapods and fish in the Mediterranean (Masssutí et al., 2004). Also differences in salinity and temperature between the basins (12.8 – 13ºC in the Mediterranean and 10 – 4ºC in the eastern Atlantic) have been indicated as causes yielding to morphological differences (Ellett et al., 1986; Hopkins, 1985; Rice et al., 1991).

Massutí and coworkers (2004) detected differences in the biomass structure between Mediterranean and Atlantic deep-sea fish assemblages using data derived from a series of bottom trawl surveys carried out between 1978 and 1998. In their study, they observed that when the same species occurred both in the Mediterranean and the Atlantic, those in the Mediterranean tended to attain a smaller adult size.

Intraspecific studies have shown a reduction of gene flow between the two basins, but 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). Thus, the differentiation pattern between the Atlantic and Mediterranean cannot be considered of general validity, not even for species with comparable ecological features. Clear examples of this are the inconsistencies that have been observed within sparids (Bargelloni et al., 2003). Experimental data (genetic and morphometrics) from some sparids (e.g. *Dentex dentex* and *Lithognathus mormyrus*) lend strong support to the presence of a phylogeographical boundary between the Atlantic and the Mediterranean located in the Almeria- Oran oceanographic front (Tintoré et al., 1988). For other sparids, like *Spondyliosoma cantharus*, *Pagrus pagrus* and *Pagellus bogaraveo*, genetic data provided little evidence for a separation between the two basins.

Inconsistencies between different methods used in stock identification have also been observed. A recent morphometric study of horse mackerel showed a clear distinction between Atlantic and Mediterranean samples (Murta et al., 2008). However, when this results where compared to the genetic results of an integrated study using other stock identification approaches, no genetic differences were found between horse mackerel from the two basins (Abaunza et al., 2008).

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 Finisterrae (Sánchez and Gil, 2000; Sánchez and Serrano, 2003; Serrano et al. 2008), and in the western Mediterranean, the Almeria-Oran front (Tintoré et al., 1988; Roldán et al., 1998, Naciri et al., 1999).

4.1.2 Bluemouth population structure in the NE Atlantic and western Mediterranean

At the present, the population structure of bluemouth in terms of age and size is relatively well studied in the following areas: a) the Mediterranean (western Mediterranean: Massutí et al., 2000a and b, 2001 and Ribas et al., 2006; central Mediterranean: Consoli et al., 2010 and Pirrera et al., 2009); b) the Northeast Atlantic (Rockall Trough: Kelly et al., 1999; North Sea: Heessen et al., 1996, Portuguese waters including Azores Archipelago, mainland Portugal and Madeira: Esteves et al., 1997, Abecasis et al., 2006, Sequeira et al., 2009) and c) the Northwest Atlantic (off the coast of the Carolinas, USA: White et al., 1998).

In most of these studies, differences in the composition/structure of bluemouth populations inhabiting different geographical sectors have been found. For example, in the western Mediterranean, Massutí et al. (2001) and Ribas et al. (2006) have observed that in the Alboran Sea a mature population is found in high densities, but as we move away from this nucleus, the density decreases, the mortality increases and the mean total length decreases and approaches to the first sexual maturation (13 – 14.5 cm). A bathymetrical distribution pattern has also been observed in the same studies, where the recruits of the year inhabit preferentially the shallower waters; juveniles occupy intermediate depths and then, at greater depths, the larger specimens (which make up the reproductive stock) are found. The differences in the population structure and distribution patterns of *H. dactylopterus* along the western Mediterranean, related to bathymetrical and latitudinal trends have been related to variety of direct and indirect factors of biogeographic, environmental and anthropogenic origin (Massutí et al., 2001).

A similar structure has been observed in the northeastern fishing ground (Galicia-Cantabria), where the most heavily exploited area, below 400m, has small-sized fish, while in the deep, less exploited areas and in the submarine canyons like Le Danois Bank, considerably larger fish are found (e.g. Sánchez et al., 2008 and Serrano et al., 2008).

However, the population structure in terms of stock components (phenotypic and/or genotypic) of bluemouth in the NE Atlantic and the Mediterranean has received less attention. Virtually all studies that have been focused on delineation of bluemouth stocks have been carried out in only in Portuguese waters (i.e. Azores archipelago, Madeira and mainland Portugal). Thus, the information of the population structure of bluemouth in the NE Atlantic and the Mediterranean is still limited.

To our knowledge, only one study has compared bluemouth populations from the NE Atlantic and Mediterranean with the aim of stock identification by analyzing otolith composition. In that study, few identifiable trends were detected between the ocean basins and it was concluded that the composition of the *H. dactylopterus* otolith nuclei was not sufficiently different for consistent discrimination between fish from the different sampling sites (Swan et al., 2006).

In the studies carried out in Portugal, a variety of techniques have been used to identify bluemouth stocks, and in all of them, some degree of differentiation between the studied bluemouth populations has been found: Mitochondrial DNA markers revealed strong genetic differentiation between the NE Atlantic populations in Portuguese waters (Azores, Madeira and Peniche), the populations from Cape Verde Islands and the NW Atlantic, but the evidence of genetic differentiation within the NE Atlantic region (i.e., between the island groups and seamounts in the Azores, Madeira and the Portuguese continental slope) was weak (Aboim et al., 2005). However, when microsatellites were used, the data revealed isolation of Peniche and some differentiation at the local scale within the Azores archipelago (Aboim, 2005). Unfortunately, no genetic studies have been carried out using other bluemouth samples from the continental slopes of Spain and Portugal that could clarify the extent of genetic differentiation of this species around the Iberian Peninsula.

The growth rates for bluemouth from the Portuguese continental slope were determined by Sequeira et al. (2009). When comparing their results with those published from other areas, differences among all the estimated growth parameters were evident. Several factors that could have affected growth rates were identified in that study (e.g., the different method used for reading otoliths, the heterogeneity of the size composition in the samples, the different environmental conditions, different latitudes and different fishing pressures). Nevertheless, the authors point out that the differences may be attributable to the fact that *H. dactylopterus* dwell mostly around submarine mountains in

the neighbourhood of deep canyons and lead a rather sedentary existence and may constitute local populations.

The comparison of body shape of bluemouth from Portuguese waters (i.e., Azores, Madeira and mainland Portugal) also showed that different stocks may exist (Sequeira et al., 2011a). The body shapes of bluemouth differed significantly among the studied areas and a considerable morphological heterogeneity within the Azores group was observed, which could reflect a substructure of the bluemouth population within this area. The similar conclusions were drawn from a study using otolith shape analysis in the same areas (Neves et al., 2011).

Finally, the macroparasite assemblage infecting bluemouth in three different areas off the Portuguese coast was evaluated by Sequeira et al. (2010) in order to assess their use as biological tags in stock identification. Anisakidae larvae presented different prevalence and mean abundance levels between the three areas and a multivariate discriminant analysis applied to the macroparasites species revealed a high differentiation among the three sampled areas suggesting at least three different bluemouth stocks in Portuguese waters. The differences found in parasite assemblages between localities might, therefore, be due to differences in the type and quantity of prey consumed, suggesting the ecological differentiation of *H. dactylopterus* populations inhabiting the three areas, i.e. the existence of ecological stocks.

In Chapter 3, differences among growth trajectories of bluemouth were detected. These differences in the way bluemouth grow could be an indicator that different populations exist in the NE Atlantic and Mediterranean. In this chapter, the population structure is investigated using geometric morphometrics and meristics.

4.2 Objectives

In this study, it was hypothesized that morphological differences could have arisen in bluemouth populations around the Iberian Peninsula (NE Atlantic and Mediterranean) as a result of:

(1) Isolation (at least partially) due factors like boundary effects of biogeographical limits (e.g., the Strait of Gibraltar between the NE Atlantic and Mediterranean), the species' sedentary behavior (Uiblein et al., 2003, Pakhorukov, 2008), a limited larval dispersal in the pelagic stage (Aboim, 2005) and the geographical distance existing between the different study areas.

(2) Environmental differences between the studied locations such as temperature, salinity, quantity and type of prey available, etc.

(3) Anthropogenic factors like different fishing pressure on bluemouth populations in the different locations.

Thus, a landmark-based geometric morphometric analysis was conducted to assess body shape variation among specimens of bluemouth, in order to identify phenotypic stocks around the Iberian Peninsula. The locations included were: the Cantabrian Sea, Galicia, Portugal (Peniche), and the Gulf of Cadiz in the Northeast Atlantic; and the Alboran Sea (subareas A1 and A2), Alicante and Catalonia in the Mediterranean Sea. Additionally two reference areas were sampled as well, one in the Atlantic, the Porcupine Bank and another in the Central Mediterranean, Sicily. Body shape differences between males and females within each study area were also analyzed to determine if overall sexual dimorphism exists. Meristic characters were analyzed to complement the information provided by morphometric characters.

4.3 Results

4.3.1 Sexual dimorphism

The comparison of mean body shapes between males and females using the parametric Hotelling's T^2 test showed significant differences for some of the populations, i.e., Porcupine Bank, Portugal and both subareas in the Alboran Sea, but not for the remaining areas (Table 4-1). The shape comparison between males and females using permutation tests with Procrustes and Mahalanobis distances also yielded heterogeneous results (Table 4-2). The results of the permutation tests using Mahalanobis distances were in accordance with the results of the parametric T^2 test. However, the results between Procrustes and Mahalanobis distances did not coincide in many cases. No significant morphometric differences were detected in some of the studied populations (i.e., Galicia, the Gulf of Cadiz and Alicante); the results of the tests were conflicting in the case of the Cantabrian Sea, Portugal, subarea A2 and Sicily; and significant differences between sexes were detected in both tests in the Porcupine Bank and subarea A1. For these two areas, the shape changes that characterized females and males were explored (Figure 4-1 and 4-2 respectively). The deformation of the mean shape of females into the mean shape of males for the Porcupine Bank showed displacements of landmarks 1 (tip of the snout), 3 (insertion of the second dorsal fin) and 9 (insertion of pectoral fin). Thus, subtle shape differences are visible in the head and in body depth. For subarea A1, the figure shows relative displacements of landmarks 3, 7 (insertion of anal fin) and 12 (end of mandible), indicating differences in the mouth and, more evidently, in body depth.

Nevertheless, based on the evidence provided by the analyses in this section, it is difficult to determine if overall sexual shape dimorphism exists in the bluemouth populations under study. In addition, the robustness of these results is questionable because a reduced sample size was used in most of the areas (Portugal, Gulf of Cadiz, subarea A2 in the Alboran Sea, Alicante and Sicily) (Tables 4-1 and 4-2) and the comparison for Catalonia was not performed due to a very low number of sexed specimens in the area (only 6 males and 9 females). Consequently, it was decided to use the samples with pooled sexes in population structure analysis in the next section.

Table 4-1. Results of the parametric Hotelling's T^2 tests carried out to compare mean shape between males and females in each location. The comparison for Catalonia was not performed due to insufficient sexed specimens in the area.

Area	Sample size	Hotelling's T^2	F (d. f.)
NE Atlantic			
Porcupine Bank	m = 108/ f = 67	100.7254	4.023 (22,152)**
Cantabrian Sea	m = 48/ f = 64	34.6633	1.275 (22,89) $^{n.s.}$
Galicia	m = 75/ f = 87	30.5123	1.205 (22,139) $^{n.s.}$
Portugal	m = 26/ f = 34	77.4510	2.246 (22,37)*
Gulf of Cadiz	m = 31/ f = 35	34.0005	1.038 (22,43) $^{n.s.}$
Mediterranean			
Alboran Sea –A1	m = 60/ f = 54	86.3003	3.187 (22, 91)**
Alboran Sea –A2	m = 29/ f = 24	76.2182	2.038 (22,30)*
Alicante	m = 27/ f = 35	48.5218	1.434 (22,39) $^{n.s.}$
Sicily	m = 29/ f = 19	58.0363	1.636 (22,25) $^{n.s.}$

m = males/ f = females

** Significant at the 1% level ($p < 0.01$)
* Significant at the 5% level ($p < 0.05$)
$^{n.s.}$ Not significant at the 5% level ($p > 0.05$)

Table 4-2. Results of the comparisons between the mean shapes of males and females within each area, based on Procrustes and Mahalanobis distances. Permutation tests with 10,000 runs were used to test the null hypothesis of no mean difference between sexes. The comparison for Catalonia was not performed due to insufficient sexed specimens in the area.

Area	Sex	N	Procrustes distance	Mahalanobis distance
NE Atlantic				
Porcupine Bank	Females Males	67 108	0.01210436**	1.5608**
Cantabrian Sea	Females Males	64 48	0.01353000**	1.1242 n.s.
Galicia	Females Males	87 75	0.00544723 n.s.	0.8704 n.s.
Portugal	Females Males	34 26	0.01039709 n.s.	2.2928*
Gulf of Cadiz	Females Males	35 31	0.00713610 n.s.	1.4381 n.s.
Mediterranean Sea				
Alboran Sea-Subarea A1	Females Males	54 60	0.00922679*	1.7426**
Alboran Sea-Subarea A2	Females Males	24 29	0.01105004 n.s.	2.4091*
Alicante	Females Males	35 27	0.01170293 n.s.	1.7842 n.s.
Catalonia	Females Males	9 6	–	–
Sicily	Females Males	19 29	0.01474672*	2.2485 n.s.

** Significant at the 1% level ($p < 0.01$)
* Significant at the 5% level ($p < 0.05$)
n.s. Not significant at the 5% level ($p > 0.05$)

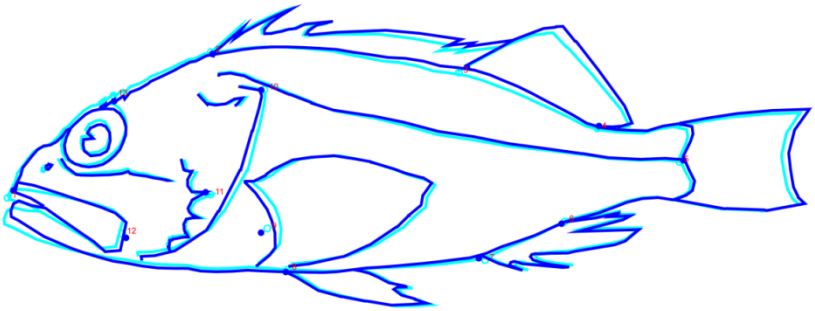


Figure 4-1. Visualization of the transformation from the mean shape of females (light blue outline) to the mean shape of males (dark blue outline) in the Porcupine Bank. Shape changes have been exaggerated three-fold for better visualization.

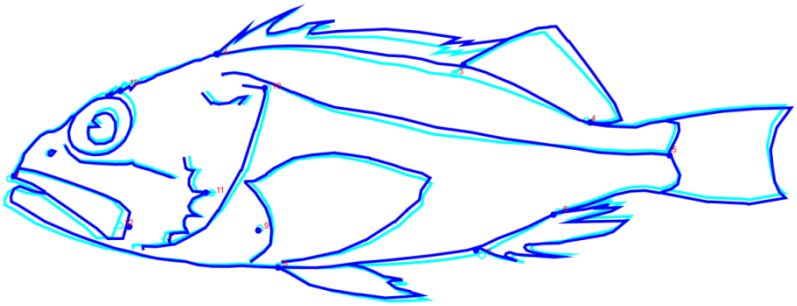


Figure 4-2. Visualization of the transformation from the mean shape of females (light blue outline) to the mean shape of males (dark blue outline) for subarea A1 in the Alboran Sea. Shape changes have been exaggerated three-fold for better visualization.

4.3.2 Overall morphometric analysis

Significant differences ($p < 0.05$) among the mean shapes of bluemouth specimens from all the sampled areas were found (Table 4-3). In the CVA, overall differences among the bluemouth samples were also detected. The value of Wilks' Lambda was 0.07658 and it was statistically significant: $F_{(242,12436)} = 15.26413$ $p < 0.0001$. The plot of the first three canonical variables is shown in Figure 4-3. The first CV (39.36 % of the variance explained) sets the four samples from Galicia and the Cantabrian sea apart from the remaining study areas. However, contrary to what was expected, significant shape differences were detected between the samples from 2006 and 2007 for both Galicia and the Cantabrian Sea. Since the samples for these two areas were obtained within the DEMERSALES survey with a time difference of one year only, the differences between them were analyzed separately to investigate if biological factors such as migration (mixing of the bluemouth specimens) or methodological issues (problems during the processing of the samples) had taken place. Thus, the samples from Galicia and the Cantabrian Sea caught in 2006 were excluded, and the overall analysis repeated with the remaining 10 areas. The results are presented below.

Table 4-3. Procrustes distances among the mean shapes of bluemouth of the studied areas. All of them are significant at $p < 0.05$.

Location	**Porc.**	**Cant. 07**	**Cant. 06**	**Gal. 07**	**Gal. 06**	**Portugal**	**Cadiz**	**A1**	**A2**	**Alicante**	**Catalonia**	**Sicily**
NE Atlantic												
Porc.	0											
Cant. 07	0.0213	0										
Cant. 06	0.0251	0.0187	0									
Gal. 07	0.0200	0.0097	0.0142	0								
Gal. 06	0.0278	0.0154	0.0132	0.0129	0							
Portugal	0.0205	0.0306	0.0360	0.0319	0.0384	0						
Cadiz	0.0218	0.0177	0.0297	0.0223	0.0245	0.0285	0					
Mediterran Sea.												
A1	0.0333	0.0256	0.0344	0.0278	0.0290	0.0443	0.0202	0				
A2	0.0174	0.0227	0.0289	0.0239	0.0304	0.0268	0.0195	0.0264	0			
Alicante	0.0238	0.0151	0.0237	0.0179	0.0194	0.0347	0.0140	0.0146	0.0185	0		
Catalonia	0.0367	0.0248	0.0300	0.0265	0.0237	0.0488	0.0252	0.0129	0.0323	0.0162	0	
Sicily	0.0196	0.0254	0.0352	0.0290	0.0335	0.0174	0.0165	0.0332	0.0197	0.0254	0.0392	0

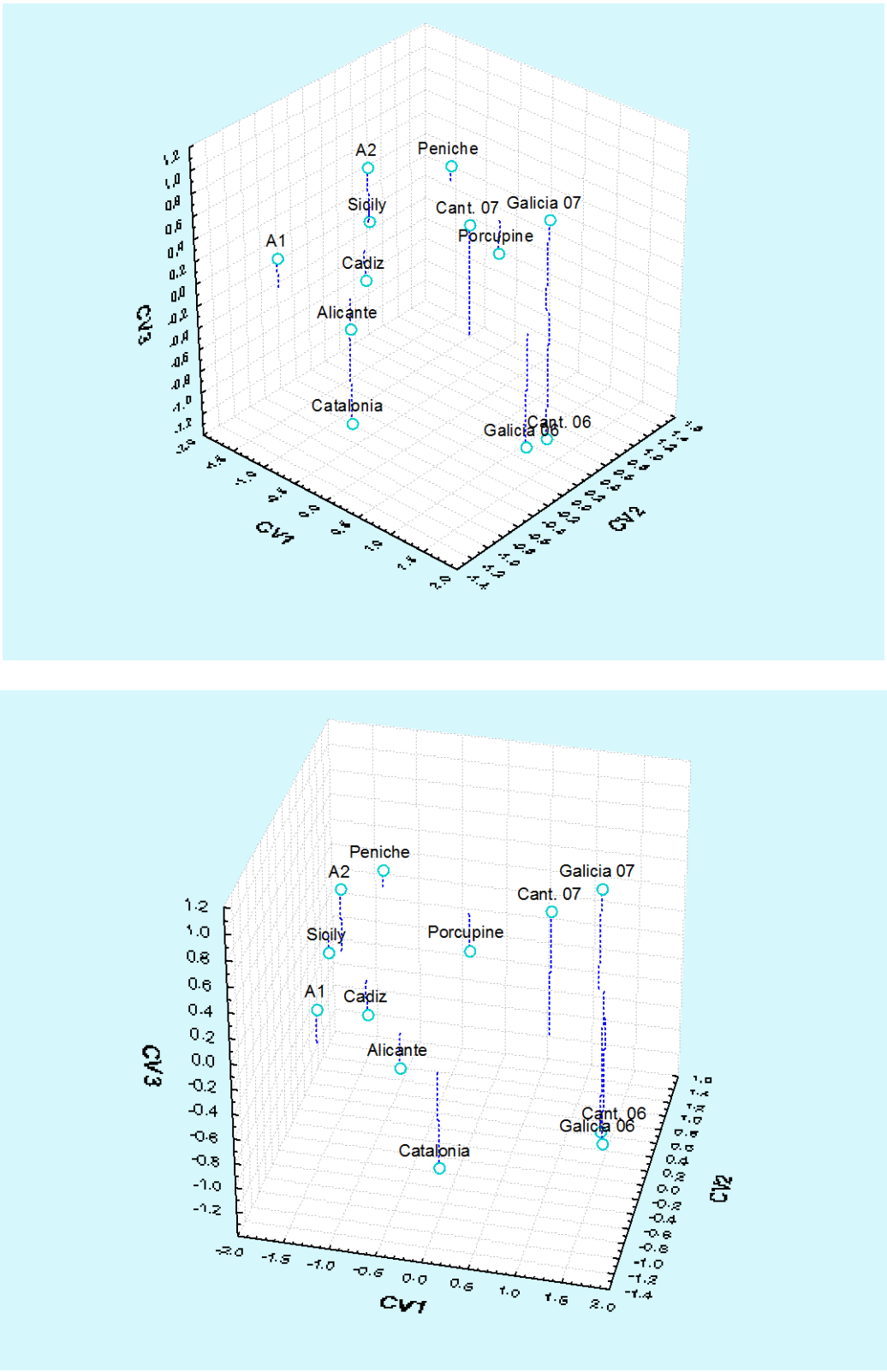


Figure 4-3. Two different views of the CVA plot showing the discrimination of the 12 bluemouth samples. The first three canonical variates shown in this graph accounted for 69.1% of the among-group variation: CV1 (39.4%), CV2 (17.5%) and CV3 (12.2%).

4.3.3 Overall morphometric analysis (excluding samples from 2006)

Significant differences ($p < 0.05$) among the mean shapes of bluemouth from the 10 areas were found (Table 4-4). Procrustes distances between the mean shapes ranged from 0.0100 to 0.0489 and the largest body shape difference was found between bluemouth from Portugal and Catalonia. In fact, the amount of shape variation between the mean shape for Portugal and those of the remaining areas (mean Procrustes distance of 0.0314 units), is the highest of all, indicating a strong morphological differentiation of bluemouth from this area.

The pairwise comparisons based on the Mahalanobis distances also revealed significant morphological differences among bluemouth from all of the 10 locations (all of p-values < 0.05). 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 & Monteiro, 2005). Therefore, the relationship among the bluemouth groups was investigated using these distances and the results were depicted with a dendrogram using an average linkage method (i.e., UPGMA) (Figure 4-4). In the dendrogram, three small clusters can be observed. The first cluster shows that Alicante and Catalonia are the most similar groups and they cluster closely to 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 seems to be well separated from the two previous clusters. Interestingly, subarea A2 in the Alboran Sea clustered to the other Mediterranean locations at higher distance, and seems to define a distinct area. Similarly, Portugal did not cluster together with any other locations, and it was located between the Galicia- Cantabrian Sea cluster and the rest of locations.

Table 4-4. (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) Location	**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) Location	**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

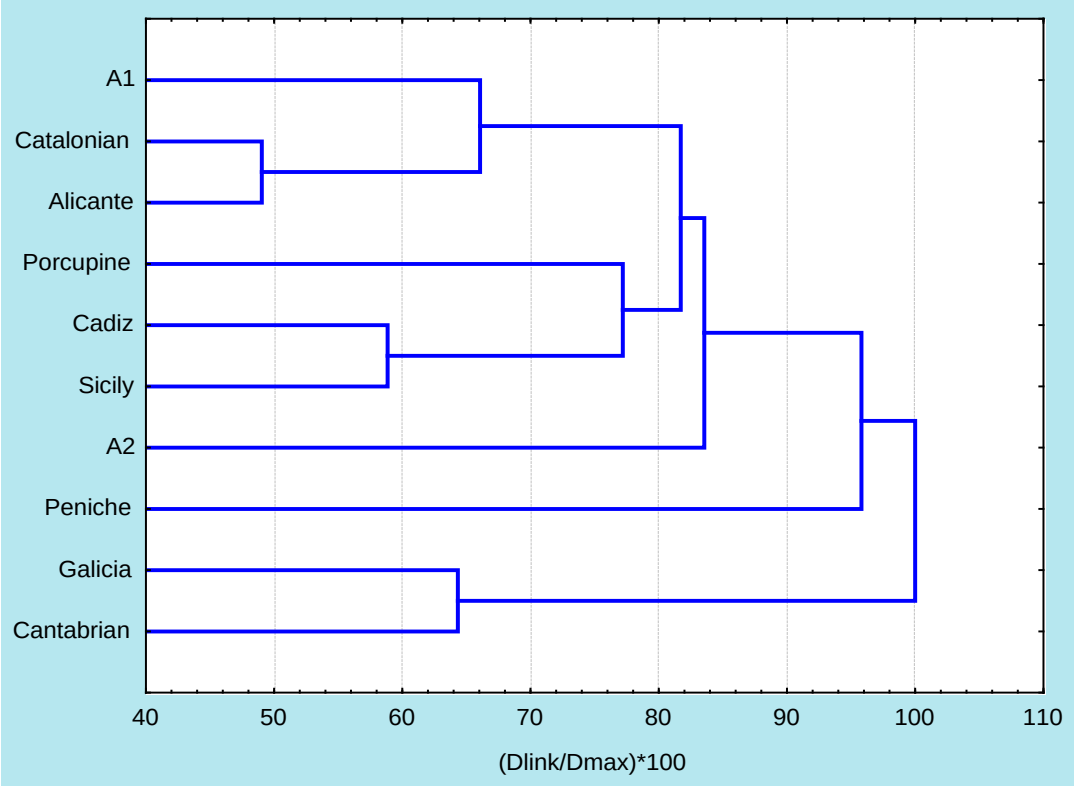


Figure 4-4. Cluster analysis linkage distance plot based on the Mahalanobis distances among the 10 groups of bluemouth considered in this study.

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 4-5) showed a similar picture as the one observed in the Cluster analysis, however, it was more difficult to see the degree of separation for some areas. The first canonical variable separated Galicia and the Cantabrian Sea from the rest of locations while the second canonical variable allowed the distinction between: i) Alicante, Catalonia and subarea A1, ii) the Porcupine Bank and Portugal and iii) Sicily, subarea A2 and the Gulf of Cadiz. Finally, CV3 differentiated each population within the above described groups (Figure 4-5). Overall, the largest differences were observed between Galicia-Cantabrian Sea and the rest of locations, and the most similar locations were Cadiz and A2. Thus, contrary to what was shown in the cluster analysis, the CVA plot showed a different relationship among Cadiz, subarea A2 and Sicily.

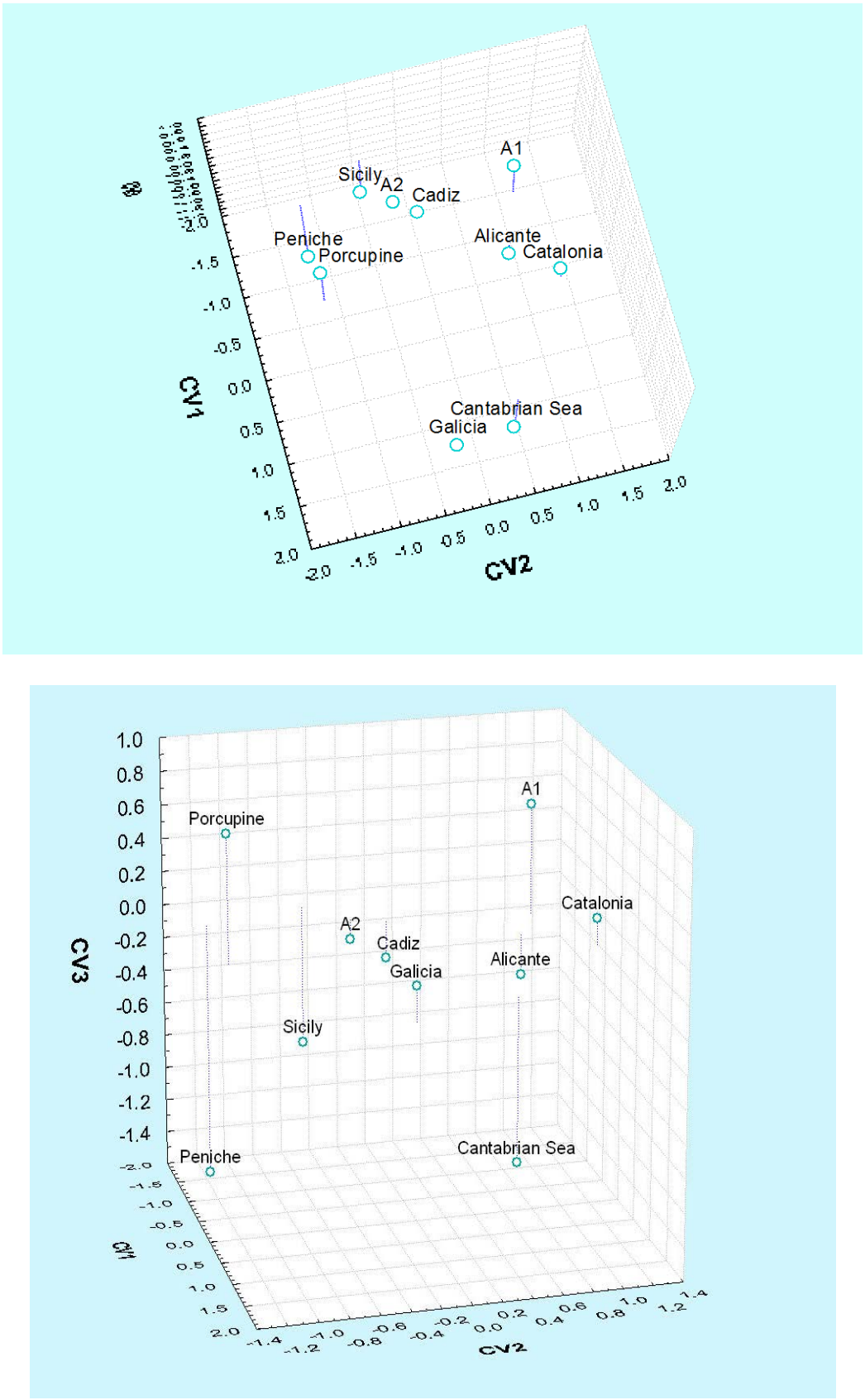


Figure 4-5. Three different views of the CVA plot showing the discrimination of the 10 bluemouth samples (continued on the next page). The first three canonical variates shown in this graph accounted for 72.9 % of the among-group variation: CV1 (37.7%), CV2 (21.6 %) and CV3 (13.6 %).

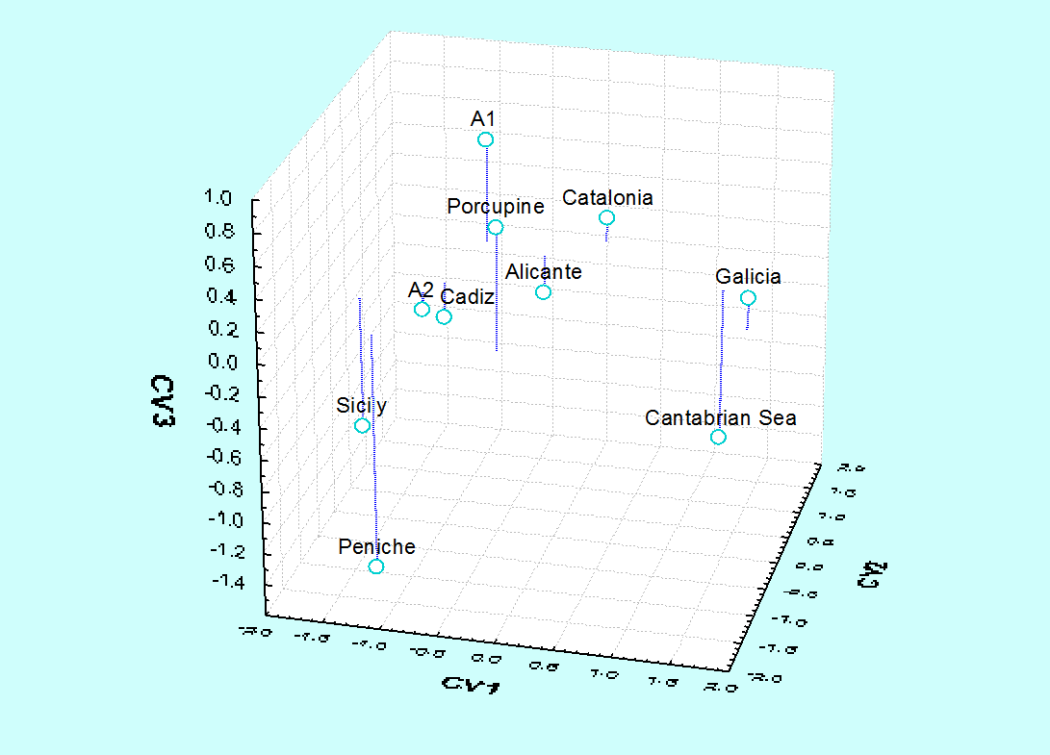


Figure 4- 5. (continued) Three different views of the CVA plot showing the discrimination of the 10 bluemouth samples. The first three canonical variates shown in this graph accounted for 72.9 % of the among-group variation: CV1 (37.7%), CV2 (21.6 %) and CV3 (13.6 %).

Table 4-5. 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	**71.20**	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**

The CVA yielded only a 57.6 % of correct classification (Table 4-5), 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 analyzing the classification matrix by areas, correct classification rates varied considerably (from 71.20 to 28.77 %). From the NE Atlantic, the highest correct classification rates were obtained by Galicia and Portugal (71.20 % and 70.00 % respectively). From the Galician sample, most of the misclassified individuals were either assigned to the Porcupine Bank (8.90%) or to the Cantabrian Sea (9.42%). Moreover, a considerable proportion of specimens from the Cantabrian Sea confounded with the Galician sample. Thus, the close relationship between these areas (Galicia-Cantabrian Sea), as shown by the cluster analysis and CVA plot was also confirmed by the CVA classification rate. 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).

In the Mediterranean, subarea A1 in the Alboran Sea had the highest correct classification rate (67.84 %). Specimens from this area were confounded mostly into the neighbouring areas in the Mediterranean (especially with Alicante), but in low proportions. Also, almost 20% of the samples from Alicante were incorrectly classified into subarea A1. Subarea A2 was also confounded with subarea A1 and Alicante (20% of the samples), but a reasonable proportion of individuals (around 12%) was assigned to the Porcupine Bank. The lowest correct classification rates correspond to the remaining areas in the Mediterranean, Catalonia and Sicily. Although Catalonia was mostly confounded with Alicante (almost 30%), a considerable number of individuals from this area was incorrectly classified into Atlantic locations. Finally, 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). Thus, it seems that the specimens from areas with high percentages of misclassification share features regarding body shape with other areas, which complicates the classification of the individuals.

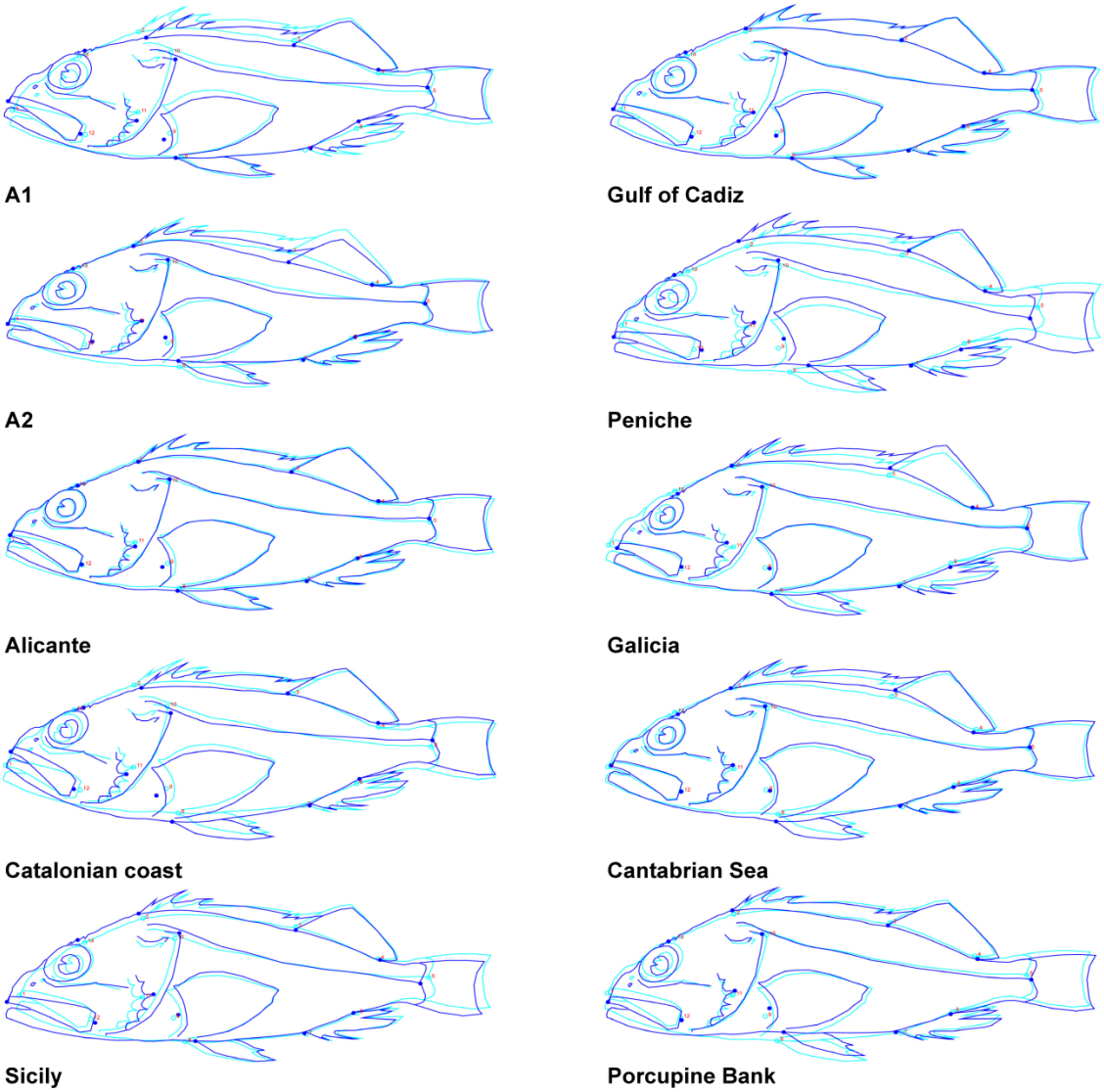


Figure 4-6. Mean shapes for bluemouth from the different locations. The figures represent the transformation from the overall mean shape (light blue outline) to the mean shape for each location (dark blue outline). Shape changes have been exaggerated three-fold for better visualization. The mean shapes were calculated using the size-corrected shape variables.

The mean shapes did not show any specific trends of landmark displacements between

Atlantic and Mediterranean locations (Figure 4-6). 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 analyzed separately.

According to the results of all of the analyses, bluemouth from Portugal was more differentiated morphologically from the rest of locations. For the remaining locations a general separation between Atlantic and Mediterranean seems to exists, but the pattern was unclear and some inconsistencies occurred. For example, according to the Mahalanobis distances in the dendrogram (Figure 4-4), subarea A2 in the Alboran Sea was not closely related to subarea A1, but in the classification matrix, the highest number of misclassified specimens from subarea A2 (9 out of 67 specimens, equivalent to 13.43 % of the sample) were those allocated into subarea A1. Another point to notice is that the results did not support the hypothesis of the Strait of Gibraltar acting as a barrier between Atlantic and Mediterranean bluemouth populations that would cause a clear morphological differentiation. In fact, the patterns of morphological variation observed in this study were diverse, in some cases consistent with the geographical situation of sampling sites, with bluemouth from closely located areas being morphologically related (e.g., Alicante and Catalonia or Galicia and the Cantabrian Sea) and in other cases, morphological similarity was found for bluemouth from distant areas, as in the case of the Porcupine Bank, the Gulf of Cadiz and Sicily. In the next sections, the locations in the Mediterranean and the NE Atlantic are analyzed separately to clarify the population structure within each basin. For the NE Atlantic, the analysis was first done excluding the samples from Galicia and the Cantabrian Sea collected in 2006 and then the analyses were repeated, this time including the samples from 2006.

4.3.4 Morphometric analysis in the Mediterranean

The population structure in western Mediterranean was investigated considering the following areas: 1) the Alboran Sea, which was divided into subareas A1 and A2 considering the growth differences that were found during the analysis of allometry (Chapter 3); 2) Alicante; and 3) Catalonia. Two reference areas were included: Sicily (located in the Central Mediterranean) and the Gulf of Cadiz, to investigate the connection between the Alboran Sea and the Atlantic.

The results of the analyses considering only the Mediterranean locations and the Gulf of Cadiz were similar to the ones obtained in the overall analysis. Body shape differences indicated by Procrustes and Mahalanobis distances (Table 4-6) were significant between all of the locations (at $p<0.005$). The relationship between locations according to the Cluster analysis (Figure 4-7) showed a similar pattern as in the overall analysis, i.e. two clear clusters are defined, one with Catalonia, Alicante and the Subarea A1 of Alboran sea and the other with the two references areas and subarea A2. In the dendrogram, two small clusters were observed: the first cluster contained Alicante and the Catalonian coast grouped together, with subarea A1 in the Alboran Sea joining these two locations. The other cluster grouped the two reference areas: the Gulf of Cadiz and Sicily, while subarea A2 did not group with any location, but it was more similar to the second cluster. However, both Alboran Sea areas clustered in their respective groups at high distance, especially A2 that could easily be considered as a separate group.

According to the results, subarea A1, Alicante and Catalonia seem to be morphologically related. Although the Procrustes and Mahalanobis distances between their mean shapes were significant, these three populations obtained the lowest values for both distances, indicating shape similarity.

In the CVA (Figure 4-8), the two large groups were separated by the first CV (which accounted for 41.2 % of variation between groups). Additionally, subarea A1 was separated from Alicante and Catalonia on the second CV (30.2 % of between-group variation) and third CV (which accounted for 20.9% of variation between groups). These two CVs also showed the separation of A2 from within its group or cluster. Thus, there seems to be a substructure within the western Mediterranean samples, and despite the shape similarities that were detected between subarea A1 and Alicante/Catalonia according to Procrustes and Mahalanobis distances, subarea A1 seems to be a different group. This is also reflected in the results of the classification matrix (Table 4-7), where

this area showed the highest correct classification rate (69.59 %), although with a 11.7 % of the specimens being classified as belonging to Alicante. On the other hand, the results of the classification matrix showed a considerable confusion of specimens between Alicante and Catalonia. The percentage of the specimens from Catalonia that were allocated to Alicante reached 36.99%, (equivalent to 27 out of 73 specimens), and 13.40 % of the bluemouth from Alicante, (equivalent to 18 out of 134 specimens) were misclassified into the sample from Catalonia. Yet, bluemouth from Alicante were mostly confounded with bluemouth from subarea A1 (23.88 %), and also a 17.8% of the fish from Catalonia were allocated in subarea A1, indicating that these areas are connected to some extent.

The mean shape of bluemouth from subarea A1 (Figure 4-9) 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 Catalonian 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 4-9).

As described before, subarea A2 did not cluster with the closest location to it (subarea A1) or with the other neighboring location, Alicante. The separation was also evident in the CVA plot (Figure 4-8), where subareas A1 and A2 were separated by the first two canonical variates and subarea A2 was separated from Alicante by the first CV. However, the CVA showed that specimens from this area were mostly misclassified into subarea A1 (16.42 %) and Alicante (13.43 %), indicating that bluemouth from subarea A2 do share some shape features with bluemouth from surrounding areas or that some specimens move between these areas.

As mentioned above, the two reference areas (Gulf of Cadiz and Sicily) were related morphologically according to the dendrogram using Mahalanobis distances. When the mean shapes of these two locations were examined (Figure 4-9), some similarities were observed. In both mean shapes, there was an upward shift of landmark 2 (insertion of the dorsal fin), a right-upwards movement of landmark 11 (the second preopercular spine) and a left-downwards movement of landmark 1 (the tip of the snout). However, the changes on the mean shape of Sicily were visibly larger and included displacements of other landmarks, such as in landmark 8, 5 and 13 (insertion of the ventral fin, the end of the hypural plate and the second spine above the eye).

The classification results, in addition, showed that bluemouth from the Gulf of Cadiz were confounded mostly with bluemouth from the neighboring area, subarea A1, and to a lesser extent, from Alicante. This evidence weakens the hypothesis that the Strait of Gibraltar acts as a barrier isolating Atlantic and Mediterranean bluemouth populations, because it indicates that shape similarities exist between bluemouth from the Gulf of Cadiz and the close Alboran Sea. Actually, despite being from a distant region, bluemouth from Sicily were also confounded with bluemouth from Alicante, subarea A1 and especially the Gulf of Cadiz. In this case, morphological adaptations to similar environmental factors can lead to shape similarities between distant fish populations (phenotypic plasticity).

Table 4-6. (a) Procrustes distances and (b) Mahalanobis distances among the mean shapes of bluemouth from the Mediterranean locations and the Gulf of Cadiz. All of them are significant ($p < 0.05$).

a) Location	A1	A2	Alicante	Catalonia	Sicily
A2	0.0270				
Alicante	0.0157	0.0182			
Catalonia	0.0131	0.0313	0.0156		
Sicily	0.0330	0.0197	0.0248	0.0379	
Gulf of Cadiz	0.0198	0.0203	0.0142	0.0240	0.0167

b) Location	A1	A2	Alicante	Catalonia	Sicily
A2	2.4338				
Alicante	1.7551	2.2127			
Catalonia	2.0334	3.1663	1.5253		
Sicily	2.6503	2.3243	2.5876	3.358	
Gulf of Cadiz	1.9879	2.5505	2.0671	2.5838	1.7829

Table 4-7. Jackknifed classification matrix from the CVA performed on the bluemouth samples from the locations in the Mediterranean. Wilks' Lambda: 0.2059, $F_{(110,2655)} = 9.1874$, $p < 0.05$. The overall correct classification rate was of 57.2 %. Observed classifications are shown in rows; predicted classifications are shown in columns.

Location	A1	A2	Catalonia	Alicante	Sicily	Gulf of Cadiz
A1	**69.59**	7.02	3.51	11.70	2.34	5.85
A2	16.42	**58.21**	1.49	13.43	8.96	1.49
Catalonia	17.81	0.00	**41.10**	36.99	0.00	4.11
Alicante	23.88	2.24	13.43	**50.75**	2.24	7.46
Sicily	14.58	6.25	0.00	14.58	**45.83**	18.75
Gulf of Cadiz	13.33	6.67	2.67	9.33	5.33	**62.67**

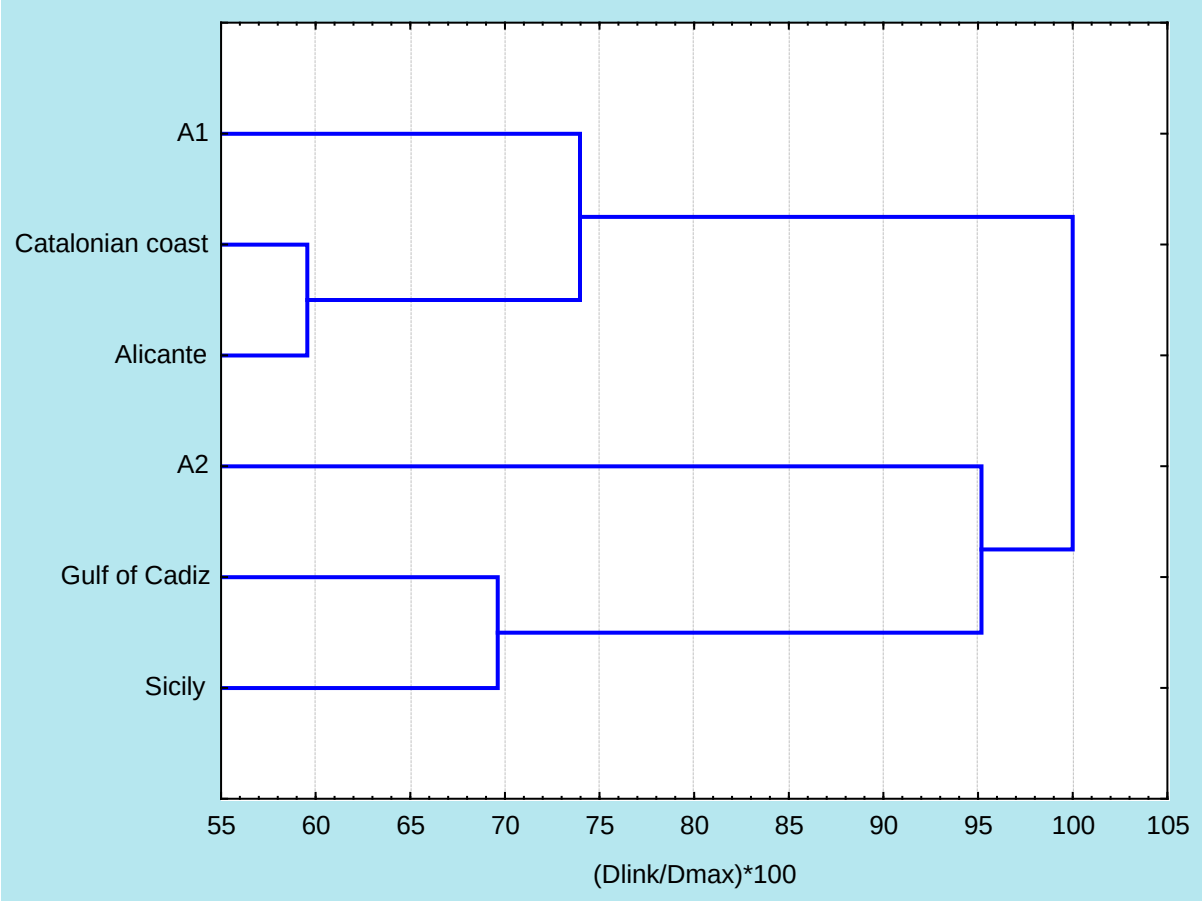


Figure 4-7. Cluster analysis linkage distance plot for the bluemouth from the Mediterranean locations. (UPMGA; Based on Mahalanobis distances).

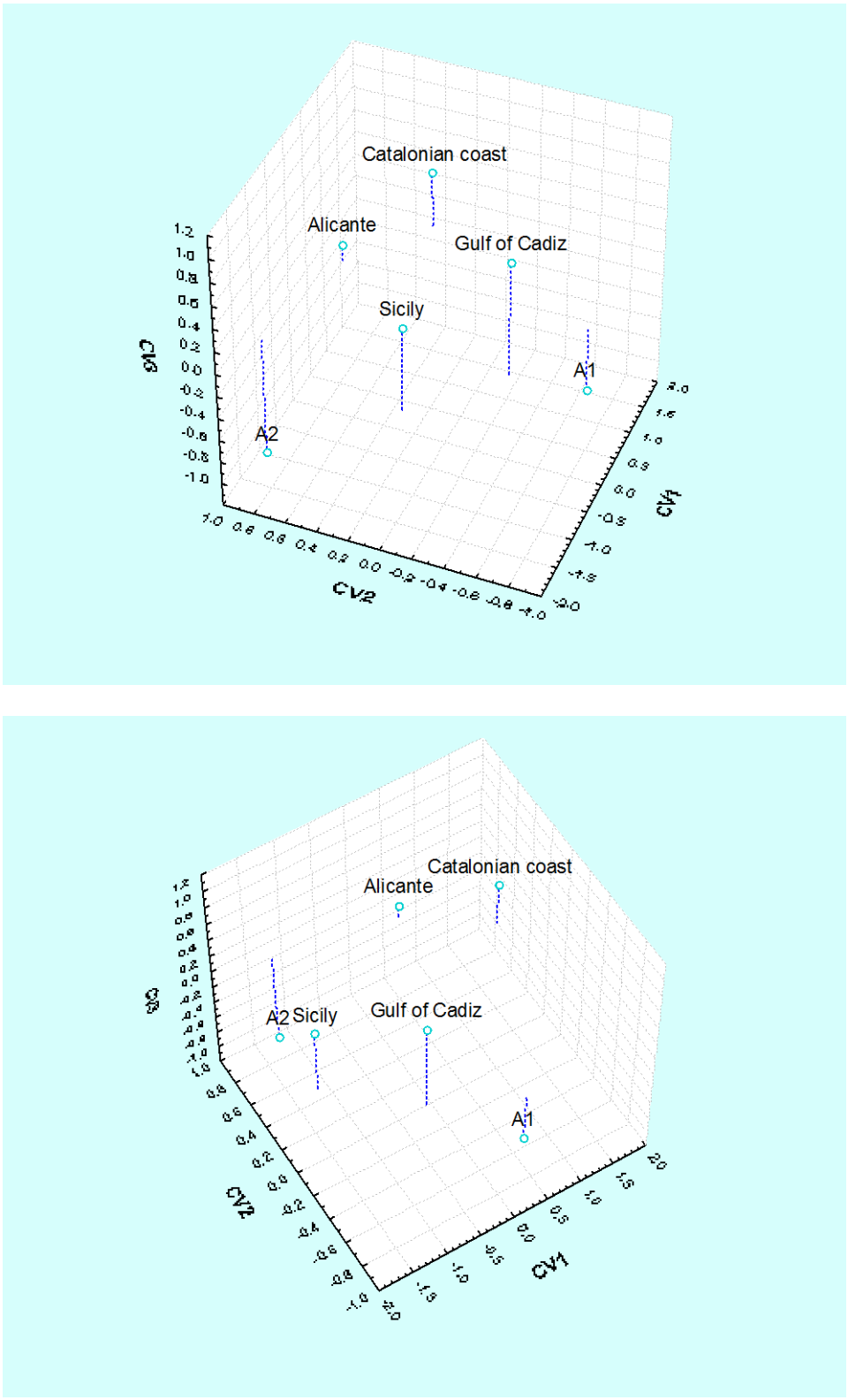


Figure 4-8. Two different views of the CVA plot showing the discrimination of the Mediterranean bluemouth samples. The first three canonical variates shown in this graph accounted for 93.5 % of the among-group variation: CV1 (41.2%), CV2 (30.4%) and CV3 (21.9%).

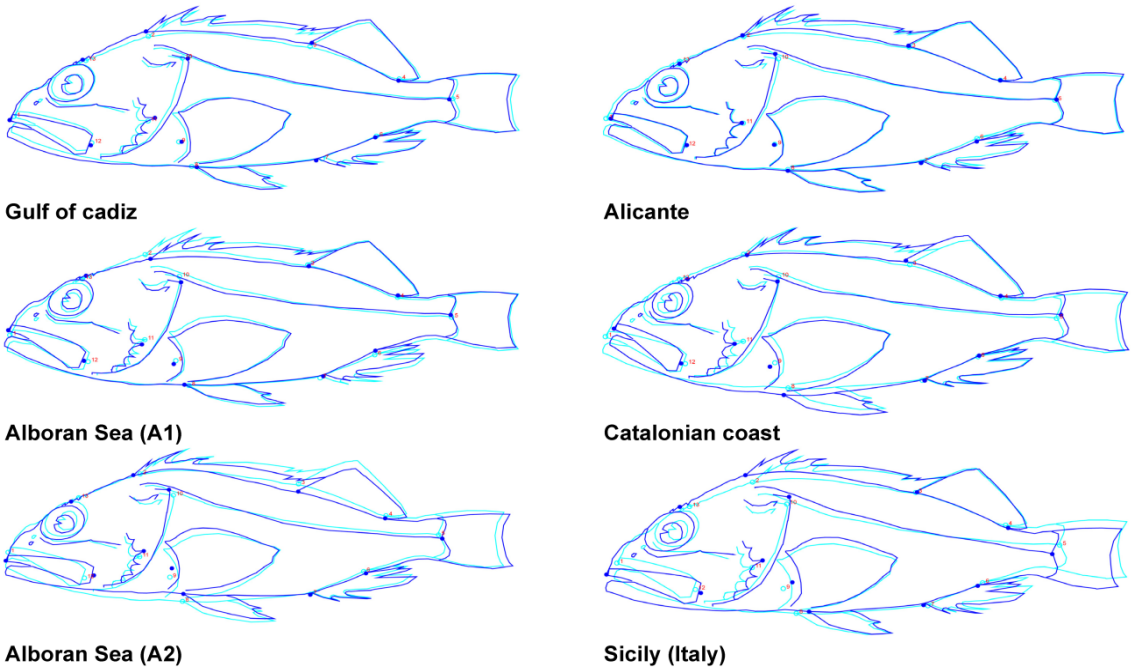


Figure 4-9. Mean shapes for bluemouth from the different locations. The figures represent the transformation from the overall mean shape (light blue outline) to the mean shape for each location (dark blue outline). Shape changes have been exaggerated three-fold for better visualization. The mean shapes were calculated using the size-corrected shape variables.

4.3.5 Morphometric analysis in the NE Atlantic (excluding 2006 samples)

The results showed significant differences in the two distances analyzed among bluemouth from all of the NE Atlantic locations (Table 4-8). Considering both the Procrustes and Mahalanobis distances, the greatest amount of morphological differentiation was found for bluemouth from Portugal. The mean Procrustes distance with respect to the other locations was of 0.0286 units and the mean Mahalanobis distance of 3.1845, which in both cases were the highest values obtained in the comparison of all of the studied locations. The cluster analysis based on Mahalanobis distances showed more clearly that Portugal did not cluster with any other location (Figure 4-10).The shape differentiation of bluemouth was reflected as well in CVA analysis (Wilks' Lambda: 0.1387 $F_{(88,2378)} = 17.5142$ $p < 0.05$) as overall significant differences were found. On the CVA plot (Figure 4-11) Portugal was well separated from Galicia (the neighboring location to the North) by the first and second CV's. It was also separated from the Cantabrian Sea by the first CV and from the Porcupine Bank by the second CV. In addition, Portugal was the group with the highest classification success (80 %) in the classification matrix (Table 4-9). The greatest number of misclassified specimens from Portugal was assigned to the Porcupine Bank (13.33 %) and only a small amount of individuals were incorrectly assigned to Galicia and the Gulf of Cadiz (3.33% in both cases). Thus, these results indicate that bluemouth from Portugal are well differentiated of bluemouth from neighboring locations, Galicia and the Gulf of Cadiz, and can be considered as a separate population regarding body morphology. Actually, the body shape changes that characterized bluemouth from Portugal included relative displacements of landmarks in most of the body (Figure 4-12). Shape changes were detected mainly at the insertion of the fins: the first dorsal fin (landmark 2), the ventral fin (landmark 8), the end of the hypural plate (landmark 5) and the pectoral fin (landmark 9) and in the head region: at the tip of the snout (landmark 1), the tip of mandible (landmark 12) and the second preopercular spine (landmark 11).

In spite of the significant distances between the mean shapes of bluemouth, morphological differentiation was not so clear for bluemouth from the rest of the studied locations in the NE Atlantic. In fact, a close morphological relationship between bluemouth from Galicia and the neighboring location to the Northeast, the Cantabrian

Sea, was observed. 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 (1.9986) between any two groups. The shape differences between bluemouth from these locations were basically indicated by the displacement of landmarks in the head region and the insertion of the ventral fin (Figure 4-12). Bluemouth from Galicia showed a smaller and more contracted head than bluemouth from the Cantabrian Sea, and bluemouth from the Cantabrian Sea showed a deeper body, related to the displacement of landmark 8 at the ventral fin.

The observed similarity of the bluemouth from Galicia and the Cantabrian Sea might have been due to the misidentification of the geographical boundary between the two populations, which could have led to the incorrect assignment of specimens to each of the populations during the analysis. Two important biogeographical limits (with boundary effects for fish populations) have been identified between Galicia and the Cantabrian Sea: Cape Estaca de Bares and Cape Finisterrae (Figure 2-2) (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 in a CVA. 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 results of the CVA and the classification matrix showed that the morphological variation among the four subareas followed a gradient, with no clear boundary between Galicia and the Cantabrian Sea (Figure 4-13 and Table 4-10). The misclassification rate between G1 and G2 was very 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 4-10).

On the dendrogram (Figure 4-10), Galicia and the Cantabrian Sea clustered together and appeared separated from Portugal and the cluster formed by the Gulf of Cadiz and the Porcupine Bank. In the CVA plot (Figure 4-11), 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 (23.53 %; i.e., 28 out of 119

specimens), and 11 % (i.e., 21 out of 191 specimens) of the bluemouth from Galicia was incorrectly assigned to the Cantabrian Sea (Table 4-9). However, in the case of Galicia, a slightly higher number of bluemouth specimens was misclassified into the Porcupine Bank (12.57%) and the number of bluemouth from the Porcupine Bank that was confounded with that of Galicia was also appreciable. Therefore, if we consider the results for these three areas, a gradient of morphological variation can be observed: Bluemouth from the Cantabrian Sea are more similar to Galicia than to the Porcupine Bank, and bluemouth from Galicia are more similar to the Porcupine Bank. However, according to the classification matrix, appreciable numbers of bluemouth specimens from the Gulf of Cadiz (18.67 %; 14 out of 75) and Portugal (13.33 %, 8 out 60) were misclassified into the Porcupine Bank group, indicating that shape similarities also exist with bluemouth from areas other than Galicia. Moreover, as mentioned before, the Porcupine Bank clustered together with the Gulf of Cadiz and this could indicate a case of phenotypic plasticity instead of morphological variation related to the geographical situation of the areas (i.e., areas located closely would show a more similar body shape than distant areas).

Table 4-8. (a) Procrustes distances and (b) Mahalanobis distances among the mean shapes of bluemouth of the study areas in the NE Atlantic. All of them are significant ($p < 0.05$).

(a)

Area	Porcupine Bank	Cantabrian Sea	Galicia	Portugal	Mean *
Porcupine Bank	0				0.0217
Cantabrian Sea	0.0222	0			0.0202
Galicia	0.0220	0.0100	0		0.0219
Portugal	0.0204	0.0314	0.0337	0	0.0286
Gulf of Cadiz	0.0225	0.0175	0.0221	0.0291	0.0228

*The mean Procrustes distance represents the mean amount of (absolute) shape variation between the population in question and the remaining populations.

(b)

Area	Porcupine Bank	Cantabrian Sea	Galicia	Portugal	Mean *
Porcupine Bank	0				2.5730
Cantabrian Sea	2.9376	0			2.8252
Galicia	2.3419	1.9986	0		2.7401
Portugal	2.9104	3.6071	3.6006	0	3.1845
Gulf of Cadiz	2.1021	2.7576	3.0195	2.6200	2.6248

*The mean Mahalanobis distance represents the mean difference between the population in question and the remaining populations, but unlike the Procrustes distance, the Mahalanobis distance is a relative distance that takes into account the within-group variation.

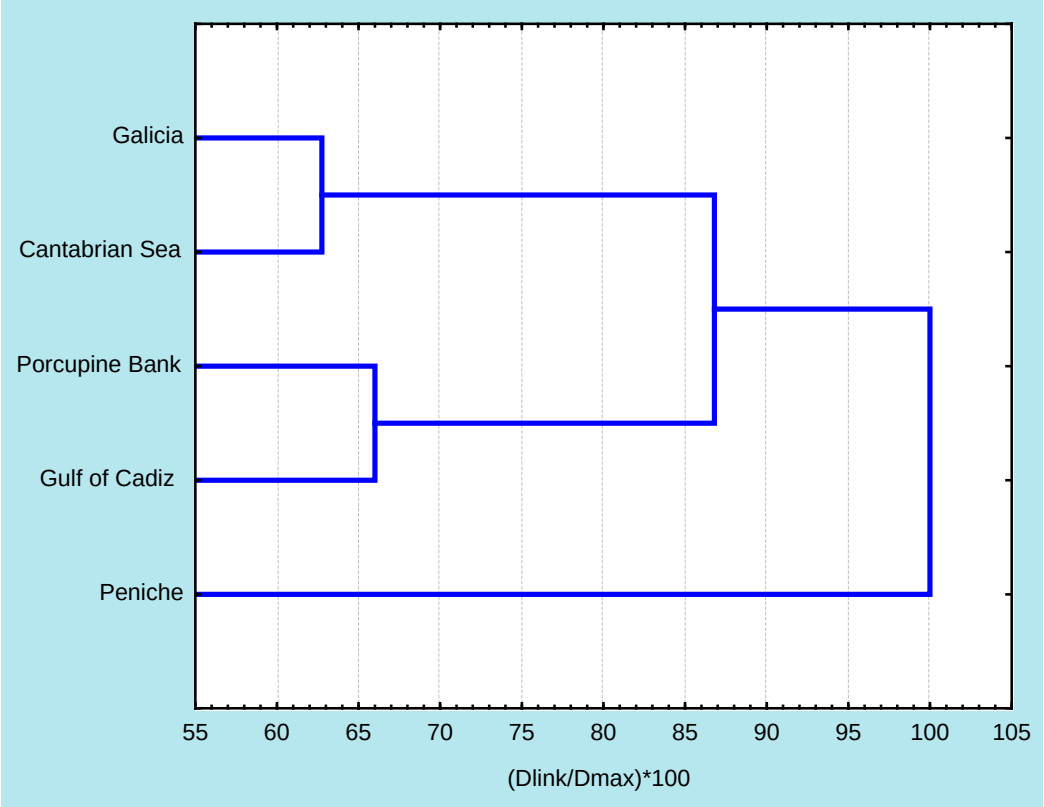


Figure 4-10. Cluster analysis linkage distance plot for bluemouth from the NE Atlantic locations. (UPMGA; Based on Mahalanobis distances).

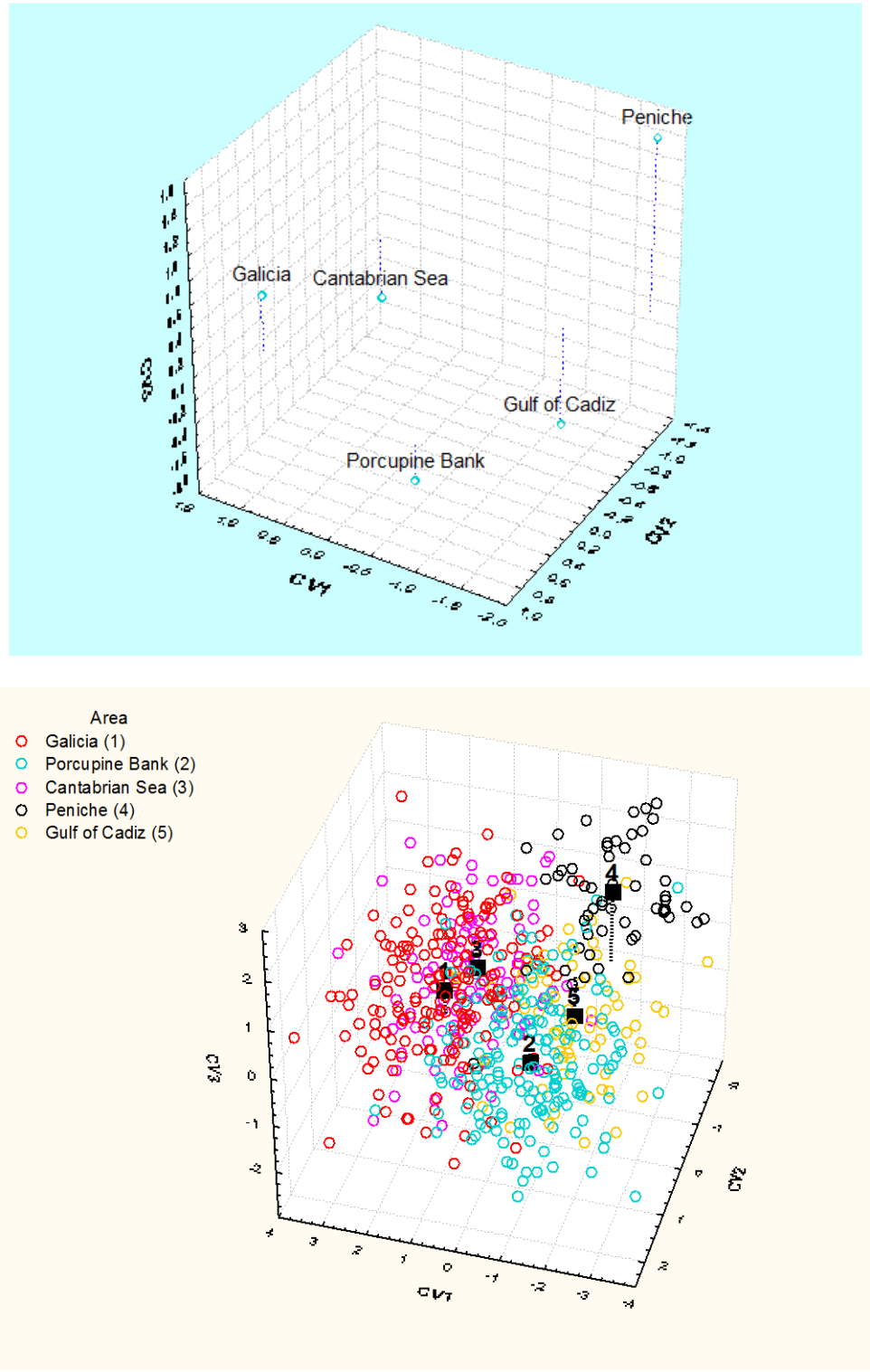


Figure 4-11. Two different views of the CVA plot showing the discrimination of the NE Atlantic bluemouth samples. The first three canonical variates shown in this graph accounted for 93.7 % of the among-group variation: CV1 (51.1%), CV2 (27.2%) and CV3 (15.4%).

Table 4-9. Jackknifed classification matrix from the CVA performed on the bluemouth samples from the NE Atlantic study areas. Wilks' Lambda: 0.1387 $F_{(88,2378)}$ = 17.5142 $p < 0.05$. The overall correct classification rate was of 71.60 %. Observed classifications are shown in rows; predicted classifications are shown in columns.

Location	Porcupine Bank	Cantabrian Sea	Galicia	Portugal	Gulf of Cadiz
Porcupine Bank	**77.47**	1.65	13.19	1.65	6.04
Cantabrian Sea	5.04	**63.87**	23.53	2.52	5.04
Galicia	12.57	10.99	**74.35**	0.52	1.57
Portugal	13.33	0.00	3.33	**80.00**	3.33
Gulf of Cadiz	18.67	10.67	9.33	5.33	**56.00**

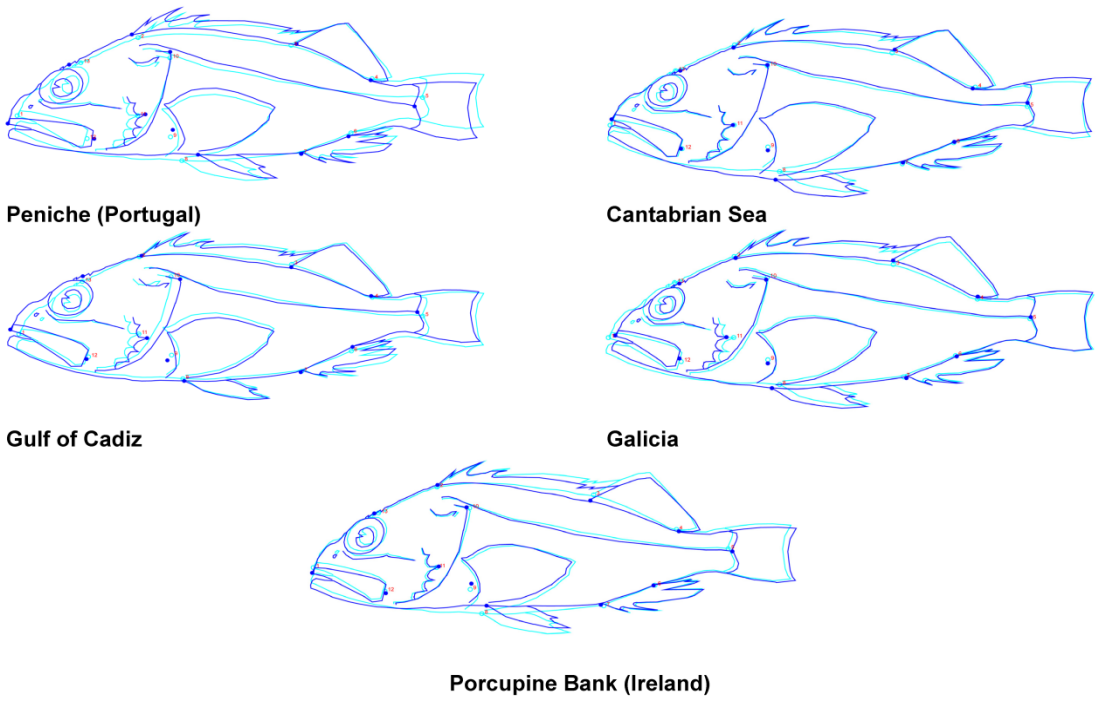


Figure 4-12. Mean shapes for bluemouth from the NE Atlantic locations. The figures represent the transformation from the overall mean shape (light blue outline) to the mean shape for each location (dark blue outline). Shape changes have been exaggerated three-fold for better visualization. The mean shapes were calculated using the size-corrected shape variables.

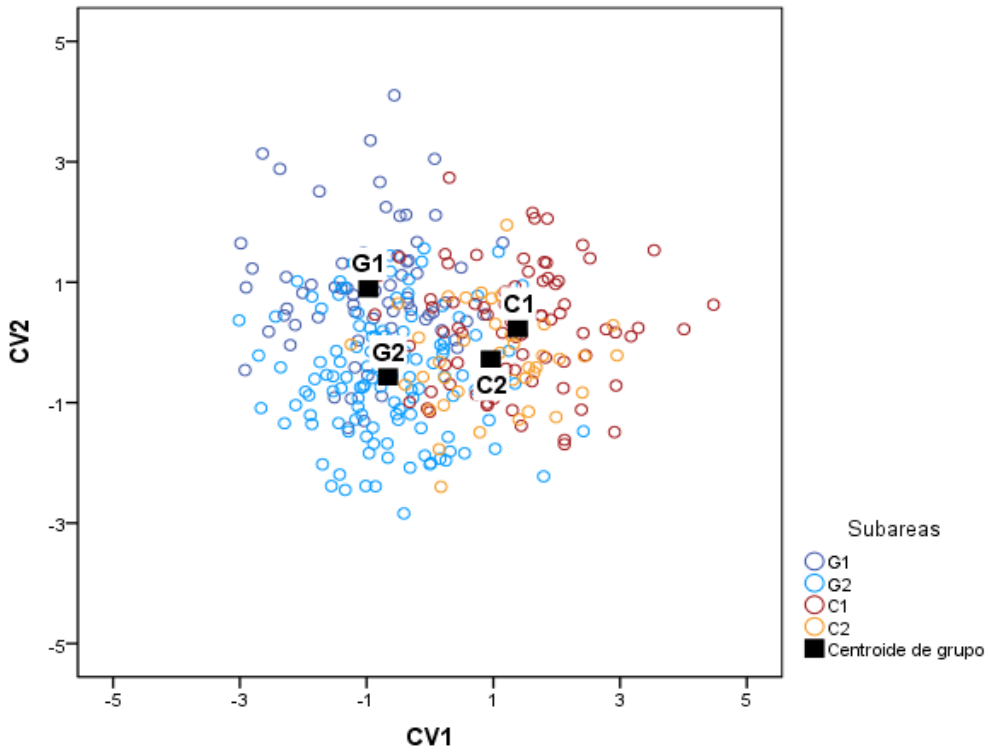


Figure 4-13. CVA plot showing the discrimination of the bluemouth samples from the four subareas in Galicia and the Cantabrian Sea. The three canonical variates shown in this graph accounted for 100.0 % of the among-group variation: CV1 (65.8%), CV2 (22.0%) and CV3 (12.2%).

Table 4-10. Jackknifed classification matrix from the CVA performed on the bluemouth samples from the four subareas in Galician waters and the Cantabrian Sea. Wilks' Lambda: 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**

4.3.6 Morphometric analysis in the NE Atlantic (including 2006 samples)

Coinciding with all of the previous analyses, the Mahalanobis and Procrustes distances (Table 4-11) were significantly different ($p<0.05$) among all of the samples from the NE Atlantic locations once the 2006 samples from Galicia and the Cantabrian Sea were included. The lowest Procrustes distances were obtained between Galicia and the Cantabrian Sea within each year and then between years, indicating the greatest absolute body shape similarity between these locations. The cluster analysis based on Mahalanobis distances (Figure 4-14), revealed that Galicia and the Cantabrian Sea clustered together first within years and only at greater distance the two years clustered. These two clusters were separated from the rest of the locations. Again, the Porcupine Bank grouped with the Gulf of Cadiz and Portugal joined this cluster later.

The CVA also detected overall shape differences (Wilks' Lambda = 0. 1086, $F_{(132, 4474)} = 15.7510$, $p < 0.05$). As it was noticed in the overall population structure analysis, with the 12 bluemouth samples included, bluemouth from Galicia and the Cantabrian Sea caught in 2006 were different from those caught in the same locations a year later (Figure 4-15). In the analysis of the population structure of bluemouth from the NE Atlantic including the 2006 samples, the separation of the 2006 and 2007 samples was clear as well, while for the rest of the locations (i.e., the Porcupine Bank, Portugal and the Gulf of Cadiz) the resulting structure was basically the same as in the previous analysis carried out with the NE Atlantic locations without the 2006 samples.

On the CV plot (Figure 4-15), the first CV also showed the separation of the four samples from Galicia and the Cantabrian Sea from the remaining samples of the NE Atlantic. Thus, these results indicate that despite the differences between the Galician and Cantabrian 2006 and 2007 samples, bluemouth from these locations are still more related between them than to the three other NE Atlantic locations (the Porcupine Bank, Portugal and the Gulf of Cadiz). The results of the classification matrix (Table 4-12) confirmed these findings: Bluemouth from Galicia 2006 were mainly confounded with bluemouth from Galicia 2007 (17.65 %, 21 out of 119), and then with the Cantabrian Sea sample from 2006 (10.08 % or 12 specimens), although a similar percentage was assigned to the Porcupine Bank (9.24 % or 11 specimens). Likewise, bluemouth from the Cantabrian Sea 2006 were not well differentiated from those of Galicia caught in the same year, because

24 % of the specimens were misclassified (12 out of 50). Moreover, 16 % of the specimens (8 out of 50) from this area were incorrectly assigned to Galicia 2007, indicating that shape similarity exists between bluemouth these areas.

The main features involved in the body shape differentiation pattern between bluemouth from 2006 and bluemouth from 2007 were relative displacements of landmarks 1 and 13, which define head height and the length of the snout; and of landmark 9, at the insertion of the pectoral fin (Figure 4-16). Regarding bluemouth from 2006, the displacements resulted in a relative contraction of the head region and the pectoral area, very evident in the case of the Cantabrian Sea. The opposite shape changes were observed in the case of bluemouth from 2007, where the head and pectoral areas showed a relative expansion.

For the remaining locations (the Porcupine Bank, Portugal and the Gulf of Cadiz), the results were similar to the ones presented in the previous analysis of the NE Atlantic locations without the 2006 samples (see section 4.3.2.4 above).

Table 4-11. (a) Procrustes distances and (b) Mahalanobis distances among the mean shapes of bluemouth of the study areas in the NE Atlantic including the samples from Galicia and Cantabrian Sea from 2006. All of them are significant ($p < 0.05$).

(a) Location	Porcupine	Cant.07	Cant.06	Gal.07	Gal.06	Portugal	Cadiz
Porcupine Bank	0						
Cantabrian Sea 2007	0.0218	0					
Cantabrian Sea 2006	0.0279	0.0187	0				
Galicia 2007	0.0210	0.0096	0.0140	0			
Galicia 2006	0.0277	0.0151	0.0117	0.0123	0		
Portugal	0.0205	0.0311	0.0395	0.0329	0.0380	0	
Gulf of Cadiz	0.0224	0.0175	0.0292	0.0217	0.0241	0.0289	0

(b) Location	Porcupine	Cant.07	Cant.06	Gal.07	Gal.06	Portugal	Cadiz
Porcupine Bank	0						
Cantabrian Sea 2007	2.9264	0					
Cantabrian Sea 2006	2.9331	2.9260	0				
Galicia 2007	2.2716	1.8705	2.4917	0			
Galicia 2006	2.5969	2.5152	1.8839	2.0107	0		
Portugal	2.8052	3.6070	3.8114	3.5473	3.8117	0	
Gulf of Cadiz	2.0835	2.7815	3.5674	2.9837	2.8670	2.6004	0

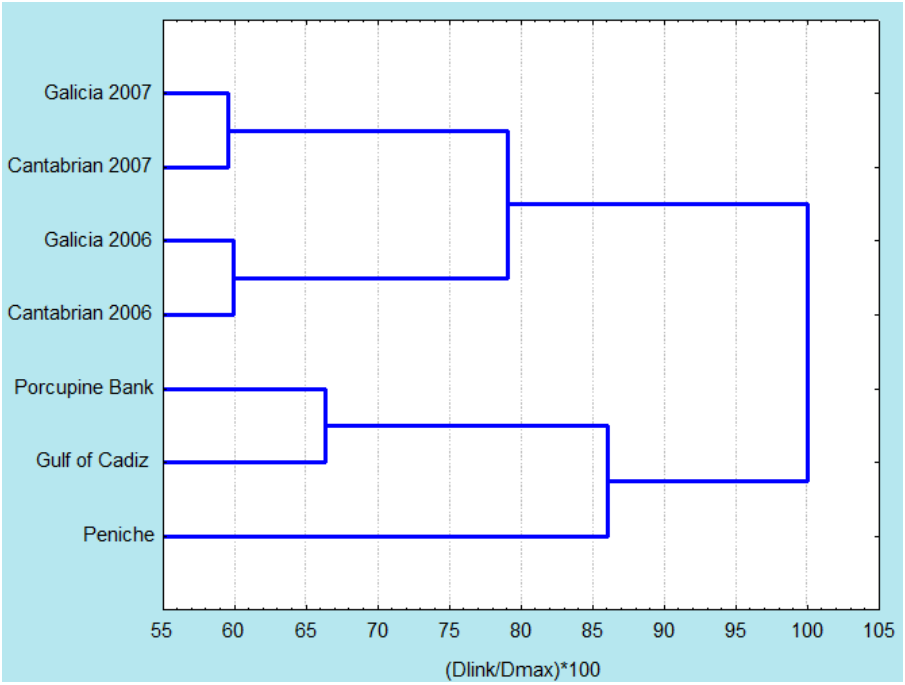


Figure 4-14. Cluster analysis linkage distance plot for bluemouth from the NE Atlantic including the samples from Galicia and Cantabrian Sea from 2006. (UPMGA; Based on Mahalanobis distances).

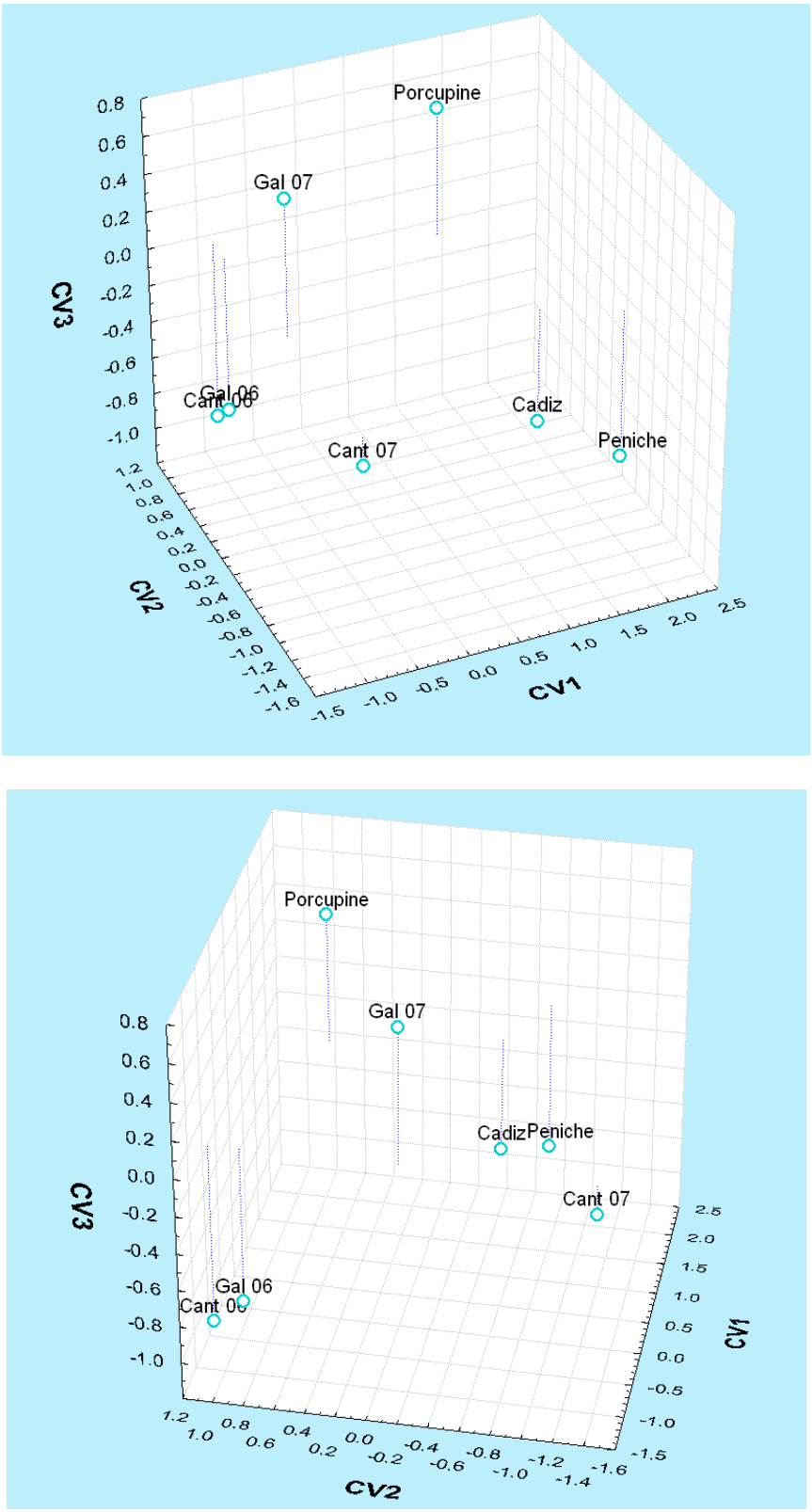


Figure 4-15. Two different views of the CVA plot showing the discrimination of the NE Atlantic bluemouth samples including those from Galicia and Cantabrian Sea from 2006. The first three canonical variates shown in this graph accounted for 93.5 % of the among-group variation: CV1 (41.2%), CV2 (30.4 %) and CV3 (21.9 %).

Table 4-12. Jackknifed classification matrix from the CVA performed on the bluemouth samples from the NE Atlantic study areas including the samples from Galicia and Cantabrian Sea from 2006. Wilks' Lambda: 0.1086 $F_{(132,4474)} = 15.7510$ $p < 0.05$. The overall correct classification rate was of 63.70 %. Observed classifications are shown in rows; predicted classifications are shown in columns.

Location	Porcupine	Cant.07	Cant.06	Gal.07	Gal.06	Portugal	Cadiz
Porcupine Bank	**75.82**	1.65	1.10	10.99	3.85	1.10	5.49
Cantabrian Sea 07	5.04	**60.50**	0.00	21.01	6.72	2.52	4.20
Cantabrian Sea 06	4.00	8.00	**48.00**	16.00	24.00	0.00	0.00
Galicia 07	8.90	9.42	2.09	**64.92**	12.04		2.09
Galicia 06	9.24	5.88	10.08	17.65	**52.10**	1.68	3.36
Portugal	11.67	0.00	0.00	5.00	1.67	**76.67**	5.00
Gulf of Cadiz	22.67	6.67	0.00	5.33	8.00	2.67	**54.67**

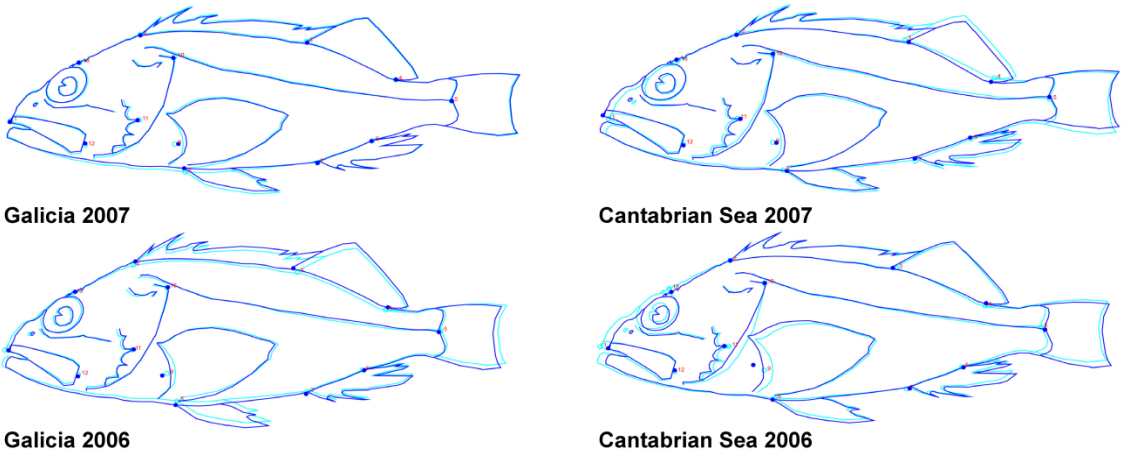


Figure 4-16. Mean shapes for bluemouth from Galicia and the Cantabrian Sea from 2006 and 2007. The figures represent the transformation from the overall mean shape (light blue outline) to the mean shape for each location (dark blue outline). Shape changes have been exaggerated three-fold for better visualization.

4.3.7 Meristics

The meristic characters that were used in this study were: the number of spines of the first dorsal fin (SDF1), the number of rays of the second dorsal fin (RDF2), the number of rays of the pectoral (RPF), ventral (RVF) and anal (RAF) fins, and gill rakers of the horizontal (GRH) and vertical (GRV) segment of the gill.

In general, meristic counts were very stable among the different locations (Tables 4-13 – 4-19, Figures 4-17 – 4-23). The greatest variability was detected in the number of gill rakers of the vertical and horizontal branches.

No differences between sexes or size dependency were observed in the meristic variables of bluemouth from the locations in the NE Atlantic. The Kruskal-Wallis H test showed overall significant differences ($p < 0.05$) only for 3 of the 7 meristic variables: the number of rays of the pectoral fin (RPF), the number of gill rakers on the horizontal branch (GRH) and the number of gill rakers on the vertical branch (GRV). The results of the Kruskal-Wallis tests and the post-hoc pairwise comparisons between the locations are shown in Table 4-20.

The differences detected between locations were not consistent among the meristic variables. The number of gill rakers of the horizontal branch was different between the Porcupine Bank and most locations in the NE Atlantic (i.e., the Gulf of Cadiz, Portugal and Galicia) but regarding the number of gill rakers of the vertical branch, only bluemouth from Galicia were different from those of the Gulf of Cadiz.

No significant differences between any of the locations were detected in the number of rays of the pectoral fin in the post-hoc comparisons, although the Kruskal-Wallis test revealed overall significant differences ($H(4) = 15.61$, $p = 0.004$). It is likely that significant differences in RPF between the locations were not detected because of the loss of statistical power due to the correction of the level of significance in the multiple comparisons (see Chapter 2 - Material and Methods).

No differences in meristic variables were detected between bluemouth males and females from the Mediterranean locations. In the Catalonian sample, a significant relationship between the number of gill rakers (GRH and GRV) and size (CS) was observed only ($r_s = 0.402$; $p < 0.05$ and $r_s = 0.259$; $p < 0.05$ respectively) (Figure 4-24). It is possible that the correlation between the gill rakers and size in bluemouth from Catalonia indicates the presence of different year classes in the sample, where each cohort underwent different environmental conditions.

The meristic variables were quite stable in the Mediterranean locations. The only significant difference detected ($H(4) = 13.96$, $p < 0.05$) was in the number of gill rakers of the vertical branch (GRV) between Alicante and subarea A1 of the Alboran Sea (Table 4-21a).

In general, no differences were observed between the locations on both sides of the Strait of Gibraltar (the Gulf of Cadiz and subareas A1 and A2). The Kruskal-Wallis test showed that only the number of gill rakers of the vertical branch was different among these locations ($H(2) = 8.30$, $p = 0.016$) (Table 4-21b). In spite of this, none of the differences between locations were significant in the post-hoc comparisons, but the rank difference between the Gulf of Cadiz and subarea A2 (36.05) was very close to the value of the critical difference (36.06). A Mann–Whitney test using the two locations was carried out to confirm whether the difference was significant or not. The results of this test ($U = 1902.00$, $p = 0.006$) confirmed that a significant difference in the number of RPF existed between the Gulf of Cadiz and subarea A2.

Table 4-13. Results regarding the number of spines of the first dorsal fin (RDF1) in the 10 locations.

RDF1					
Area	**N**	**Mean**	**Mode**	**Min.**	**Max.**
Porcupine Bank	176	12.03	12	11	13
Cantabrian Sea	108	12.01	12	12	13
Galicia	191	12.01	12	10	13
Portugal	60	12.00	12	12	12
Gulf of Cadiz	74	12.00	12	11	13
Alboran Sea - A1	168	12.03	12	11	13
Alboran Sea - A2	67	12.00	12	12	12
Alicante	132	12.00	12	11	13
Catalonia	73	11.99	12	11	12
Sicily	48	12.06	12	12	14

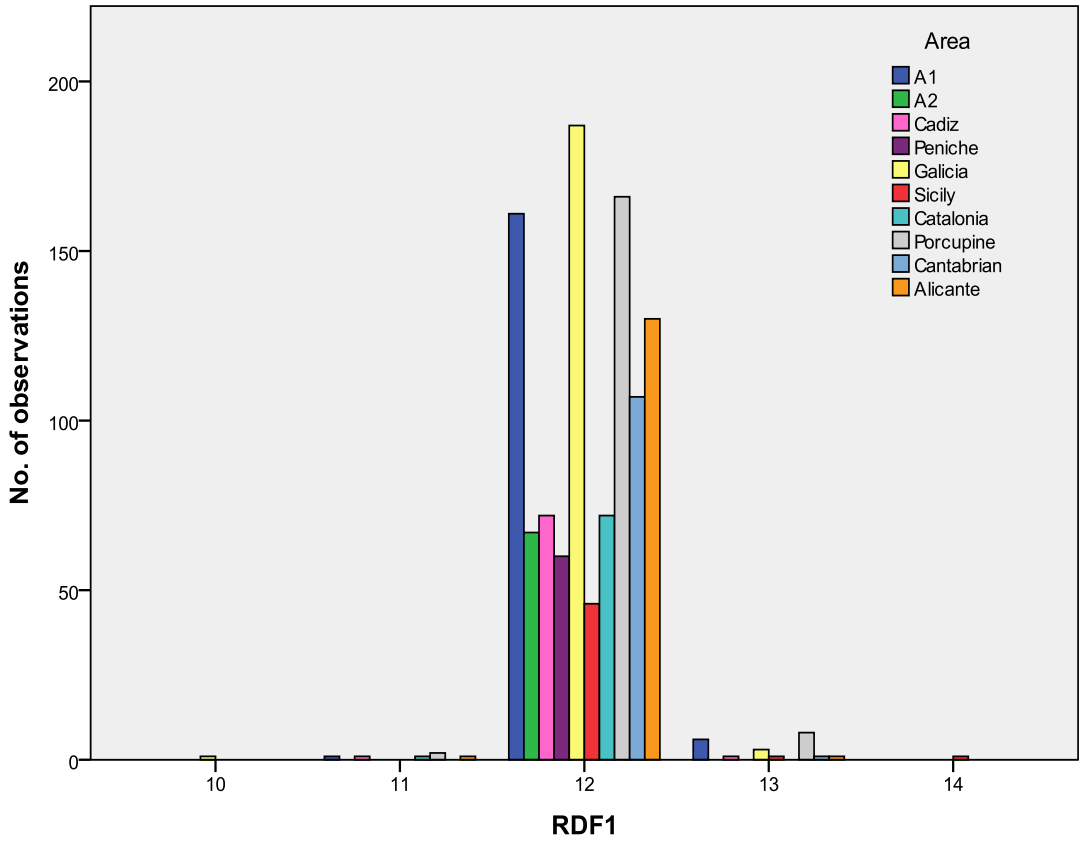


Figure 4-17. Frequency of spines of the first dorsal fin (RDF1) in the 10 locations.

Table 4-14. Results regarding the number of rays of the second dorsal fin (RDF2) in the 10 locations.

RDF2					
Area	**N**	**Mean**	**Mode**	**Min.**	**Max.**
Porcupine Bank	176	13.04	13	11	14
Cantabrian Sea	108	13.11	13	12	14
Galicia	191	13.12	13	11	14
Portugal	60	13.05	13	12	14
Gulf of Cadiz	74	12.96	13	10	14
Alboran Sea - A1	168	13.10	13	12	14
Alboran Sea - A2	67	13.09	13	12	14
Alicante	132	13.04	13	12	14
Catalonia	73	13.19	13	12	14
Sicily	47	13.04	13	11	14

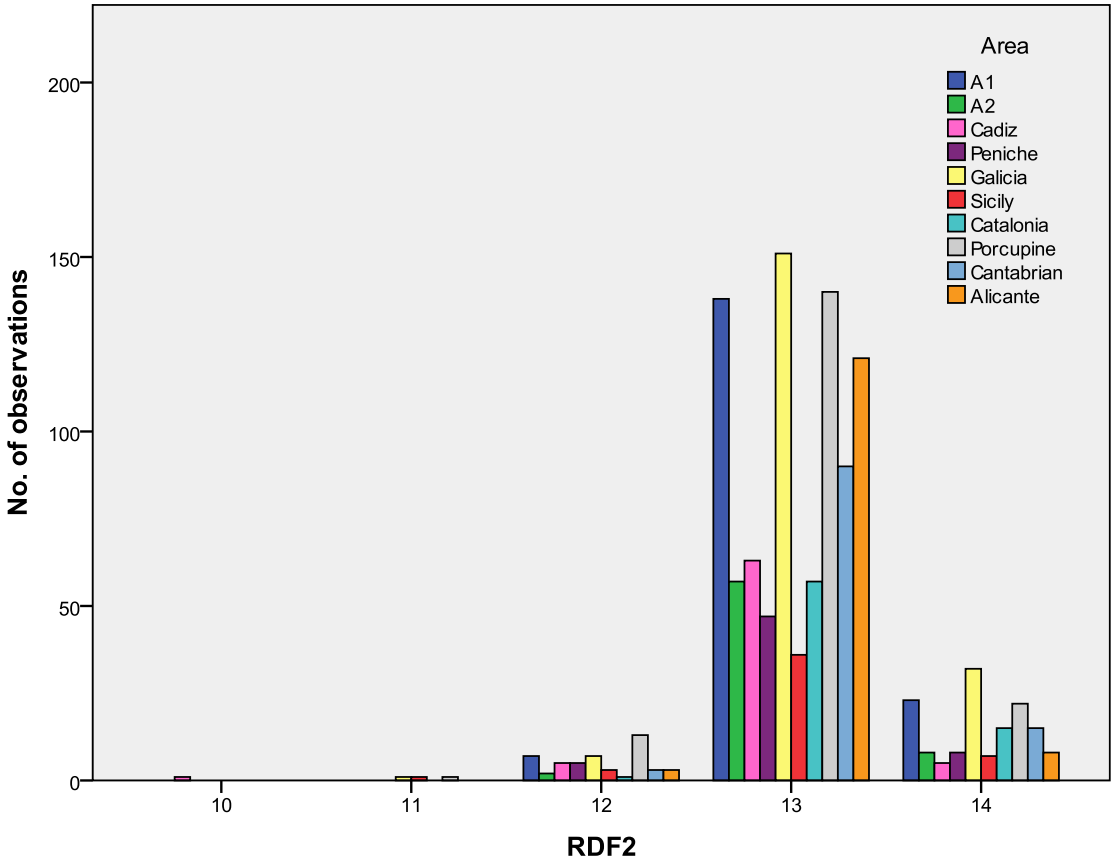


Figure 4-18. Frequency of rays of the second dorsal fin (RDF2) in the 10 locations.

Table 4-15. Results regarding the number of rays of the anal fin (RAF) in the 10 locations.

RAF					
Area	**N**	**Mean**	**Mode**	**Min.**	**Max.**
Porcupine Bank	176	7.97	8	7	9
Cantabrian Sea	108	7.98	8	7	8
Galicia	191	7.99	8	7	9
Portugal	60	8.00	8	8	8
Gulf of Cadiz	74	8.03	8	8	9
Alboran Sea - A1	168	8.00	8	7	9
Alboran Sea - A2	67	8.00	8	7	9
Alicante	132	8.00	8	7	9
Catalonia	73	8.00	8	8	8
Sicily	48	8.00	8	8	8

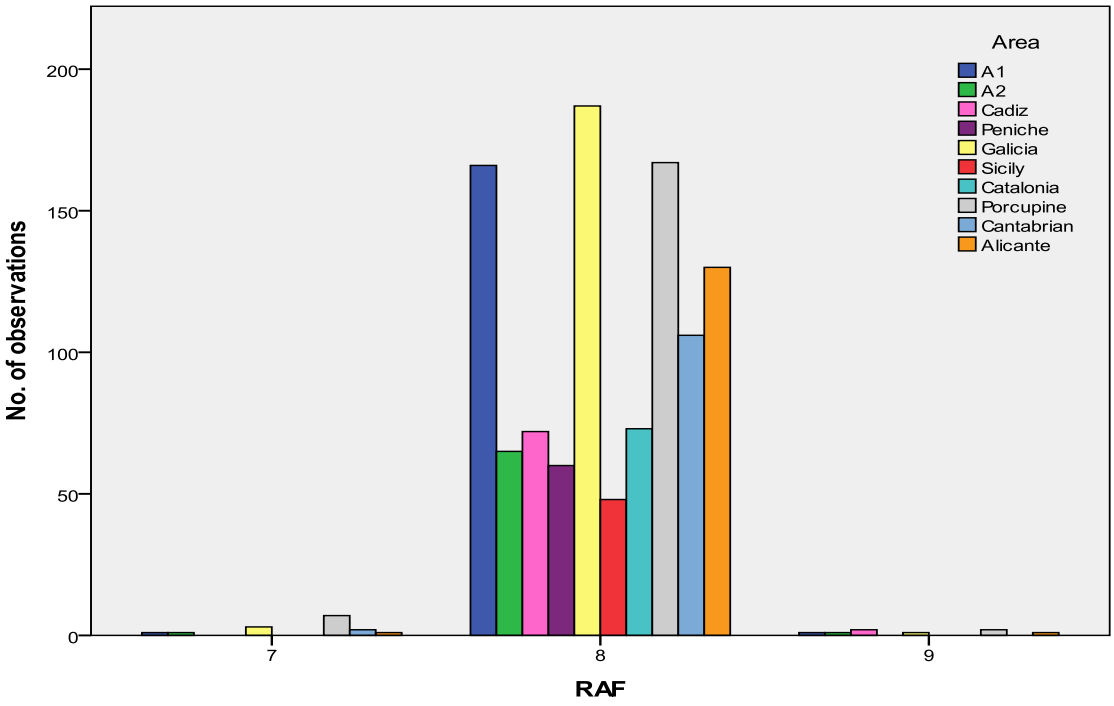


Figure 4-19. Frequency of rays of the anal fin (RAF) in the 10 locations.

Table 4-16. Results regarding the number of rays of the pectoral fin (RPF) in the 10 locations.

RPF					
Area	**N**	**Mean**	**Mode**	**Min.**	**Max.**
Porcupine Bank	176	18.14	18	17	19
Cantabrian Sea	108	17.99	18	16	19
Galicia	190	18.01	18	17	19
Portugal	60	17.97	18	17	19
Gulf of Cadiz	74	18.04	18	17	19
Alboran Sea - A1	168	17.98	18	17	19
Alboran Sea - A2	67	17.94	18	16	19
Alicante	132	17.94	18	11	19
Catalonia	73	17.99	18	17	19
Sicily	48	17.94	18	17	19

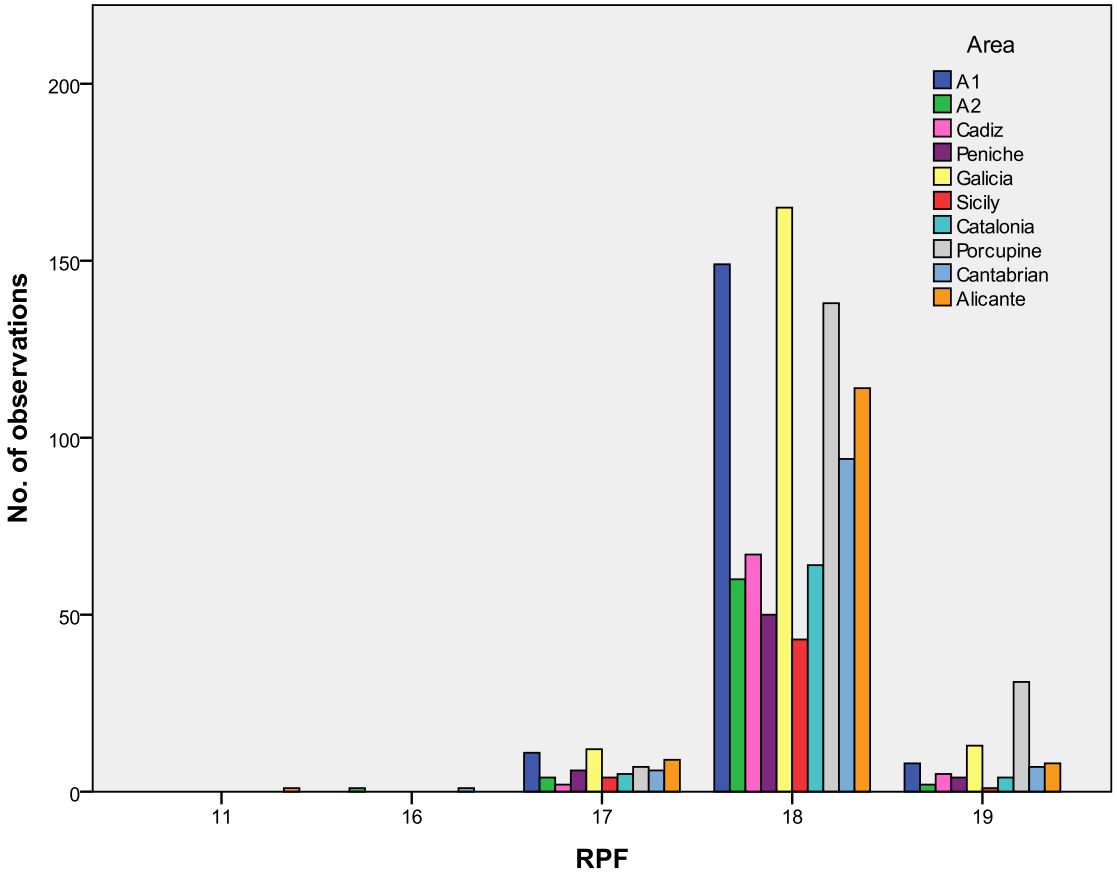


Figure 4-20. Frequency of rays of the pectoral fin (RPF) in the 10 locations.

Table 4-17. Results regarding the number of rays of the ventral fin (RVF) in the 10 locations.

RVF					
Area	**N**	**Mean**	**Mode**	**Min**	**Max**
Porcupine Bank	176	5.99	6	5	6
Cantabrian Sea	108	6.00	6	6	6
Galicia	191	5.99	6	5	6
Portugal	60	6.02	6	6	7
Gulf of Cadiz	74	5.99	6	5	6
Alboran Sea - A1	168	6.00	6	6	6
Alboran Sea - A2	67	6.00	6	6	6
Alicante	132	6.00	6	6	6
Catalonia	73	6.00	6	6	6
Sicily	48	6.00	6	6	6

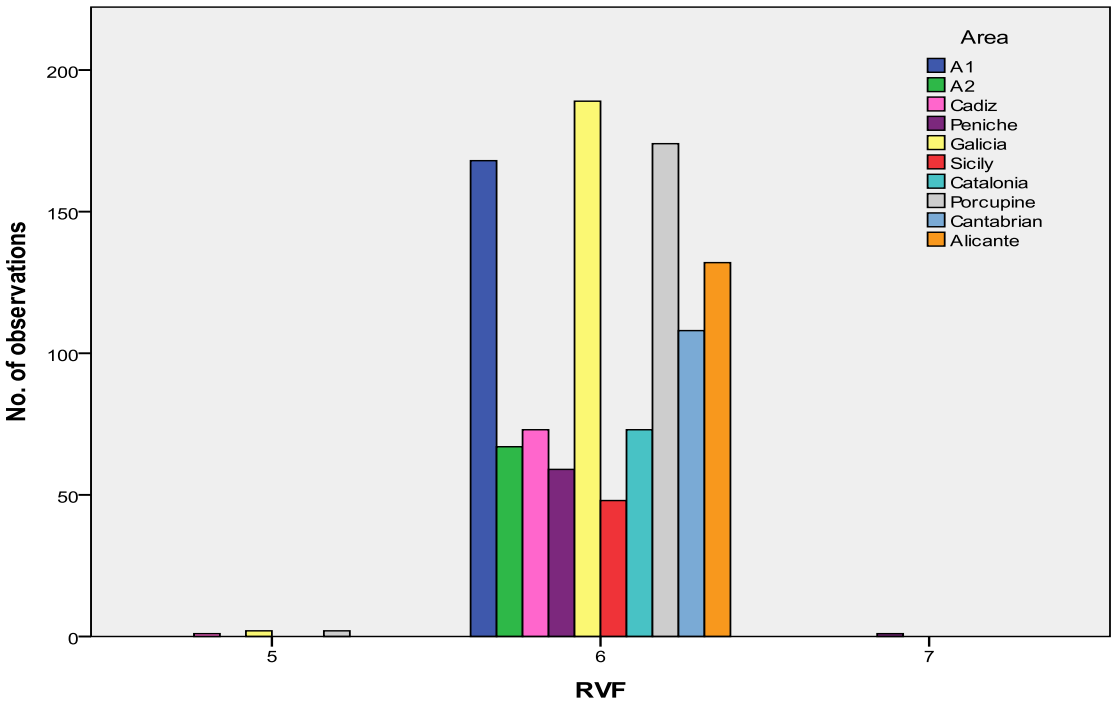


Figure 4-21. Frequency of rays of the ventral fin (RVF) in the 10 locations.

Table 4-18. Results regarding the number of gill rakers of the vertical branch (GRV) in the 10 locations.

GRV					
Area	**N**	**Mean**	**Mode**	**Min**	**Max**
Porcupine Bank	176	8.47	8	7	10
Cantabrian Sea	108	8.49	8	8	9
Galicia	185	8.36	8	7	9
Portugal	60	8.45	8	8	9
Gulf of Cadiz	74	8.70	9	8	10
Alboran Sea - A1	168	8.54	9	8	9
Alboran Sea - A2	67	8.46	8	8	9
Alicante	132	8.33	8	8	9
Catalonia	73	8.47	8	8	9
Sicily	48	8.54	9	8	9

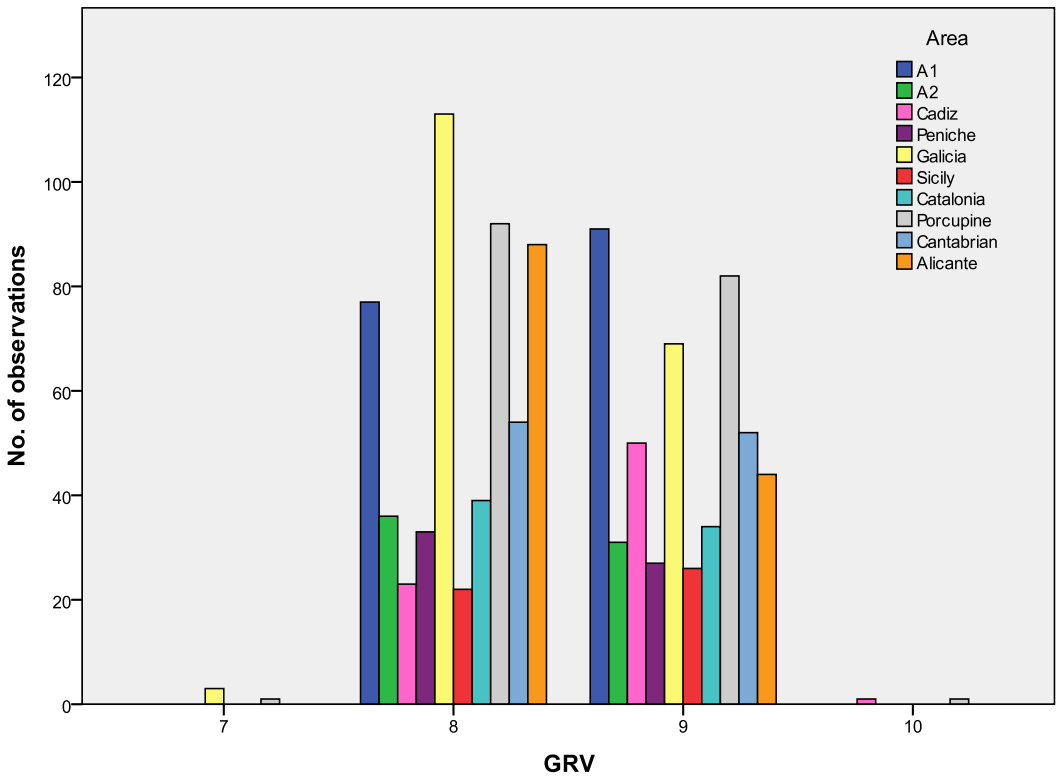


Figure 4-22. Frequency of the gill rakers of the vertical branch (GRV)in the 10 locations.

Table 4-19. Results regarding the number of gill rakers of the horizontal branch (GRH) in the 10 locations.

GRH					
Area	**N**	**Mean**	**Mode**	**Min**	**Max**
Porcupine Bank	176	16.85	17	15	19
Cantabrian Sea	108	16.67	17	16	18
Galicia	188	16.49	16	15	18
Portugal	60	16.43	16	15	18
Gulf of Cadiz	74	16.45	17	15	18
Alboran Sea - A1	167	16.53	17	15	18
Alboran Sea - A2	67	16.54	17	15	18
Alicante	132	16.39	16	14	18
Catalonia	73	16.60	17	15	18
Sicily	48	16.52	17	14	18

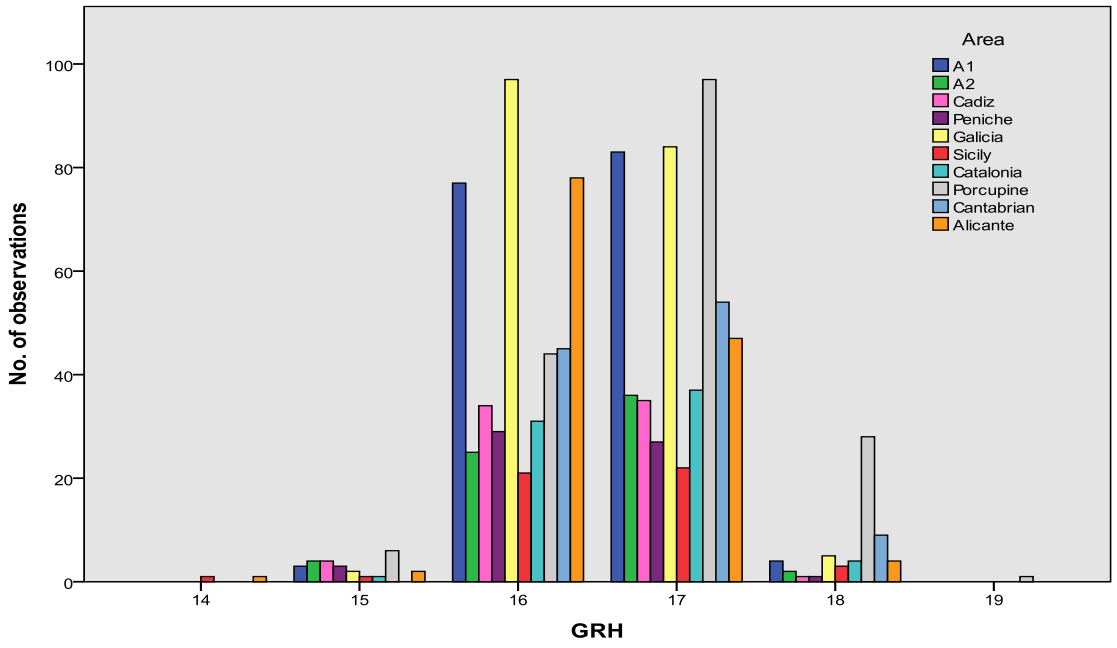


Figure 4-23. Frequency of the gill rakers of the horizontal branch (GHV)in the 10 locations.

Table 4-20. Kruskal-Wallis H-test for the meristic variables and mean rank differences between all pairs of locations in the NE Atlantic (highlighted in light grey). Significant differences are shown in bold. The critical difference values used in each comparison are shown below the diagonal. RPF = number of rays of the pectoral fin; GRH = Number of gill rakers on the horizontal branch and GRV = number of gill rakers on the vertical branch.

RPF

H (4) = 15.61** N		**Mean rank**	**Porcupine**	**Cantabrian**	**Galicia**	**Portugal**	**Cadiz**
Porcupine	176	331.17	0	38.96	37.31	47.89	27.67
Cantabrian	108	292.22	60.27	0	1.65	8.93	11.28
Galicia	190	293.86	51.59	59.42	0	10.58	9.64
Portugal	60	263.64	73.47	73.71	73.02	0	20.22
Cadiz	74	303.50	68.31	68.31	67.57	85.66	0
Total	608						

GRH

H (4) = 37.22** N		**Mean rank**	**Porcupine**	**Cantabrian**	**Galicia**	**Portugal**	**Cadiz**
Porcupine	176	359.16	0	45.71	**86.80**	**95.52**	**91.19**
Cantabrian	108	313.46	60.07	0	41.09	49.82	45.48
Galicia	188	272.37	51.55	59.37	0	72.87	67.44
Portugal	60	263.64	73.47	79.13	72.87	0	4.34
Cadiz	74	267.98	68.09	74.16	67.44	85.38	0
Total	606						

GRV

H (4) = 22.94**** N		**Mean rank**	**Porcupine**	**Cantabrian**	**Galicia**	**Portugal**	**Cadiz**
Porcupine	176	302.19	0	5.75	31.82	6.32	66.75
Cantabrian	106	307.94	59.92	0	37.57	12.07	61.00
Galicia	185	270.37	51.32	59.37	0	25.50	**98.57**
Portugal	60	295.88	72.86	78.74	72.41	0	73.06
Cadiz	74	368.94	67.53	73.83	67.04	84.67	0
Total	601						

** Significant at the 5% level ($p<0.05$)

Table 4-21. Kruskal-Wallis H-test for the meristic variables and mean rank differences between a) all pairs of locations in the Mediterranean (highlighted in light grey) and b) between the Gulf of Cadiz and the Alboran Sea. Significant differences are shown in bold. The critical difference values used in each comparison are shown below the diagonal. GRV = number of gill rakers on the vertical branch.

a)

GRV							
H (4) = 13.96**	**N**	**Mean rank**	**A1**	**A2**	**Alicante**	**Catalonia**	**Sicily**
A1	167	262.73	0	18.56	**50.06**	17.82	0.67
A2	67	244.16	57.12	0	31.50	0.75	19.23
Alicante	132	212.67	46.01	59.26	0	32.24	50.73
Catalonia	73	244.91	55.43	66.83	57.62	0	18.48
Sicily	48	263.40	64.69	74.70	66.58	73.41	0
Total	487						

** Significant at the 5% level (p<0.05)

b)

GRV					
H (2) = 8.30**	**N**	**Mean rank**	**A1**	**A2**	**Cadiz**
A1	168	151.92	0	12.16	23.89
A2	67	139.75	30.91	0	36.05
Cadiz	74	175.80	29.84	36.06	0
Total	309				

** Significant at the 5% level (p<0.05)

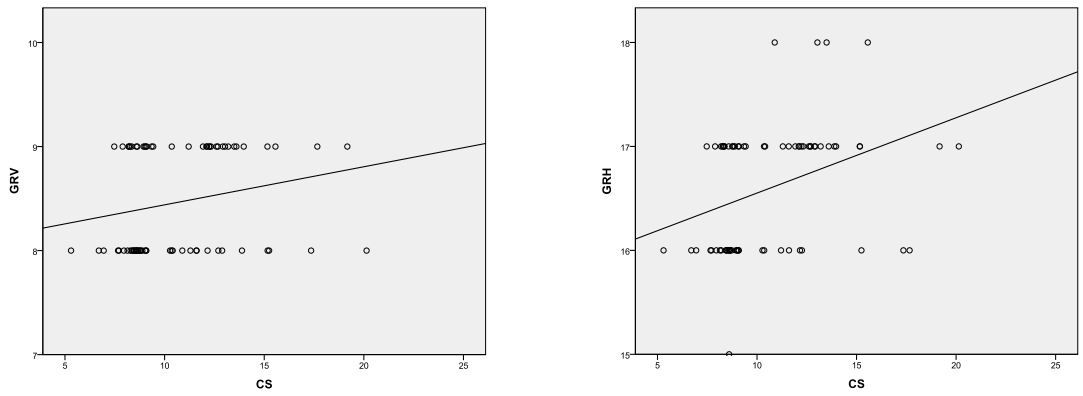


Figure 4-24. Relationship between the number of gill rakers of the horizontal (GRH) and vertical (GRV) branch for bluemouth from Catalonia.

4.4 Discussion

4.4.1 Sexual dimorphism

Studies regarding sexual dimorphism of bluemouth have focused only in age and growth differences between males and females (e.g., Massutí et al., 2000; Ribas et al., 2006; Abecasis et al., 2006; Sequeira et al., 2009), but the results among the different works are not consistent. Some of these authors have found that females grow faster and achieve a larger asymptotic length, others have found the opposite and in some studies no differences in growth rates were detected at all. However, to our knowledge, no studies have specifically investigated body morphology of male and female bluemouth to determine if there are differences between them. During the processing of the bluemouth samples used in this study, there were no evident external differences between sexes such as coloration (in fresh specimens during the sampling surveys) and body shape, or appreciable modifications of body parts (e.g. thicker or longer fins in males, stronger jaws in males, etc.). In addition, none of these features, that could indicate sex dimorphism in spawning bluemouth, have been reported in the literature (e.g., Muñoz et al., 1999; Mendonça et al., 2006 and Muñoz et al., 2010), with the exception of one study that indicated that males have an evident conical urogenital papillae that is absent in females (Sequeira et al., 2012).

Considering body shape, this morphometric study did not give a clear picture of whether bluemouth is a sexually dimorphic species because there were contradictions in the results. While no significant morphometric differences were observed in some of the studied populations (Galicia, the Gulf of Cadiz and Alicante), significant differences between sexes were detected within subarea A1 and the Porcupine Bank. Moreover, the results of the tests were contradictory in the case of Subarea A2, Sicily, the Cantabrian Sea and Portugal.

It is improbable that bluemouth males and females differ in some populations and in other not, but the presence of sexual dimorphism cannot be discarded based only on these results. However, there are cases were sexual dimorphism has been found for some populations within a species and not for others. For example, a similar situation occurred in a morphometric study where the structure of 11 populations of redfish (*Sebastes* spp.) in the north-west Atlantic was investigated (Saborido-Rey, 1994). Among them, only two populations, *S. fasciatus* from Newfoundland Grand Banks and *S. marinus* from St Pierre

Bank, exhibited sexual dimorphism. Since in both cases, sampling time corresponded with the period of larval pre-extrusion or extrusion in these areas, the first hypothesis was that sex differences were caused by spawning-related deformations of the ventral area. However, significant differences between sexes were found in morphometric variables such as eye diameter or head length, and it was concluded that proper sexual dimorphism existed in these two populations. Thus, besides the population structure analysis using pooled sexes, complementary analyses were carried out with males and females separately.

In our study, spawning-related ventral deformations were unlikely, because the influence of gonad size was eliminated when the specimens were eviscerated prior to the morphometric analysis. In addition, the shape differences between males and females of the areas were significant sexual dimorphism was detected, i.e., the Porcupine Bank and subarea A1 were related mainly to the mouth region and body depth.

Unfortunately in this study, the incongruence of the results did not allow us to draw solid conclusions regarding the existence of sexual dimorphism. In this case, the best strategy would be to analyze males and females separately in the population structure analysis (e.g., Saborido-Rey, 1994; Valentin et al., 2002), because if shape differences related to sex truly exist, the differences among the populations can be masked. However, in our case, the reduced sample size that results from dividing the sample into males and females would have prevented a representative sample for most areas and what is more, a large number of undifferentiated/unsexed specimens would be left out of the analysis, reducing the natural variability in each area.

4.4.2 Overall population structure

According to the results of this study, morphological differences were observed 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 (Aboim, 2005; Aboim et al., 2005). MtDNA markers revealed strong genetic differentiation between the NE Atlantic populations (Azores, Madeira and Portugal) and the populations from Cape Verde Islands and the NW

Atlantic, but the evidence of genetic differentiation within the NE Atlantic region (i.e., between the island groups and seamounts in the Azores, Madeira and the Portuguese continental slope) was weak (Aboim et al., 2005). However, when microsatellites were used, the data revealed isolation of Portugal and some differentiation at the local scale within the Azores archipelago (Aboim, 2005). Based on the results of the microsatellites, Aboim (2005) 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 (as no significant correlation was found between geographic distance and genetic distances of populations), and (2), that 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 could help in the interpretation of the morphometric differences between bluemouth populations and will be considered.

In addition to the genetic component, there are several environmental factors that can modify body shape in fishes i.e., temperature, water velocity, quantity of food, type of food and feeding mode, since 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 percoides*) in the Fiordland region in New Zealand, highlighting the importance of the local habitat quality and food resources for subpopulation structure.

In this study, bluemouth were sampled from a considerable number of locations in the NE Atlantic and the Mediterranean. Although the deep-sea (depth > 200 m) has been regarded as a relatively uniform environment with respect to some variables such as temperature and salinity (Hopkins, 1985; Merrett & Haedrich, 1997), the upper slope (200 – 500m), where the bluemouth is abundant (Massutí et al., 2001; Sánchez et al., 2008; Romeo et al., 2009), shows a variety of habitats, e.g. deep coral reefs, off-reefs habitats and transition habitats, with different bottom types, e.g. rocks, sand, mud or a combination of these, that support different fish and crustacean assemblages (Fariña et al. 1997, Demestre et al. 2000, Roberts et al. 2008, Sánchez et al., 2009; Ross and Quattrini, 2007, 2009). In a recent study, Ross and Quattrini (2007) observed that bluemouth was

very abundant on the deep coral reefs of the southeastern USA continental slope and the Rockall banks off Ireland, but they also mention that bluemouth was regularly observed away from reef habitat, associated to other structures (e.g. burrows or anemones). These observations indicate that bluemouth indeed use a diversity of habitats with different characteristics. Benthic habitats on the continental slope not only differ in their physical structure, but also in the faunal assemblages associated to them. As many demersal fish species, the bluemouth has a benthopelagic diet. The bluemouth can be considered specialized on medium sized benthic decapods (Natantia, Brachyura and Macrura), but it also feeds on demersal fish and sometimes pyrosomes, polychaetes and echinoderms (Macpherson, 1979, 1985; Nouar & Maurin, 2000; Serrano et al., 2003). Thus, the morphological differences detected in this study may be linked to differences in foraging behavior, depending on the food type and availability in the studied areas. In this case, morphometric differences would result from bone remodeling in response to differences in loading regime induced by the diet or feeding mode in a particular area, a phenomenon known as trophic morphological plasticity (Swain et al., 2005 and references therein). On the other hand, habitat and food similarity can also produce morphological similarities among distant populations (Swain et al, 2005), such as the ones found for bluemouth from the Porcupine Bank, the Gulf of Cadiz and Sicily.

Intra-specific phenotypic differences can also arise due to preferences regarding habitat use of different populations (e.g., coastal *vs* oceanic or pelagic *vs* demersal). For example, morphometric differences between deep-sea and oceanic phenotypes of beaked redfish (*Sebastes mentella*) have been described (Garabana, 2005). In that study, beaked redfish from the Irminger Sea were more fusiform than those from other areas, and the orientation of third and fifth pre-opercular spines was more forward-pointing in deep-sea types. In the case of bluemouth, it is possible that the use of the different habitats (described above) could produce morphometric differences affecting overall body shape (e.g., more fusiform vs more robust body), but further studies would be required to confirm this.

Nevertheless, in all cases, the population structure should be confirmed using a multidisciplinary (i.e. holistic) approach, because the investigation of any single characteristic will not necessarily reveal stock differences even when 'true' stock differences exist or may not be enough to delineate different stocks (Begg and Waldman, 1999). Morphometric variation in fish populations has not only been used as biological markers in stock identification studies. Actually, it is recognized that morphometric

research is more biologically meaningful if coupled with functional hypotheses regarding the adaptive significance of differences in body shape (Cadrin, 2005). However, in this study, the body shape characteristics for the different locations could not be interpreted directly to point out functional or adaptive implications, limiting to a certain degree the biological meaningful conclusions that can be drawn from our results. Moreover, it is difficult to measure all of the environmental variables (physical and biological) that may be influencing body shape in bluemouth to determine which factors are indeed causing morphological variation between areas. Another aspect that must be taken into account is that an experimental approach (e.g., "common garden experiments" and reciprocal transplants) would be needed to disentangle the genetic and environmental components of phenotypic plasticity (see Swan et al., 2005, for a detailed discussion on this topic).

The Strait of Gibraltar has been proposed to be the division between two important marine biogeographical regions, the Mediterranean Sea and the Northeast Atlantic (Borsa et al., 1997). 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 as it was previously thought. In that study, the sample from Malaga, lying in the Alboran Sea, was found to be genetically closer to the Atlantic rather than the Mediterranean gene pool, while the neighboring samples from Alicante and Balearic islands had Mediterranean features. Based on their results, these authors concluded that the Alboran Sea can be a zone of steep transition between Atlantic and Mediterranean Sea populations. In fish populations, the genetic differentiation between Atlantic/Alboran and Mediterranean populations has been explained not only by means of passive retention of larvae but through mechanisms of selection and/or homing of spawners, or else due to a secondary

contact between Pleistocene divergent forms that are now merging to produce temporary clines (Naciri et al., 1999; Cimmaruta et al., 2005).

On the other hand, some species show no differentiation at all between Atlantic and Mediterranean populations (Bargelloni et al., 2003). Thus, the differentiation pattern between the Atlantic and Mediterranean cannot be generalized, not even for species with comparable ecological features. For example, Bargelloni et al. (2003) observed inconsistencies within sparids. Their experimental data (genetic and morphometrics) from some sparids (e.g. *Dentex dentex* and *Lithognathus mormyrus*) lend strong support to the presence of a phylogeographical boundary between the Atlantic and the Mediterranean. Yet, for other sparids, like *Spondyliosoma cantharus*, *Pagrus pagrus* and *Pagellus bogaraveo*, genetic data provided little evidence for a separation between the two basins.
In this context, the observed pattern of morphological variation bluemouth populations (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) is compatible with the idea of the Almeria-Oran Front having some boundary effect for bluemouth.

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 their study, otolith elemental composition of bluemouth populations was investigated with the aim of stock discrimination. However, in that study, no clear differences in otolith composition were found between Atlantic and Mediterranean samples, because samples from northern Portugal were similar in composition to the Mediterranean groups (Alboran Sea and the Catalan slope). Thus, based on this result, it seems that neither the Strait of Gibraltar or the AOF are effective boundaries between Atlantic and Mediterranean bluemouth populations, which is not completely consistent with the results of the present study. Thus, it would be interesting to compare gene frequencies between Atlantic and Mediterranean bluemouth populations in future studies to determine whether the AOF (or the Strait of Gibraltar) 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.

4.4.3 Population structure of bluemouth in the Mediterranean

In the western Mediterranean, three sectors have been described as well-defined areas in terms of oceanographic conditions (Massutí et al., 2001): 1) the south-western basin (Alboran Sea), the north-western basin (Balearic Sea and Catalonia) and 3) the transition zone, which goes from Cape Palos to Sagunto (Alicante sector).

In this study, bluemouth from the Alboran Sea (subarea A1) were differentiated from the other two locations in the western Mediterranean (Alicante and Catalonia), showing the highest correct classification rate. On the other hand, bluemouth from Alicante and Catalonia were closely related in terms of body shape. Thus, there is evidence that at least two bluemouth populations exist in the western Mediterranean: one bluemouth population in the south-western basin (Alboran Sea) and another in the north-western basin (Balearic Sea and Catalonia) that extends to the transition zone in the Alicante region.

An important departure from the morphological pattern of bluemouth populations described above was observed in bluemouth from subarea A2, a group of 67 specimens caught to the east of the Alboran Island in the Alboran Sea (35º58.44´N, 2º49.53´W). Bluemouth from subarea A2 presented important morphometric differences with respect to bluemouth from the neighboring areas, even with bluemouth caught in two hauls to the west of the Alboran Island (35º59.61´N, 3º04.33´W and 35º55.70´N, 3º08.48´W). 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 following the established morphometric protocol, by the same person and using the same equipment. Several possibilities could explain the strong 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).

It has been suggested that the Alboran Sea should be considered a distinct separate management unit when dealing with demersal fisheries from an ecosystem point of view

(Abelló et al., 2002), which is in agreement with our results. Several studies have reported differences in life history parameters and distribution of bluemouth between the two basins of the western Mediterranean, i.e., the Alboran Sea and the Balearic Sea-Catalonia (Massutí et al., 2000b; Massutí et al., 2001; Ribas et al., 2006; Abad et al., 2007) . These studies have revealed the existence of a well-developed bluemouth spawning stock in the Alboran basin, contrary to what is found in the areas with a high fishing pressure in the northern sectors of the western Mediterranean (Balearic Sea and Catalonia) where older fish are poorly represented. In addition, in a stock discrimination study based on otolith chemistry, it was found that the otolith elemental composition of adult *H. dactylopterus* from the Alboran Slope differed from those of the Catalan slope (Swan et al., 2006).

Besides physical factors (i.e., hydrography and geomorphology), the biological characteristics of the faunal communities (e.g., the species composition and distribution) in the different sectors could also play an important role influencing the bluemouth population structure in the western Mediterranean. For example, the different food availability in the continental slope ecosystems of the Alboran Sea and the Balearic Sea could be responsible for the observed differences in age composition and growth parameters between bluemouth from the two basins (Massutí et al., 2000a). Also, the high abundance of fish like the bluemouth in the small seamount Seco de los Olivos in the eastern Alboran Sea may be related to the high food availability at this site caused by strong localized currents and upwellings (Abad et al., 2007). Likewise, the morphological differentiation shown by bluemouth from the Alboran Sea is probably linked to the type and the availability of prey. Several studies regarding demersal and epibenthic communities in the shelf and upper slope of the western Mediterranean indicate that the Alboran Sea, the Alicante sector and the Catalonian sector support different faunal assemblages (Cartes et al., 1994; Demestre et al. 2000; Abelló et al. 2002; Abad et al. 2007). According to the results of a study by Abelló et al. (2002), the crustaceans considered to be the main prey items of *H. dactylopterus* in the western Mediterranean according to Macpherson (1979) (i.e., *Alpheus glaber*, *Calocaris macandreae*, *Goneplax rhomboides* and *Munida* spp.), showed different distributions along the western Mediterranean (from the Strait of Gibraltar to the northern Catalonia).

Nevertheless, despite the morphological differences found along the western Mediterranean, the two bluemouth populations, Alicante-Catalonia and the Alboran Sea, 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 and thus share the same genetic component. The Gulf of Vera (between the eastern Alboran sector and the Alicante sector) was not sampled in this study, but perhaps samples from this area could be used to determine the extent of morphological similarity between the Alboran Sea and the Alicante sector.

Bluemouth from the two reference areas (i.e., the Gulf of Cadiz and Sicily) were morphologically similar, according to the cluster and CVA analyses, where a considerable number of fish from Sicily were assigned to the Gulf of Cadiz. Considering that stock units are distributed in space as gradients, it is unlikely that fish from locations far apart from each other could belong to the same stock unit (Murta et al., 2008). However, it is possible that fish from distant areas resemble each other morphologically. In this case, body shape similarity between individuals from distant locations 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).

The fact that the greatest number of misclassified bluemouth specimens from the Gulf of Cadiz were allocated to the Alboran Sea weakens the hypothesis that the Strait of Gibraltar acts as a barrier isolating Atlantic and Mediterranean bluemouth populations. However, relative separation of bluemouth from the Alboran Sea and Alicante/Catalonia could indicate a boundary effect by the Almeria-Oran Front that limits the connection between bluemouth populations within the western Mediterranean (see Section 4. 4. 2. – Overall population structure).

4.4.4 Population structure of bluemouth in the NE Atlantic

The bluemouth population structure in the NE Atlantic was studied based on samples from four areas around the Iberian Peninsula (The Gulf of Cadiz, Portugal (Portugal), Galicia and the Cantabrian Sea) and one reference area located to the west of Ireland, at the Porcupine Bank. The results showed significant body shape differences among bluemouth from all of the NE Atlantic locations, but the greatest morphological differentiation was found 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.

The genetic structure of bluemouth from Portugal (Peniche) has been studied by Aboim et al. (2005), who found that bluemouth from Portugal showed a high degree of genetic isolation with respect to bluemouth from the Azores, and to a lesser extent, with bluemouth from Madeira. Unfortunately, that study did not include other locations on the continental margin that could be compared to Portugal in order to clarify the genetic population structure of bluemouth on the Iberian Peninsula. Nevertheless, 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 in Murta et al., 2008) or a high segregation of Portuguese samples (e.g., black anglerfish in Duarte et al., 2004).

The sample from Portugal was obtained in waters in the vicinity of Peniche, that is situated in the Lisbon continental margin region. This region is enclosed by several extensive submarine canyons, namely, the Nazaré Canyon to the north and the Cascais and Setúbal-Lisbon canyons to the south. The Nazaré Canyon (latitude 39° 35' N, longitude 9° 25' W) is the biggest in Europe: it cuts the full width of the shelf and slope, extending from shallow water less than 1 km off the coastline to a depth of 5000 m at 210 km offshore (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). However, Sousa et al. (2005) consider that the biological discontinuity is not exclusively due to the Nazaré canyon, but also due to differences in shelf and coastal morphology, bathymetry, river runoff, and ocean currents along the north and southern parts of the shelf. Thus, it is possible that the particular body shape of bluemouth from Portugal

(Peniche) reflects the relative isolation of the region due to the canyons and the other characteristics listed above.

In spite of the significant distances between the mean shapes of bluemouth, morphological differentiation was not so clear for bluemouth from the rest of the studied locations in the NE Atlantic. A close morphological relationship between bluemouth from Galicia and the neighboring location to the northeast, the Cantabrian Sea, was observed (low Mahalanobis and Procrustes distances between the mean shapes; some confusion of specimens between these areas in the classification matrix). The overall morphological variation along the Cantabrian Sea and Galicia seemed to follow a gradient, with no clear breakpoints that could indicate the isolation between bluemouth from these regions. Two important biogeographical limits (with boundary effects for fish populations) have been identified between Galicia and the Cantabrian Sea: Cape Estaca de Bares and Cape Finisterrae (Sánchez & Gil, 2000; Sánchez & Serrano, 2003; Serrano et al., 2008), but neither of them seems to have a noticeable (boundary) effect on bluemouth populations at the morphological level. 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.

However, the environmental conditions between and within the two areas are fairly variable (Sánchez & Serrano, 2003; Serrano et al., 2008), and this is certainly reflected in the different morphological characteristics of bluemouth along the northern margin of the Iberian Peninsula.

For example, the middle and outer shelves (100 – 500 m depth) of the Galician study area are covered with sand, whereas in the Cantabrian Sea the outer shelf is characterized by muddy bottoms; within Galicia, the southern shelf is covered with sediments that are enriched by the organic matter advected from the Rías Baixas, while the northern and western shelves are covered with sands with lower organic content (Serrano et al., 2008 and references therein).

The different bottom types, in turn, determine the type, quantity and distribution of crustaceans in each area, which affects the composition and distribution of fish assemblages as well (Fariña et al., 1997; Serrano et al., 2008). In the Cantabrian Sea, at the summit of Le Danois Bank where bluemouth is more abundant (400 – 550 m depth), some of the decapods which are considered to be the main prey of adult bluemouth (i.e. the crab *Goneplax rhomboides*, and the shrimps *Calocaris macandreae* and *Alpheus*

glaber) are scarce or even absent due to the low proportion of mud in the sediments that is needed by these burrowing species (Cartes et al., 2007). In contrast, these decapods are very abundant in other areas considered in this study, like the southern part of the Galician shelf and upper slope, where there are fine sediments due to outwelling from the Rías Baixas (Fariña et al., 1997).

Thus, the differences in the head and snout of bluemouth from Galicia and the Cantabrian Sea could represent morphological adaptations of the snout, reflecting the way bluemouth use food resources in these areas.

Fishing pressure is another factor that can have an impact on fish growth (Law, 2000) and thus, on body morphology (Swain et al., 2005). At some points in the Cantabrian Sea (e.g., Le Danois Bank), no regular fishery operates and a well-preserved bluemouth spawning stock has been observed (Sánchez et al., 2008), whereas strong fishing effort takes place over the entire Galician shelf (Serrano et al., 2008).

Thus, the combination of these factors (i.e. food type and availability, fishing mortality and environmental conditions) may explain the gradient of morphological variation exhibited by bluemouth along the northern regions of the Iberian Peninsula.
Regarding bluemouth from the reference area in the NE Atlantic, the Porcupine Bank, around 13 % of the specimens were incorrectly assigned to Galicia, indicating some morphological similarity between these two areas. However, the cluster analysis showed that the Porcupine Bank more related with the Gulf of Cadiz and in the classification matrix almost 19 % of the fish from Cadiz were assigned to the Porcupine Bank. 13.33 % of the fish from Portugal were also assigned to the Porcupine Bank.

Considering that adult specimens of bluemouth are thought to be sedentary (Uiblein et al., 2003; Pakhorukov, 2008; Aboim, 2005 and references therein), that fertilization is internal (Muñoz et al., 1999) and that the larval dispersive phase is generally not sufficient to allow gene-flow between distant bluemouth populations (Aboim, 2005), it would be unlikely that the bluemouth populations in the Porcupine Bank, Galicia and the Gulf of Cadiz were effectively connected.

The offshore banks to the west of Ireland have been described as an important spawning and larval ground for the bluemouth (Dransfeld et al., 2009). Moreover, the retention of eggs and larvae over these offshore banks has been suggested to occur as a consequence of the formation of Taylor columns above the Rockall bank and Porcupine Bank (Dransfeld et al., 2009). Thus, the characteristics of these banks could promote the formation of local fish populations, possibly resulting in genetic differentiation as well. In

the case of bluemouth, this phenomenon has been observed already in the Azores archipelago, where the genetic distance between the Azores Bank population and the populations around the Azores islands was found to be striking (Aboim et al., 2005).

However, there is evidence that when conditions are adequate, bluemouth can spread over considerable distances. For example, Heessen et al. (1996) reported an invasion of the North Sea by juvenile bluemouth in 1991, a region where the species was hardly recorded before this date. The invasion was attributed to either a change in larval drift or a migration of juveniles (by swimming), indicating that in some occasions, bluemouth can travel relatively long distances (Mamie et al., 2007).

Besides the possible gene exchange between different populations, it is also possible that body shape similarities are the product of phenotypic plasticity, which is the ability of a genotype to produce different phenotypes across an environmental gradient (Swain et al., 2005 and references therein), but the morphological results alone, however, do not allow determining with certainty the relationship between bluemouth populations in the Porcupine Bank and the continental slope. 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.

As it was noticed in the overall population structure analysis, with the 12 bluemouth samples included, bluemouth from Galicia and the Cantabrian Sea caught in 2006 were different from those caught in the same locations a year later (i.e., 2007). In the analysis of the population structure of bluemouth from the NE Atlantic including the 2006 samples, the separation of the 2006 and 2007 samples was clear as well.

First of all, the possibility of a methodological error was considered as a potential explanation for the observed differences. The fish caught in the DEMERSALES 2006 survey were the first samples obtained for this study, when the sampling protocol was still being developed. In many cases, the specimens were eviscerated with a cut on the left side of the fish, instead of the right side that was later established in the morphometric protocol. In addition, at this early stage of the study, several people participated during the collection of the samples, which may have introduced more errors than when the sampling protocol was fully developed in 2007. Since no significant body shape differences between the left and right sides of uncut fish were observed (see Material and Methods – Bilateral symmetry section), it is likely that the way the fish from the DEMERSALES 2006 survey were cut and eviscerated (before the protocol was established) affected their body morphology. Unfortunately, it was not possible to

determine exactly how much did the processing of these specimens affected their body shape and if this was enough to produce the observed differences between samples from 2006 and 2007.

Another possibility that has to be considered is that bluemouth distribution on the Galician and Cantabrian slopes changed in 2006 and 2007. Although adult bluemouth are mostly sedentary (Uiblein et al., 2003; Pakhorukov, 2008; Aboim, 2005 and references therein), in some cases they can spread over considerable distances (see Discussion above). In 2006, the differences between bluemouth from Galicia and the Cantabrian Sea were much less apparent than in 2007, and despite the differences between the 2006 and 2007 samples, bluemouth from these locations were still more related between them than to the three other NE Atlantic locations (the Porcupine Bank, Portugal and the Gulf of Cadiz).

In any case, the temporal and spatial stability of the bluemouth population structure for these areas must be confirmed using a multidisciplinary (i.e. holistic) approach over a longer period of time.

4.4.5 Meristics

In general, meristic variables were stable among bluemouth from the different locations, suggesting a more or less homogeneous stock structure of this species in the NE Atlantic in the Mediterranean. The only evident variability regarding meristic traits was observed in the counts of gill rakers (GRV and GRH), and particularly, between populations in the NE Atlantic like the Porcupine Bank. In the Mediterranean, only Alicante and subarea A1 differed significantly in the counts of gill rakers of the vertical branch (GRV).

The developmental environment (e.g. temperature, salinity, dissolved oxygen, etc.) can have a great effect on the number of parts formed in fish, however, there appears to be a strong genetic component to meristic variation within populations (Barlow, 1961; Swain et al., 2005). Therefore, the overall stability in meristic counts could be reflecting genetic and/or environmental homogeneity among bluemouth from the different locations.

Nevertheless, some meristic variables are thought to have a stronger genetic component than others, because they are linked to fitness through effects on survival under predation (e.g. spines and lateral plates) or feeding efficiency (e.g. gill rakers), and for gill rakers, controlled rearing experiments have generally confirmed a genetic component to differences between populations (Swain et al., 2005 and references therein). Thus, the differences in the number of gill rakers observed between bluemouth from the Porcupine Bank and Galicia, Portugal and the Gulf of Cadiz or between Galicia and the Gulf of Cadiz could be reflecting some degree of genetic differentiation. In the particular case of the Porcupine Bank, this results support the hypothesis (based on morphometric data) that the characteristics of the Porcupine Bank could promote the formation of local fish populations, resulting in genetic differentiation (see section “Population structure in the NE Atlantic” in the discussion above). However, the overall homogeneity among populations suggested by the meristic data does not coincide with the population structure that was observed based on the morphometric data. This situation stresses the importance of using other complementary techniques to determine accurately the stock structure of bluemouth. In particular, a genetic approach would be very useful to contrast the results of this study and understand better the underlying causes of the differences in meristic characters and morphology of bluemouth in the NE Atlantic and the Mediterranean.

4.5 Concluding remarks

4.5.1 Overall population structure

According to the results of this study, the morphology of bluemouth from all of the sampled areas in the NE Atlantic and the Mediterranean differed significantly. However, when all of the results were taken into account the differences between some of the areas were not as solid as they appeared at first. In fact, the pattern of morphological variation related to the geographical location of the samples was diverse: in some cases, morphological variation seemed to be gradual; with bluemouth from closely located areas being relatively similar (e.g., Alicante and Catalonia or Galicia and the Cantabrian Sea) and in other cases, morphological similarity was found for bluemouth from distant areas, as in the case of the Porcupine Bank, the Gulf of Cadiz and Sicily.

There was no clear morphological distinction between bluemouth from the Atlantic locations and that of the Mediterranean locations, and 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 that would cause a strong morphological differentiation.

4.5.2 Population structure in the Mediterranean

The analysis of body shape showed that at least two bluemouth populations exist in the western Mediterranean: one bluemouth population in the south-western basin (Alboran Sea) and another in the north-western basin (Balearic Sea and Catalonia) that extends to the transition zone in the Alicante region. Bluemouth from subarea A2 in the Alboran Sea presented important morphometric differences with respect to bluemouth from the neighboring areas, indicating that there could be a third population in the western Mediterranean (or a sub-population as in the case of bluemouth studied by Aboim (2005) in the Azores archipelago). However, further samplings within the Alboran Sea should be carried out to determine the spatial and temporal stability of our results, and possibly, the new samples should be analyzed using other techniques for stock identification at the same time.

Bluemouth from the reference area (Sicily) presented shape similarities with bluemouth from the Gulf of Cadiz, and to a lesser degree, with bluemouth from Alicante

and subarea A1. Based on our results and the available literature, we suggest that bluemouth from the reference area (Sicily) belongs to a different population unit with respect to those in the western Mediterranean. In this case, we tend to favor the hypothesis that body shape similarity between these locations is caused by coincident environmental factors or even by genetic similarities that remained during the evolution, because it is less likely that a significant rate of interbreeding exists between these populations considering the geographical distance that separates them. However, it is not possible to say if there is a clear boundary between bluemouth populations in the western and central Mediterranean or if body shape varies gradually until more sampling locations are included to determine how body shape varies between these regions.

4.5.3 Population structure in the NE Atlantic

The greatest morphological differentiation was found 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.

The overall morphological variation along the Cantabrian Sea and Galicia seemed to follow a gradient, with no clear breakpoints that could indicate the isolation between bluemouth from these regions. The two biogeographical limits located between Galicia and the Cantabrian Sea (i.e., Cape Estaca de Bares and Cape Finisterrae) did not seem to have a noticeable (boundary) effect on bluemouth populations at the morphological level

Chapter 5

General Discussion and Conclusions

5.1 Methodology and limitations to work

Several aspects determine the quality, reliability and usefulness of the results of morphometric studies aimed at stock identification. The sampling design, the procedure to acquire data and the choice of statistical methods to analyze the data are all crucial to accomplish dependable results from which the stock structure can be inferred (e.g., Cadrin, 2010; Strauss, 2010). Thus, errors or problems in each of these aspects are likely to have negative effects on the outcome of the study. For example, if the sampling design is not appropriate and does not cover the whole range of distribution of a species, spurious differences may result between specimens collected at the extremes of the distribution (Bowering, 1988 in Garabana, 2005). Likewise, if fish specimens are not processed adequately, deformations that affect body shape can take place and the results can be misleading (e.g., arching effect in Valentin et al., 2008). Therefore, it was important to identify methodological problems and limitations along this study in order to assess the possible impacts on the results and amend the situation whenever it was possible. In the next sections, the limitations regarding sampling, data acquisition and morphometric and statistical analyses are discussed.

5.1.1 Sampling

When little information is available about the stock structure of a species, the optimal sampling design is to obtain representative samples from its entire distribution range as well as all seasons to investigate patterns of variation and potential for mixing of the populations (Saborido-Rey & Nedreaas, 2000; Cadrin, 2005; Abaunza et al., 2008). The sampling design must consider all the information available regarding the biology, ecology and distribution of the species in question, in particular the size distribution and the stocks structure. If the location of spawning grounds and spawning season are known, these areas/seasons should be included for sampling because in this way, sampling takes place when mixing between putative stocks is minimal (Cadrin, 2005). However, as mentioned before, knowledge on the biology of the fish is essential here. For example, in the viviparous *Sebastes mentella* in Norway it was found that during parturition (release of larvae) mixing of the three observed stock components was highest, but only for females (Saborido-Rey & Nedreaas, 2000). This situation is the consequence of the

spatial-temporal variation that occurs in many fish populations due to migratory movements, which usually give rise to temporal cyclic changes in the distribution. The periodicity may be short or seasonal or it may be the length of the life span of the fish (Abaunza et al., 2008). These movements are based on three types of habitat: one suitable for reproduction, one suitable for feeding and one suitable as a refuge in periods of unfavourable conditions (Wootton, 1998). In the case of bluemouth, it has been proposed that spawning females may exhibit different behaviors associated with reproduction, such as migration to submarine canyons and abrupt bottoms (Muñoz et al., 2010). For example, in the Mediterranean, spawning season (i.e., extrusion of embryos) does not begin until December (Muñoz et al., 2010), but females contain stored spermatozoa from May onwards, indicating that copulation can take place well in advance of spawning (Muñoz & Casadevall, 1999). All of these reproductive characteristics may be drivers to some type of migratory movements related to copulation and/or spawning in bluemouth populations, but these aspects still need to be investigated. Thus, as happens with bluemouth, when there is no previous knowledge of the possible times and areas of migration, samplings should be carried out in different seasons of the year to determine possible migratory routes and identify the type of habitat that is the aim of the migration (Abaunza et al., 2008).

Moreover, it is advisable that the sampling design involves temporal replicates in the same geographical area according to the chosen time scale in order to discern signals from noise, i.e. to analyse temporal stability in the morphometric differences (Ward, 2000; Grant & Waples, 2001 in Abaunza et al., 2008), because the populations may vary over time. In a short - medium time scale, which is in the order of years, this variation mainly concerns changes in distribution, abundance and some biological parameters, because variation in relation to genetically based evolutionary processes usually occurs over a much larger time scale (Abaunza et al., 2008).

The collection of bluemouth samples for this study was not an easy task. The bluemouth, though an important by-catch in many demersal fisheries, is not a target species. This implies that specimens could not be obtained on a regular basis from the commercial fleet in most areas. In fact, most all of the samplings were carried out in bottom trawl surveys in collaboration with the Spanish Institute of Oceanography (Instituto Español de Oceanografía - IEO). In these surveys, the samplings were not specifically designed for capturing bluemouth, but were aimed at estimating the abundance of demersal resources in the study area on sea bottoms ranging from 100 to

600 m. The surveys, in any case, offered several advantages over commercial samplings. First, it was ensured that extensive areas around the Iberian Peninsula were covered. Second, it was possible to record the exact location (latitude/ longitude and depth) of the hauls where each bluemouth specimen was caught and third, a wide size range of bluemouth specimens (from juveniles to large adults) was obtained in most areas (see Chapter 2 - *Material and Methods* for the description of the samples from each area).

In this study, it was considered that the areas covered by the trawling surveys were appropriate for studying the population structure of bluemouth around the Iberian Peninsula. The DEMERSALES survey covers the whole northern platform of the Iberian Peninsula (Galicia and Cantabrian Sea), while the MEDITS survey covers the whole extent of the Iberian platform in the Mediterranean (from the Strait of Gibraltar to Cape Creus in Catalonia). The region across the Strait of Gibraltar, the Gulf of Cadiz, was covered by the ARSA survey. However, a significant limitation in this study was that the Portuguese coast could not be covered for sampling in the same way as the other regions. It was important to obtain samples from this region in order to be able to detect if patterns of morphological variation were continuous along the Iberian Peninsula or if gaps in the population characteristics existed. Thus, efforts were made to obtain samples from the Portuguese coast, but in the end, only a single sample from the commercial fleet (from Peniche) was obtained for this study. Nevertheless, it is worth mentioning that a close collaboration with Portuguese researchers from the University of Lisbon was established and that the author was able to investigate the patterns of morphological variation in Portuguese waters in another study (i.e., Sequeira et al., 2011a).

Though the spatial coverage of the samplings was very good, the temporal coverage was limited, because it was not possible to obtain samples from the same geographical areas at different times of the year and from different years. Moreover, the sampling years and periods within the year were different among most areas. For instance, the western Mediterranean (Alboran Sea, Alicante and Catalonian coast), Galicia and the Cantabrian Sea were sampled in 2007, but the Mediterranean samples were obtained in spring and Atlantic samples in autumn. Samples from the Gulf of Cadiz could not be obtained until March 2009 (late winter), and from Portugal, until the beginning of 2010 (mid-winter). The sampling years for the reference areas also differ: samples from the Porcupine Bank were obtained in autumn 2008 and from the Central Mediterranean (Italy), in autumn 2009. The only areas for which samplings were carried out in two consecutive years (2006 and 2007) and at the same period of the year (autumn) were Galicia and the

Cantabrian shelf. However, the specimens from 2006 could not be used reliably because errors during the processing of this sample were suspected to cause body deformations. Thus, the specimens from 2006 were excluded from the main analysis (see section *Data acquisition* below and Chapter 4 – section 4.4.4 for details) and were not taken into account when determining the population structure of bluemouth.

The disadvantage of such a limited temporal coverage was that it did not allow us investigating possible migratory movements (e.g., seasonal migrations) between study areas and what is more, we were not able to confirm the stability of the morphometric patterns that were observed in this study. Also, the validity of the conclusions that were reached regarding the stock structure of bluemouth could be questionable if migratory movements do exist. For example, the lack of significant morphometric differences between some areas (e.g., Galicia and the Cantabrian Sea) could indicate that bluemouth from these areas were mixed at the time of the samplings due to migratory movements, and not that the specimens belong to a single population.

Actually, difficulties to achieve a good temporal sampling coverage are common in studies for stock identification, using both morphometric and genetics, probably because extensive sampling demands considerable logistic, operational and economic efforts (e.g., Garabana, 2005; Abaunza et al., 2008b). Thus, a significant number of morphometric studies have been carried out using samples that were obtained during relatively limited or different periods of time (e.g., Turan, 2004; Cadrin & Silva, 2005; Murta et al., 2008; Sequeira et al., 2011a; Traina et al., 2011; Tripp-Valdez et al., 2012). In most of these studies, the authors recognized the problems that limited sampling causes (e.g., those described in the previous paragraph) and concluded that results from such studies shall be considered preliminary or exploratory until more extensive studies are carried out. In fact, it seems that slightly improving the sampling coverage within the year (i.e., sampling more than one season) may substantially increase the chances of uncovering migratory patterns. For example, Saborido-Rey and Nedreaas (2000) were able to distinguish a spawning migratory pattern of beaked redfish (*Sebastes mentella*) by comparing body shape of samples collected only in two seasons (spring and autumn). Thus, following this line, it seems reasonable to complement this study with more samplings carried out at different seasons and several years in order to confirm the bluemouth population structure around the Iberian Peninsula.

Another important aspect that needs to be considered at the sampling stage of stock identification studies is the number of specimens to be sampled from each location and

period. In stock identification studies using morphometrics, optimal sample sizes are a function of the degree of morphometric variation within groups and the magnitude of difference among stocks that is desired to detect (Cadrin, 2005). The statistical methods that will be used to analyze the data will also determine the minimum sample sizes that are required, and in the case of morphometrics data, these methods are inherently multivariate. The general recommendation when using multivariate techniques is that there must be at least as many sample entities (specimens) as variables, however, other specific rules may apply for the different techniques (e.g., Principal components analysis, Cluster analysis, Discriminant analysis, etc.) (McGarigal et al., 2000). When designing this study, we followed the recommendation for the minimum sample size required for the Canonical variate analysis (CVA), because this was one of the most important multivariate techniques in this study. For this method, McGarigal et al. (2000) suggests that minimum sample size should be three times the number of variables. On this matter, Cadrin (2005) also advises that in stock identification studies (using morphometrics) the number of variables must always be smaller than the number of specimens, and recommends using a minimum number of specimens of at least three times the number of variables. In our case, the number of variables was 22 (13 landmarks in 2 dimensions = 26 variables, but only 22 contain shape variation after Procrustes superimposition), so the recommended minimum sample size following this approach would be of 66 specimens. Considering this and possible sampling difficulties, we established a minimum of around 60 specimens to be sampled in each location. In most of the sampled areas, this requirement was met. The exceptions were the Cantabrian Sea in 2006 and Sicily, where only about 50 specimens per location were sampled.

It is possible to obtain useful information for stock identification through geometric morphometric studies based on sample sizes that can be considered low relative to the number of landmarks used to describe body shape. For example, in the morphometric study carried out by Murta et al. (2008) to identify horse mackerel (*Trachurus trachurus*) stocks, the sample sizes ranged from 10 to 75 specimens per year and location and body shape was described using 11 landmarks in 2 dimensions. In spite of uneven and low sample sizes in many of the study areas, the authors were able to determine the existence of at least six separate populations of horse mackerel according to body shape. In the study by Tripp-Valdez et al. (2012) carried out to identify sablefish (*Anoplopoma fimbria*) populations, only between 9 and 18 specimens were analyzed in each study area and the number of landmarks was of 14. These authors were also able to identify at least

to different populations of sablefish. The huge advantage in both of the above mentioned studies was that the authors were able to contrast their results with at least another technique for stock identification (e.g., genetics, parasite tags and life history traits). It demonstrated that the stock structure of the studied species using low sample sizes can be determined reliably.

Nevertheless, the reliability of the results in studies using low sample sizes can be questionable and it would be advisable to confirm the population structure using larger samples that represent better the studied populations. In fact, sample size has been found to have a profound effect on the shape and size parameters that are estimated in geometric morphometric studies (e.g. Cardini & Elton, 2007). Although not specifically applied to fish populations, the study carried out by Cardini and Elton (2007) on vervet monkeys can give us an idea of how sample sizes affect shape estimates in morphometric studies. In their study, the impact of sampling error on analytical results was assessed by using repeated randomized selection experiments to build progressively smaller samples from an original dataset of ~400 vervet monkey skulls. They found that relatively small samples ($N > 10$) could provide fairly accurate estimates of the magnitude of shape variation, provided the specimens were sampled over all or most of the distribution range of a species. However, the accuracy of the sample mean shape rapidly deteriorated when sample size decreased. For example, in samples of less than 30 specimens, the error in the mean shape estimate was found to reach between 20–37% of the interspecific distance between mean shapes of two vervet monkey species that diverged about 8 million years ago and which have profound differences in their ecology and behaviour. Thus, they suggest that when selecting a sample for inferential purposes, a judgment must be made as to whether the data sampled reflect the variability within the population as a whole, and if it is possible to make generalizations on the basis of the chosen sample.

In the case of morphometric studies, the size composition of the samples (i.e. the proportion of small, medium and large specimens in a sample) is also important, because the amount of allometric shape variation in a sample is related to its size composition. Thus, samples with different size compositions will exhibit different amounts of size-related shape variation and there is the risk of confounding this variation with real morphometric differences between fish populations. Because of this, the ideal situation in studies for stock identification would be that the samples of the populations to be compared had similar size compositions (e.g., Valentin, 2006; Costa & Cataudella, 2007). Otherwise, allometric corrections such as those carried out in Chapter 3 will be needed

before the populations can be compared and these procedures may not be always straightforward (e.g., see Introduction and Discussion in Chaper 3).

However, it is not easy to obtain homogeneous samples from different areas, because the size composition of samples is affected by factors such as the sampling method, the characteristics of the fishing ground (e.g., depth, type of bottom, fishing pressure, etc.) and the population structure itself in a particular area (Seki & Tagami, 1986, Demestre et al., 2000; Massutí et al., 2001; Santos et al., 2002). In this study, the size composition of bluemouth samples from different areas varied considerably. For example, the samples from the Portuguese coast did not include juveniles. In this case, the fishing method had a great influence on the size composition of the sample, because Portuguese samples were obtained from commercial longliners. With this fishing method, only the larger bluemouth specimens are caught (Sequeira V., pers. comm.), first because of longline gear selectivity and second, probably, because longliners are able to operate on rougher and steeper (untrawlable) grounds and have access to less exploited areas where larger specimens have not been removed (e.g., Seki & Tagami, 1986). Accordingly, we have observed that bluemouth specimens caught with trawling tend to be smaller; probably because trawlable areas are much more exploited and also because the largest individuals seem to inhabit deep untrawlable bottoms (e.g., Massutí et al. 2001; Ribas et al. 2006). A similar situation, where the fishing method largely determined the size composition of the samples, was observed by Conolly and Kelly (1996) in experimental surveys carried out in the Rockall Trough (NE Atlantic). In their study, larger fish specimens (for many species) were caught in surveys where longlines were used instead of trawls, even in the same heavily exploited areas. Likewise, Klingenberg et al. (2003) suggested that strong size selectivity of gill nets caused narrow size spectrum within samples in their study of morphological variation in cichlids of Lake Nicaragua. Thus, considering the factors which affect size composition when the sampling strategy is being designed can be very useful for obtaining more balanced (and comparable) samples among study areas.

5.1.2 Data Acquisition

The heart of any morphometric study is good morphometric data, that is, data that represent the shape of the objects of interest appropriately. To guarantee the acquisition of good morphometric data, a series of guidelines must be followed, ensuring that deformations of the specimens are avoided, the quality of the images is adequate and the different sources of measurement error are assessed (e.g., Arnqvist & Mårtensson, 1998; Garabana, 2005; Valentin et al., 2008; Cadrin, 2010; Fruciano et al., 2011).

For this thesis, avoiding deformations of the bluemouth specimens was the top priority when the samplings were carried out. For our study, almost all of the specimens were caught in trawling research surveys. Most fish were still alive when they arrived onboard and no deformations or damage to the body or fins were observed. They were killed as quickly as possible and eviscerated through a small opening on the right side of the fish, so the left side remained intact for the images. As a result, there was no influence of the gonad size or stomach fullness on the shape of the fish. Then, the specimens were immediately placed in plastic bags (to avoid desiccation) and stored in a horizontal position in the freezer of the vessel. All samples were stored at -20ºC until the time of the analysis. Storage duration ranged from a few months to 2 years. However, we did not observe any deformation of body shape caused by the duration of the storage. In our experience, desiccation can cause deformations only in specimens that are stored in the freezer without using a plastic bag. Moreover, other studies (e.g., Valentin et al., 2008) have also concluded that freezing/ thawing the specimens does not generate deformations.

One common shape deformation that can be seen in fish specimens is body arching, i.e., specimens showing a slight “U” or inverted “U” shape (Valentin et al., 2008). Body arching can be caused by rigor mortis itself and it can be aggravated by the preservation method of the specimens (e.g., formaldehyde solution), which can cause further stiffness, flexion and shrinkage of the tissues (Cavalcanti et al., 1999; Valentin et al., 2008). In our experience, this type of deformation can be very evident in fish species with thin and elongated bodies, such as anchovies. However, this type of deformation has also been detected in species with more robust bodies, such as several serranid fishes (Cavalcanti et al., 1999), redfish - *Sebastes* spp. (Valentin et al., 2008), and in the bluemouth specimens used in this study. The main problem with the arching effect is that it introduces spurious shape variability in the dataset that can be confounded with real shape variability and influence the significance of statistical tests. Despite the undesirable

effects on shape that this type of artifact can produce, few morphometric studies investigating shape variability in fish populations have actually considered this issue (e . g., Valentin et al., 2008; Haas et al., 2010; Fruciano et al., 2011, Faccenda et al., 2011). Several actions can be taken to avoid the negative effects of body arching. For example, arching can be minimized if specimens are placed with a proper orientation along a selected axis, for example, the lateral line of the fish (e.g., this study; Muir et al., 2012), or by introducing a needle along the body of the specimen to keep it straight (e.g., Fruciano et al., 2011). It is also possible to correct it at the data acquisition stage if body arching is suspected to exist in the specimens. For example, Haas et al., 2010 placed additional landmarks along the midline of each specimen to implement the 'Unbend specimens' feature in TpsUtil software (Rohlf, 2010) for removing the effects of any bending of specimens owing to preservation. The last resort is to correct the dataset using analogous procedures to those used to correct allometry. For instance, Valentin et al. (2008) and Fruciano et al. (2011) used Burnaby's projection successfully to remove the arching effect on fish samples.

In this study, body arching was investigated in depth in our bluemouth samples (Appendix I). In spite of the careful positioning of the specimens on the board when taking the pictures, such deformations were detected in bluemouth images used in this study. Thus, Burnaby's projection was used to remove this artefact from the shape variables. However, no improvement was observed in the results of the analyses (i.e., Canonical variate analysis) after the correction of the dataset. Besides, there was the risk of losing valuable shape information contained in the dimension that was removed with Burnaby's projection. In addition, we noticed that the arching effect was more or less evenly distributed in all of the bluemouth samples, which reduced the probability of incorrectly discriminating the populations based on this effect. Thus, based on these observations, we decided to use the original dataset to determine the bluemouth population structure.

Overall, we carefully followed a protocol to minimize the effect of possible deformations of the body. Nevertheless, the first samples used in this study were taken before the protocol was fully established (i.e., bluemouth caught in the DEMERSALES 2006 survey). In many cases, these specimens were eviscerated with a cut on the left side of the fish, instead of the right side that was established in the morphometric protocol. In addition, several people participated during the collection of these samples, which may have introduced more errors than when the sampling protocol was fully developed in

2007. Thus, an ad hoc study was carried out for determining if significant body shape differences between the left and right side of the fish existed (see Material and Methods section). The results of that study indicated that there were no significant differences between the sides of the fish. However, it is probable that the way the fish from the DEMERSALES 2006 survey were cut and eviscerated affected their body morphology. Unfortunately, it was not possible to determine exactly how much did the processing of these specimens affect their body shape. Thus, these specimens were included in the study with some reservations but the results were not taken into account when determining the final population structure of bluemouth.

Three sources of measurement error were assessed in this study, in order to evaluate the quality of the morphometric data that was produced: the error when locating landmarks on the specimens, the orientation error (error derived from the positioning of the specimen when the photograph is taken) and the digitization error (error during the digitization of landmarks on the image). It is important to know how large these errors are with respect to the natural variability between specimens, because the lower the error, the more realistic the discriminative power of the set of landmarks will be during further analysis (Adriaens, 2007). Thus, the chances of finding no group differences when they really exist (i.e. type II error) increase when the measurement error is high (Yezerinac et al., 1992; Arnqvist and Mårtensson, 1998). In this study, measurement error was evaluated following the protocol developed by Adriaens (2007). For that purpose, a small subset of specimens was used, considering different study areas, sizes and sexes in order to represent as best as possible the range of natural variation in the dataset (see Chapter 2 - *Materials and Methods* for details).

For our test dataset, the total error accounted for was 33.65% of the total shape variability (13.52% due to digitization error and 20.13% due to landmark location and orientation of specimens). This amount of error can be worrisome if we consider that the dataset contains only 66.35 % of natural shape variability and that in some of the samples, allometry accounted for another 20% (approx.) of total shape variation (e.g., Gulf of Cadiz or subarea A2 in the Alboran Sea). In other landmark-based geometric morphometric studies where digitization and orientation errors were evaluated following the same protocol as in this study, similar percentages of variation due to measurement errors were reported. For instance, in a study in which head shape was compared between different cichlid species, Tkint et al. (2012) found that the percentage of shape variability due to digitization and orientation errors was of 25.4%. Also, Verhaegen et al. (2007)

found that between 34.14 – 37.24% of total shape variability was due to measurement errors in their study of head deformities in larval gilthead seabream (*Sparus aurata*). In these two studies, although measurement error was evaluated, there were no judgements on whether the amount of shape variability due to measurement error could affect their results negatively or not. In other studies, actions have been undertaken to reduce measurement error, but the actual amount of error has not been evaluated (e.g., Frédérich & Adriaens, 2008; Frédérich & Sheets, 2010 and Leysen et al., 2011). Moreover, in most of the landmark-based geometric morphometric studies on fish populations no specific assessment of measurement errors related to orientation of specimens and digitization of landmarks were carried out (e.g. Valentin et al., 2002; Garabana, 2005; Murta et al., 2008; García-Rodríguez et al., 2011; Sequeira et al., 2011a, Tripp-Valdez et al., 2012). Thus, it is difficult to say what level of measurement error is necessary to truly compromise the results of a study, especially because there are no hard-and-fast rules in the literature.

In order to assess better the impact of measurement error, several authors recommend that at least two repeated measures of all the specimens should be taken in morphometric studies (e.g., Yezerinac et al., 1992 and Arnqvist & Mårtensson, 1998). According to Arnqvist and Mårtensson (1998), this enables a quantification, by estimating repeatabilities from nested analyses of variance (type II), and a reduction, by averaging repeated measures, of the relative impact of measurement error. Considering this, it is evident that to have better estimates of measurement error and assess properly its impacts on the results of this study, replicate measurements of the specimens in this study would have been needed. Indeed, there are some studies were measurement error has been evaluated in this way. For instance, Fruciano et al. (2011) used an experimental design in which every specimen had two presentations (two pictures) and two digitizations of landmarks for each presentation, resulting in a total of four sets of coordinates that were later averaged. Unfortunately, in the present study this would have implied a significantly higher effort during the samplings, and this was not possible due to time and economic constraints. Nevertheless, our estimate of measurement error does not necessarily mean that the measurement error in the whole population structure dataset reaches this magnitude because it is difficult to say to what extent the results of the test dataset can be extrapolated to the whole dataset, which is much larger in number of specimens and probably more variable. As already mentioned, the presence of measurement errors introduces spurious shape variability in the dataset that can be

confounded with real shape variability and influence the significance of statistical tests. If these errors are distributed randomly in all of the populations, the probability of finding significant differences among populations may be reduced. Thus, if this were the case with measurement error in the dataset used in this study, our results would be conservative.

Optical distortions (e.g., pincushion, barrel and perspective distortions) are another source of error that may affect the quality of the data obtained from photographs. Optical distortions are produced by lenses of bad quality, but also when short or long focal lengths are used when taking photographs of the specimens. To avoid problems related to bad quality of the camera, it is advisable to use digital cameras with exchangeable lenses, i.e., SLR, that normally are of better quality. But even good quality cameras produce distortions related to the focal length (Garabana, 2005). The quality of the images (in terms of optical distortions) was not evaluated in this thesis. However, the images produced with the digital camera used in this study, a NIKON D1X, were tested for optical distortions using different focal lengths by D. Garabana in 2005. In that study, the images obtained with a 35mm focal length (equivalent to a 50 mm lens in traditional photography) did not show barrel or pincushion distortion. However, other anomalies occurred when the camera was not positioned parallel to the plane where the fish were being photographed. Thus, Garabana (2005) highlighted the importance of using a lens without aberration, and that it is placed parallel to the fish.

The use of two-dimensional images to represent three-dimensional objects, such as fish, could cause some problems as well. Fish width (i.e. fish height when laying down ready for the picture) will drastically affect to the distances between landmarks when the landmarks are not in the same plane (Arnqvist & Mårtensson, 1998; Garabana, 2005). This issue is difficult to control especially if there is a wide range of sizes in the specimens. For example, in larger specimens, the head and pectoral area are much wider than the tail, and the specimens do not lie flat on the board when the photographs are taken. In this thesis, a board of expanded polystyrene was used to display the fish. Then, tissue paper or narrow wedges of polystyrene were placed under the head and tail (and sometimes abdomen) of the fish to lift up these body parts until the sagittal plane of the fish was parallel to the camera plane. However, there are other creative solutions to achieve this need to minimize the effects of the size of fish when acquiring the images. For example, Muir et al. (2012) have suggested the use of a mesh cradle to display large fish (>300 mm), so that heavier and thicker parts of the animal can sag down while

thinner and lighter parts remain elevated. In this way, a planar imaging surface with respect to the camera lens is created. Other good materials that can be used for placing the fish are rubber or foam boards (Muir et al., 2012) and glass pearls (Frédérich & Sheets, 2010).

Unfortunately, as in the case of the other types of measurement error, the effects of optical distortions and use of two-dimensional images have not been evaluated in most morphometric studies. However, in many of them (including this study) actions such as that specimens are positioned in comparable lateral planes and flattened (e.g., Frédérich & Adriaens, 2008; Frederich & Sheets, 2010; Leysen et al., 2011; Muir et al., 2012, this study) and that good quality lenses are used with adequate focal lengths (e. g, Muir et al., 2012; this study) have been undertaken to reduce this type of measurement error.

5.1.3 Morphometric and statistical analyses

The value of morphometric analysis for determining stock structure relies on appropriate methodology. Although traditional morphometrics have been useful for discriminating fish populations and other organisms (e.g., Saborido-Rey, 1994; Murta, 2000; Turan, 2004), several studies have demonstrated that geometric morphometrics are more sensitive and overall perform better (e.g., Trapani, 2003; Garabana, 2005; Maderbacher et al., 2008; Evin et al., 2012). For example, Maderbacher et al. (2008) concluded that both traditional and geometric methods were able to discriminate populations of cichlids of the genus *Tropheus*. However, the differences could be visualized and quantified much better by coordinate-based methods, and they recommended the use of geometric morphometrics for differentiation of closely related entities (i.e., intra-specific studies). Moreover, the newer geometric morphometric techniques for shape extraction coupled to multivariate methods have been useful tools for separating populations of a variety of marine fish, such as grey mullet (Corti & Crosetti, 1996), the silverside *Atherinops affinis* (O'Reilly and Horn, 2004), sardine (Silva, 2003), horse mackerel (Murta et al., 2008), scorpaenids like *Sebastes* spp. (Valentin et al., 2002; Garabana, 2005) and bluemouth in Portuguese waters (Sequeira et al., 2011a). Thus, in this thesis, the landmark-based geometric morphometric approach was preferred over the more traditional methods to analyze morphological variation of bluemouth populations. As in the studies mentioned above, geometric morphometrics

enabled us to quantify and visualize morphometric differences among bluemouth from different areas. Hence, it was concluded that these techniques clearly fulfilled their function in this study.

The choice of statistical methods to analyze geometric morphometric data is also important, because in geometric morphometrics the variables used to measure shape have particular characteristics. Namely, all of variables are expressed in the same units so that analyses must be based on the covariance matrix (instead of the correlation matrix) and the shape space where these variables are has a well-defined metric, the Procrustes metric. Thanks to this, results of multivariate methods that preserve this metric, such as Principal component analysis (PCA) or multivariate regression, can be visualized as actual shapes or shape deformations in the geometry of the original specimens (Mitteroecker & Gunz, 2009). Because of these characteristics, PCA and multivariate regression are standard methods for the analysis of geometric morphometric data.

Principal components analysis was used mainly as a method for size correction in this thesis (see Chapters 2 and 3), however, this technique has played a major role in many other studies using geometric morphometrics. For example, the ordination plots of the first few PC's have been used for visualization and exploration of patterns of shape variation (e.g., Cavalcanti et al., 1999; Trapani, 2003; Collyer et al., 2005; Seiler et al., 2007; Viscosi & Cardini, 2011) and for detecting outliers (e.g., Viscosi & Cardini, 2011). PCA has also been used for reducing dimensionality of the data in order to adjust for the loss of dimensions that results from the Procrustes analysis (Valentin et al., 2002; Baylac et al., 2003;). Perhaps, the only limitation of using this method in the context of geometric morphometrics is that interpretation of the PC coefficients or loadings is not meaningful as in traditional morphometrics, where these coefficients can be used to determine which morphometric variables contribute most in each axis of variation. Thus, to determine which features of shape are associated to each PC axis, one must use diagrams to visualize this (Viscosi & Cardini, 2011).

Like PCA, multivariate regression is one of the most useful techniques to study shape variation. With this method, the relationship between shape variation and one or more independent variables can be determined (Monteiro, 1999). This technique also has the advantages that it is not sensitive to the number of dependent shape variables or to their covariance structure and the resulting vector of regression coefficients (quantifying the average effect on shape) can be visualized as shape deformation (Mitteroecker & Gunz, 2009).

In geometric morphometrics, the regression of shape on logarithm of Centroid Size is considered the optimal measure of allometry (Mitteroecker et al., 2004). Therefore, this technique has been widely used for two purposes: 1) to study allometric trajectories and growth of organisms (e.g., Hood & Heins, 2000; Klingenberg et al., 2003; Trapani, 2003; Mitteroecker et al., 2004; Drake & Klingenberg, 2008 and Frédérich & Sheets, 2010) and 2) to eliminate the effects of size on the shape variables (e.g., Jørgensen et al., 2008; Sequeira et al., 2011a and Fruciano et al., 2011). In this thesis, multivariate regression was used with both of these purposes, and it clearly outperformed other methods (such as those related to PCA), for determining ontogenetic allometry of bluemouth.

In the context of stock identification, morphological discrimination among groups of fish is often difficult because samples usually differ in size composition and allometric growth is therefore present. As it was mentioned earlier in this discussion (Section 5.1.1 – *Sampling*) and in Chapter 3, there is a risk of confounding accidental differences in size composition of the samples with real morphometric differences between fish populations. In studies using traditional morphometrics, there has been no question in that correcting morphometric variables is a crucial step before multiple groups of fish can be compared (e .g., Saborido-Rey, 1994; Murta, 2000; Turan, 2004; Cadrin & Silva, 2005; Traina et al., 2011). However, in many studies using geometric morphometrics to identify fish stocks, the effects of allometric size on shape have not been investigated (e.g., Vasconcellos et al., 2008; García-Rodríguez et al., 2011; Tripp-Valdez et al., 2012). Thus, there is a risk that possible size-induced morphometric differences have contributed to the discrimination of fish populations in these studies. This issue is especially concerning when the putative populations are sampled in areas with essentially different size structure. For example, in the case of the bluemouth, several studies have demonstrated differences in the size composition/structure of bluemouth populations inhabiting different geographical sectors. The northeastern fishing ground (Galicia-Cantabria) is a heavily exploited area, and bluemouth in these areas tends to be considerably smaller than in the deep, less exploited areas surrounding Le Danois Bank (Sánchez et al., 2008; Serrano et al., 2008). Thus, if we compared body shape between these areas without considering the effects of size, it is likely that a spurious discrimination of the two samples would result. In this sense, Loy (1996) concluded that intra-specific size variation often represents morphometric noise, and can mask geographically-related shape variability that is of interest in stock identification (Cadrin, 2000).

Moreover, in some studies where both traditional and geometric morphometrics have been used, size correction has been carried out only in traditional morphometric variables (i.e., Parsons, 2003; Garabana, 2005; Murta et al., 2008). Perhaps, in all of these studies, it was assumed that the superimposition procedure used in geometric morphometrics removed both isometric size (scale) and allometric size. However, this is not the case and allometric shape variation may be still present after re-scaling step of the Procrustes analysis. Thus, it is important to emphasize that allometric effects should always be explored when morphological comparisons of fish populations (and other organisms) are to be carried out using geometric morphometrics. If allometry is found to be significant, a size-correction of the shape variables should be performed. Based on the results of this study and the numerous studies that have used this technique successfully (mentioned above), multivariate regression is recommended as the first option to explore allometry and correct shape variables if certain assumptions are met (See *Material and Methods – Multivariate regression* and *Discussion* in Chapter 3).

In the context of geometric morphometrics, this technique has been explained recently in more accessible ways (e.g., Zelditch et al., 2004; Drake & Klingenberg, 2008 and Viscosi & Cardini, 2011), and what is more, it is now available in user-friendly software such as PAST (Hammer, 2001), IMP series (Sheets, 2011), TpsRegr (Rohlf, 2011), and MorphoJ (Klingenberg, 2011). Thus, it is probable that allometric size-correction using multivariate regression in fish stock identification studies based on geometric morphometrics will become more common in the future.

Unlike PCA and multivariate regression, discriminant analysis (DFA or CVA) presents more limitations for the analysis of geometric morphometric data. Some morphometricians, like Fred Bookstein (the pioneer of geometric morphometrics), consider that discriminant analysis is not the most appropriate technique to analyze morphometric data (e.g., Bookstein, 1991). The controversy is caused mainly because some of the mathematical operations (i.e. inversion of the landmark variance-covariance matrix) needed to compute the discriminant functions destroy the special properties of shape space (see Rohlf, 1996, for details) and the direct link to the original landmark configurations. Thus, it was traditionally considered that discriminant functions are not vectors in shape space (Klingeneberg & Monteiro, 2005), which means that discriminant functions (or canonical variates) could not be visualized as shape deformations directly. Because of this, the typical method for visualizing the shape features associated with the factor(s) of interest consists of a regression of the shape variables on the discriminant (or

canonical variate) scores, which yields the expected shapes of specimens with low and high discriminant scores (Rohlf, 1996; Klingenberg & Monteiro, 2005). Indeed, this visualization method is the one used by the MorphoJ software that was used for analyzing bluemouth shape data in this thesis. However, a recent revision on this topic has shown that discriminant functions are indeed vectors in the shape space and can be visualized as shape deformations (Mitteroecker & Bookstein, 2011). Thus, it is possible that direct visualization of shape deformations will be soon incorporated into morphometric software, for example, by showing the deformation of the reference configuration into specific specimens located in the CV ordination plot as it is done for PCA (i.e., Relative warps analysis) in the TpsRewl software (Rohlf, 2010).

The other issue is that discriminant analysis is not based on biologically meaningful hypotheses and discriminant axes often cannot be interpreted as "biological factors" – landmark displacements consequent to a common cause. It is considered that biological factors are better estimated by regressing shape variables on the measured causes, such as environmental, functional or genetic determinants (Bookstein, 1991; Mitteroecker & Bookstein, 2011). As already mentioned, discriminant analysis also requires the number of specimens to be much larger than the number of variables in order to provide a stable solution, that is, one that would not change very much if a new set of samples from the same populations were taken. However, the minimally reasonable sample size depends on how distinctive the groups are (because subtle differences require more statistical power to detect). In addition, it requires larger sample sizes to determine the nature of the differences among groups than just to demonstrate that the difference is significant (Strauss, 2010). Moreover, when the number of variables is close to the number of cases – a common situation in geometric morphometrics – CVA will always separate groups even if they actually have the same mean (Mitteroecker & Gunz, 2009).

If parametric tests are used to test for group separation (e.g., Wilk's lambda), it is important to check normality, homogeneity of variance and outliers for each shape variable using exploratory data analysis procedures. However, transformations that change the scale of measurement of the data (e.g., log or square root) should not be performed on shape variables that need to be normalized, because this would alter the uniformity of the set of variables. Multivariate parametric tests are especially sensitive to heteroscedasticity, however, there is no straightforward way for testing this assumption (Quinn & Keough, 2002). Thus, the best option to test hypothesis with geometric morphometric data is perhaps to use resampling techniques such as the bootstrap or

permutations tests. These techniques do not make specific assumptions concerning the shape of the distributions from which observations were drawn. Thus, the comparisons between the mean shapes of bluemouth from different areas or sexes were carried out using permutation tests.

Despite its limitations, discriminant analysis has been a widely used technique in geometric morphometrics and continues to be one of the most useful techniques for separating populations of organisms according to shape. The examples in the literature are abundant (e.g., Rohlf et al., 1996; Corti & Rohlf, 2001; Dos Reis et al., 2002; Klingenberg et al., 2003; O'Reilly & Horn, 2004; Murta et al. 2008; Fruciano et al., 2011 and Sequeira et al., 2011a). The value of this technique relies in that once the limitations and underlying assumptions are understood it can provide a good picture of the degree of separation and relationships among groups according to shape.

In this thesis, the interpretation of discrimination analyses was not straightforward. The interpretation of the CVAs was based on both the CV plot and the classification matrix validated by a jackknife procedure in each case. To the present, there are no conclusive criteria to make the correspondence between the statistical result of classification success and the qualitative (graphical) separation, so the final interpretation of the results was rather empirical and attempted to take both into account. Additionally, dendrograms obtained from a cluster analysis using Mahalanobis distances (from the CVA results) were used to show in a clearer way the relationships among groups of bluemouth.

In spite of all of the limitations mentioned in this section, discriminant analysis was a key and irreplaceable technique that allowed distinguishing bluemouth populations according to patterns of shape variation.

5.2 Population structure of bluemouth

5.2.1 Contributions to the knowledge of bluemouth stock structure

Compared with pelagic species, demersal fish normally present more restricted geographic distributions, reduced mobility and limited dispersive larvae. Nevertheless, many demersal species retain strong migratory capacity and spawn pelagic eggs that are subject to passive dispersal (White et al., 2009 and references therein). However, the actual degree of dispersal and structuring of the populations within a species is affected by several factors, such as the length of time of the pelagic larvae and juvenile stages, behavioural mechanisms, and physical barriers such as gyres, ocean fronts and seamounts (Zardoya, 2004; Smith, 2007). In fact, genetic studies have shown that most species present intra-specific genetic differentiation and some degree of isolation between populations at oceanic, regional and even local scales (Aboim, 2005).

The study of the population structure of bluemouth in the NE Atlantic and other species of *Helicolenus* in the South Pacific (e.g., *H. percoides* and *H. barathri*) began recently, but the focus of most of these studies has been typically the age and size structuring of bluemouth populations in the NE Atlantic or the Mediterranean (e.g., Kelly et al., 1999; Massutí et al. 2001; Abecasis et al., 2006; Ribas et al., 2006). In many of these studies, some differences in the distribution, age and size composition and growth parameters of bluemouth populations were observed, suggesting that some structuring of bluemouth populations exist. However, none of these studies investigated the stock structure of bluemouth. In 2005, a series of genetic studies directed towards determining the population structure of bluemouth in terms of stock components or discrete populations was carried out (Aboim, 2005; Aboim et al., 2005). In these studies, several populations in Portuguese waters (i.e. Azores archipelago, Madeira and mainland Portugal) were compared, and the results indicated that population structure existed at different levels. The genetic analyses based on mtDNA sequences provided evidence of population structuring at a NE Atlantic regional scale, indicating little or no effective gene flow between Cape Verde bluemouth and others from the Mid-Atlantic Ridge (Azores), Madeira and European Continental slope (Portugal). Microsatellite analysis revealed population differentiation not detected by mtDNA markers, demonstrating isolation between the European continental slope population (Peniche) and the Azores archipelago.

That study was followed by other studies which were aimed at identifying bluemouth stocks in Portuguese waters (using mostly the same locations as in the genetic studies). In these newer studies, phenotypic and ecological approaches were applied, and methods such as the analysis of body shape variation (Sequeira et al., 2011a), otolith shape (Neves et al., 2011), and parasite tags (Sequeira et al., 2010) were used. It is interesting to note that with all the approaches, population structuring of bluemouth was observed.

For *Helicolenus,* the analysis of morphological characters has provided a valuable tool for studying their population structure. In Portuguese waters (NE Atlantic), at least three different bluemouth populations have been identified based on morphological characteristics, in the Azores archipelago, Madeira and Peniche (Portuguese continental coasts) (Sequeira et al., 2011). In New Zealand, Lawton and collaborators (2010) found evidence for discrete subpopulations of sea perch (*Helicolenus percoides*) across four fjords that are closely situated. These subpopulations were characterized by distinct morphological patterns and other indicators at the scale of individual fjords.

In this thesis, the stock structure of bluemouth around the Iberian Peninsula was basically inferred from the results of the analysis of body shape variation using geometric morphometrics (Chapter 4). The results indicated that at least four phenotypic stocks can be identified in the Mediterranean. There was evidence of the existence of two bluemouth populations in the western Mediterranean; one in the south-western basin (Alboran Sea) and another in the north-western basin (Balearic Sea and Catalonian coast) that extends to the transition zone in the Alicante region. A third population might be present in the western Mediterranean (subarea A2 in the Alboran Sea), however, further samplings within the Alboran Sea should be carried out to confirm the local stock structure found in the area. Finally, bluemouth from Sicily (central Mediterranean) would belong to a fourth population, assuming that the distance separating the Iberian coast and the Sicilian coast does not allow for bluemouth populations to be effectively connected. However, it is not possible to say if there is a clear boundary between bluemouth populations in the western and central Mediterranean or if body shape varies gradually until more sampling locations are included to determine how body shape varies between these regions.

In the NE Atlantic, there is evidence that at least four phenotypic stocks/populations exist in the studied regions. Bluemouth from Galicia and the Cantabrian Sea seem to belong to the same population. Although there is some degree of morphological variability within this population, no clear breakpoints between the two areas were identified. Bluemouth around Peniche (off the Portuguese coast), on the other

hand, can be considered as a separate population regarding body morphology. Bluemouth from the Gulf of Cadiz was found to be morphologically related to bluemouth from Alboran Sea. Thus, it is likely that bluemouth from the Gulf of Cadiz are effectively connected to the populations in the western Mediterranean. This also makes unlikely that the Strait of Gibraltar acts as a barrier isolating Atlantic and Mediterranean bluemouth populations, however, it is possible that the oceanographic front near Almeria-Oran (Tintoré et al., 1988) can have some effect in the differentiation of bluemouth within the western Mediterranean (i.e., between the Alboran Sea and Alicante/Catalonian coast) (see Chapter 4, section 4.4.2). Finally, we suggest that bluemouth from the Porcupine Bank belong to a different population from the ones observed in Iberian waters, in spite of some morphological similarities that were detected between them. The main arguments that favored this conclusion were many: the geographical distance that separates the Porcupine Bank from the continental slopes, the use of this bank as an important spawning ground by bluemouth and the particular characteristics of the bank (e.g. larval retention areas) (Dransfeld et al. 2009), the sedentary behavior of adult bluemouth (Uiblein et al., 2003; Pakhorukov, 2008; Aboim, 2005 and references therein), the differences in meristic characters (i.e. number of gill rakers), etc., (See Discussion in Chapter 4). However, more samples from locations in between Porcupine Bank and Iberian northern waters would be needed to see if bluemouth follow a gradient of morphological variation or if there is a clear boundary between these populations.

Despite phenotypic stocks can be determined based on morphometric results, variation in meristic variables (Chapter 4) and growth patterns (Chapter 3) of bluemouth from the different areas were taken into account, because they can contribute valuable information to the understanding of stock structure, ecology and biology of the bluemouth. For example, the existence of two different growth patterns in the Alboran Sea made us suspect that two different bluemouth populations were present in this area. Accordingly, these two putative populations were analyzed separately in the discriminant analyses in Chapter 4 and the separation according to body shape was indeed confirmed.

In stock identification, however, it is common that different approaches yield different pictures of the stock structure (e.g., Abaunza et al. 2008), and integrating the results can be difficult (e.g., Cadrin et al., 2010). For instance, in the NE Atlantic, morphometric variation of bluemouth along the Cantabrian Sea and Galicia followed a gradient; with no clear breakpoint that could indicate the isolation between bluemouth from these regions. Likewise, the results of analyzing meristic variables offered a similar

picture, because no differences were detected between bluemouth from Galicia and the Cantabrian Sea. However, the growth trajectory of bluemouth from the Cantabrian Sea was very different from the one determined for bluemouth in Galicia, suggesting that these two populations develop differently. Body form in fishes is a product of their ontogeny (Cadrin, 2000, 2005). Thus, we would have expected that differences in ontogenetic shape changes would have led to overall morphometric differences between the two populations, as in the case of the populations in the Alboran Sea. It is difficult to say why different growth patterns do not lead to detectable morphometric differences, because many factors are involved in determining body shape. Nevertheless, when this type of inconsistency is observed, the analysis of more samples from these regions would be of great help to confirm the accuracy and reliability of the available growth and morphometric data.

Based on the results in this thesis, the usefulness of meristic variables to identify bluemouth phenotypic stocks is questionable, because we observed almost no variation in these characters across the studied bluemouth populations. As discussed in Chapter 4, the overall stability in meristic counts could be reflecting genetic homogeneity of the bluemouth populations studied here, because there appears to be a strong genetic component to meristic variation within populations (Barlow, 1961; Swain et al., 2005). It appears that in *Helicolenus*, the study of meristic characters may be more useful at the interspecific level rather than at the intraspecific level. For example, in a study where genetic and morphological divergence among sea perches (*Helicolenus spp.*) was investigated in Australia and New Zealand, a systematic modal difference in the number of dorsal fin rays (12 for *H. percoides* and 13 for *H. barathri*) was observed between these sympatric species. In the same study, differences in mtDNA markers, morphometric characters and color and banding pattern on the body were found.

Again, this situation stresses the importance of using other complementary techniques to determine accurately the population structure of bluemouth. In particular, a genetic approach would be very useful to contrast the results of this study and understand better the underlying causes of the differences in meristic characters and morphology of bluemouth in the NE Atlantic and the Mediterranean. Moreover, genetic analyses are the most rigorous technique among the suite of approaches for stock identification to test for reproductive isolation among population components (Begg & Waldman, 1999). Thus, these techniques would be very useful to determine gene flow between the phenotypic stocks that were identified in this study. For example, an examination of gene frequencies

between blumeouth from the Alboran Sea and Alicante/ Catalonian coast would allow determining if the Almeria-Oran Front has a boundary effect that is reflected in the observed morphometric differences between these areas in the western Mediterranean, or if these differences are environmentally induced. However, although genetic analyses would offer a deeper insight regarding the degree of isolation among bluemouth populations, the combination of the genetic and phenotypic approaches (such as morphometrics) would provide the necessary information to achieve the best management approach. For instance, yellowtail flounder off northeastern USA appears to comprise a single genetic stock, however, some of the populations exhibit differences in developmental rates and in response to fishing, and are actually managed as three different stocks (Cadrin, 2003; Cadrin, 2010).

Connectivity of bluemouth populations could also be evaluated by modelling the dispersal of early life stages, mark-recapture analysis of artificial tags, or examination of natural tags (e.g. otolith chemistry and parasite infestation) (Cadrin et al., 2010). The study by Sequeira et al. (2010) is an example that corroborates the value of using parasite tags in the study of populations, since differences found in the number of individuals of different *Anisakis* species and their infection levels allowed a clear distinction of *H. dactylopterus* from the Azores, Madeira and mainland Portugal.

There is no doubt that a multidisciplinary approach is needed to really understand the population structure and dynamics of marine fish. However, a multidisciplinary approach generally requires collaboration of different experts (e.g., Sequeira, 2010; Cadrin 2003; Abaunza et al., 2008). To give an idea of the number of experts that can be required to apply multiple techniques, some of the newest studies following a multidisciplinary approach can be examined. For example, in the identification of horse mackerel stocks in European waters, 23 experts collaborated (Abaunza et al., 2008), and in the identification of bluemouth populations in Portuguese waters by Sequeira (2010), up to 15 experts participated in different ways.

Multidisciplinary information on population structure can also be synthesized to form a holistic view and provide clear advice for fishery science and management (Cadrin et al., 2010). These studies, where the results of different studies using various techniques are collated in order to identify and delineate stocks, can be very extensive and can provide a good overview of the population structure of a resource. For example, Cadrin (2010) recently reviewed the available information from a wide variety of approaches to determine the stock structure of yellowtail flounder off USA and Canada. In his review,

he analyzed the evidence from 14 different methods used to identify and delineate stocks, which included: phenotype (meristics and morphometrics), life history parameters (growth, spawning period, age and size at maturity), distribution (fishery, resource surveys, eggs and larvae), movement (tagging and parasites) and demographics (size structure, age structure and abundance trends). A similar study, where all the available information on stock structure was collated, including genetic analyses, was carried to determine the population structure of beaked redfish (*Sebastes mentella*) (Cadrin et al., 2010). Thus, both studies allowed a revision of the stock structure and new management advice was provided.

Yet, even if a full multidisciplinary approach cannot be implemented due to economic, logistic or other constraints, it is still possible to apply a few (two or three) complementary techniques to the same individuals (e.g., morphometrics and genetics) without much complication. The combination of morphometrics and genetics in stock identification studies is common, because with these techniques one can obtain information regarding phenotypic and genetic stocks at the same time. This has been done in a number of stock identification studies (e.g., Vasconcellos et al., 2008; García-Rodríguez et al., 2011; Tripp-Valdez et al., 2012), and a considerably better overview of the population structure of the analyzed species was achieved. In fact, some authors consider that simultaneous collection of genetic and phenotypic information is critical for stock structure analysis (Carvalho & Hauser, 1994). In Cadrin (2003), the same individual were used for genetic and morphometric analysis, though research on genetic variation was carried out by Kuzirian & Chickarmane (2004) in collaboration with the author of that study.

Actually, the research carried out in this thesis was linked to a larger Spanish research project[1] carried out in the period 2006 - 2009. The objective of this project was to analyze the population structure of bluemouth by studying morphology, genetics and reproductive features in five well-differentiated sectors of the Iberian coast (the Cantabrian Sea, Galicia, the Alboran Sea, Alicante and Catalonian coast) with particular environmental and exploitation characteristics. The final goal of this research was to provide useful information for the management of this species.

Thus, in that project, the same bluemouth samples were used to carry out morphometric and genetic analyses. Unfortunately, the study of genetic variation in

[1] , "Population structure and reproductive ecology of *Helicolenus dactylopterus* in the Iberian platform" (CTM2006-13964-C03-00/MAR - Spanish Ministry of Science and Innovation)

bluemouth populations is still being carried out by researchers at the University of Vigo (Spain) and the results were not available at the moment when this thesis was written. However, it is expected that results of the genetic analyses will be soon available, and that a comparison between morphometric and genetic approaches will be carried out.

The above project also envisaged a comparative study of reproductive features of bluemouth in the Atlantic and Mediterranean, which could have been used as a complement to morphometric and genetic analysis for the task of determining the bluemouth stock components. However, since bluemouth is not a target species in many areas, Atlantic samples could not be obtained on a regular basis and reproductive parameters could not be estimated in this region. Thus, it was not possible to determine if reproductive differences exist among bluemouth populations in the NE Atlantic and Mediterranean.

A recent study carried out in Portuguese waters (Azores archipelago, Madeira and continental Portugal) showed that some life history parameters were indeed useful for establishing differences between bluemouth populations (Sequeira, 2010). For example, results on age and growth showed significant differences in the comparison of the mean total length and the mean length-at-age among the three areas. On the other hand, reproductive features (i.e., annual sexual cycle and maturity ogives) did not show a clear differentiation among the studied populations. Nevertheless, the authors concluded that even if the analysis based on life history parameters was not conclusive for the bluemouth stock structure, the results did provide a comprehensive understanding of this species life history, which is an essential requirement for successful stock identification.

From this discussion, it is clear that despite phenotypic stock were identified in this study, more work needs to be done to get a clearer picture of the bluemouth stock structure in the Atlantic and Mediterranean. A multidisciplinary study, covering both basins, would certainly be the best option to finally identify the stock components and understand the dynamics of bluemouth populations.

5.2.2 Implications for fisheries management and conservation

In general, there is a considerable mismatch of the level of knowledge about the population dynamics and structure of deepwater species and their current exploitation. The level of exploitation of deep-sea species is variable in the NE Atlantic, ranging from about 40 000 t (e.g., great silver smelt – *Argentina silus*) to a few hundred tons each year (e .g. Alfonsinos – *Beryx* spp.) (ICES, 2012). In the Mediterranean, between 500 and 1600 t of deepwater marine fish have been landed yearly in the past decade (FAO, 2012). Resources in the Mediterranean Sea are managed by the Scientific Advisory Committee (SAC) of the General Fisheries Commission for the Mediterranean (GFCM). To the present, SAC provides assessments and management advice only in a few deep-sea species (e.g., blackspot seabream – *Pagellus bogaraveo* and red shrimp – *Aristeus antennatus*). In the North Atlantic, assessments and management recommendations for deep-sea stocks are provided each year by different working groups of the International Council for the Exploration of the Seas (ICES), the Working Group on the Biology and Assessment of Deep-sea fisheries Resources (WGDEEP), the North-Western Working Group (NWWG) and the Arctic Fisheries Working Group (AFWG). Up to now, ICES has been able to carry out assessments and provide advice for many deepwater species (e.g., Greenland halibut, great silver smelt, tusk, ling, orange roughy and Alfonsinos). For bluemouth stocks, no advice has been required to the present from the WGDEEP. Moreover, the information available to the assessment group on bluemouth is limited, and only includes landings data for the NE Atlantic, abundance indices and length composition from the Spanish survey on the Porcupine Bank (ICES area VII) and trends in mean length from Azorean surveys (ICES, 2012).

Thus, from the management perspective, bluemouth has not received as much attention as other species caught in demersal fisheries in the NE Atlantic. The fact that bluemouth are mainly caught as by-catch in many fisheries and no directed fisheries for this species exist could be a plausible explanation for overlooking the management of this species. Additionally, the importance of bluemouth in the fisheries in terms of volume of catches could be underestimated, because data on landings are not consistent. For example, ICES estimated that in the last few years (2007 – 2010), between 408 and 951 t of bluemouth were landed in the NE Atlantic (ICES, 2012). However, FAO has estimated that bluemouth landings in the NE Atlantic are much higher, ranging from 2129 to 3961 t in the same period of time. Moreover, only in Galician ports, official landings data for

bluemouth range between 1059 t in 2007 to 2932 t in 2010 (Xunta de Galicia, 2012). In these ports, bluemouth come mainly from Spanish trawl fisheries targeting hake and anglerfish in the NE Atlantic (ICES areas VI, VII and VIII) (ICES, 2012; Vázquez-Rowe et al., 2011). Additionally, the amount of bluemouth landed in Galician ports could be even larger, because according to our experience, this species is often misidentified and reported as other scorpaenids (e.g., *Scorpaena scrofa*). Thus, it seems that landings data for bluemouth could be much higher than those estimated by ICES and that the actual exploitation rates may not be sustainable. Moreover, bluemouth discards data are not available (ICES, 2012), which makes even more difficult to assess the actual level of exploitation of this species.

Reliable commercial data are key to most stock assessments and to the understanding of the current status of the stock, relative to the past. But besides the lack of landings and effort data, a clear issue for managing most deep-sea species is that their stock structure is unknown (ICES, 2010). Because of that, identification of stocks is based on either theoretical considerations on the mixing of populations in relation to the hydrological and geological characteristics of fishing grounds, or comparison of trends in catch rates, or consistency with management units (ICES, 2010). Thus, for many deepwater species managed by ICES, stocks units used for assessment purposes are currently individual or groups of ICES subareas or divisions, which do not consider the "real" stock structure. Since these areas were devised for the fisheries on the continental shelf, they can be inappropriate for deepwater fisheries (Large et al., 2001; Hammer a& Zimmermann, 2005). Thus, there is an urgent need to reconfigure some existing ICES areas to become biologically meaningful in terms of the distribution of deepwater species (Hammer & Zimmermann, 2005). Moreover, determining the actual stock structure of these species should become a priority, because failure to recognize the stock structure of a resource may lead to ineffective fisheries management. This can result in dramatic changes in the biological attributes and productivity rates of a species, as well as significant loss of genetic diversity of a species (Begg et al., 1999 and references therein; Cadrin et al., 2010).

When the stock structure is not completely clear (e.g., results from different techniques for stock identification are not consistent), it might be more adequate to follow a precautionary approach and manage each putative population separately. Waples et al. (2008) illustrate this using the example of school shark (*Galeorhinus galeus*) in the southwestern Pacific, which is considered overfished off Australia but sustainably

harvested off New Zealand. In this case, although genetic analyses could not reject the hypothesis of a single stock (panmixia), the results did show some genetic differentiation (using allozymes and mtDNA). Moreover, tagging experiments have shown low rates of movement of school sharks between Australia and New Zealand. Thus, treating Australian and New Zealand school sharks separately is more precautionary for the species in Australia because if the assumption were made that there is only one stock, there might have been no reason to reduce harvest rates in Australia. However, it should also be noted that always treating putative population as separate stocks for assessment and management purposes is not always precautionary and that doing so unnecessarily can lead to loss of yield (Waples et al., 2008).

In the latest report, WGDEEP stated that no information is available regarding stock identity for bluemouth (ICES, 2012). From this perspective, the work carried out in this thesis is a major 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. Phenotypic stocks are defined as groups of fish with similar growth, mortality, and reproductive rates, and morphometric stock identification can discriminate such groups because morphology is directly related to these features (Cadrin, 2000). Moreover, growth, mortality, and reproductive rates influence a stock's response to exploitation. Thus, phenotypic stocks 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 thesis, there is evidence that the bluemouth population that exists near Peniche (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 IXa, 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 VIIIc and IXa.

It is necessary to incorporate the newest information on stock structure into management advice as it becomes available in order to achieve better management of resources. For instance, after a decade of researching the stock structure of beaked redfish (*Sebastes mentella*) near the Irminger Sea, ICES revised the form of its advice to fishery managers to account for genetic differences between the deep-pelagic and shallow-pelagic stocks. Advice for the shallow-pelagic stock was "given the very low state of the stock, the directed fishery should be closed", and for the deep-pelagic stock "given the reduced abundance of this stock in recent years, a total catch limit of no greater than 20 000 tonnes should be implemented in 2010" (ICES, 2009; Cadrin et al., 2010). Hopefully, since more information on biological and population aspects of bluemouth is becoming available, and data from fisheries and surveys are being collected at the European level (i.e., landings, discards and in many areas, variables such as age, weight, sex and maturity), it will soon be possible to carry out the first assessments of bluemouth stocks and implement management recommendations in the region.

5.3 Future work

The work in this thesis gave new insights on the population structure of bluemouth around the Iberian Peninsula. By analyzing morphological characters, several phenotypic stocks were identified. However, the process of stock identification does not end here. Typically, stock identification has several stages that move from exploratory to confirmatory (Cadrin, 2010). The last stage, known as stock discrimination, is reached once significant and meaningful differences are confirmed to exist among stocks and they allow classifying individuals to a particular stock. Consequently, stock discrimination can be used to delineate geographic (and possibly seasonal) boundaries among stocks or to determine stock composition in a mixture, e.g., a mixed-stock fishery (Fabrizio, 2005; Cadrin, 2010).

In this context, the 'preliminary' bluemouth stocks determined in this study can be used as the baseline to carry out a confirmatory analysis of the stock structure of bluemouth in European waters. To do this, 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. 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 thesis are affected by temporary differences in the environment and the picture of this stock structure is not definitive.

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. Once the results regarding genetic variability of bluemouth populations using mtDNA and microsatellites become available (from the Spanish research project described in section II above), a comparison of the resulting stock structure from both the genetic and morphometric approaches must be done. 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 meristic characters and morphology. This type of study will also help to determine the level of genetic differentiation between populations and their connectivity. Additionally, a comparative study of life history traits among bluemouth populations in the NE Atlantic and Mediterranean (i.e., distribution, abundance, age, growth, mortality, reproduction, spawning and larval distribution) could shed light on the bluemouth population dynamics and structure.

Finally, the study of the population structure of bluemouth should be extended to other areas in European waters where this species is exploited. For example, it is necessary to study bluemouth variation in areas between the Iberian Peninsula and the Porcupine bank. In addition to that, a study on the local bluemouth populations in the surroundings of the Porcupine bank can be carried out, since a large proportion of bluemouth are caught in the Celtic Seas (ICES area VII). The offshore banks to the west of Ireland have been described as an important spawning and larval ground for the bluemouth (Dransfeld et al., 2009). Moreover, the retention of eggs and larvae over these offshore banks has been suggested to occur as a consequence of the formation of Taylor columns above the Rockall bank and Porcupine Bank (Dransfeld et al., 2009). Thus, the characteristics of these banks could promote the formation of local fish populations, possibly resulting in genetic differentiation as well (e.g., Lawton et al., 2010).

5.4 Conclusions

5.4.1 Conclusions drawn from the methodological implementation

1. The sampling carried out in research surveys was appropriate for studying the population structure of bluemouth around the Iberian Peninsula, because extensive areas were covered and it made possible to record the exact location where the bluemouth specimens were caught. Also, a wide size range of bluemouth specimens (from juveniles to large adults) was obtained in most areas.
2. The size composition of the samples (i.e. the proportion of small, medium and large specimens in a sample) is important, because the amount of allometric shape variation in a sample is related to its size composition. If sample size composition differs among areas, there is the risk of confounding this variation with real morphometric differences between fish populations. In those cases, allometric corrections such as those carried out in Chapter 3 will be needed before the populations can be compared and these procedures may not be always straightforward.
3. Avoiding deformation of the fish specimens in the morphometric study needs to be considered a priority during the processing of the samples. In this study, freezing the specimens in plastic bags was an appropriate method to store samples until further processing and storage duration of frozen samples did not seem to cause deformations of body shape.
4. Although body arching was minimized during data acquisition, an arching effect was detected on the dataset. Discrimination did not improve when this effect was removed, and considering it was homogeneously distributed in all of the samples, it was preferred to use the original dataset for determining the population structure of bluemouth.
5. There were no significant morphometric differences between the left and right sides of the fish.
6. For our test dataset, the total error accounted for 33.65% of the total shape variability (13.52% due to digitization error and 20.13% due to landmark location and orientation of specimens respectively). Despite this amount of error, the natural variability in the dataset allowed discrimination of bluemouth stocks in this study.

13. The landmark-based geometric morphometric approach enabled us to quantify and visualize morphometric differences among bluemouth from different areas.
14. Multivariate regression was used to study allometric growth of bluemouth and to eliminate the effects of size on the shape variables. This method outperformed the other size-correction methods, and is recommended as a first option to study allometry and corrects its effects in shape variables in stock identification studies.
15. In spite of all of the limitations that discriminant analysis presents for analyzing geometric morphometric data, discriminant analysis was a key technique that allowed distinguishing bluemouth populations according to patterns of shape variation.

5.4.2 Conclusions drawn from the study of ontogenetic allometry in bluemouth populations

16. The general pattern of ontogenetic changes seemed to be related to the changing ecology of the species (i.e. ontogenetic diet and habitat adaptations) and consisted of a relative expansion of the area between the second preopercular spine and the pectoral fin, a relative deepening and shortening of the body and an upward shift of the snout as the head becomes more compact in relation to the body.
17. Growth trajectories were not homogeneous among bluemouth populations. A complex combination of factors such as food availability, fishing pressure and other environmental conditions can produce the distinctive growth patterns that were observed. These differences could be an indicator that different populations exist and should be further studied.
18. The pooled within-group regression method yielded the best results for size-correction of the shape variables. Despite this method requires equal growth trajectories to remove allometry, it provided a compromise estimate of allometry that was useful for size-correction of the bluemouth population structure dataset.

5.4.3 Conclusions drawn regarding the population structure of bluemouth in the NE Atlantic and Mediterranean and the implications for management and conservation of this resource.

19. Statistically significant differences in body shape were found among bluemouth samples. However, discriminant analyses showed that in some cases the differences between the populations were not strong enough to consider these populations as separate phenotypic stocks.
20. Geographical distance between sampling locations did not always result in morphological differentiation. In some cases, morphological similarity was found for bluemouth from distant areas, and could be caused by coincident environmental factors or even by genetic similarities that remained during the evolution, rather than by a significant rate of interbreeding between these populations.
21. 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 that would cause a strong morphological differentiation. However, the relative separation of bluemouth from the Alboran Sea and Alicante/Catalonia could indicate a boundary effect by the Almeria-Oran Front that limits the connection between bluemouth populations within the western Mediterranean. This aspect needs further study.
22. In the western Mediterranean, there was evidence that at least two bluemouth phenotypic stocks exist: one in the south-western basin (Alboran Sea) and another in the north-western basin (Balearic Sea and Catalonian coast) that extends to the transition zone in the Alicante region. A third stock in the western Mediterranean (or a sub-population) might be present, because bluemouth from subarea A2 in the Alboran Sea presented important morphometric differences with respect to bluemouth from the neighboring areas, but his needs to be further investigated.
23. Bluemouth from the reference area (Sicily) was relatively well differentiated from bluemouth in the western Mediterranean and probably constitutes a different stock unit. However, it was not possible to determine if there is a clear boundary between bluemouth populations in the western and central Mediterranean until more sampling locations between these areas are analyzed.

24. The greatest morphological differentiation was found 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 bluemouth from Portugal can be considered as a separate phenotypic stock.
25. The overall morphological variation along the Cantabrian Sea and Galicia seemed to follow a gradient, with no clear breakpoints that could indicate the isolation between bluemouth from these regions. The two biogeographical limits located between Galicia and the Cantabrian Sea (i.e., Cape Estaca de Bares and Cape Finisterrae) did not seem to have a noticeable boundary effect at the morphological level. Thus, bluemouth from these two areas seem to constitute a single phenotypic stock.
26. In general, meristic variables were stable among bluemouth from the different locations, with the exception of the counts of gill rakers (GRV and GRH), where some variability was observed. Thus, the usefulness of meristic variables to identify bluemouth phenotypic stocks is questionable. It appears that in *Helicolenus*, the study of meristic characters may be more useful at the interspecific level rather than at the intraspecific level.
27. The work carried out in this thesis is a major contribution for understanding the population structure of bluemouth in European waters, providing evidence that different phenotypic stocks exist. These stocks can be used as a first approach to model population dynamics for fishery stock assessment and management.
28. Despite phenotypic stocks were identified in this study, more work needs to be done to understand the bluemouth stock structure in the NE Atlantic and Mediterranean. A multidisciplinary study, covering both basins, is necessary to identify correctly the stock components and understand the dynamics of bluemouth populations.

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APPENDIX I

The arching effect

I Introduction

An important source of measurement error in morphometric studies is the distortion associated with the specimen's posture when the photograph is taken, because it can strongly affect the configuration of individual landmarks (Arnqvist and Mårtensson, 1998, Valentin et al. 2008). In organisms like fish, which have flexible bodies, the arching effect (Valentin et al. 2008) refers to an upward or downward arching of the body. The problem with the arching effect is that it produces undesirable shape variability in the dataset that can obscure true shape variation related to biological or ecological factors, or introduces bias if the variation caused by arching is unevenly distributed in the samples.

Thus, arching effect in the dataset used in this study was investigated using a PCA-model of the arching coupled with Burnaby's orthogonal projection, according to Valentin et al. (2008). In the present study, this information was needed to decide if the correction of the arching effect was necessary for our population structure dataset and if the Burnaby projection yielded good results in correcting this kind of artefact.

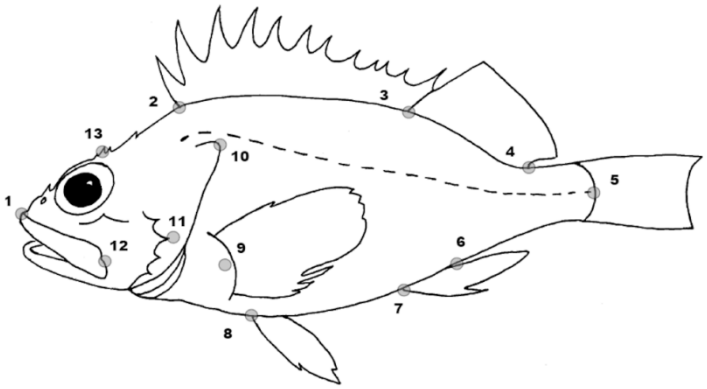
II Material and methods

The procedure to determine and remove the arching effect on fish body shape is described in detail in Valentin et al., (2008). In this study, the sample used to generate deformation models consisted of 10 specimens of bluemouth (Table 1), selected randomly from the total sample used to study population structure, which comprised 9 different areas around the Iberian Peninsula, the NE Atlantic and the Mediterranean. Size of the specimens is expressed as centroid size (CS) in cm. Although one single specimen would have been theoretically sufficient to generate the deformation model, the use of 10 specimens allowed the consistency of the model to be tested. 13 landmarks were used to define fish body shape (Fig. 1). These landmarks were marked with black-headed

entomological using the left side of the fish. Then, the same specimen was photographed 20 times in different arching postures with a digital camera Nikon D1X using a focal length of 35 mm (Fig. 2). As explained in Valentin et al., (2008), the rationale behind the generation of the deformation models is simply trying to capture the range of shape changes due to bending, so the particular choices of the degrees of bending are not important. In this way, 10 independent deformation models were produced in which shape variation is related only to body posture. Then, landmarks were digitized using the tpsDig software (Rohlf, 2008) and the coordinates where then submitted to a generalized Procrustes analysis (GPA) with the MorphoJ software (Klingenberg, 2011). The first step of this procedure is to scale all the specimens to unit centroid size. The landmark configurations are then superimposed to have a common centroid and rotated to minimize the distances between the corresponding landmarks of all the configurations. Once the specimens are aligned, the mean configuration of landmarks is computed and the specimens are projected to a linear shape tangent space. (Rohlf, 1999; Rohlf and Slice, 1990; Slice, 2001). The coordinates of the aligned specimens are the Procrustes coordinates. Then, the Procrustes coordinates corresponding to the deformation models were used as shape variables (separately for each model) in a principal components analysis (PCA) and the PCA eigenvectors (principal components or PC's) were saved. For each PCA, shape changes associated with each principal component were visualized. PCA and visualization of shape changes were done in MorphoJ (Klingenberg, 2011).

The consistency between the deformation models was assessed by pair-wise comparisons of the angle separating the first PC of the deformation models, where small angles would indicate that the models are similar between them. The angles were calculated using R software (R Development Core Team, 2011). Once the consistency between the models was assessed, the mean first PC was computed to describe the overall arching effect. To investigate if the arching effect was present in the population structure dataset, a PCA was carried out using the population structure dataset (consisting of 1120 specimens from different areas in the NE Atlantic and Mediterranean; see Chapter 2 for a detailed description of this dataset). Then, the angle between the first PC of the population structure dataset and the mean first PC describing the arching effect was computed. In this case, a small angle was considered to indicate a strong effect of the arching deformation on the PC and an angle close to 90 degrees independence, i. e., no arching effect present in that component (Valentin et al. 2008).

As proposed by Valentin et al. (2008), Burnaby's method was used to remove the arching artefact from the original population structure dataset. The Burnaby projection was carried out using R software (R Development Core Team, 2011). Originally, this method was designed for removal of size from a set of linear measurements to generate data in which shape variation was independent of size (Burnaby, 1966) (see Chapter 2 for details). In this study, the Procrustes coordinates were projected orthogonally to the mean first eigenvector of the deformation model. Then, a comparison of the shape variability (by means of a PCA) in the dataset was done before and after correcting arching to see the effect in the dataset of the Burnaby projection. Also, the performance of the resulting shape variables (before and after correction) as discriminators of bluemouth samples in a canonical variate analysis (CVA) was evaluated.



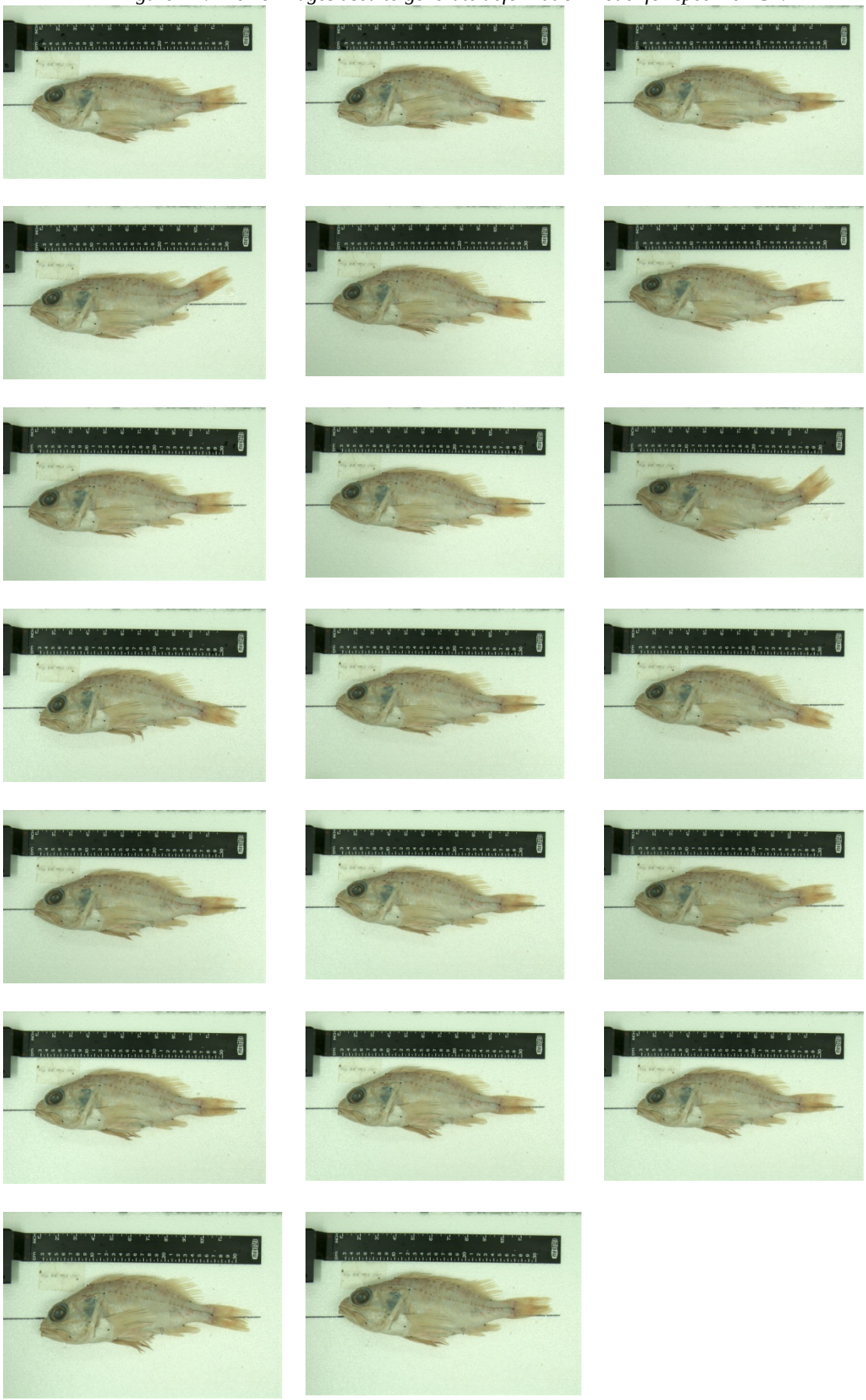
Landmark	Description
1	Marks the snout, i. e., the tip (most distal part) of the upper jaw
2	Base of the first dorsal spine
3	Point between the first and second dorsal fins
4	Marks the end of the second dorsal fin, at the base of the last soft ray
5	End of the hypurals, mid-point
6	Posterior end of the anal fin
7	Base of the first spine of the anal fin
8	Base of the first ray of the ventral fin
9	Mid-point of the insertion of the pectoral fin
10	Posterior limit of the operculum
11	Tip of the second preopercular spine
12	Mid-point of the posterior end of the upper jaw
13	Second supraocular spine

Figure I-1. Scheme showing the location of the 13 landmarks used in the analysis.

Table I-1. Specimens used to generate the 10 deformation models to describe the arching effect in bluemouth.

Specimen	Area	Size (CS, in cm)
A1	Alboran Sea	22.6297048
C1	Gulf of Cadiz	27.9633825
G1	Galicia	14.0718558
G2	Galicia	10.6644305
G4	Galicia	22.6144294
P1	Porcupine Bank	29.6489754
S1	Cantabrian Sea	16.4654939
S2	Cantabrian Sea	16.3346885
S3	Cantabrian Sea	38.3776411
S4	Cantabrian Sea	21.1573984

Figure I-2. The 20 images used to generate deformation model for specimen C1.



III Results

The deformation model

10 deformation models were obtained from the principal components analyses carried out separately on each specimen's shape data. An example of the deformation model and the shape changes associated to it is shown in Fig. 3. In this study, the variation accounted by the first eigenvector (i. e. first PC) of the deformation models ranged from 78.35 to 96.32 % (Table 2) .The first eigenvector of two of the deformation models (G1 and G2) explained only 78.35% and 85.01% of the total variation. Considering that in each model the same specimen was used in all the pictures, the other significant sources of variation were orientation error (error derived from the positioning of the specimen when the photograph is taken) and digitization error (error during the digitization of landmarks on the image), but a formal quantification of the magnitude of these errors was not carried out here (as in Chapter 2). From our experience, the larger digitizing error is related to the smaller size of the two specimens used in these models (10.66 and 14.07 cm CS), where it is more difficult to locate correctly the landmarks. Because of the lower amount of shape variation related to the arching effect, these two deformation models (G1 and G2) were excluded from further calculations, so that only the models describing more accurately the arching effect were used in this study .

The consistency between the remaining 8 deformation models was assessed by pair-wise comparisons of the angle separating the first eigenvectors of the 8 deformation models (Table 3). The angles between the first eigenvectors were small (14.79 ± 4.6), showing a general consistency of the deformation models that can also be observed in the plot of PC1 and PC2 (Fig. 4), where all models point in the same direction.

Table I-2. Variation accounted by the first eigenvector of the deformation models.

Deformation model	Percent of total variation accounted by PC1
A1	94.378
C1	96.326
G1	85.01
G2	78.352
G4	92.248
P1	96.214
S1	94.133
S2	93.436
S3	91.158
S4	95.453

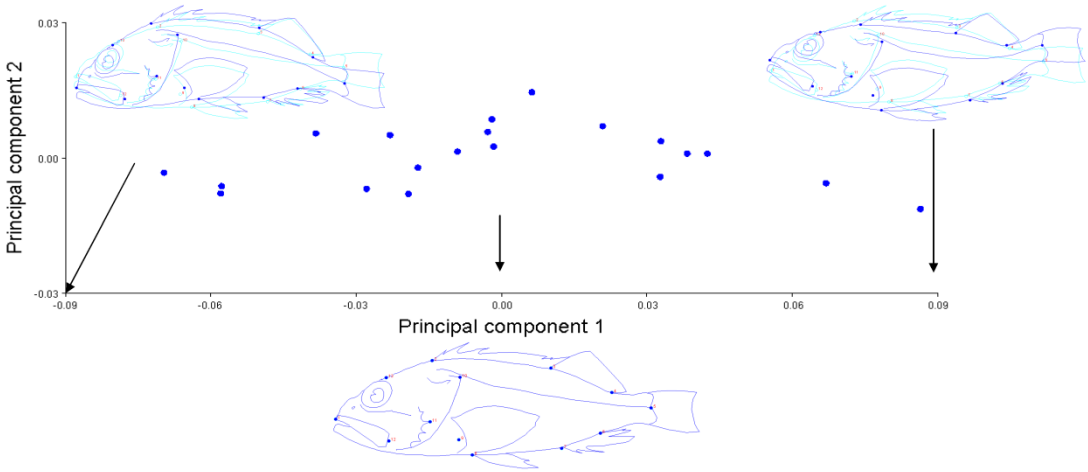


Figure I-3. Deformation model for specimen C1. Figure shows the association of the arching effect with PC1.

Table I-3. Pairwise comparison of the angle separating the first eigenvectors of the 8 deformation models. Angles are in degrees.

Deformation model	A1	C1	G4	P1	S1	S2	S3	S4
A1	0	8.78	19.51	14.24	20.84	21.05	11.44	12.20
C1	8.78	0	21.32	16.19	23.04	22.41	9.49	16.59
G4	19.51	21.32	0	8.42	10.65	10.95	16.71	12.66
P1	14.24	16.19	8.42	0	12.13	11.47	13.02	10.68
S1	20.84	23.04	10.65	12.13	0	7.04	21.36	12.79
S2	21.05	22.41	10.95	11.47	7.04	0	20.44	14.05
S3	11.44	9.49	16.71	13.02	21.36	20.44	0	14.55
S4	12.20	16.59	12.66	10.68	12.79	14.05	14.55	0

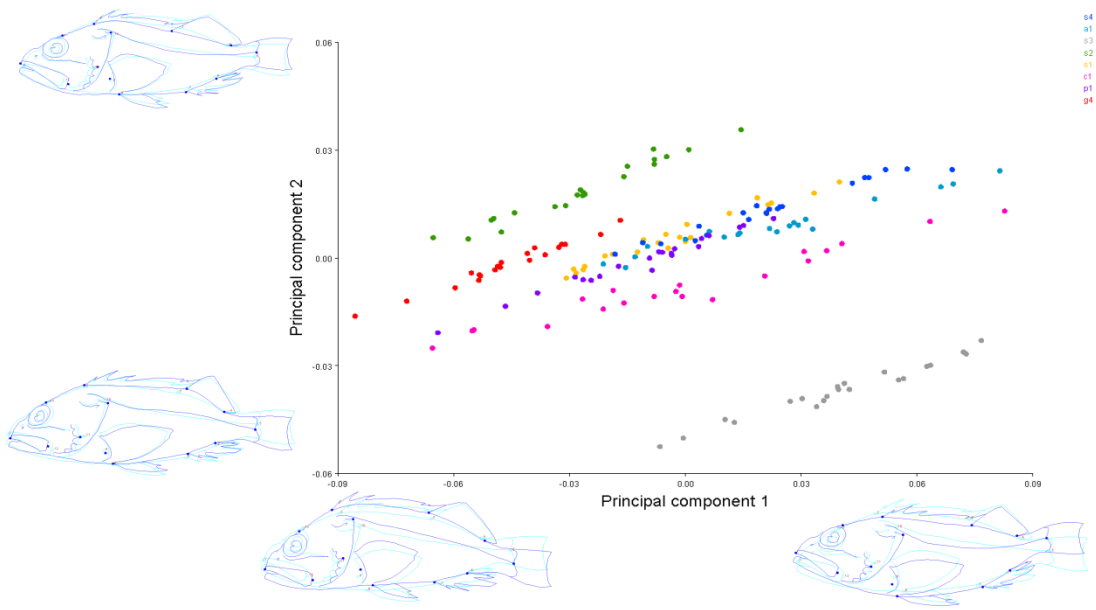


Figure I-4. Plot of the first and second PCs considering all of the 8 deformation models together. Outline drawings show the shape changes that occur along the PC axes.

Deformation models and size

Some differences in the shape patterns captured by the different deformation models were observed. In Figure 4, the variation among the deformation models can be observed mainly along the PC2 axis but some variation is also observable along PC1. In fact, a tendency can be observed, where the models produced with smaller fish are above and the models produced with larger specimens below. To test if PC1 and/or PC2 were related to the size of the specimens used to calculate the deformation models, a regression between each PC and CS was done. To test the null hypothesis of independence, a permutation test with 10.000 replicates was used. Both regressions were significant ($p < 0.01$). The regression of the first PC on CS accounted only for 13.60% of the variance, however, the regression of the second PC revealed a strong relationship between the deformation models and size, because the regression accounted for 65.8% of the variation.

Thus, according to these results, body arching is related to the size of the specimens used in the deformation models. According to our observations during the generation of the deformation models, medium-sized specimens (20 - 25 cm of CS) seemed to be more flexible than either large or small specimens. Moreover, very large specimens tend to be significantly u-shaped and rigid. The differences in flexibility are likely to be causing differences in the deformation models. Besides differences in arching due to the flexibility of the fish of different sizes, the deformation models could be also capturing shape differences due to allometry and/or geographical origin of the specimens.

The arching effect in the population structure dataset

To see if the relationship between the mean first eigenvector of the deformation model and the first eigenvector of the population structure dataset, the angle between these two eigenvectors was computed. This angle was of 40.54 degrees, suggesting that the deformation model describes a considerable amount of the arching deformation associated with the first PC of the population structure dataset.

The mean angle between the mean first eigenvector and the rest of the PC's (PC2 to PC22) was of 91.15 ± 7.93. These values close to 90 degrees mean independence between PC1 of the deformation model and these components.

Shape variation in the original dataset was investigated using a PCA. In this analysis, PC1, PC2 and PC3 accounted for 41.81, 9.59 and 8.75% respectively of the total

variability in the original dataset. In the PCA plot (Fig. 5), no particular pattern or grouping of specimens was observed along PC1, however, a slight differentiation was evident for some groups along PC2. By examining the shape changes associated to PC1, we observed an arching effect, where the specimens at one extreme of the axis were bent slightly downwards and those at the other end of the axis were bent slightly upwards. However, all of the bluemouth samples (from the different locations) were distributed similarly along PC1, indicating that this effect was present more or less evenly in all samples. We conducted a canonical variate analysis (CVA) based on these variables (i.e. PC's from the original dataset) to see if this analysis was able to discriminate bluemouth populations from different areas in spite of the presence of an arching effect, and which of these shape variables contributed the most to the discrimination of bluemouth populations. In the CVA, Wilks' Lambda value was: 0.1129, significant at the 5 % level ($F_{(176,8284)} = 15.7640$, $p<0.05$), indicating overall overall shape differences among the bluemouth groups from different areas. The jackknifed correct classification rate was relatively low (59.7%), indicating that despite the shape differences detected among groups, the canonical functions were not able to separate bluemouth specimens effectively at least in some of the areas. The structure matrix indicated that PC3 and PC2 were the variables that contributed the most to the group discrimination of bluemouth samples according to their location of origin, while the contribution of PC1 to group separation was negligible (Table 4). This indicates that the arching effect, contained in PC1, was "ignored" in the CVA, and those shape variables that contained relevant information on geographical shape variation (e. g., PC2 and PC3) were used instead to construct the canonical variates.

Population structure data set after the Burnaby projection

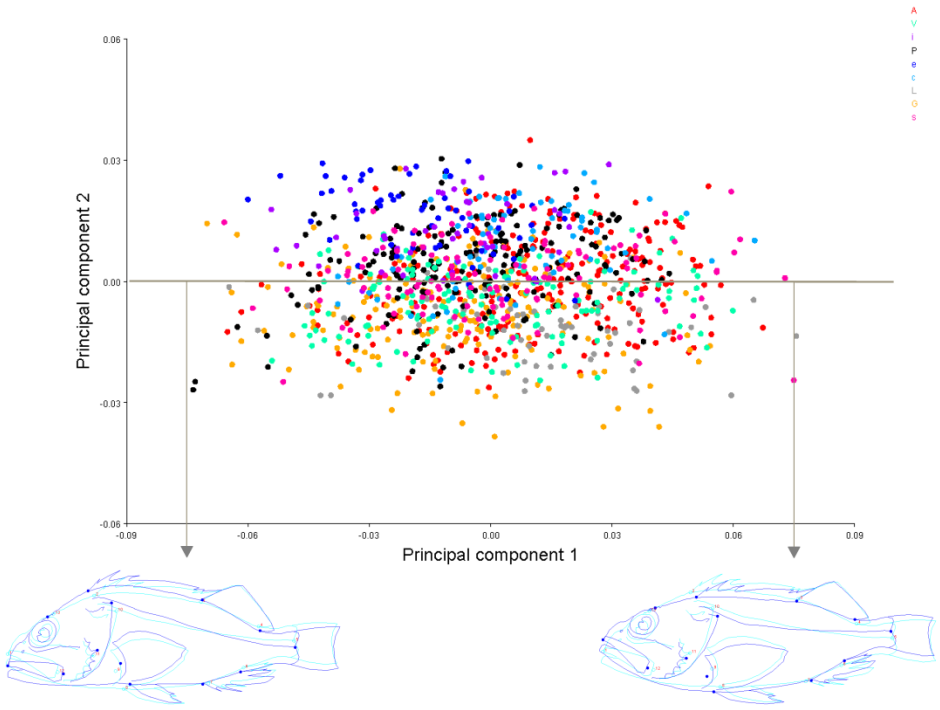
The percentage of total variance accounted for by the first four PC's of the PCA carried out on the Burnaby adjusted coordinates was 26.22, 12.95, 11.56 and 10.03% respectively. If we compare the amount of variance explained by PC1 on the original dataset (41.81 %) with that of the PC_{B1} (26.22 %), we can see that there is a notable reduction in the amount of variance after the Burnaby projection. This is also evident in the PCA plot (PC_{B1} vs PC_{B2}) after correction of the arching effect; where the observations form a tighter cluster than when the original data were used (Fig. 5). To see if the shape variation contained in PC_{B1} was related to allometry or the geographical

shape variation, we conducted a regression of PC_{B1} on geographical area and centroid size. This model had an r^2 of 0.249 and both centroid size and area were significant at the 5 % level ($F_{1, 1107}$ = 137.0968 and $F_{8,1107}$ = 24.5202, respectively). Though this indicates that size and geographical area had an effect on the shape changes described by PC_{B1}., the percentage of shape variation explained by these variables (i. e. 24.9 %) was relatively low. Therefore, PC_{B1}.seems to be mainly gathering shape information that is not useful for determining the population structure of bluemouth, but in this case, a canonical variate analysis (CVA) would be more appropriate to evaluate the role of PC_{B1} in the discrimination of bluemouth samples from the different areas (see results below). Actually, the shape changes associated with PC_{B1} are related to a pronounced upward/downward bending of the tail (Fig. 5), which could be indicating that the Burnaby projection did not eliminate all the shape information related to the arching effect on the bluemouth specimens and/or that the deformation model that we used to describe the arching effect was not good enough. If we examine Figure 4, we can see that despite most shape variability related to the arching effect in our deformation models is summarized by PC1, a slight bending of the tail (upwards at one end of the axis and downwards at the other) is captured by PC2. These shape changes (related to the tail area) are the ones that can be actually observed in PC_{B1}, (Fig. 5) indicating that not all of the arching effect was removed from the dataset by the Burnaby projection.

Both PC_{B2} and PC_{B3} were associated with allometric and geographical shape variation, explaining approximately 45% percent of the shape variation in each PC (r^2 of 0.4474 and 0.4600 respectively). In both cases, centroid size and area were significant at the 5 % level (PC2: $F_{1, 1107}$ = 559.2398 and $F_{8,1107}$ = 37.7226, respectively; PC3: $F_{1, 1107}$ = 559.2398 and $F_{8,1107}$ = 37.7226, respectively).

The results of the CVA performed on the Burnaby adjusted coordinates were very similar to those using the original Procrustes coordinates: Wilks' Lambda value was 0.1196 (significant at the 5% level, approx. $F_{(168,8248)}$ = 16.0414) indicating also overall shape differences among the bluemouth groups from different areas and the jackknifed correct classification rate was of 58.2%. The structure matrix also indicated that PC3 and PC2 were the variables that contributed the most to the group discrimination of bluemouth samples, while the contribution of PC1 to group separation was again negligible (Table 5).

a)



b)

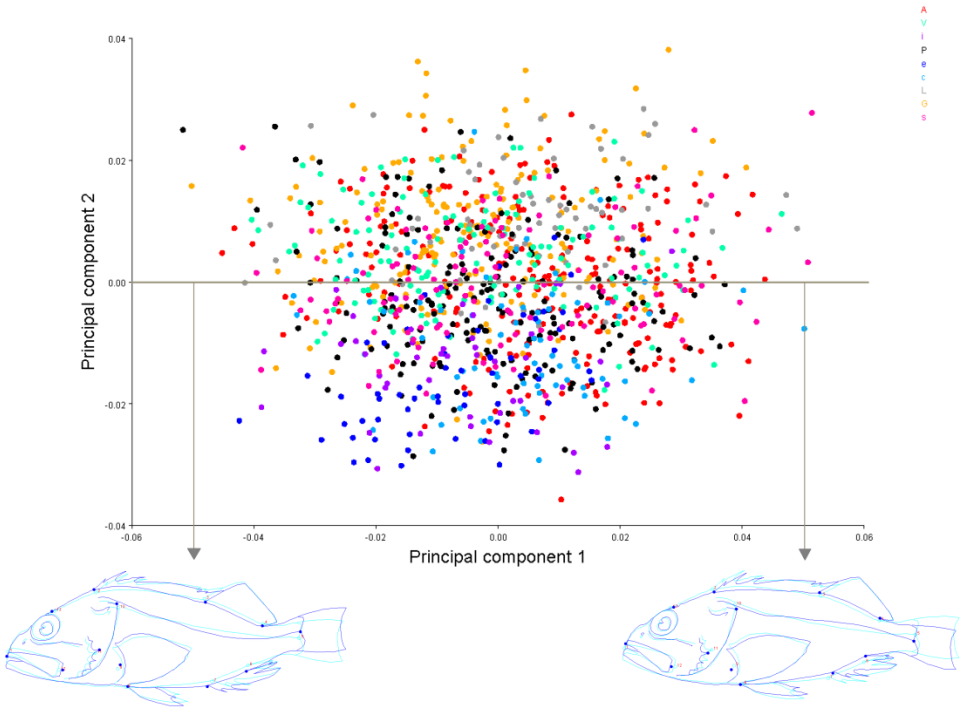


Figure I-5. PCA plots for the population structure dataset (a) prior to correction with the Burnaby projection, PC1 accounts for 41.81% and PC2 for 9.59% of the total variation and (b) after correction with the Burnaby projection, PC1 accounts for 26.21 % and PC2 for 12.95 % of the total variation. The outline drawings show the shape changes along PC1 axis.

Table I-4. Canonical variate correlation coefficients (structure matrix) of the CVA performed on the original population structure dataset. These coefficients show the relative contribution of each shape variable to group separation. Larger values indicate a larger contribution. Only the coefficients for the first four canonical variates are shown here.

Shape variable	CV1	CV2	CV3	CV4
PC3	0.5119	-0.2826	0.0158	0.0494
PC2	-0.3203	-0.6495	0.2323	0.0949
PC11	0.1927	0.0499	0.4222	0.3114
PC13	0.0751	-0.1162	-0.2114	0.1346
PC14	0.0240	0.1629	0.0014	0.3960
PC12	0.1459	0.1645	-0.2157	0.3444
PC6	0.2379	-0.0537	-0.1567	-0.3279
PC22	-0.0488	0.0110	-0.0590	0.2515
PC1	-0.0866	0.3108	0.0657	0.0411
PC21	-0.0280	0.0499	-0.0001	0.0321
PC7	0.0080	0.0198	-0.1084	0.0315
PC16	-0.0685	-0.0620	0.0393	0.1061
PC4	0.1721	0.0578	0.4379	-0.0520
PC20	-0.1046	0.0126	-0.2289	0.2221
PC9	-0.1063	0.1547	0.1367	-0.1211
PC15	-0.1486	0.1313	0.2594	0.0636
PC18	0.0131	0.0602	0.2002	-0.1214
PC10	0.0506	-0.1885	0.1247	0.3349
PC5	-0.0839	-0.0456	-0.0460	0.0561
PC17	0.0267	0.0555	0.2079	-0.2075
PC8	-0.0673	0.0516	-0.0061	0.0149
PC19	-0.0134	-0.0334	-0.1632	-0.1599

Table I-5. Canonical variate correlation coefficients (structure matrix) of the CVA performed on Burnaby corrected dataset. These coefficients show the relative contribution of each shape variable to group separation. Larger values indicate a larger contribution.

Shape variable	CV1	CV2	CV3	CV4
PC3	0.5192	-0.3810	-0.0929	0.0508
PC2	0.3743	0.6468	0.1130	-0.1481
PC12	0.1267	-0.0709	0.3025	0.1754
PC14	0.1310	-0.1361	0.2892	-0.1161
PC22	-0.0131	0.0476	0.2183	0.1352
PC13	0.0424	0.1538	0.0546	0.3838
PC9	-0.0889	0.2116	0.2026	-0.3469
PC11	-0.1459	-0.2342	0.0221	-0.3192
PC5	0.2119	-0.0610	0.1581	-0.2965
PC21	-0.0482	0.0199	0.0826	0.2742
PC7	0.0545	-0.0272	-0.0078	0.0174
PC20	-0.0280	0.0500	0.0376	0.0823
PC15	0.0776	0.0585	0.1134	-0.0360
PC4	-0.0939	-0.1085	0.4224	0.0482
PC8	0.1087	-0.1315	0.1790	0.1421
PC6	0.1187	0.0856	0.0392	-0.1215
PC17	-0.0082	-0.0351	0.1926	0.1061
PC1	-0.0834	0.1792	0.0188	0.1335
PC10	0.1586	0.0080	-0.3223	0.2430
PC19	-0.0864	0.0536	0.1157	0.1646
PC16	0.0228	0.0384	-0.2568	-0.2388
PC18	-0.0178	-0.0199	0.1975	-0.1263

Discussion and Conclusions

In Valentin et al. (2008), the authors reported that the first eigenvectors derived from the 10 deformation models accounted for 97.3 ± 1.1% of the total variation, while the variation accounted by the first eigenvector of the 10 deformation models in the present study was lower: 91.67 ± 5.7%, probably indicating higher digitization and orientation errors. Moreover, the bluemouth specimens used to produce the deformation models in the present study were very variable in size (as in the population structure dataset, where the size composition of the samples varied among areas) and the effect of size on the deformation models was explored. Body arching was found to be related to the size of the specimens used in the deformation models and it is probable that the digitization and orientation errors are also related to the size of the fish in some way. In our experience, it is more difficult to locate correctly the landmarks in small fish, and it is more difficult to place the larger fish in an adequate position when the photographs are taken. Valentin et al. (2008) also noted this issue, and indicate that for specimens >35 cm FL, size might influence the fish's posture during landmark capturing because these specimens are wider in the head-pectoral area, relative to the posterior part of the body, than smaller specimens.

As in Valentin et al. (2008), in this study, slight random posture differences between bluemouth specimens during landmark capture generated higher shape variability than the shape variability accounted for by geographical area or allometric size (the biological factors of interest). The arching effect dominated PC1 while shape variability related to biological factors was mostly distributed in the following PC's (PC2 - PC4). However, it seemed that the canonical variate analysis (CVA) was able to discriminate bluemouth populations from different areas in spite of the presence of an arching effect. In the comparison of the results of the CVA performed on the original data set and the Burnaby corrected dataset, there was no improvement in group discrimination in terms of Wilk's lambda values and correct classification rate, that is, both analyses yielded very similar results. This situation can be explained by the fact that the effect of body arching is orthogonal in shape space relative to the effect of the biological factors of interest and the discriminat analysis (CVA) is very efficient in removing this artefact by giving very low weight to the component summarizing the arching (i. e., PC1) (Valentin et al., 2008). Moreover, one entire dimension was eliminated from the dataset after the Burnaby

correction and it is possible that some amount of shape variation not related to the arching effect was eliminated as well in this procedure.

Thus, despite the presence of an arching effect, we decided to use the original dataset to determine the bluemouth population structure.

APPENDIX II

Resumen

La gallineta, *Helicolenus dactylopterus* (Delaroche 1809), es un pez marino demersal, cuya distribución geográfica abarca grandes áreas en el océano Atlántico y el mar Mediterráneo. La gallineta está clasificada actualmente en la familia Sebastidae dentro del orden Scorpaeniformes (Eschmeyer y Fricke, 1998; 2010). Sin embargo, algunos autores han incluido a esta especie dentro de la familia Scorpaenidae (Eschmeyer, 1969; Nelson, 1984; Hureau y Litvinenko, 1986) y parece ser que esta clasificación es todavía comúnmente usada. En ambos casos, el género *Helicolenus* está incluido en la subfamilia Sebastinae.

Los adultos de gallineta tienen un cuerpo robusto pero flexible, típico de los depredadores bénticos que esperan al acecho a sus presas (Webb, 1984; Uiblein et al., 2003). Los peces del género *Helicolenus* pueden alcanzar tallas de alrededor de 50 cm de longitud (Paul y Horn, 2009). El espécimen de gallineta de mayor talla reportado en la literatura científica tenía 47 cm de longitud (Abecasis et al., 2006).

La gallineta ha sido descrita como una especie de crecimiento lento y de longevidad considerable, que puede llegar a vivir más de 30 años (Massutí et al., 2000a; Abecasis et al. 2006, Sequeira et al., 2009). De hecho, se ha encontrado que la especie de *Helicolenus* que habita en aguas neozelandesas (*H. percoides*), puede llegar a vivir incluso más años. El espécimen más longevo que se ha capturado de esa especie en Nueva Zelanda era un macho de 50 cm de longitud y unos 59 años de edad.

El comportamiento de los adultos parece ser sedentario, ya que en varios estudios se ha observado que permanece inmóvil en el fondo la mayor parte del tiempo (Pakhorukov, 2008; Uiblein et al., 2003; Ross y Quattrini, 2007) y en un estudio de marcaje-recaptura, se han capturado los peces en los mismos sitios en donde se habían marcado un año antes (Menezes datos personales, en Aboim, 2005). En general, la dieta de la gallineta consiste en crustáceos decápodos bénticos (Natantia, Brachyura y Macrura), peces demersales y algunas veces pirosomas, poliquetos y equinodermos (Macpherson, 1979, 1985; Nouar y Maurin, 2000; Serrano et al., 2003).

La especies dentro de la familia Sebastinae son principalmente vivíparas (Wourms, 1991). Dentro del género *Helicolenus*, la estrategia reproductiva abarca desde el zigoparismo (embriones liberados al medio ambiente en los estados tempranos de desarrollo) que caracteriza a la gallineta, *H. dactylopterus* (Muñoz y Casadevall, 2002;

Sequeira et al., 2003), hasta el viviparismo (liberación de larvas) en *H. percoides* (Wourms, 1991). En el género *Helicolenus*, la fertilización ocurre internamente y las hembras de gallineta pueden almacenar el esperma dentro de los ovarios hasta por 10 meses (Muñoz et al., 1999, 2000). De esta manera, las células espermáticas se mantienen viables y quedan protegidas del sistema inmunológico de la hembra hasta que los ovocitos maduren (Muñoz et al., 2002; Vila et al., 2007). La información más reciente sobre la biología reproductiva de la gallineta proviene de un estudio llevado a cabo en el Mediterráneo occidental (Muñoz et al., 2010). Aquí, los autores encontraron que la gallineta es una especie con una fecundidad relativamente alta, teniendo en cuenta sus otras características reproductivas (por ejemplo, la fertilización interna y zigoparidad). A pesar de su alta fecundidad, se considera que esta especie es vulnerable a la pesca, ya que su potencial reproductivo se ve afectado por su compleja estrategia reproductiva (zigoparismo con fertilización interna) y ciclos reproductivos asincrónicos entre machos y hembras y por la relación que existe entre el tamaño del pez y su potencial reproductivo (Muñoz et al., 2010).

La gallineta está considerada como una especie de aguas profundas, pero en realidad tiene un amplio rango de distribución batimétrica, que va desde los 62 m hasta los 1135 m de profundidad según diversos estudios. Precisamente por esta característica, la gallineta es capturada en un gran número de pesquerías dirigidas a otras especies que habitan en la plataforma continental y las aguas profundas. En el Atlántico Noreste, la gallineta es capturada en pesquerías de arrastre de merluza (*Merluccius merluccius*), rape (*Lophius* spp.), gallo (*Lepidorhombus whiffiagonis*) y cigala (*Nephrops norvegicus*), llevadas a cabo por la flota española en los Bancos de Porcupine, Rockall y Gran Sol además del Golfo de Vizcaya (subáreas VI, VII y VIII del Consejo Internacional para la Exploración del Mar - ICES/CIEM). En aguas portuguesas, esta especie es capturada en las pesquerías de arrastre dirigidas a crustáceos (por ejemplo, de cigala - *Nephrops norvegicus* y gamba rosada - *Aristeus antennatus*) y en las de palangre (ICES, 2011; Vázquez-Rowe et al., 2011). En el Mediterráneo occidental, la gallineta aparece frecuentemente como captura incidental en las pesquerías de gamba rosada (Sardà et al., 2004). En el Mediterráneo central, esta especie es capturada en pesquerías de gambas de aguas profundas que utilizan diversos artes de pesca, como el arrastre, palangre y redes de enmalle y trasmallo (Romeo et al., 2009).

A partir de los años 90, la biología de la gallineta ha sido estudiada en algunas áreas del Atlántico Noroeste y Noreste y en el Mediterráneo. Estos estudios se han

centrado principalmente en la distribución geográfica y batimétrica de la especie, la estimación de la edad y el crecimiento en distintas áreas y de sus características reproductivas. Sin embargo, al igual que ocurre con otras especies de aguas profundas, aún quedan muchas incógnitas en lo que respecta a su biología, ecología y dinámica poblacional.

En la actualidad se reconoce que la información sobre la estructura poblacional de los recursos marinos es de especial importancia para el desarrollo de una estrategia óptima de gestión (Coyle, 1998). Así pues, el objetivo general de este estudio fue el de aportar información básica sobre la estructura poblacional de la gallineta, *Helicolenus dactylopterus*, en aguas de la Península Ibérica. Además, en esta tesis se ha llevado a cabo el primer estudio comparativo de las poblaciones de gallineta en el Atlántico Noreste y el Mediterráneo.

La determinación de la estructura poblacional de la gallineta fue llevada a cabo desde un punto vista morfológico, ya que el análisis de caracteres morfométricos y merísticos ha demostrado ser útil para este propósito en numerosas poblaciones de peces marinos (Swain et al. 2005).

Actualmente, existen dos enfoques principales hacia el análisis de la variación morfológica de los organismos (Adams y Rohlf, 2004; Mitteroecker y Gunz, 2009; Strauss, 2010). El primero es conocido como morfometría tradicional, y consiste en la aplicación de técnicas estadísticas multivariantes a un conjunto de variables morfológicas como mediciones de distancias, proporciones y ángulos. El segundo y más reciente enfoque es conocido como "morfometría geométrica". En este enfoque, la información sobre la forma de los individuos es caracterizada mediante un conjunto de puntos homólogos llamados "landmarks" o mediante contornos, de tal manera que la geometría de las estructuras morfológicas es preservada a lo largo de todos los análisis (Adams y Rohlf, 2004). En esta tesis, la forma corporal de la gallineta de las diversas áreas fue analizada mediante un análisis de morfometría geométrica basado en landmarks.

Para llevar a cabo este estudio, nueve áreas (principalmente alrededor de la Península Ibérica) fueron muestreadas. En el Atlántico Noreste, los especímenes fueron capturados en aguas del banco de Porcupine (situado en el margen continental de Irlanda), Galicia, el mar Cantábrico, el golfo de Cádiz y Portugal (Peniche). Los especímenes del Mediterráneo fueron muestreados en el mar de Alborán, en las costas cercanas a Alicante (suroeste del mar Baleárico), Cataluña y Sicilia (Italia). Las áreas de Porcupine y Sicilia fueron usadas como referencia, para comprender la estructura poblacional de la gallineta

a mayor escala y para relativizar las posibles diferencias que se encontraran entre las poblaciones de gallineta alrededor de la Península Ibérica. A excepción de las áreas de Portugal y Sicilia, todas las muestras fueron obtenidas en campañas de investigación oceanográfica. Las otras muestras se obtuvieron de la pesca comercial.

El primer objetivo específico de esta tesis fue analizar la alometría ontogénica de la gallineta en el Atlántico Noreste y el Mediterráneo mediante técnicas de morfometría geométrica. En esta parte del estudio, los cambios en la forma corporal que ocurren durante el crecimiento de la gallineta fueron caracterizados para comprender mejor la biología y ecología de la especie. Las trayectorias alométricas en cada área fueron determinadas usando una regresión multivariante (en cada grupo por separado). Al analizar estas trayectorias, se observó un patrón general de cambios ontogénicos que parece estar relacionado con la ecología cambiante de la especie, por ejemplo, con las adaptaciones en la dieta y hábitat que tienen lugar durante su crecimiento. Conforme los individuos crecen, se observó una expansión relativa del área comprendida entre la segunda espina preopercular y la aleta pectoral, una compresión longitudinal y ensanchamiento del cuerpo (es decir, se vuelve más robusto) y un desplazamiento vertical de la boca a la vez que la cabeza se vuelve más compacta. Sin embargo, algunos patrones específicos fueron observados en las distintas áreas de muestreo, indicando así que las trayectorias de crecimiento no son homogéneas entre las poblaciones de gallineta muestreadas.

El estudio de la alometría también fue utilizado para determinar el mejor método para eliminar el efecto del tamaño (alométrico) sobre las variables morfométricas. Este tipo de corrección es esencial cuando se llevan a cabo comparaciones morfológicas de varios grupos de especímenes en los que existen diferencias en la composición de tallas. Esto es debido a que existe el riesgo de que la variación morfométrica causada por las diferencias de tallas enmascare la variación morfométrica real entre los grupos que se están comparando. En este estudio, se compararon cinco métodos multivariantes comúnmente utilizados para corregir las variables morfométricas: a) el Análisis de Componentes Principales (Jolliffe, 2002), b) el método de Burnaby (Burnaby, 1966; Rohlf y Bookstein 1987), c) el método de Análisis de Componentes Principales calculado a partir de las variables morfológicas y el tamaño simultáneamente, conocido en inglés como “Size-and-shape PCA” (Mitteroecker et al., 2004) d) la regresión multivariante usando una recta única para todo el conjunto de datos (sin considerar la estructura de

grupos) y e) la regresión multivariante calculando una recta de regresión para cada grupo pero ajustándolas a una pendiente común (considerando la estructura por grupos).

A pesar de las pequeñas diferencias detectadas entre las trayectorias de crecimiento en las distintas áreas, el método de regresión en el que se tomó en cuenta la estructura por grupos fue el que dio mejores resultados en la eliminación del efecto del tamaño sobre las variables morfométricas. Por lo tanto, este método se utilizó para corregir el conjunto de datos usado en el análisis de la estructura poblacional de gallineta.

El segundo objetivo específico de esta tesis fue la identificación de stocks fenotípicos de gallineta en el Atlántico Noreste y el Mediterráneo mediante técnicas de morfometría geométrica y el análisis de variables merísticas. La hipótesis que se formuló es que posibles diferencias morfológicas podrían haber surgido entre las poblaciones de gallineta alrededor de la Península Ibérica (Atlántico Noreste y Mediterráneo) como resultado de:

1) El aislamiento entre poblaciones (por lo menos parcial) debido a factores como el efecto de límites biogeográficos, como el estrecho de Gibraltar entre el Atlántico y el Mediterráneo), el comportamiento sedentario de la especie (Uiblein et al., 2003; Pakhorukov, 2008), una dispersión larvaria limitada (Aboim, 2005) y la distancia geográfica que existe entre las áreas de estudio muestreadas.
2) Diferencias medioambientales entre las áreas de estudio, como la temperatura, salinidad, disponibilidad y tipo de alimento, etc.
3) Factores antropogénicos como diferente presión pesquera sobre las poblaciones de gallineta en las distintas áreas.

También se analizaron las diferencias morfológicas entre machos y hembras dentro de cada área de estudio para determinar si hay dimorfismo sexual o no en la gallineta.

Para determinar las diferencias morfológicas de la gallineta entre las distintas áreas muestreadas se utilizaron las distancias de Mahalanobis y las distancias de Procrustes. Éstas últimas son las distancias usadas en morfometría geométrica para medir la magnitud absoluta de la desviación entre dos formas (Slice et al., 1998). Para representar visualmente la forma media del cuerpo en cada área se utilizaron dibujos deformados usando la técnica de interpolación de placa delgada (Thin-plate splines) (Bookstein, 1989). Además, las variables morfométricas corregidas fueron utilizadas en una serie de análisis discriminantes (Análisis de Variables Canónicas o CVA por sus siglas en inglés). Finalmente, las relaciones entre las distintas áreas, basadas en las

distancias de Mahalanobis, fueron representadas mediante dendrogramas (Análisis de Cluster).

En este estudio se encontraron diferencias estadísticamente significativas respecto a la forma del cuerpo entre la gallineta de distintas áreas muestreadas. A pesar de esto, los análisis discriminantes mostraron que en algunos casos, estas diferencias inter-poblacionales no fueron lo suficientemente robustas como para considerar a estas poblaciones stocks fenotípicos diferentes.

La distancia geográfica entre áreas muestreadas no siempre resultó en diferenciación morfológica. En algunos casos, se observó una similitud morfológica entre muestras de gallineta provenientes de áreas muy separadas entre sí, que podría estar causada por factores medioambientales coincidentes o por similitudes genéticas que quedaron durante la evolución, más que por una tasa significativa de reproducción entre estas poblaciones.

Los resultados de este estudio no corroboraron la hipótesis de que el Estrecho de Gibraltar actuara como una barrera entre las poblaciones de gallineta del Atlántico y el Mediterráneo que causara una diferenciación morfológica marcada. Sin embargo, la separación relativa entre las poblaciones del mar de Alborán y las áreas de Alicante/Cataluña podrían indicar que el Frente de Amería-Orán (Tintoré et al., 1988) pudiera ser una barrera que limita la conectividad de las poblaciones de gallineta dentro del Mediterráneo occidental. Este aspecto, sin embargo, requiere más investigaciones para ser confirmado.

La mayor diferenciación morfológica fue observada entre las áreas de Portugal y las áreas colindantes hacia el norte (Galicia y el mar Cantábrico) y el sur (golfo de Cádiz). Esto indica que la gallineta de Portugal puede considerarse como un stock fenotípico por sí mismo. La variación morfológica entre Galicia y el mar Cantábrico parecía seguir un gradiente, es decir, que no se observó ningún punto de separación evidente entre estas poblaciones. Por lo tanto, la gallineta de estas dos áreas geográficas parece pertenecer a un mismo stock fenotípico.

En el Mediterráneo occidental, se encontró evidencia de que al menos dos stocks fenotípicos existen: uno en la cuenca suroeste (mar de Alborán) y otro en la cuenca noroeste (mar Baleárico y costa de Cataluña), que se extiende hasta la zona de transición en las aguas cercanas a Alicante. Es posible que además exista un tercer stock en el Mediterráneo occidental, ya que se observaron diferencias morfométricas importantes entre la gallineta proveniente de una pequeña zona en el mar de Alborán y la de zonas

circundantes (es decir, las demás zonas muestreadas en el mar de Alborán, Alicante, etc.). Sin embargo, para comprobar este último hallazgo se requieren más investigaciones.

Las variables merísticas se refieren al número de caracteres discretos, repetidos en serie y que se pueden contar, como por ejemplo, el número de vértebras, espinas y radios en las aletas, branquispinas, etc. (Swain et al. 2005). Las variables merísticas usadas en este trabajo fueron las siguientes: a) el número de espinas de la primera aleta dorsal (SDF1), b) el número de radios de la segunda aleta dorsal (RDF2), c) el número de radios de la aletas pectoral (RPF), ventral (RVF) y anal (RAF), d) el número de branquispinas en el segmento horizontal (GRH) y vertical (GRV) de la branquia.

Estos caracteres merísticos fueron analizados mediante métodos estadísticos no paramétricos, ya que estas variables no siguen una distribución continua. Entre los métodos usados están el test de Mann – Whitney, el coeficiente de correlación de Spearman y el test de Kruskal-Wallis. En general, se observó muy poca variabilidad en las variables merísticas, con la excepción de los conteos de branquispinas (GRH y GRV). Así pues, la utilidad de este tipo de variables para la identificaión de stocks fenotípicos de gallineta es cuestionable. Parece ser que en el género *Helicolenus*, el estudio de los caracteres merísticos tiene mayor utilidad a nivel inter-específico que intra-específico, es decir dentro de poblaciones de una misma especie.

En relación a la gestión de esta especie, la información obtenida en esta tesis sobre la estructura poblacional de la gallineta puede ayudar a definir áreas significativas en las que pueda ser llevada a cabo la recopilación de datos para realizar evaluaciones preliminares de los stocks de gallineta. Por ejemplo, de acuerdo a los resultados de esta tesis, existe evidencia de la existencia de un stock fenotípico de gallineta en las aguas cercanas a Peniche (Portugal) y que este stock es diferente al que existe en Galicia o el golfo de Cádiz. Esto implica que hay por lo menos tres poblaciones distintas que cohabitan en la división estadística IXa del ICES/CIEM, y que a la hora de recopilar datos para formular recomendaciones para la gestión de la especie, esta estructura debería ser tomada en cuenta. La situación contraria, cuando una población homogénea se distribuye en dos divisiones estadísticas diferentes, también debe ser considerada. Este podría ser el caso del población de Galicia, que estaría distribuida en las divisiones VIIIc y IXa del ICES/CIEM.

Cada vez hay más información disponible sobre la biología y otros aspectos poblacionales de la gallineta, además de que actualmente ya se están recopilando datos provenientes de las pesquerías y campañas de investigación a nivel de la Unión Europea

(por ejemplo, datos de descargas, descartes y algunas variables como la edad, peso, sexo y estado de madurez). Por esa razón, esperamos que en un futuro próximo puedan llevarse a cabo las primeras evaluaciones de los stocks de gallineta y entonces puedan formularse algunas recomendaciones para gestionar esta especie en la región.

El trabajo desarrollado en esta tesis ofrece una contribución importante para la comprensión de la estructura poblacional de la gallineta en aguas europeas, ya que ha aportado evidencias de que existen diferentes stocks fenotípicos. Estos stocks pueden ser usados preliminarmente en modelos de dinámica poblacional para ser usados en gestión pesquera. Sin embargo, aún queda mucho trabajo por hacer para poder comprender a fondo la estructura poblacional de la gallineta en el Atlántico Noreste y el Mediterráneo.

Primeramente, la estabilidad temporal y espacial de la estructura poblacional determinada en este estudio debe ser evaluada más a fondo. Esto es importante porque la variación morfológica es fenotípica y los caracteres morfométricos tiende a tener una heredabilidad moderada o baja (Swain et al. 2005). De esta manera, podría ser posible que las poblaciones de gallineta estudiadas en este trabajo pudieran ser afectadas por fluctuaciones temporales en el ambiente y que la imagen de la estructura poblacional obtenida aquí no sea definitiva. Además, es necesario contrastar los resultados de este estudio con marcadores genéticos y ecológicos, que pueden ofrecer otra perspectiva de la estructura y dinámica poblacional de la gallineta.

Particularmente, un enfoque genético podría ser muy útil para complementar los resultados de esta tesis, porque permitiría comprender mejor las causas subyacentes de las diferencias morfológicas. Este tipo de estudio también ayudaría a determinar el grado de diferenciación genética entre las poblaciones y su conectividad. Además, un estudio comparativo de los parámetros vitales entre las poblaciones de gallineta en el Atlántico Noreste y el Mediterráneo (por ejemplo, la distribución, abundancia, edad, crecimiento, mortalidad, reproducción, época de puesta y distribución larvaria) podría aportar información imprescindible sobre las poblaciones de gallineta. Es por ello que lo más adecuado en el futuro es llevar a cabo un estudio multidisciplinar que cubra ampliamente áreas en el Atlántico Noreste y el Mediterráneo, para así esclarecer definitivamente la estructura y dinámica poblacional de la especie.