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Discrimination of red mullet populations (Teleostean, Mullidae) along multi-spatial and ontogenetic scales within the Mediterranean basin on the basis of otolith shape analysis*

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Abstract – Otolith shape analyses were conducted on three different species of Mullidae (*Mullus barbatus barbatus*, *M. b. ponticus* and *M. surmuletus*). The otolith shape was described by 19 harmonics from elliptic Fourier descriptors. In a first step, their comparison through canonical discriminant analyses (CDA) was run for all fish with right otoliths, left otoliths and both otoliths pooled. The latter possibility had a higher discriminating power and allowed much more visually explicit results. This implied that the two otoliths were not similar as often claimed, and had each their specific information. In a second step, the CDA demonstrated strong spatial discrimination of local populations from various areas within the Mediterranean basin, i.e. NW Mediterranean, Aegean Sea and Black Sea, and between sites within each area. The percentage of well classified individuals of *M. barbatus* in predefined groups varied between 78 to 100% depending on sites, and even reached 100% for each site for *M. surmuletus*. These spatial patterns were most likely linked to differences in environmental conditions between areas and sites, such as effects of strong river runoffs and differences in depths and/or habitat types. In a third step, CDA also evidenced ontogenetic discriminations of mullet populations that could be linked (i) to influence of diet of fish of various sizes and (ii) to changes in physiological conditions according to the stage of development of the fish.

Key words: Otolith / Shape analysis / Environmental influences / Fluctuating asymmetry / Mullidae / Mediterranean

1 Introduction

Bony fish have three pairs of otoliths (*sagittae*, *asterisci* and *lapilli*) which are mineral inert components consisting of calcium carbonate without any dissolution or resorption ; thus their component material being unlikely to be resorbed or altered (Campana and Neilson 1985; Campana and Casselman 1993). They are located within the inner ear of teleosts where they contribute to several physiological processes, such as audition, mechano-reception and equilibration (Popper and Combs 1980; Popper et al. 2005). Otoliths are used in a wide range of studies, such as species identification (Aguirre and Lombarte 1999; Parmentier et al. 2001), age and growth assessment (Cardinale et al. 2004), stocks assessment (Friedland and Reddin 1994; Gonzalez-Salas and Lenfant 2007) and determination of the diet of top predators (Lilliendahl and Solmundsson 2006). Within a species, the otolith shape may vary according to fish size, site, depth and/or other environmental factors (Begg et al. 2001; Stransky and MacLellan 2005; Hüssy 2008). Several studies have shown that shape analysis allowed discrimination of local fish stocks according to ecological factors (Smith 1992; Friedland and Reddin 1994; Bolles and Begg 2000; Cardinale et al. 2004). The ecomorphological link hypothesis of otolith shape was highlighted in relation to life history traits, biological

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and behavioural characteristics of fish species (Gauldie and Crampton 2002; Volpedo and Echeverria 2003) and even the type of swimming activity (Lychakov and Rebane 2000).

Red mullets (Mullidae) are species of major commercial interest in the Eastern Atlantic and in the Mediterranean. Nevertheless, catches of these species remain moderate in the NW Mediterranean, mainly because these species are not the most targeted fish but also because catches by recreational and some artisanal fishermen cannot be yet assessed. For the French Mediterranean coasts, annual catches range between 300–800 tonnes (Campillo 1992). It is usually considered that there is no true fishery management strategy for the Mediterranean basin as a whole, for demersal stocks or for the red mullets in particular. Gaertner et al. (2005) analyzing spatial variations of fish assemblages at large scale in the NW Mediterranean, suggested the existence of three main fishing grounds from the Strait of Gibraltar to the Gulf of Lions, but also showed a clear subdivision between the eastern and western parts of the Gulf of Lions. That study, along with a few others (Farrugio et al. 1993; Aldebert 1997), is among the first attempting to provide a basis for the definition of spatial units for fisheries management purposes in the NW Mediterranean. Although our sampling does not offer a suitable basis for taking part in this debate, we however can offer some contribution through local populations, as has been successfully done for *Solea solea* in that region (Mérigot et al. 2007). Therefore, it can be hypothesized that otolith shape could be a good marker allowing the discrimination of local populations of mullids in the NW Mediterranean, Aegean and Black Seas. The aim of this study was thus to identify different local populations of three species of Mullidae through otolith shape analysis and also to assess the possible role of fish size on otolith shape as a discriminant factor. To this end, canonical discriminant analysis was used. This method always implied the use of only one of the two otoliths. There was indeed a preconceived idea because there was no reason a priori to consider that the right otolith might be different from the left one, for a given roundfish species at population level. For roundfish, similarity in physical and chemical processes involved in the growth of the right and left otoliths is suggested to explain the maintenance of symmetry between both otoliths (Lychakov and Rebane 2005; Lychakov et al. 2008). However, one exception to this consensus may concern flatfish due to their form resulting from the shift from pelagic larval life to benthic life after metamorphosis, and then adopting a typical flat-form after lying on its left (or right) side (Graf and Baker 1983). For roundfish, most otolith shape analyses considered only one of the otoliths, without always testing for differences between sides. Nevertheless, some recent studies have shown that both otoliths of a roundfish may present differences in their shape, a phenomenon named fluctuating asymmetry, defined as random deviations from ideal, perfect symmetry (van Dongen and Lens 2000; Palmer et al. 2010). The possible consequences of fluctuating asymmetry on otolith shape analyses was however not well documented and, to our knowledge, never assessed in a context of characterization of local populations. In the present paper, all three possibilities, i.e. analyzing only the right otoliths, only the left otoliths and analyzing both otoliths pooled together were explored in order to capture any effect of asymmetry on population separation of the investigated species. In addition to the objective of spatial and ontogenetic discrimination mentioned above, this aspect will enable to clarify the preconceived idea of whether using only one of the two otoliths is justified or not.

2 Material and methods

2.1 Species studied

Mullus barbatus barbatus Linnaeus, 1758, *M. barbatus ponticus* Essipov, 1927 and *M. surmuletus* Linnaeus, 1758 belong to Mullidae and usually inhabit coastal shelf ecosystems from 0 to ~200 m depth. *M. barbatus barbatus* is widely distributed in the Eastern Atlantic Ocean, from the British Isles and Scandinavia in the North to Senegal in the South, also including the Mediterranean (Fisher et al. 1987). *M. barbatus ponticus* is restricted to the Black and Azov seas, whereas *M. surmuletus* has a geographical range similar to that of *M. barbatus barbatus* (Fisher et al. 1987). These three species are gregarious, demersal and live on continental shelf soft-bottoms. *M. surmuletus* can also occur on rocky bottoms and *Posidonia oceanica* meadows in the Mediterranean. Their diet is mainly composed of small crustaceans and polychaetes (Bautista-Vega et al. 2008). The maximum adult size is ~30 cm (total length, *TL*) for *M. barbatus barbatus* and *M. b. ponticus*, and ~40 cm (*TL*) for *M. surmuletus* (Fisher et al. 1987).

2.2 Studied areas and sampling

Sampling was conducted in three main areas, i.e. the French coasts of the NW Mediterranean, the Romanian coasts of the Black Sea, and around Lesvos Island, Greece, in the Aegean Sea (Fig. 1). Within each of these areas, several sites were sampled (Table 1). Sites off the Rhône river mouth are subject to strong variations in environmental conditions, mainly within the 0–50 m depth range, due to river runoffs. The mean yearly river discharge is about 1700 m^3 s^{-1}. These freshwater inputs have an influence not only directly off the river mouth, but also as far as the Spanish border due to the Northern current which transports the particular and dissolved organic matter river plume to the SW of the Gulf of Lions (Millot 1999). On average, 7.4×10^6 tonnes $year^{-1}$ of terrestrial particulate material is discharged off the river mouth (Pont et al. 2002) and is of particular importance both for pelagic (Sempéré et al. 2000) and benthic production (Salen-Picard et al. 2002). Thus, sites sampled in the NW Mediterranean included sites subjected to river inputs (i.e. within the Gulf of Lions) and sites located easterly not subject to these inputs (Marseille, Cassis and La Ciotat).

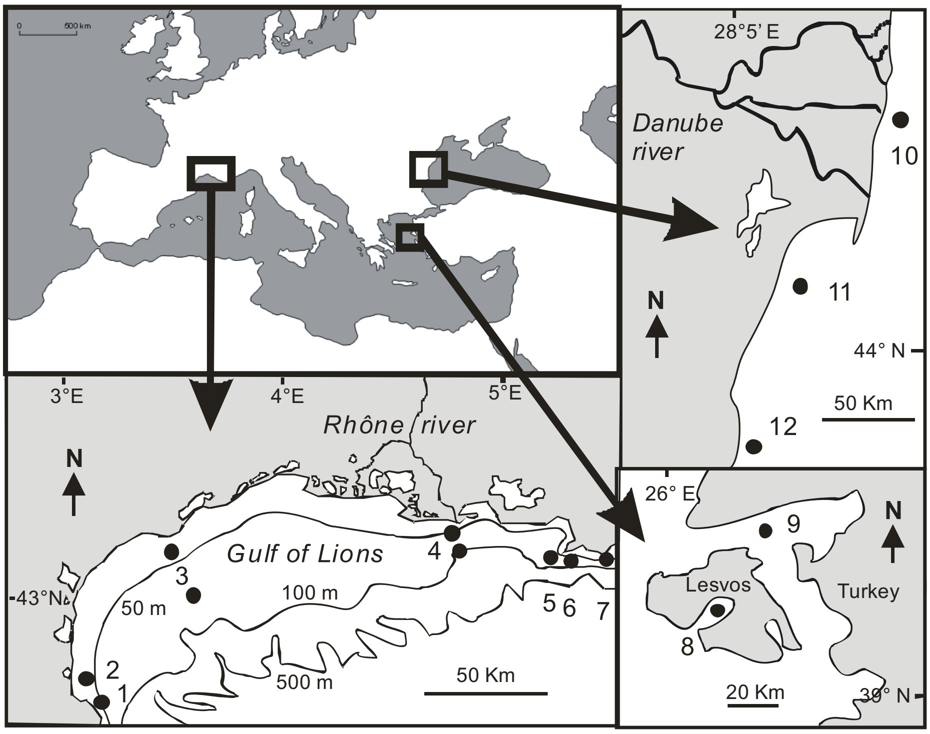


Fig. 1. Location of the three areas studied within the Mediterranean basin, and of the different sites sampled within each of the three areas (black dots), i.e. the NW Mediterranean, Aegean Sea and Black Sea.

Table 1. Number of individuals of the three size classes caught for each species and in various sites (see codes of locations, Fig. 1).

Species and site	Code	Depth (m)	Date	Size class (TL)			Total
				Small (<12 cm)	Medium (12–17 cm)	Large (> 17 cm)	
Mullus barbatus barbatus							
Banyuls-sur-mer	1	30–40	Jan. 2007	3	14	5	22
Port-Vendres	2	30–40	Oct. 2004	15	15	15	45
Agde	3	30–50	Oct. 2004	15	10	15	40
		80–100	Oct. 2004	–	15	15	30
Rhône river mouth	4	30–50	Oct. 2004	15	15	15	45
		80–100	May 2005	–	11	3	14
Marseille	5	60–80	Oct. 2004	12	14	15	41
Cassis	6	60–80	Oct. 2004	–	15	9	24
NE Lesvos	8	60–80	Sept. 2006	–	23	15	38
Gulf of Kalloni	9	10–20	Sept. 2006	–	5	22	27
Total				60	137	129	326
Mullus barbatus ponticus							
Sulina	10	10–20	May 2005	42	4	–	46
Midia	11	20–30	Oct. 2005	14	1	–	15
Costinesti	12	10–20	May 2005	44	10	–	54
Total				100	15	–	115
Mullus surmuletus							
Port-Vendres	2	30–40	Oct. 2004	15	15	16	46
Agde	3	30–50	Oct. 2004	–	15	14	29
Rhône river mouth	4	30–50	Oct. 2004	15	19	2	36
Cassis	6	60–80	Oct. 2004	–	–	32	32
La Ciotat	7	20–30	June 2004	–	15	15	30
NE Lesvos	8	60–80	Sept. 2006	–	18	21	39
Gulf of Kalloni	9	10–20	Sept. 2006	7	14	–	21
Total				37	96	100	233

Sites along the Romanian coasts are also subject to strong variations in environmental conditions due to the Danube river inputs. The mean yearly discharge is about 6 400 $m^3 s^{-1}$ (Winkels et al. 1998) and is partly responsible for the low salinity, from 2 to 18, of Romanian coastal waters which could, in addition, display strong variations in sea-surface temperatures ranging from 0 °C in winter to 30 °C in summer. Sites were mainly characterized by a sandy/muddy bottom (Banaru and Harmelin-Vivien 2007).

Sites in the Aegean Sea are subject to a relatively weak range of environmental variations, although the Gulf of Kalloni is impacted by human activities such as the presence of several small towns without any sewage treatment and seasonal (December and January) organic matter waste from olive oil factories (Mérigot et al. 2006). Salinity within the gulf usually remains very close to that of the open sea, i.e. ~37.5 in this region. The site in the NE of Lesvos Island is located in a channel separating the island from the coasts of Turkey and is composed of sandy and muddy bottoms partly covered by a *Posidonia oceanica* bed.

In total, 674 individuals were caught by bottom trawl and/ or gillnet depending of sites, all species and sites pooled together. Individuals were analysed according to three size-classes, small ($TL \leq 12$ cm), medium ($12 < TL \leq 17$ cm), and large fish ($17 \text{ cm} < TL$) (Table 1). The limits of these size-classes result from a diet analysis study that evidenced the main shifts in diet (Bautista-Vega et al. 2008).

2.3 Otolith extraction and shape analysis

The right and left *sagittae* of 674 individuals were extracted (total of 1 348 otoliths) for analysis, cleaned with distilled water, and stored dry in tubes. Each fish was measured to the nearest mm (*TL*). Each otolith, systematically placed with the *sulcus acusticus* oriented towards the observer, was examined under a stereomicroscope (Leica MZ16) fitted with a digital camera (Mediacybernetic evolution LS color) linked to a computer. Digital images were then acquired with the software Visilog 6.1. An episcopic light through optical fibres allowed optimization of direction and intensity of light to obtain the most highly contrasted image.

The shape of each otolith was assessed with the elliptic Fourier analysis (Stransky and MacLellan 2005). This technique describes the outline on the basis of several components named harmonics. Each harmonic is characterized by 4 coefficients, resulting from the projection of each point of the outline on axes (x) and (y). The higher the number of harmonics, the greater the accuracy of the outline description (Kuhl and Giardina 1982). For each numerical image, the software Shape 1.2 (Iwata and Ukai 2002) calculated the Fourier coefficients in order to make them invariants to the otolith size and its orientation (and position) regarding the beginning of the outline, which is arbitrarily defined. In addition, the Fourier power (FP) spectrum was calculated in order to determine the sufficient and necessary number of harmonics for the best reconstruction of the otolith outline (Crampton 1995). The Fourier power of a harmonic is proportional to its amplitude and provides a measure of the amount of “shape information” described by this harmonic. For the *n*th harmonic, Fourier power (FP_n) is given by the expression:

$$FP_n = (A^2{}_n + B^2{}_n + C^2{}_n + D^2{}_n) / 2$$

where A_n, B_n, C_n and D_n are the Fourier coefficients of the *n*th harmonic.

Then, we can calculate the cumulated power percentage (FP_c) defined by:

$$FP_c = \Sigma_1{}^n FP_n$$

For this purpose, two sub-samples of 40 otoliths each (the first for right otoliths, the second for left otoliths) were randomly chosen for the three species, and the threshold of 99.99% of the mean cumulated Fourier power was chosen to define the suitable number of harmonics to be considered in the analyses (Crampton 1995). As the first 20 harmonics reached 99.99% of the cumulated power for both right and left otoliths, the Fourier analysis indicated that the otolith shape of the studied species could be summarized by these 20 harmonics, i.e. 80 Fourier coefficients. However, the coefficients derived from the 1st harmonic were not taken into account, because the outline reconstructed with these coefficients is a simple ellipse resulting in maximum Fourier power and then mask the information derived from the other harmonics (Crampton 1995). So, 19 harmonics and thus 76 Fourier coefficients were used for the data analyses.

2.4 Data analysis

The analytical design was built in order to determine differences between species, areas (and sites) and size-classes through canonical discriminant analysis (hereafter CDA) (Fig. 2). The CDA was performed with the 76 Fourier coefficients. The objective of the CDA is to investigate the integrity of pre-defined groups, i.e. individuals belonging to a given group such as species, geographical areas, regional sites or size-classes, through finding linear combinations of descriptors that maximize the Wilks lambda (λ) (Ramsay and Silveman 2005). The Wilks λ allows assessment of the performance of the discriminant analysis. This statistic is the ratio between the intra-group variance and the total variance, and provides a means of calculating the chance-corrected percentage of agreement between true and predicted group membership. The values of λ range from 0 to 1, and the closer the λ is to 0, the better is the discriminating power of the CDA.

Several CDA were performed (Fig. 2), first to search the best subset of data in order to increase the quality of the discrimination process. CDA were performed with data from the right otoliths, with data from the left otoliths, and with data from both otoliths pooled. The first step was to determine of the relative positions of the three species. The second step was to discriminate local populations of each species at large (i.e. NW

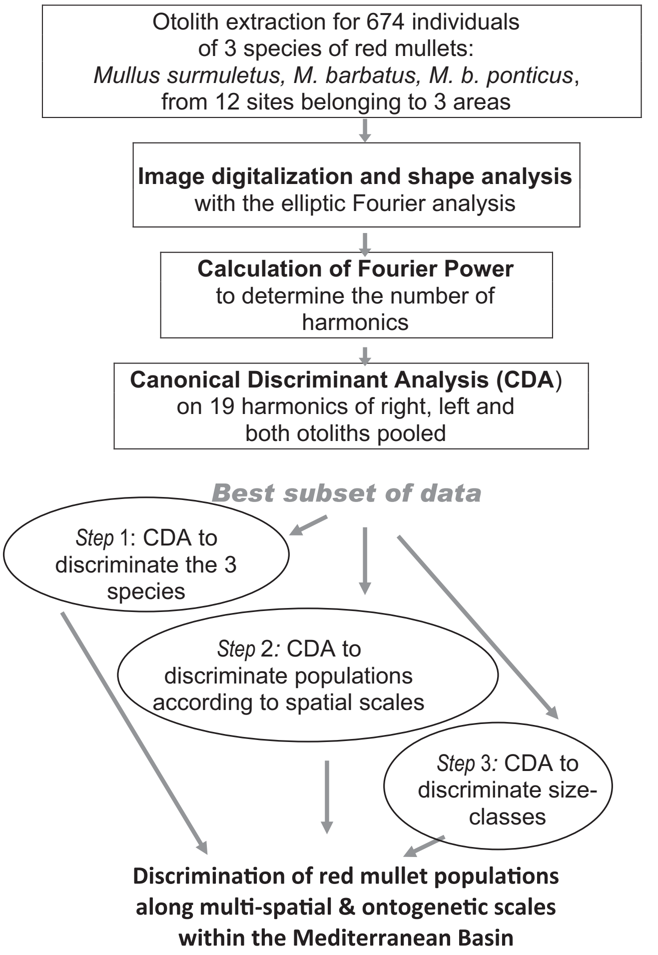


Fig. 2. Analytical framework of otolith shape analyses run on the three species of red mullets from the Mediterranean basin.

Mediterranean Sea, Aegean Sea and Black Sea) and regional (i.e. various sites within a given area) scales. Finally, three size classes were also distinguished to look at the possible ontogenetic influence on discrimination of local populations.

Euclidian distances (chosen as they allow graphic representation of the observations according to the first discriminant scores from a discriminant analysis) between the barycentre of different groups were calculated to quantify the dissimilarity between groups, and the reclassifying rate of individuals within the pre-defined groups was determined with a Cohen's kappa test, that expresses the average proportion of individuals with index agreement, after removing the effect of chance agreement (Titus et al. 1984).

3 Results

3.1 Quality of the discrimination

The Fourier coefficients for right and left otoliths, and also for both otoliths pooled, were used to show the relative positions of the three species. The CDA performed on these data allowed to clearly separate the three species (Fig. 3). However, a decrease in the value of the Wilks λ from 0.282 (right otolith) or from 0.252 (left otolith) to 0.152 (both otoliths pooled) on the first function was noted. A similar decrease in the Wilks λ was also shown for function 2 (Fig. 3). This decrease of the Wilks λ when using both otoliths is accompanied by an increase in the Euclidian distance between groups (Table 2) and by a concomitant increase in the well reclassified individuals. These different types of results indicate that an individual is better characterized by its two otoliths rather than by only one of them. This was also clearly found for all the CDAs we performed in this work, thus justifying why all the following results presented herein are based on analyses done with both otoliths pooled together.

Table 2. Euclidian distances between barycentres of groups resulting from CDA performed on the right otolith, the left otolith or both otoliths.

Species	Type of CDA on the otoliths	*Mullus b. barbatus*	*Mullus b. ponticus*
Mullus barbatus ponticus	Right	2.14	
	Left	2.49	
	Both	3.12	
Mullus surmuletus	Right	2.64	2.41
	Left	2.55	2.70
	Both	3.24	3.48

Individuals of *Mullus barbatus barbatus* were well classified with a percentage of 91.5%, and 86.6% and 87.7% for *M. b. ponticus* and *M. surmuletus*, respectively. The general reclassification assessed with the Cohen's kappa test was 82.6 ± 4%. Function 1 of the CDA separates *Mullus surmuletus* from the two other species, whereas the function 2 distinguishes *M. barbatus barbatus* from *M. b. ponticus* (Fig. 3). In addition, Euclidian distances indicated that *M. surmuletus* was more distant from the other species, than the latter were between themselves (Table 2).

3.2 Discrimination over large spatial scale

For *Mullus barbatus* (both subspecies pooled together), the CDA clearly discriminated the three main areas, i.e. the NW Mediterranean, the Aegean Sea and the Black Sea (Fig. 4), with a reclassification of 89.0%, 93.3% and 90.7%, respectively, allowing the assessment of the general reclassification of 82.4 ± 5.3% through the Cohen's kappa test. Individuals from the Aegean Sea and the Black Sea were more distant from each other ($d = 4.70$) than from those of the NW Mediterranean ($d = 3.24$ and 3.22, respectively). A very similar pattern was obtained when considering all sites (CDA thus not shown herein but available from the first author), with sites from the Black Sea and the Aegean Sea markedly separated from the NW Mediterranean sites (Table 3). Some sites from

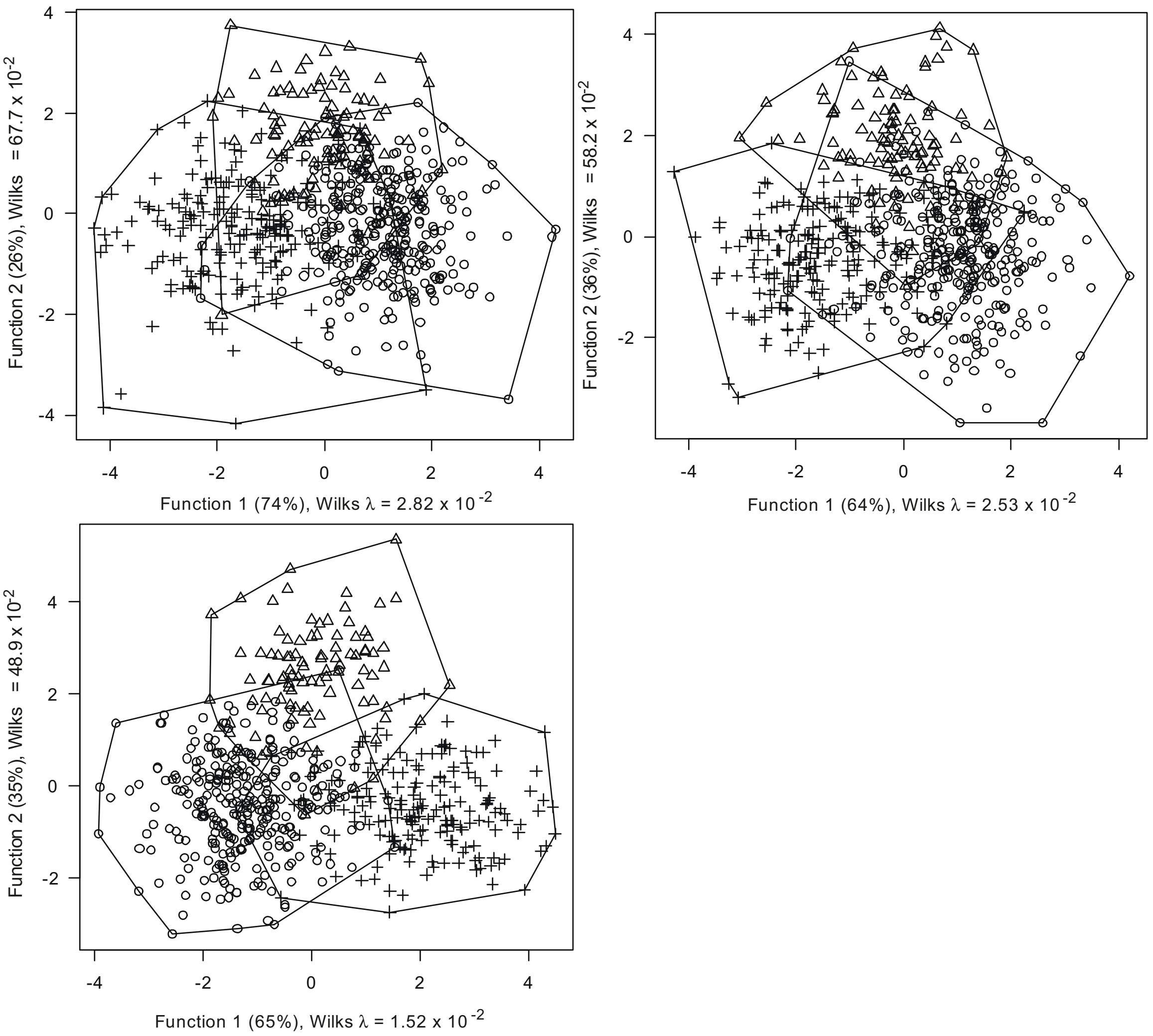


Fig. 3. First plan resulting from the CDA between the three mullid species run with right otoliths (top left), left otoliths (top right) and with both otoliths (below). O *Mullus barbatus barbatus*, + *M. surmuletus* and Δ *M. barbatus ponticus*.

Table 3. Euclidian distances between barycentres of groups resulting from the CDA performed with both otoliths for *Mullus barbatus* (both sub-species pooled).

Site	Banyuls	Port-Vendres	Agde 30–50 m	Agde 80–100 m	Rhône 30–50 m	Rhône 80–100 m	Marseille	Cassis	Gulf of Kalloni	NE Lesvos	Sulina	Midia
Port–Vendres	1.20											
Agde 30–50 m	0.86	0.39										
Agde 80–100 m	2.45	3.63	3.25									
Rhône 30–50 m	1.76	0.72	1.09	4.20								
Rhône 80–100 m	1.13	2.31	1.94	1.32	2.88							
Marseille	1.64	2.84	2.48	0.86	3.37	0.57						
Cassis	2.51	3.52	3.12	1.15	4.19	1.55	1.50					
Gulf of Kalloni	3.82	3.37	3.62	5.78	2.74	4.69	4.95	6.24				
NE Lesvos	1.48	2.37	2.17	2.36	2.58	1.55	1.58	3.04	3.45			
Sulina	2.27	2.29	2.06	3.38	2.97	2.52	3.02	2.56	5.66	3.70		
Midia	4.59	3.81	3.89	6.27	4.08	5.26	5.80	5.47	6.61	6.04	2.91	
Costinesti	3.17	3.23	3.00	3.84	3.89	3.23	3.66	2.83	6.60	4.54	0.94	2.86

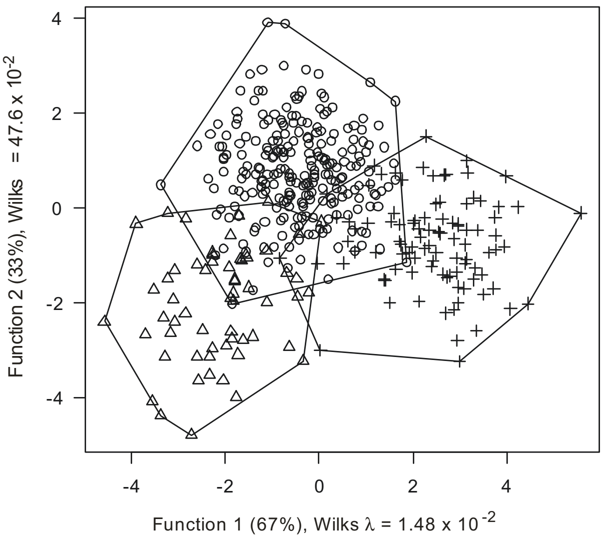


Fig. 4. First plan resulting from the CDA between the 3 geographical areas, run with both otoliths (left and right) for *Mullus barbatus*. O NW Mediterranean Sea, + Black Sea and Δ Aegean Sea.

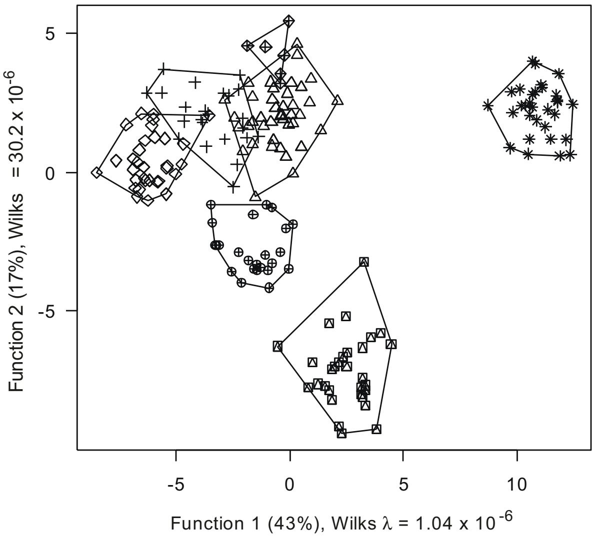


Fig. 5. First plan resulting from the CDA between the different sites of the north-western Mediterranean Sea and the Aegean Sea for *Mullus surmuletus*. ∗ Cassis, ▨ La Ciotat, + Agde (30 m), Δ Port Vendres, ◇ Rhône (30 m), ⊕ NE Lesvos and ⟐ Gulf of Kalloni.

the same area were strongly separated, such as the two sites from the Aegean Sea ($d = 3.45$), or the site of Midia *vs.* the two other sites from the Black Sea ($d > 2.86$) for instance. The reclassification percentage of individuals among sites varied between 77.8 and 100% (Table 4), with an average reclassification of 89.6 ± 3.2%.

For *Mullus surmuletus*, the large scale approach concerned the NW Mediterranean and the Aegean Sea as this species is absent from the Black Sea. A CDA performed only with the two main areas did not show any clear pattern, but another CDA performed with all sites from these two areas separated the groups, mainly discriminating the sites of Cassis and La Ciotat (both in the NW Mediterranean), whereas the two sites from the Aegean Sea were not strongly distinct from the other sites of the NW Mediterranean (Fig. 5). Euclidian distances have also shown than fish from the sites of Cassis and La Ciotat were very distinct from each other ($d = 12.66$; Table 5) but also from each of the other sites ($d > 6.00$). Although fish from the two sites of the Aegean Sea were well separated from the other sites ($d > 2.20$), they did not constitute a clear 'Aegean' group as they were markedly separated ($d = 7.08$). For all sites, the reclassification of individuals was 100%.

Table 4. Percentages of correctly classified individuals within each sampling site for *Mullus barbatus,* both subspecies pooled together.

NW Mediterranean		Aegean Sea		Black Sea	
%		%		%	
Banyuls	77.8	Gulf of Kalloni	100.0	Sulina	90.0
Port-Vendres	81.8	NE Lesvos	94.6	Midia	90.9
Agde 30–50 m	88.2			Costinesti	93.3
Agde 80–100 m	90.0				
Rhône 30–50 m	92.9				
Rhône 80–100 m	100.0				
Marseille	87.2				
Cassis	95.8				

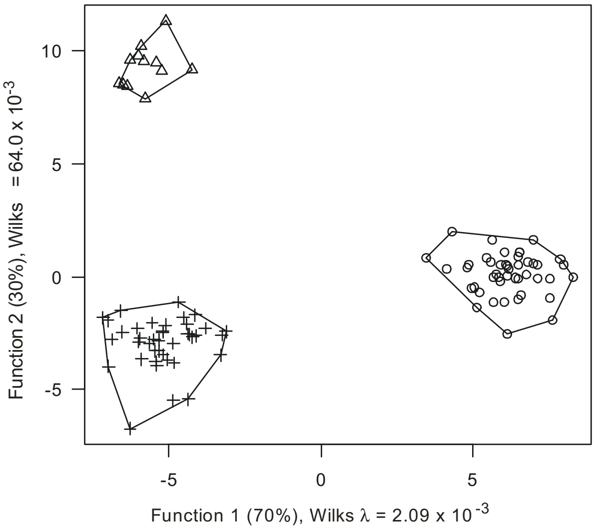


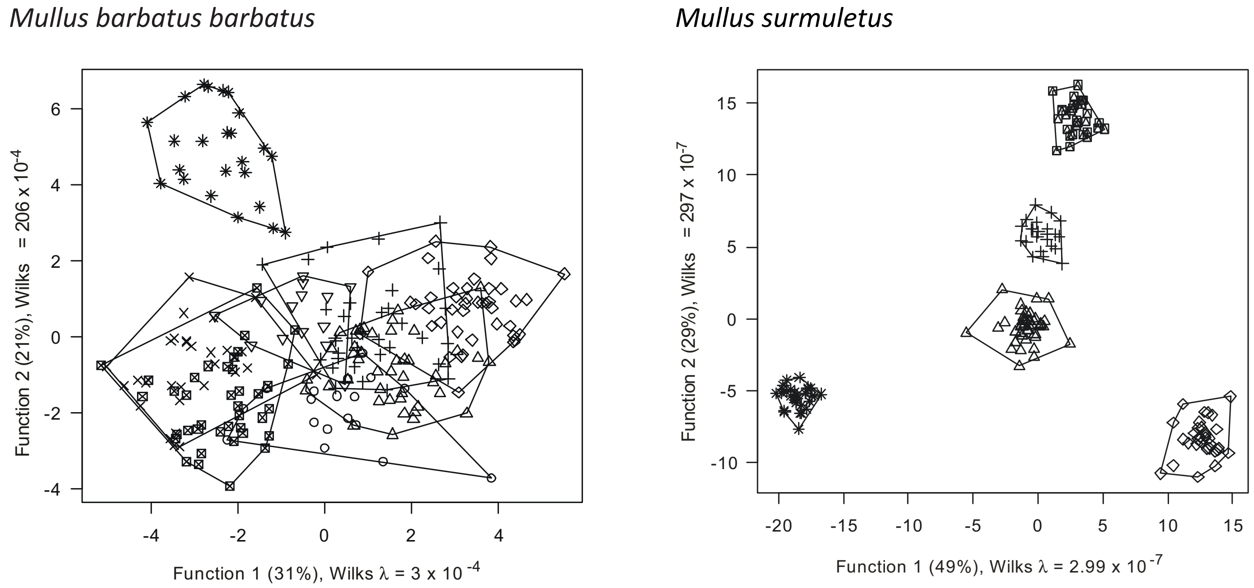
Fig. 6. First plan resulting from the CDA between the 3 sites in the Black Sea for *Mullus barbatus ponticus*, i.e. Sulina in the north (+), Midia in the center (Δ) and Costinesti in the south (O).

3.3 Discrimination over regional scales

For the Black Sea, the CDA clearly separated individuals of *Mullus barbatus ponticus* of the three sites (Fig. 6). The first function of the CDA separated individuals from Costinesti, at

Table 5. Euclidian distances between barycentres of groups resulting from the CDA performed with both otoliths for *Mullus surmuletus*.

Site	Port-Vendres	Agde 30–50 m	Rhône 30–50 m	Cassis	La Ciotat	Gulf of Kalloni
Agde 30–50 m	2.98					
Rhône 30–50 m	5.98	3.20				
Cassis	11.45	14.43	17.29			
La Ciotat	9.68	10.92	11.54	12.66		
Gulf of Kalloni	2.20	3.58	6.77	11.80	11.83	
NE Lesvos	4.96	5.12	5.60	13.54	6.00	6.00



(a) (b)

Fig. 7. First plan resulting from the CDA between the different sites of the NW Mediterranean Sea; (a) *Mullus barbatus barbatus*, ∗ Cassis, + Agde (30 m), Δ Port Vendres, ◇ Rhône (30 m), O Banyuls, ⊠ Marseille, × Agde (100 m) and ▽ Rhône (100 m); (b) *Mullus surmuletus*, ∗ La Ciotat, + Rhône (30 m), Δ Agde (30 m), O Port Vendres and × Cassis.

Table 6. Euclidian distances between barycentres of groups resulting from the CDA performed with both otoliths for *Mullus barbatus barbatus.*

Site	Banyuls	Port-Vendres	Agde 30–50 m	Agde 80–100 m	Rhône 30–50 m	Rhône 80–100 m	Marseille
Port–Vendres	1.68						
Agde 30–50 m	2.35	1.22					
Agde 80–100 m	3.53	4.61	4.24				
Rhône 30–50 m	4.04	2.36	2.30	6.54			
Rhône 80–100 m	2.65	2.57	1.67	2.80	3.91		
Marseille	2.69	4.04	3.98	1.13	6.20	2.89	
Cassis	7.32	6.93	5.73	5.72	7.01	4.67	6.60

Table 7. Euclidian distances between barycentres of groups resulting from the CDA performed with both otoliths for *Mullus surmuletus* in the NW Mediterranean.

Site	Port-Vendres	Agde 30–50 m	Rhône 30–50 m	Cassis
Agde 30–50 m	6.46			
Rhône 30–50 m	15.70	18.82		
Cassis	18.33	21.96	31.43	
La Ciotat	14.99	8.56	24.31	28.98

Table 8. Euclidian distances between barycentres of groups resulting from the CDA performed with both otoliths for *Mullus surmuletus* according to size-classes in the NW Mediterranean; (–) Unsufficient number of individuals for distance calculations.

Site	Size-class	Port-Vendres	Agde 30–50 m	Rhône 30–50 m	Cassis
Agde 30–50 m	Medium	8.12			
	Large	14.86			
Rhône 30–50 m	Medium	23.46	15.38		
	Large	–	–		
Cassis	Medium	–	–	–	
	Large	10.42	16.76	–	
La Ciotat	Medium	17.34	15.88	21.55	
	Large	4.07	11.94	–	12.91

the southern Romanian coast, from those from Sulina, off the Danube mouth ($d = 11.78$) and from Midia, close to the city of Constanta ($d = 15.07$), whereas function 2 separated individuals from Sulina from those from Midia ($d = 12.26$). The reclassification of individuals was 100% for all sites.

In the NW Mediterranean, the mean reclassification of individuals of *M. b. barbatus* was of 96.7 ± 2.5%, ranging from 93.2% at Port-Vendres to 100% at Cassis, Rhône river mouth (100 m) and Banyuls. The first function of the CDA indicated a distribution according to depth, with deep sites (Marseille, Cassis, Rhône mouth at 80–100 m and Agde at 80–100 m) discriminated from shallower sites (Port-Vendres, Banyuls, Agde at 30–50 m and Rhône mouth at 30–50 m) (Fig. 7a). The function 2 displayed a clear distinction of individuals from Cassis which constituted a group strongly different from the others ($d > 4.67$; Table 6). For *M. surmuletus*, the reclassification of individuals into the predefined groups was 100% for each site, accompanied by high values of Euclidian distances (Table 7). The CDA has shown a clear distinction of groups, with the function 1 separating individuals from Cassis from those Rhône mouth at 30 m, whereas function 2 separated the three other sites (Fig. 7b).

3.4 Discrimination between size classes

This approach only concerned the NW Mediterranean because we did not have a sufficient number of individuals for each of the three size classes from the two other areas. For *Mullus barbatus barbatus*, considering individuals from a given size class resulted in a reclassification of 100% for each site and in an increase in the quality of the discrimination, as shown by the decrease in the Wilks' λ values (Fig. S1). For each of the three size classes, individuals from Marseille and from Rhône mouth at 30 m were the most distinct groups (Fig. S1 and Table S1). Although the other sites were well separated for each size class, no clear common spatial pattern appeared.

For *Mullus surmuletus*, a clear discrimination between sites according to fish size (medium and large sizes) was also shown (Fig. S2), with a reclassification of individuals of 100% for each site. However, the patterns found for medium and for large individuals were not consistent, without any common spatial pattern. The CDA performed on medium-sized fish indicated a clear distinction of individuals from Rhône mouth at 30 m from those of La Ciotat (Fig. S2a), whereas the CDA performed on large sized fish indicated a clear distinction of individuals from Cassis from those from Agde at 30 m (Fig. S2b, Table 8).

4 Discussion

4.1 Which otolith should be used: the right, the left or both?

The results for right and for left otoliths showed clear and interpretable patterns in both cases. However, our results clearly evidenced that taking into account both otoliths was the best option as (i) it increased the discrimination between groups, (ii) generating a decrease in the value of the Wilks λ on the two first canonical functions, (iii) an increase in the Euclidian distance between groups and (iv) a concomitant increase in the well-classified individuals. We therefore strongly recommend that both otoliths pooled together be used for further research on otolith shape using Fourier series and canonical discriminant analyses, rather than only one otolith at has been systematically the case to date. It can be thus hypothesized that unclear results in a given case based on the right (or the left) otoliths will be easier to interpret with shape analyses based on both otoliths. More importantly, our results mainly imply that information contained by the right and the left otoliths for a given individual are not identical. By contrast, this information must be considered as complementary in order to better take into account all shape characteristics at the fish and population levels. This reinforces the finding of Morat et al. (unpublished data) who have highlighted that the mean otolith outlines for left and right otoliths of *M. b. barbatus* were different. This also implies that the preconceived idea previously evoked for using only one otolith for shape analyses for practical/pragmatic reasons does not have any serious mathematical justification and needs at least to be tested for each species and for each case studied, even if similarity in the processes involved in the growth of the right and left otoliths remains highly plausible (Lychakov and Rebane 2005). Several studies have already evoked the asymmetry of otoliths for flatfish for their mass (Toole et al. 1993; Mérigot et al. 2007) or their chemical composition and isotopic signature

(Loher et al. 2008). For such species, it thus appears that both otoliths have clearly and obviously to be taken into account for shape analyses. Even if it may not be immediately obvious in an *a priori* approach, our results have evidenced that fluctuating asymmetry also should concern roundfish.

4.2 Spatial patterns

A strong specificity of mullids' local populations was noted according to areas, with a high value of % of reclassification at a large spatial scale (NW Mediterranean, Aegean Sea and Black Sea). However, these three groups do not reflect a geographical gradient in their distribution. For *M. barbatus* (two subspecies pooled), the NW Mediterranean group has a central position between the two other areas, meaning that otolith shape cannot be used as a mean to evidence a potential spatial gradient at this scale. These results however allowed discrimination of the local populations, suggesting a marked role of environmental factors and thus corroborate the hypothesis of ecological and morphological differences in otolith shape depending on the type of environment used by fish. Several authors have shown that environmental factors *sensu lato* are the most influential determinants of otolith shape, which changes in response to differences in growth rate (Smith 1992; Campana and Casselman 1993; Friedland and Reddin 1994; Hüssy 2008). The otolith shape also depends on the behaviour of the species (Cardinale et al. 2004) and may be associated with differences in food and spatial-temporal niches (Aguirre and Lombarte 1999; Mérigot et al. 2007) as well as with differences in abiotic factors such as temperature and/or salinity (Gonzales-Salas and Lenfant 2007) or short-term variations in diet characteristics (Gagliano and McCormick 2004).

Variations in otolith shape of fish from different geographic areas are at least partly expressed during the life history, thereby representing a phenotypic measure of stock identification. It is however unclear how to use this information in a fisheries management perspective yet. At the current stage of our knowledge on *Mullus* spp. in the NW Mediterranean, Aegean Sea and Black Sea, it remains difficult to consider otolith shape as a useful tool to contribute to the debate regarding the definition of spatial units for fisheries purposes. Indeed, the spatial scales at which fisheries work differ from those evidenced here. Gaertner et al. (1997) suggested that the local populations of *Mullus barbatus* in the Gulf of Lions (westwards from the Rhône river mouth) likely belong to the same stock, but our results highlight that several local and close populations can be distinguished within this stock. In addition, the case of *M. surmuletus* is interesting because the two most distinct groups were those of Cassis and La Ciotat, two sites which are very close and present very similar oceanic conditions but differing in depth. The home range and mobility of mullids are not well known but these species are usually considered as mobile fishes that are not particularly bound to a given area irrespective of the depth range used. If so, otoliths from fish of close sites should have been similar or at least weakly distinct but our results suggest the opposite, i.e. mullids being much less mobile than previously thought and/or remaining in a more or less restricted zone at least for several weeks or even months. It should be checked by further research but this hypothesis might imply that fishery management for mullids should be conducted at a small spatial scale within a given geographical area. It also highlights that otolith shape cannot be used as a mean to evidence a potential spatial gradient at a small spatial scale, as evoked above for a larger spatial scale.

An alternative/complementary hypothesis can be proposed to explain the difference found at such a small spatial scale. If mullids are highly mobile species potentially using different sites during their life-span, the marked differences in shape characteristics can be explained only by short-term responses of these descriptors to variations in environmental conditions. Gagliano and McCormick (2004) evidenced short-term responses in otolith shape characteristics linked to variations in diet characteristics and it thus remains possible to suggest that processes other than feeding might be implied in a short-term response of otolith shape. Such a short-term response could imply efficient and rapid chemical and/or physiological processes acting on otolith shape. The variations found in otolith outlines near the *sulcus acusticus* and at the dorsal face in *M. barbatus* (Morat et al., unpublished data) suggest that the metabolic activity of macula cells around these two areas could be the key process determining a short-term response in otolith shape variation. The case of the two close Aegean sites is similar but it is not clear whether the reasons for the difference found are related to the previous hypotheses. The two sites are clearly subjected to different environmental and oceanographic conditions, i.e. the NE of Lesbos is a usual marine area for this region whereas the Gulf of Kalloni is a semi-closed area exposed to eutrophication and seasonal discharge of waste from olive-oil factories (Mérigot et al. 2006).

Strong differences also appeared between sites at similar depths (Cassis and NE Lesvos for *Mullus surmuletus* or Black Sea sites for *M. b. ponticus* for instance) and between sites at different depths (Cassis and Gulf of Kalloni for *M. surmuletus*). Oceanographic characteristics are obviously different between areas and thus can be evoked to explain the differences found between local populations at this large spatial scale (Volpedo and Echeverria 2003). The Romanian coasts are subject to very strong runoffs from the Danube river, some sites from the NW Mediterranean are affected by high runoff from the Rhône river, whereas Aegean sites are not affected by river runoff. The substrate characteristics can be considered as broadly homogeneous within a given area, such as the NW Mediterranean. So other factors should have an impact on such differences at the regional scale. In the NW Mediterranean, the diet of the European hake *Merluccius merluccius* markedly changes at a spatial scale similar to those studied here, with a dominance of euphausids as the main prey in the eastern part of the Gulf of Lions, and of mysids off the Rhone river mouth (Ferraton et al. 2007). Similar results were found for *M. b. barbatus* and *M. surmuletus*, both having different prey in the eastern and western parts of the Gulf of Lions (Bautista-Vega et al. 2008). The diet of these mullids also changes eastwards from the Rhône river mouth and according to depth (Bautista-Vega et al. 2008) and thus can also explain the difference in otolith shape. Fluctuations of the diet in terms of quantity and frequency (Gagliano and McCormick 2004) or linked to depth or site (Tuset et al. 2003; Mérigot et al. 2007; Bautista-Vega et al. 2008) can be proposed to explain changes in otolith shape.

4.3 Ontogenetic influences

The otolith shapes of the three species of mullids highlighted marked differences linked to fish size. Tuset et al. (2003) using shape indices found similar results for three species of the genus *Serranus* and explained this pattern by the depth range of the species. They also highlighted that the most visible change in otolith shape occurred for a size corresponding to the onset of sexual maturity. This is the size at which the metabolism changes markedly, sexual maturity influences fish growth and consequently may have an impact on otolith morphology. For the mullids, this size-range is not known in details but could be assumed as roughly ranging between 10 and 15 cm (TL) (Quéro and Vayne 1997). Significant discriminations were found between sites for the three size-classes for *Mullus barbatus barbatus* and between medium and large individuals for *M. surmuletus*. The most marked changes were found between small/medium and large fish, except at the Rhône mouth at 30 m where the most distinct groups were the small and the medium sized-fish. However and in all cases, large individuals for both species had reached the adult stage, and our results thus corroborate those of Tuset et al. (2003) for *Serranus* spp. and those of Mérigot et al. (2007) for *Solea solea*. Several other authors have provided evidence of the role of fish size and thus fish age to explain variability in otolith outline (Campana and Casselman 1993; Begg et al. 2001; Cardinale et al. 2004). Our results not only confirm but also reinforce this view.

The diet of *Mullus* spp. displays marked changes according to individual size (Bautista-Vega et al. 2008). In the NW Mediterranean, small fish mainly consume small crustaceans whereas large fish mainly eat polychaetes and gastropods (Bautista-Vega et al. 2008). The diet also changes when fish live in areas close to river runoffs, such as at Rhône mouth at 30 m and/or Sulina, Black Sea, probably reflecting changes in invertebrate community structure and composition in these sites (Banaru and Harmelin-Vivien 2007; Bautista-Vega et al. 2008). The role of diet has already been evoked above as a possible determinant of otolith shape, and the ontogenetic shift in diet as a complementary explanation to the patterns found can be thus mentioned (Campana and Casselman 1993; Cardinale et al. 2004).

Interestingly, the differences between sites were not all consistent according to fish size. For instance, small individuals of *M. b. barbatus* were clearly separated between Cassis and Agde 30 m, but large individuals from both sites were very close. A similar result was found for medium and large fish of *M. surmuletus* between Port-Vendres and La Ciotat. This kind of result suggests either that an ontogenetic shift in diet between fish size-classes can be more pronounced at some sites than at other ones depending on local characteristics, but ending up with similar diet for large fish (Bautista-Vega et al. 2008), or other constraints might influence the processes involved in otolith shape formation. As they remain as yet largely poorly understood despite a few works (Borelli et al. 2003; Payan et al. 2004), studies on the processes responsible for otolith shape formation would require further research mostly oriented towards chemistry and physiology at the endolymph and macula levels.

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Supporting information

Table S1. Euclidian distances between barycentres of groups resulting from the CDA performed with both otoliths for *Mullus barbatus barbatus* according to size-classes in the NW Mediterranean. See Figure 1 for locations of sites; (–) Insufficient number of individuals for the calculation of distance.

Fig. S1. First plan resulting from the CDA between various sites of the NW Mediterranean Sea for *Mullus barbatus barbatus* according to fish size: small (top left), medium (top right) and large individuals (below). ◊ Rhône (30 m), Δ Port-Vendres, + Agde (30 m), ⊠ Marseille, × Agde (100 m), o Banyuls, ∗ Cassis and ▽ Rhône (100 m).

Fig. S2. First plan resulting from the CDA between various sites of the NW Mediterranean Sea for *Mullus surmuletus* according to fish size: medium (left) and large individuals (right). Δ Port-Vendres, + Agde (30 m), ◊ Rhône (30 m), ▽ La Ciotat and ∗ Cassis.

Supporting information is only available in electronic form at www.alr-journal.org.

References

Aguirre H., Lombarte A., 1999, Ecomorphological comparison of sagittae of *Mullus barbatus* and *M. surmulletus*. J. Fish Biol. 55, 105–114.

Aldebert Y., 1997, Demersal resources of the Gulf of Lions (NW Mediterranean). Impact of exploitation on fish diversity. Vie Milieu 47, 275–284.

Banaru D., Harmelin-Vivien M.L., 2007, Variations spatio-temporelles de la signature en isotopes stables (δ^{13}C et δ^{15}N) des eaux du Danube et des communautés marines sur les côtes roumaines de la mer Noire. Cybium 31, 167–174.

Bautista-Vega A.A., Letourneur Y., Harmelin-Vivien M.L., Salen-Picard C., 2008, Difference in diet and size-related trophic level in two sympatric fish species, the red mullets *Mullus barbatus* and *M. surmuletus*, in the Gulf of Lions (NW Mediterranean). J. Fish Biol. 73, 2402–2420.

Begg G.A., Overholtz W.J., Munroe N.J., 2001, The use of internal otolith morphometrics for identification of haddock (*Melanogrammus aeglefinus*) stocks on George Bank. Fish. Bull. 99, 1–14.

Bolles K.L., Begg G.A., 2000, Distinction between silver hake (*Merluccius bilineariz*) stocks in U.S. waters of the northwest Atlantic using whole otolith morphometric. Fish. Bull. 98, 451–462.

Borelli G., Guibbolini M.E., Mayer-Gostan N., Priouzeau F., De Pontual H., Allemand D., Puverel S., Tambutte E., Payan P., 2003, Daily variations of endolymph composition: relationship with the otolith calcification process in trout. J. Exp. Biol. 206, 2685–2692.

Campana S.E., Casselman J.M., 1993, Stock discrimination using otolith shape analysis. Can. J. Fish. Aquat. Sci. 50, 1062–1082.

Campana S.E., Neilson J.D., 1985, Mircrostructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42, 1014–1031.

Campillo A., 1992, Les pêcheries françaises de Méditerranée : synthèse des connaissances. IFREMER, RIDRV-92/019- RH Sète.

Cardinale M., Doering-Arjes P., Kastowsky M., Mosegaard H., 2004, Effects of sex, stock, and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths. Can. J. Fish. Aquat. Sci. 61, 158–167.

Crampton J.S., 1995, Elliptic Fourier shape analysis of fossil bivalves, practical considerations. Lethaia 28, 179–186.

Farrugio H., Olivier P., Biagi F., 1993, An overview of the history, knowledge, recent and future research trends in Mediterranean fisheries. Sci. Mar. 57, 105–119.

Ferraton F., Harmelin-Vivien M.L., Mellon-Duval C., Souplet A., 2007, Does spatio-temporal variation in diet affect condition and abundance of European hake (*Merluccius merluccius*) juveniles in the Gulf of Lions (NW Mediterranean)? Mar. Ecol. Prog. Ser. 337, 197–208.

Fisher W., Bauchot M., Scheinder M., 1987, Fiches FAO d'identification des espèces pour les besoins de la pêche (Révision 1). Méditerranée et mer Noire, zone de pêche 37, Vol. 2, CEE, FAO publ., Rome, 761–1530.

Friedland K.D., Reddin D.G., 1994, Use of otolith morphology in stock discrimination of Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 51, 91–98.

Gagliano M., McCormick M.I., 2004, Feeding history influences otolith shape in tropical fish. Mar. Ecol. Prog. Ser. 278, 291–296.

Gaertner J.C., Bertrand J.A., Gil de Sola L., Durbec J.P., Ferrandis E., Souplet A., 2005, Large spatial scale variation of demersal fish assemblage on the continental shelf of the NW Mediterranean Sea. Mar. Ecol. Prog. Ser. 297, 245–257.

Gaertner J.C., Chessel D., Bertrand J.A., 1997, Stability of spatial structures of demersal assemblages: a multitable approach. Aquat. Living Resour. 11, 75–85.

Gauldie R.W., Crampton J.S., 2002, An eco-morphological explanation of individual variability in the shape of the fish otolith: comparison of the otolith of *Hoplostethus atlanticus* with other species by depth. J. Fish Biol. 60, 1204–1221.

Gonzalez-Salas C., Lenfant P., 2007, Interannual variability and intraannual stability of the otolith shape in European anchovy *Engraulis encrasicolus* (L.) in the Bay of Biscay. J. Fish Biol. 70, 35–49.

Graf W., Baker R., 1983, Adaptive changes of the vestibulo-ocular reflex in flatfish are achieved by reorganization of central nervous pathways. Science 221, 777–779.

Høie H., Otterlei E., Folkvord A., 2004, Temperature-dependent fractionation of stable oxygen isotopes in otoliths of juvenile cod (*Gadus morhua* L.). ICES J. Mar. Sci. 61, 243–251.

Hüssy K., 2008, Otolith shape in juvenile cod (*Gadus morhua*): Ontogenetic and environmental effects. J. Exp. Mar. Biol. Ecol. 364, 35–41.

Iwata H., Ukai Y., 2002, Shape: a computer program package for quantitative evaluation of biological shapes based on Elliptic Fourier descriptors. J. Hered. 93, 384–385.

Kuhl F.P., Giardina C.R., 1982, Elliptic Fourier features of a closed contour. Comput. Graph. Image Process 18, 236–258.

Lilliendahl K., Solmundsson J., 2006, Feeding ecology of sympatric European shags *Phalacrocorax aristotelis* and great cormorants *P. carbo* in Iceland. Mar. Biol. 149, 979–990.

Loher T., Wischniowski S., Martin G.B., 2008, Elemental chemistry of left and right sagittal otoliths in a marine fish *Hippoglossus stenolepis* displaying cranial asymmetry. J. Fish Biol. 73, 870–887.

Lychakov D.V., Rebane Y.T., 2000, Otolith regularities. Hear. Res. 143, 83–102.

Lychakov D.V., Rebane Y.T., 2005, Fish otolith mass asymmetry: morphometry and influence on acoustic functionality. Hear. Res. 201, 55–69.

Lychakov D.V., Rebane Y.T., Lombarte A., Demestres M., Fuiman L.A., 2008, Saccular otolith mass asymmetry in adult flatfishes. J. Fish Biol. 72, 2579–2594.

Mérigot B., Batjakas I.E., Letourneur Y., 2006, Fish community structure of two Greek close gulfs (Lesvos Island, Aegean Sea). Cybium 30, 79–81.

Mérigot B., Letourneur Y., Lecomte-Finiger R., 2007, Characterization of local populations of the common sole *Solea solea* (Pisces, Soleidae) in the NW Mediterranean through otolith morphometrics and shape analysis. Mar. Biol. 151, 997–1008.

Millot C., 1999, Circulation in the Western Mediterranean Sea. J. Mar. Syst. 20, 423–442.

Palmer M., Linde M., Morales-Nin B., 2010, Disentangling fluctuating asymmetry from otolith shape. Mar. Ecol. Prog. Ser. 399, 261–272.

Parmentier E., Vandewalle P., Lagardère F., 2001, Morpho-anatomy of the otic region in carapid fishes: ecomorphological study of their otoliths. J. Fish Biol .58, 1046–1068.

Payan P., De Pontual H., Edeyer A., Borelli G., Boeuf G., Mayer-Gostan N., 2004, Effects of stress on plasma homeostasis, endolymph chemistry, and check formation during otolith growth in rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 61, 1247–1255.

Popper A.N., Combs S., 1980, Auditory mechanisms in teleost fishes. Am. Sci. 68, 429–440.

Popper A.N., Ramcharitar J., Campana S.E., 2005, Why otoliths? Insights from inner ear physiology and fisheries biology. Mar. Freshw. Res. 56, 497–504.

Pont D., Simonnet J.P., Walter A.V., 2002, Medium-term Changes in Suspended Sediment Delivery to the Ocean: Consequences of Catchment Heterogeneity and River Management (Rhône River, France). Estuar. Coast. Shelf Sci. 54, 1–18.

Quéro J.C., Vayne J.J., 1997, Les poissons de mer des pêches françaises. Delachaux & Niestlé, Paris.

Ramsay J.O., Silverman B.W., 2005, Functional Data Analysis, Springer, New-York.

Salen-Picard C., Darnaude A., Arlhac D., Harmelin-Vivien M.L., 2002, Fluctuations of macrobenthic populations: a link between climate-driven river run-off and sole fishery yields in the Gulf of Lions. Oecologia 133, 380–388.

Sempéré R., Charrière B., Van Wambeke F., Cauwet G., 2000, Carbon inputs of the Rhône river to the Mediterranean Sea: biogeochemical implications. Glob. Biogeochem. Cycle 14, 669–681.

Smith M.K., 1992, Regional differences in otolith morphology of the deep slope red snapper *Etelius carbunculus*. Can. J. Fish. Aquat. Sci. 49, 795–804.

Stransky C., MacLellan S.E., 2005, Species separation and zoogeography of redfish and rockfish (genus *Sebastes*) by otolith shape analysis. Can. J. Fish. Aquat. Sci. 62, 2265–2276.

Titus K., Mosher J.A., Williams B.K., 1984, Chance-corrected classification for use in discriminant analysis : ecological applications. Am. Midl. Nat. 111, 1–7.

Toole C.L., Markle D.F., Harris P.M., 1993, Relationship between otolith microstructure, microchemistry, and early life history events in Dover sole, *Microstomus pacificus*. Fish. Bull. 91, 732–753.

Tuset V.M., Lozano I.J., Gonzalez J.A., Pertusa J.F., Garcia-Diaz M.M., 2003, Shape indices to identify regional differences in otolith morphology of comber, *Serranus cabrilla* (L., 1758). J. Appl. Ichthyol. 19, 88–93.

van Dongen S., Lens L., 2000, Symmetry, size and stress. Trends Ecol. Evol. 15, 330–331

Volpedo A., Echeverria D.D., 2003, Ecomorphological patterns of the sagitta in fish on the continental shelf off Argentine. Fish. Res. 60, 551–560.

Winkels H.J., Kroonenberg S.B., Lychagin M.Y., Marin G., Rusakov G.V., Kasimov N.S., 1998, Geochronology of priority pollutants in sedimentation zones of the Volga and Danube delta in comparison with the Rhine delta. Appl. Geochem. 13, 581–591.