



# Anatomical differences among Atlantic and Mediterranean populations of *Trisopterus luscus* (Pisces)

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**ABSTRACT:** The aim of this study was to see if a simple, low-cost anatomical method could be used to identify genetic differences among different populations of pouting *Trisopterus luscus*. We based our study on 8 anatomical units of the pouting skeleton in 170 specimens from 4 populations (3 Atlantic, 1 Mediterranean). Significant variations in the relative size of the respiratory anatomical structures and other cranial regions were present. These differences are thought to be a consequence of the different environmental conditions to which the fish are exposed. This is most noticeable along the Mediterranean coasts of the Alborán Sea, where pouting show a significant increase in the size of skeletal structures involved in respiration, whereas the Atlantic populations showed no interpopulation variation, suggesting the existence of a genetic barrier between the Mediterranean and Atlantic populations. Further research is needed to identify possible environmental factors involved.

**KEY WORDS:** Pouting · Atlantic · Mediterranean · Skeleton · Anatomy

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## INTRODUCTION

The pouting *Trisopterus luscus* (Linnaeus 1758) is distributed throughout the Atlantic Ocean, from the Skagerrak and the British Isles to southern Morocco, and into the western Mediterranean. Its preferred habitat consists of rocky and sandy bottom areas on the continental shelf at depths of 30 to 100 m (Wheeler 1978). The pouting belongs to the gadoid family and is of major commercial importance for the artisanal fleets of France, Portugal, and Spain (Alonso-Fernández et al. 2008).

The Mediterranean area is peculiar from the biodiversity point of view, as it includes tropical and temperate zones. The high species richness characterising this area is largely due to its complex history through the Tertiary and post-Pliocene diversity pump from the Atlantic, and to Lessepsian migration through the Suez Canal (Bianchi & Morri 2000). The Mediterranean salt tongue is a conspicuous feature of North Atlantic

hydrography. At a depth of 1000 to 1200 m, it extends westward from the Gulf of Cadiz almost across the entire Atlantic basin. Along the eastern margin it can be traced far into the northern North Atlantic (Gerdes et al. 1999). This has a significant influence on the environmental conditions found in 2 of our study areas: the Ría de Vigo and Huelva.

The NW Iberian Peninsula is the northern limit of the eastern boundary of the large marine ecosystem that extends along the Atlantic coasts of Africa and Europe from 10° 8' N to 44° 8' N (Wooster et al. 1976, Bakun & Nelson 1991). Four fjords known as Rías Baixas occupy the Spanish coast in the 42° 8' N to 43° 8' N latitudinal range. The Rías Baixas function as an extension of the shelf during the upwelling season, with northerly winds enhancing the positive residual circulation pattern. On the other hand, during the downwelling season, the outer part of the Rías Baixas circulates like a negative estuary forced by the dominant southerly winds, whereas the inner part behaves as a positive

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estuary forced by continental runoff. This unique combination of wind patterns and coastal morphology makes the Rías Baixas an exceptional site for fish exploitation and study (Álvarez-Salgado et al. 2000, Piedracoba et al. 2005).

Since the structure of the marine community on the western Iberian margin is also under the influence of seasonal upwelling (Sousa et al. 2005), we have further investigated whether anatomical features of *Trisopterus luscus* change among the eastern and western Iberian Peninsula margins.

## MATERIALS AND METHODS

**Study sites.** The study was carried out in 2003 and 2004 in 3 Atlantic populations (2 from the Ría de Vigo and 1 from Huelva) and 1 Mediterranean population (Alborán Sea).

The Rías Baixas (NW Iberian Peninsula) are 4 flooded tectonic valleys that behave as partially mixed estuaries (Beer 1983) with a 2-layered positive residual circulation pattern (Fraga 1981). The density-driven flow (gravitational convection) is strongly influenced by the freshwater contribution, the wind regime along the main axis of the rías and the upwelling–downwelling events (Blanton et al. 1987, Prego & Fraga 1992, Rosón et al. 1997) control the water exchange between the rías and the shelf. The Ría de Vigo, the southernmost of the Rías Baixas, is 2160 km<sup>2</sup> in area, 23.3 km<sup>3</sup> in capacity, and averages 221 m depth with a maximum depth of 245 m in the main channel.

The Huelva Estuary is a 25 km long incised valley on the southwestern coast of Spain. This area is underlain by neogene sandy-silty sediments that characterize the southwestern area of the Guadalquivir Basin. The slope of the southern part of the western Iberian Peninsula is under the influence of the flow of Mediterranean Water (MW) into the Atlantic, which tends to follow northward the contours of the Iberian Peninsula's southwestern slope. According to Ambar et al. (1986), this warmer and saltier water mass is part of an undercurrent that may extend from the bottom of the surface mixed layer down to 1500 m.

The Alborán Sea, in the southwestern Mediterranean, is located in the subtropical high-pressure cell of the NE Atlantic and experiences a markedly seasonal Mediterranean climate, with cool winters and hot, dry summers, and is highly influenced by the Atlantic Waters (AW). Most of the scarce rain (average 200 to 300 mm) is of cyclonic origin and related to prominent contrasts in temperature between land and sea.

**Anatomical features.** Samples were taken aboard commercial fishing vessels. The number of specimens analyzed was: (1) 40 (July 2003) plus 50 (May 2004)

from the Ría de Vigo; (2) 50 (May 2004) from the coast of Huelva; and (3) 30 (July 2003) from the coast of the Alborán Sea. The size of the specimens was greater than the minimum commercial size.

We adopted the bone nomenclature of Gregory (1933), Birdsong (1975), and Rojo (1976, 1985, 1991). We grouped the bones into 8 anatomical zones according to the skeletal unit to which they belonged (Fig. 1). This allowed us to distinguish the following zones (and associated measurements taken) taking in consideration Vandewalle & Chardon (1981):

(1) *Neurocranial zone*: provomer length, otolith width, frontal length, lacrimal length and width, nasal length and width, pterotic length, parietal-pterotic length, pterotic wing length, neurocranium total length, axis-end parietal length, axis-end pterotic length, axis-pterotic wing length, and sphenotic-pterotic length.

(2) *Suspensorium zone*: symplectic length, hyoman-dibular height and width, quadrate length and width, and palatine length and width.

(3) *Buccal zone*: dental length, inferior dental length and superior dental length, articular length and width, premaxillar length and width, and maxillar length.

(4) *Hyoideal zone*: distance between the hindmost point of the epihyal and the beginning of the anterior ceratohial rod, hyoideal arch total length, and 1st, 2nd, 3rd, 4th, 5th, 6th, and 7th branchiostegal ray length.

(5) *Branchial zone*: 1st, 2nd, 3rd, 4th and 5th branchial arch length.

(6) *Opercular zone*: opercle length and height, sub-opercle length and width, interopercle length and width, and preopercle length and width.

(7) *Scapular belt zone*: cleitrum length and width, supracleitrum length and width, postcleitrum length and width, posttemporal length and width, coracoid length, and scapula length.

(8) *Vertebral zone*: 1st vertebra height, spine of the 1st vertebra length, neural arc radius of the 1st vertebra, 1st vertebra total length, 10th vertebra height, 10th vertebra total length, spine of the 10th vertebra length, body of the 10th vertebra diameter, epicentrum 10th vertebra length, 17th vertebra height, spine of the 17th vertebra length, and neural arch radius of the 17th vertebra.

For each fish, we measured 137 variables, for 46 pairs of osseous elements with bilateral symmetry and 11 axial ones, i.e. a total of 23 290 measurements in 170 specimens.

**Data processing.** From the 3 repeated measurements for each variable we obtained the average absolute value. Thereafter, we used the average absolute values to obtain the relative average values as a percentage of the total fish length. From these average relative values we carried out a factorial analysis followed by a discriminant analysis for each population.

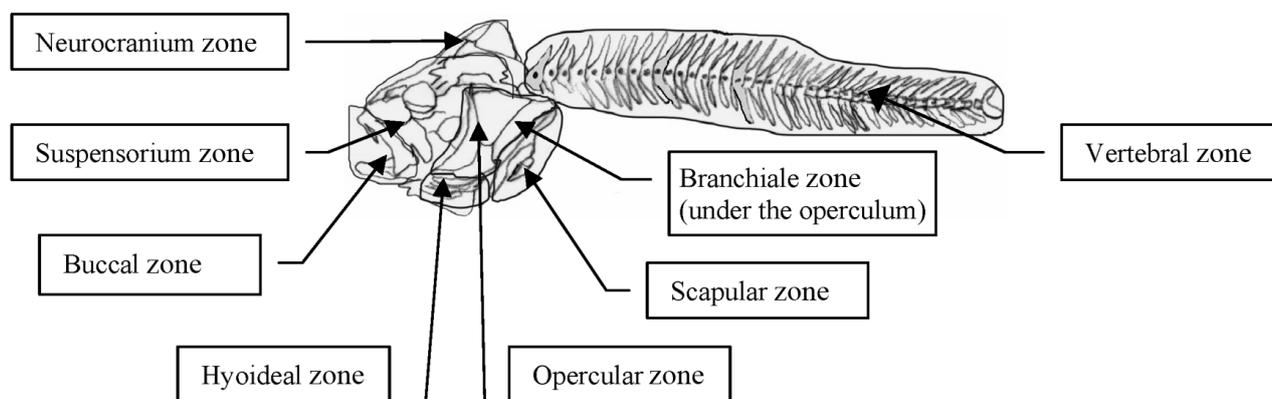


Fig. 1. *Trisopterus luscus*. Anatomical zones of the axial skeleton and scapular belt. See 'Materials and methods' for measurements

## RESULTS

### Alborán Sea, July 2003

The results of the factorial analysis indicate that the first 8 components explain up to 78.4% of the original variance in anatomical features. All the variables corresponding to the elements of respiratory function are grouped in Component 1 (Table 1). Certain elements of the neurocranium and the suspensorium also grouped in Component 1. Consequently, the respiratory structures maintained the same proportions during fish development.

### Ría de Vigo 1, July 2003

The first 5 components explain up to 73.8% of the total variance, and not all the variables corresponding to the respiratory organs are grouped in Component 1; instead, some correlate with other components. Therefore, the bones involved in respiratory function do not follow the same trends in variation during development. As pouting grow, the values of the Component 1 variables increase. The opposite is true for the elements of Component 2. This shows that during the development of pouting, while certain respiratory structures increase in relative size, others decrease.

### Ría de Vigo 2, May 2004

The first 4 components explain up to 72.7% of the total variance and the variables corresponding to the essential respiratory elements, such as those of the branchial and hyoideal zones, are all assembled in Component 1. Variables of the opercular and buccal

zones are distributed in Components 1 and 2. This means that the respiratory structures do not follow the same pattern of variation during pouting development. Certain elements of respiratory function, such as those of the branchial and hyoideal zones, decrease their relative size as total fish length increases, while others such as those of the opercular and buccal zones increase their relative size.

Table 1. Summarized matrix of principal components (a prime symbol indicates components of negative correlation)

Variables	Component
<b>Alborán Sea</b>	
Buccal, branchial, hyoideal, opercular	1
Neurocranium	1, 2, 3, 6', 8'
Suspensorium	1, 2, 4, 5'
Scapular	6, >8
Vertebral	3, 4', 7
<b>Ría de Vigo 1</b>	
Branchial, buccal	1
Opercular	1, 2, 3'
Hyoideal	1, 2, 4, 5'
Neurocranium	1, 2, 5'
Suspensorium	1, 3
Scapular	1, 1', 4
<b>Ría de Vigo 2</b>	
Branchial, hyoideal	1
Opercular, buccal	1, 2
Buccal	1, 2
Neurocranium	1, 4
Scapular	1, 3
<b>Huelva</b>	
Opercular	1
Hyoideals, buccal	1, 2
Branchial	1', 2, 3
Neurocranium	1, 2, 3'
Scapular	1'

### Huelva, May 2004

The first 5 components explain up to 76% of the total variance, illustrating that the variables corresponding to the essential parts of respiratory function are dispersed in various components, in contrast to the samples from the Alborán Sea (Table 1). The same dispersion of variables was found in the case of specimens from Ría de Vigo 1. It follows that the respiratory structures do not follow the same trends in variation during pouting development, as some elements increase in relative size during development, while others decrease.

### Huelva, Ría de Vigo and Alborán Sea

The interpopulational factorial analysis of all samples shows that Component 1 includes the respiratory variables of the branchial, opercular and buccal zones and those of the neurocranium, scapular, vertebral and suspensorium zones. Component 2 is entirely constituted of the bones of the hyoideal zone (Table 2).

### DISCUSSION

In the Atlantic specimens (Ría de Vigo and Huelva), the variables corresponding to the respiratory structures are scattered among various principal components. This type of scattered distribution of the measurement data from the respiratory bones shows that these elements grow asynchronously during pouting

Table 2. Component matrix of the samples from Ría de Vigo, the Alborán Sea and Huelva

Variables	Component
Branchial, opercular, buccal, neurocranium, scapular, vertebral, suspensorium	1
Hyoideal	2

development. In the individuals from the Alborán Sea, however, the osseous elements which actively play an important part in the respiratory function, like those of the opercular, branchial, hyoideal, and oral zones, are grouped in Component 1 (Table 1) and grow synchronously with the size of the fish. This means that they follow the same pattern of development during the growth of pouting.

These significant anatomical differences (Fig. 2) may originate from environmental differences between the Atlantic Ocean and the Mediterranean Sea. These different environmental features may produce selective pressures which lead to genetic variability. The presence in other marine species of a genetic barrier between the Atlantic and Mediterranean is supported by genetic studies (Ouagajjou et al. 2009). The anatomical methods used in the present study allow us to detect this possible genetic barrier, as well as establish a hypothesis about the potential environmental cause of this variability.

We hypothesize that differences in salinity, temperature, water currents, etc., lead to variable oxygen levels in the water, which exert a stronger selective pressure in the respiratory structures of the Mediterranean populations. The possibility of rapid changes in skeletal structures in response to environmental conditions has been suggested by Shapiro et al. (2004).

The anatomical modifications found in pouting could be derived from past migratory and colonisation processes in the Atlantic Ocean and the Mediterranean Sea. If so, the anatomical modifications we found in the present study are within the range of anatomical plasticity of *Trisopterus luscus* and are likely the result of natural selection. The present study shows that it is possible to trace and evaluate population adaptation and differentiation through the use of anatomical traits, complementing studies based on physiology and genetics.

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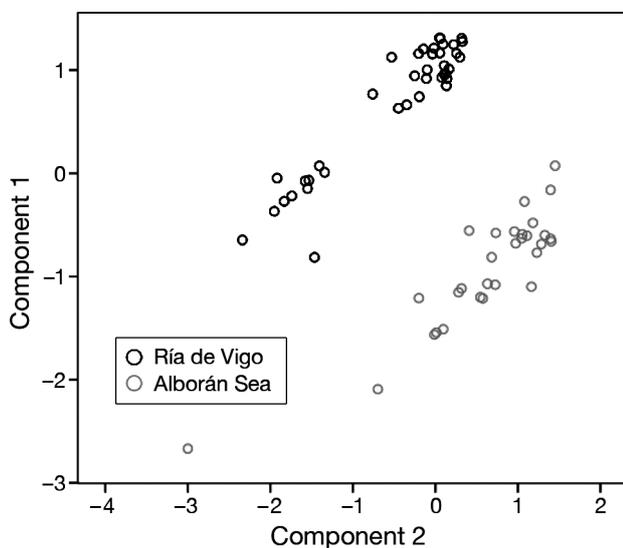


Fig. 2. Scattering of Component 1 and 2 values from *Trisopterus luscus* populations of the Alborán Sea and Ría de Vigo. Both populations are well discriminated: Wilk's lambda = 0.041,  $p = 0.001$

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