

Testing otolith morphology for measuring marine fish biodiversity

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Abstract. To check the suitability of otoliths for measuring biodiversity, the contour and shape of the sulcus acusticus of sagittal otoliths were described using geometric morphological analysis. Thirteen and fourteen points were used to define these structures respectively. Three current coastal fish assemblages of the north-western Mediterranean were selected for the present study. The results demonstrate that the relative warps generated in the geometric analysis explained both characteristics related to contour and the otolith sulcus. A comparative study with body fish shape using morphospaces and clusters revealed that otolith shape is a better variable for explaining the ecological structure of a fish assemblage. Moreover, three morphological indices (morphological richness (MR), morphological disparity and the morphogeometric index) were estimated from relative warps of otoliths and were compared with ecological, taxonomic, functional and morphological (from body shape) indices. MR increased with functional diversity and average taxonomic distinctness, reflecting the ecological and taxonomic character of otolith morphology. These findings suggest that otoliths could be a useful tool for studying the diversity of present and past fish assemblages.

Additional keywords: geometric morphology, otolith shape.

Received 7 February 2015, accepted 7 November 2015, published online 1 March 2016

Introduction

Natural changes or human impacts on ecosystems have often been quantified using indices of ecological diversity (e.g. Ungaro *et al.* 1998; Colloca *et al.* 2003; D'Onghia *et al.* 2003). Techniques for measuring diversity have varied over time, and include taxonomic, phylogenetic, morphological and functional indices (Clarke and Warwick 2001; McClain *et al.* 2004; Petchey and Gaston 2006; Vellend *et al.* 2011; Farré *et al.* 2013). Morpho-functional diversity is recognised as a key element representing the roles that organisms play within ecosystems because interspecific competition among ecologically equivalent species facilitates different life strategies (Karr and James 1975; Motta *et al.* 1995; Weissburg 2005; Villéger *et al.* 2010). In recent and fossil fish, most studies have focussed primarily on morpho-functional traits related to feeding and locomotor apparatus, because these features are considered to be indicators of resource partitioning and thus linked to species coexistence (e.g. Gatz 1979; Winemiller 1991; Wainwright *et al.* 2002). Nevertheless, body shape is also used because it is a multi-functional and single factor related to aspects of behavioural

ecology, such as the mode of feeding, predator evasion or courtship displays (Lavin and McPhail 1985; Loy *et al.* 2000; Walker 2010). In addition, body shape may also be influenced by anthropogenic activities, such as aquaculture or fishing pressure (Alós *et al.* 2014; Abaad *et al.* 2016).

The fossil record of modern bony fish is based on isolated teeth and otoliths, as well as on articulated skeletons, although otoliths are the best-preserved structures in marine fish. Otolith analysis has made important contributions to the understanding of fish evolution and phylogeny (e.g. Gaemers 1983; Nolf 1985, 2013; Reichenbacher *et al.* 2007). In addition, otoliths and fish evolution and phylogeny may also be valuable for interpretation of historical fisheries (Van Neer *et al.* 2002; Limburg *et al.* 2008). Because of their scientific relevance, collections and atlases of otoliths have been created worldwide (e.g. Nolf 1985; Smale *et al.* 1995; Volpedo and Echeverría 2000; Campana 2004; Tuset *et al.* 2008; Lin and Chang 2012). At the same time, the development of digital techniques has offered new possibilities for classification based on image handling and analysis (e.g. Gauldie and Crampton 2002; Stransky *et al.* 2008;

Parisi-Baradad *et al.* 2010; Tuset *et al.* 2012). The use of otoliths for geometric morphometric analysis was initially questioned because otoliths have limited points with biologically homologous characteristics or landmarks (Rohlf and Marcus 1993). However, more recent studies have demonstrated the suitability of this approach (Monteiro *et al.* 2005; Ponton 2006; Lombarte *et al.* 2010; Vignon and Morat 2010).

From a functional point of view, otoliths are associated with hearing and the sense of balance (Popper and Coombs 1982; Ramcharitar *et al.* 2006; Schulz-Mirbach *et al.* 2014). Numerous studies have demonstrated that otolith morphology, including the sulcus acusticus, is also linked to swimming (Volpedo and Echeverría 2003, Volpedo *et al.* 2008), feeding (Lombarte *et al.* 2010), spatial distribution (Gauldie and Crampton 2002; Lombarte and Cruz 2007; Sadighzadeh *et al.* 2014) and acoustic communication (Popper and Lu 2000; Cruz and Lombarte 2004). Moreover, otoliths have been used in taxonomic (e.g. L'Abée-Lund and Jensen 1993; Ponton 2006; Tuset *et al.* 2006) and phylogenetic studies in many groups of Teleostean fish (e.g. Gaemers 1983; Nolf 1985; Monteiro *et al.* 2005). Therefore, it is reasonable to suppose that the high morphological variability of otoliths and their specificity may be also used to measure biodiversity.

The main objectives of the present study were to: (1) develop a geometric morphometric method for combined analysis of otolith outline and sulcus acusticus features; (2) evaluate whether the spatial distribution of otolith species in a graphical illustration, a biplot called 'morphospace', has morpho-functional meaning; (3) analyse the spatial distribution of species in the biplot and morphological indices generated from otolith and body fish shapes, because body shape can be used to estimate the diversity of fish assemblages (Farré *et al.* 2013); and (4) test the potential of the otolith method for estimating the diversity of known fish assemblages.

Materials and methods

Data compilation

Three fish assemblages comprised of a total of 61 species with a variety of life histories and similar depth ranges were selected from the western Mediterranean Sea (see Farré *et al.* 2013). Two assemblages (A, sandy-rocky 20 m with 25 species; B, sandy 20 m with 27 species) were characterised by similar species richness and dissimilarities in ecological and taxonomical indices. The third assemblage selected (C, artificial reef-sandy 15–19 m with 48 species) was higher in species richness and functional diversity than the other two assemblages (see Farré *et al.* 2013).

Otolith and body shape

In all, 466 sagittal otolith images from the 61 species (see Table S1, available as Supplementary material to this paper) were obtained from the ICM Barcelona (Consejo Superior de Investigaciones Científicas, CSIC) collection, which is integrated in the AFORO (Análisi de Formes d'Otòtils, or 'Shape Analysis of Fish Otoliths') database (Lombarte *et al.* 2006; see <http://www.cmima.csic.es/aforo/>, accessed 1 January 2015). The anatomical terminology used in the present study is based on that of Tuset *et al.* (2008).

In the present study only subadult and adult specimens were analysed, avoiding the effect of ontogenetic changes in otolith shape, only samples from Mediterranean and Atlantic waters were included in the analysis because not all species used in the present study have otoliths in the AFORO database collected from other areas and we assumed that the intraspecific variability in otolith shapes using our methodology (see below) is not significant in relation to interspecific variability. This assumption is based on two reasons: (1) the contour is defined from few points, providing an otolith pattern in lesser detail in relation to other mathematical procedures; and (2) the shape of the sulcus acusticus is consistent within species (Torres *et al.* 2000; Tuset *et al.* 2008). Therefore, the otolith shape obtained for each species is a consensus representation obtained using a morphological geometric method, which can be applied for any general research (such as the present study), but not for specific studies, such as stock identification. Finally, images of body shape in the present study were only of Mediterranean fish because most of the otoliths came from this area.

The shape of the otolith outline was described using eight reference landmarks (homologous points) established in terms of straight lines according to Reichenbacher *et al.* (2007). Five semi-landmarks (non-homologous points) equidistant from particular consecutive landmarks were also added to improve the representation of otolith shape (see Fig. 1a). To characterise the sulcus acusticus contour, 14 landmarks and three semi-landmarks were selected according to the literature (Monteiro *et al.* 2005; Lombarte *et al.* 2010; see Fig. 1a). The number of points for defining the otolith and sulcus acusticus was similar to avoid unbalanced influence in the estimation and meaning of relative warps (see below). Several examples with all points considered are illustrated in Fig. 1b. Twenty-seven landmarks and semi-landmarks (Fig. 2) with anatomical, ecological and taxonomic meaning were used for the morphological analysis of the body shape of fish (for more detail, see Farré *et al.* 2013).

Geometric morphometric analysis

The morphological diversity of the otoliths and fish shapes was quantified separately using geometric morphometrics (Bookstein 1991). The digitised coordinates of the landmarks and semi-landmarks were obtained using tpsDig software (ver.2.16; Rohlf 2003a). Then, Cartesian grid coordinates for the landmarks and semi-landmarks were analysed using relative warp analysis with tpsRelw ver.1.49 (Rohlf 2003b). Essentially, relative warp (RW) analysis is a principal components analysis of the covariance matrix of the aligned specimens (e.g. rotated, translated and scaled landmark coordinates). Thus, the RW represents a set of specific morphological characteristics allowing the analysis of particular morphological attributes (Rohlf and Marcus 1993; Kassam *et al.* 2002; Zelditch *et al.* 2003). The geometric morphological analysis was performed independently for each species, obtaining a consensus (average) configuration from all otolith samples in the database. Then, the protocol was repeated for each fish assemblage considering all species and using the consensus figure for each species. The body shape analysis followed the protocols explained in Farré *et al.* (2013).

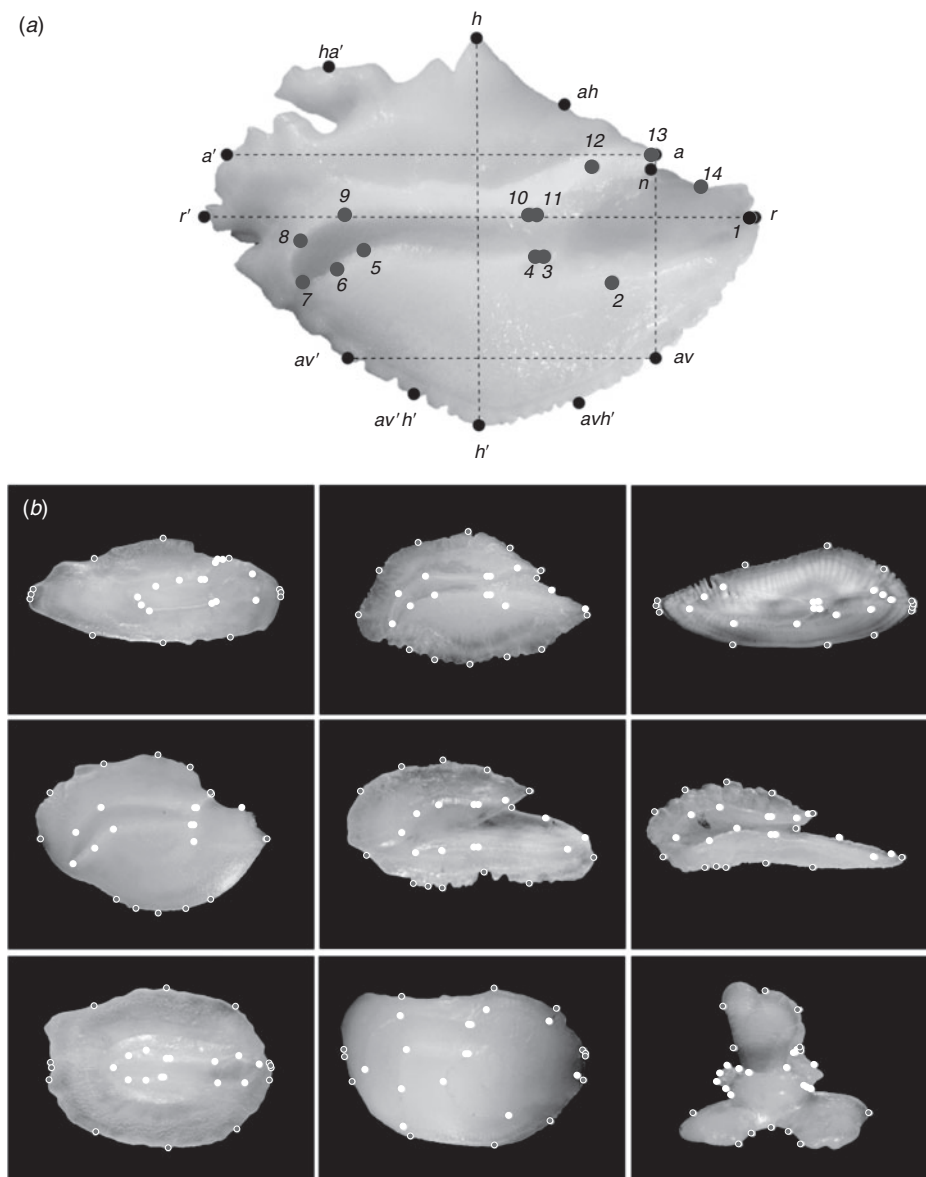


Fig. 1. (a) Medial side of the left otolith showing the landmarks and semi-landmarks used in the present study to define the otolith contour (black circles, labelled by character) and the features of the sulcus acusticus (grey circles, labelled by number). For contour: r is the most prominent point of the rostrum, r' is the posterior projection of r , a defines the point of the antirostrum and a' is the posterior projection of a (if the antirostrum were absent and the ostium typically had an ostial opening, the dorsal ending of the crista dorsal was considered a ; if the ostium was not opening, a was located together with r), av is the ventral projection of a , av' defines the posterior projection of av , $h-h'$ describes the maximum height perpendicular to lines $r-r'$ between the dorsal margin (h) and the ventral margin (h'), n defines the notch and $av'h'$, $av'h$, ha' and ah are the semi-landmarks. For the sulcus acusticus, 1 and 13 indicate the intersection between inferior and superior crista of the ostium and the excisura ostii, 2 and 12 show the place where the inferior and superior crista of the ostium change the curvature, 3–4 and 10–11 provide the position, size and symmetry of the constriction between the ostium and cauda, 5 and 9 indicate the place where the inferior and superior crista of the cauda change the curvature, 7 is the most distal point of the cauda and 6, 8 and 14 are semi-landmarks. (b) Several examples are illustrated for all points of otolith contour (dark grey circles) and sulcus acusticus (white circles) considered in (a) as follows (from left to right starting with the upper-left image): *Conger conger*, *Pagrus auriga*, *Merluccius merluccius*, *Pomadasys incisus*, *Sardina pilchardus*, *Seriola dumerili*, *Synapturichthys kleinii*, *Umbrina canariensis* and *Zeus faber*.

Morphological variations were illustrated with a ‘morphospace’, where the species distribution and occupied space indicate the structural complexity of the assemblage (Wainwright *et al.* 2002; Clabaut *et al.* 2007). The species were represented by applying a non-metric multidimensional scaling (nMDS; Tuset *et al.* 2014) from the first three RWs (representing >75% of total variability) to capture the most useful information regarding shape variation (Recasens *et al.* 2006). Locations of species clusters within morphospaces were compared graphically using contour lines from a bivariate Gaussian kernel density estimator (Werdelin and Lewis 2013). Finally, a Mantel test was used to analyse the correlation between the Euclidean morphological distances for the body characteristics and otolith characteristics. Specifically, the *P*-value obtained from the analysis served to estimate the probability of obtaining a correlation equal to or greater than the calculated value, based on 5000 random matrix permutations (Clabaut *et al.* 2007). These analyses were performed in PAST (Palaeontological Statistics, ver. 1.81; Hammer *et al.* 2001).

Diversity indices

For the otolith analysis, the first eight RW scores were selected to describe the morphological variability of shape for each species because they explained more than 90% of the total morphological variability (see Results). Three morphological indices were estimated for measuring the diversity from otoliths and body shapes (Farré *et al.* 2013): morphological disparity (MD), morphological richness (MR) and the morphogeometric

or ecomorphological index (EMI). Values of these indices were calculated as follows:

$$MD = \frac{\sum_j RW_j^2}{N - 1}$$

where *RW_j* is the RW of species *j* and *N* is the total number of species;

$$MR = \sum_j CC$$

where *CC* is the cluster coefficient, computed from the Euclidean distance matrix using the unweighted pair group method with arithmetic mean (UPGMA) method, and *j* is the species; and

$$EMI = \frac{\sum_j CC}{(N - 1)}$$

For the comparative study, ecological (Shannon’s diversity index; *H*), taxonomic (average taxonomic distinctness; Δ⁺), morphological (discussed above) and functional (*FD*) diversity indices of these fish assemblages were taken from data published by Farré *et al.* (2013; see Table 1).

Results

Otolith morphological variability

The localisation of landmarks and semi-landmarks in the consensus figure for each species is given in Figs S1 and S2 (available as Supplementary material to this paper). The first eight warps explained 91.6% of interspecific variation and each warp contributed to the description of otolith outline (shape, dorsal–ventral curvature, type of posterior zone and the presence and type of rostrum) and features of the sulcus acusticus (mode opening, relative size of the ostium and type and curvature of the cauda; Fig. 3a). The first warp explained 45.9% of the variability, clearly identifying otoliths with a mesial opening on the left side of the plot. The second warp only explained 13.5% of the variability, discriminating between rounded otoliths with a concave cauda and wider ostium (positive axis) and enlarged otoliths with a convex cauda and narrower ostium (negative axis). The third warp explained a similar degree of morphological variation (11.4%), but the otoliths with a rounded shape showed a convex cauda and narrower ostium. In general, the variation explained by the remaining warps decreased

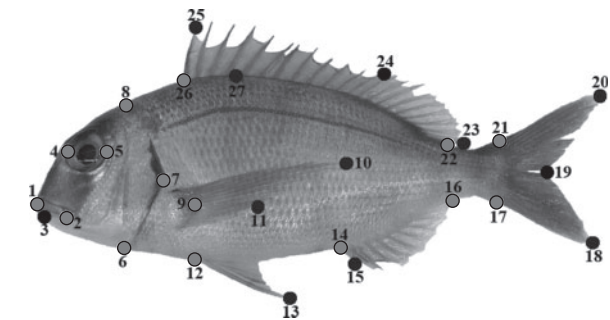


Fig. 2. Location of the selected landmarks (grey circles) and semi-landmarks (black circles) on the left side of standardised images of the fish body according to Farré *et al.* (2013). The numbers are as described in Fig. 1.

Table 1. Morphological, ecological, taxonomic and functional indices estimated for three coastal fish assemblages off the western Mediterranean Sea. All indices provided, with the exception of morphological indices for sagittal otoliths, were obtained from Farré *et al.* (2013). *EMI*, morphogeometric index; *FD*, functional diversity; *MD*, morphological disparity; *MR*, morphological richness; *H'*, Shannon’s diversity index; Δ+, average taxonomic distinctness

Assemblage	Otolith			Fish body			<i>H'</i>	Δ+	<i>FD</i>
	<i>MR</i>	<i>EMI</i>	<i>MD</i>	<i>MR</i>	<i>EMI</i>	<i>MD</i>			
A: Sandy–rocky	3.74	0.156	0.0458	4.15	0.173	0.037	2.09	85.39	64.4
B: Sandy	4.94	0.190	0.0758	6.59	0.182	0.043	3.02	85.26	68.9
C: Reef–sandy	7.31	0.156	0.0664	7.11	0.158	0.168	2.78	88.90	105.3

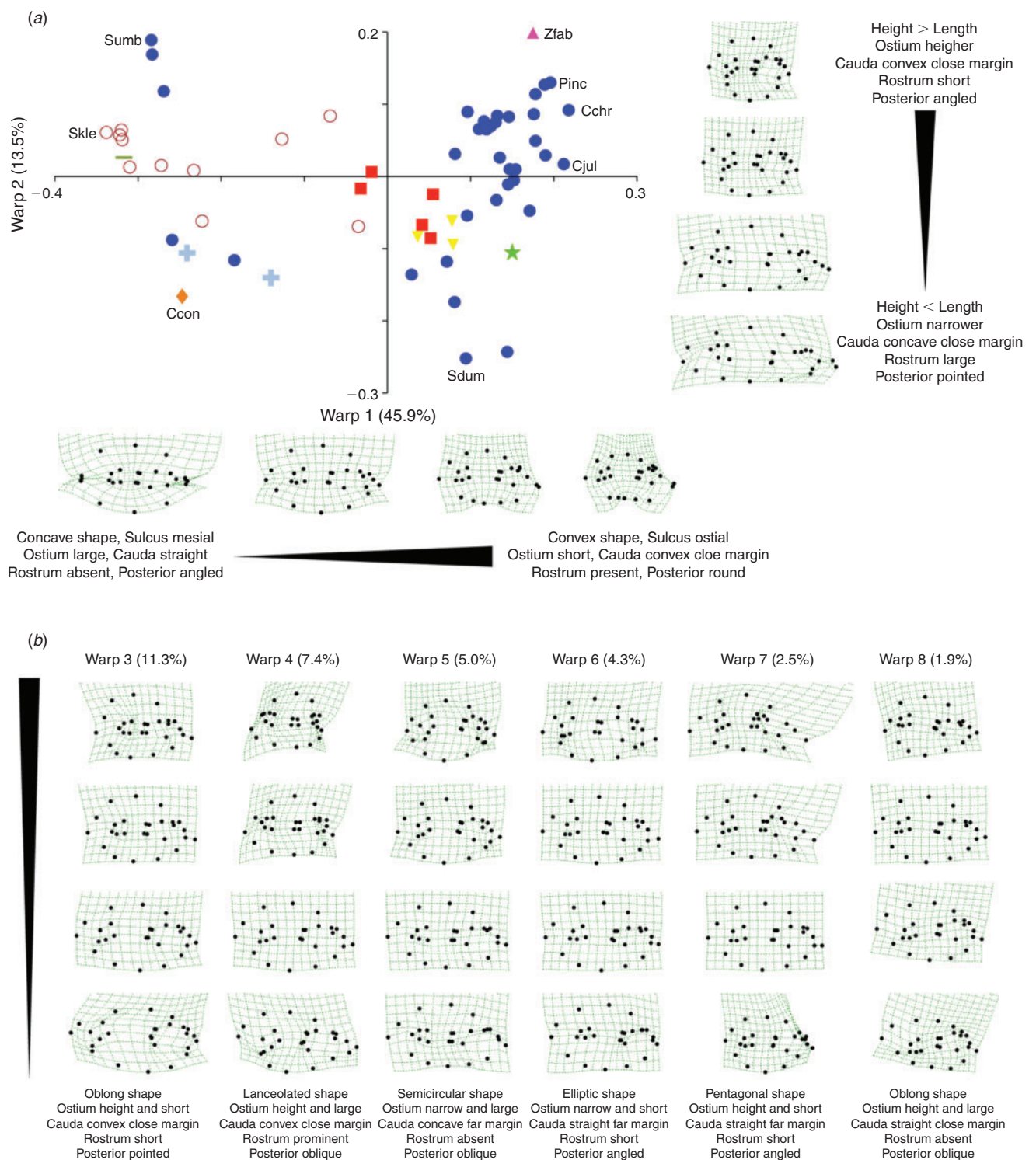


Fig. 3. (a) Morphospace and thin-plate spline images obtained from the first and second axes of warp analyses of the landmarks and semi-landmarks in otoliths. Symbols show fish Order. The species abbreviations are given for peripheral species. Cchr, *Chromis chromis*; Ccon, *Conger conger*; Cjul, *Coris julis*; Pinc, *Pomadasys inciscus*; Sdum, *Seriola dumerilii*; Skle, *Synapturichthys kleinii*; Sumb, *Sciaena umbra*; Zfab, *Zeus faber*. (b) Thin-plate spline images correspond to the variability of three to eight first warps through their axis.

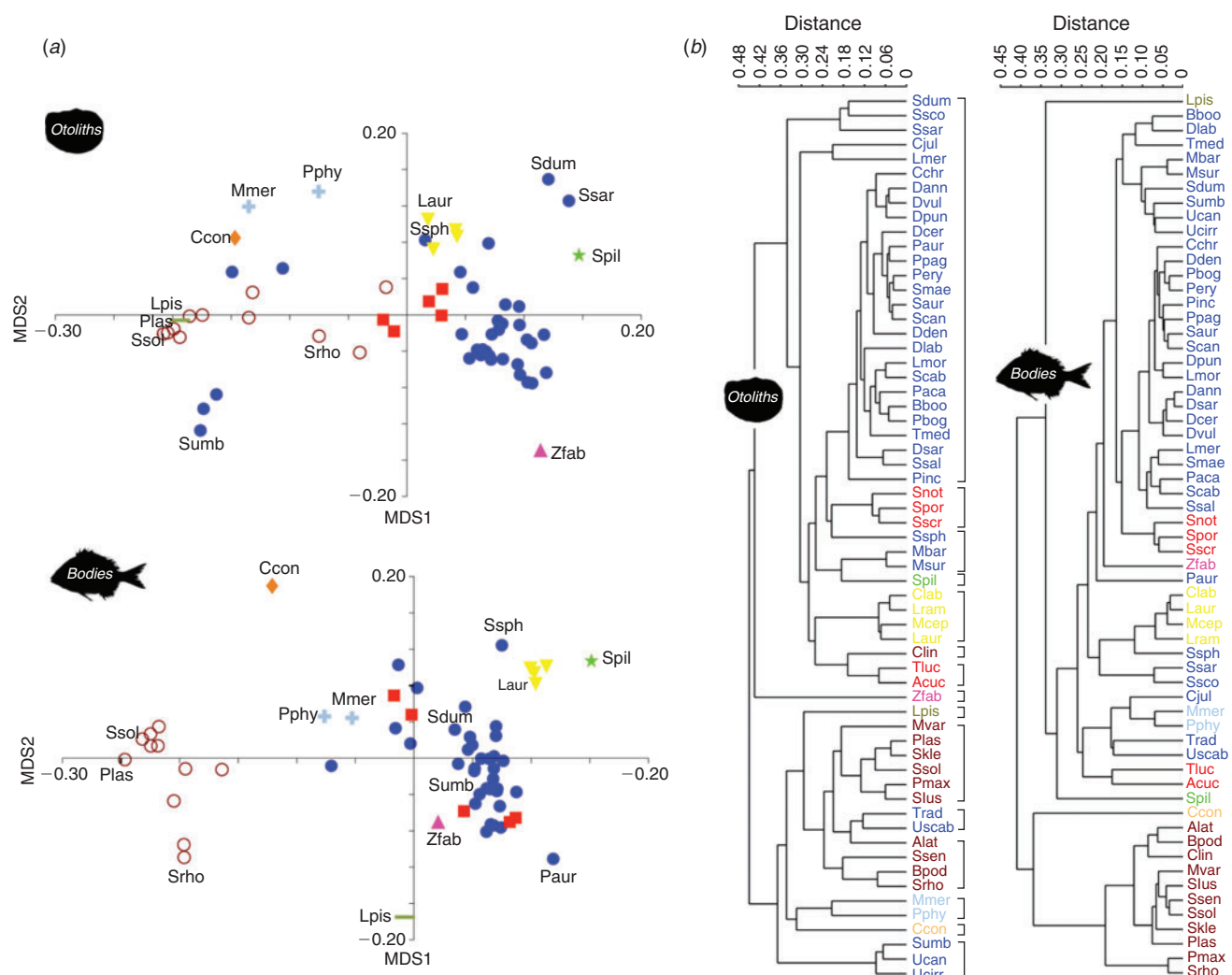


Fig. 4. (a) Morphospaces and (b) clusters obtained from the three first warps for otolith and body shape. Symbols show fish Order. Species abbreviations are given for peripheral species. Ccon, *Conger conger*; Laur, *Liza aurata*; Lpis, *Lophius piscatorius*; Mmer, *Merluccius merluccius*; Paur, *Pagrus auriga*; Plas, *Pegusa lascaris*; Pphy, *Phycis phycis*; Sdum, *Seriola dumerilii*; Spil, *Sardina pilchardus*; Ssol, *Solea solea*; Ssph, *Sphyræna sphyræna*; Srho, *Scophthalmus rhombus*; Sumb, *Sciaena umbra*; Zfab, *Zeus faber*. MDS1 and MDS2 are the axes obtained using non-metric multidimensional scaling.

gradually, verifying that each warp explained different otolith topologies (Fig. 3b). Therefore, the morphological meaning of the warps demonstrated that the points used to define the otolith morphology were appropriate.

Morphospace comparison

The body and otolith morphospaces were built using all 61 species studied (Fig. 4a). In the fish morphospace, the peripheral species presented characteristics associated with locomotion and prey capture strategies: bilateral asymmetry characterising the flatfish (*Pegusa lascaris*, *Scophthalmus rhombus* and *Solea solea*, Pleuronectiformes); flattened shapes with the first spine of the dorsal fin transformed into a luminescent sensory organ (*Lophius piscatorius*, Lophiiformes); elliptic-oval bodies, very common in most benthic fishes (*Pagrus auriga*, Perciformes); fusiform bodies specialised for rapid swimming (*Sardina*

pilchardus, Clupeiformes) or for slower movements (*Sphyræna sphyræna*, Perciformes); or eel-like bodies of fish species that use holes and caves to avoid predators and to forage (*Conger conger*, Anguilliformes). In contrast, in the otolith morphospace, only two of the previous species (*S. solea* and *S. pilchardus*) were located on the periphery. In addition, species producing sounds (*Sciaena umbra*, Perciformes), pelagic and oceanic swimmers (*Sarda sarda* and *Seriola dumerilii*, Perciformes), fish with rare otolith shapes and with particular swimming habits (*Zeus faber*, Zeiformes) or those reaching deeper waters (*Merluccius merluccius* and *Phycis phycis*, Gadiformes) together defined the limits of the convex hull. The MDS1-axis primarily separated species relative to the opening of the sulcus, whereas the MDS2-axis primarily separated species according to the otolith height : length ratio.

UPGMA cluster analysis, using Euclidean distance, for body shape clearly distinguished the flatfish according to bilateral

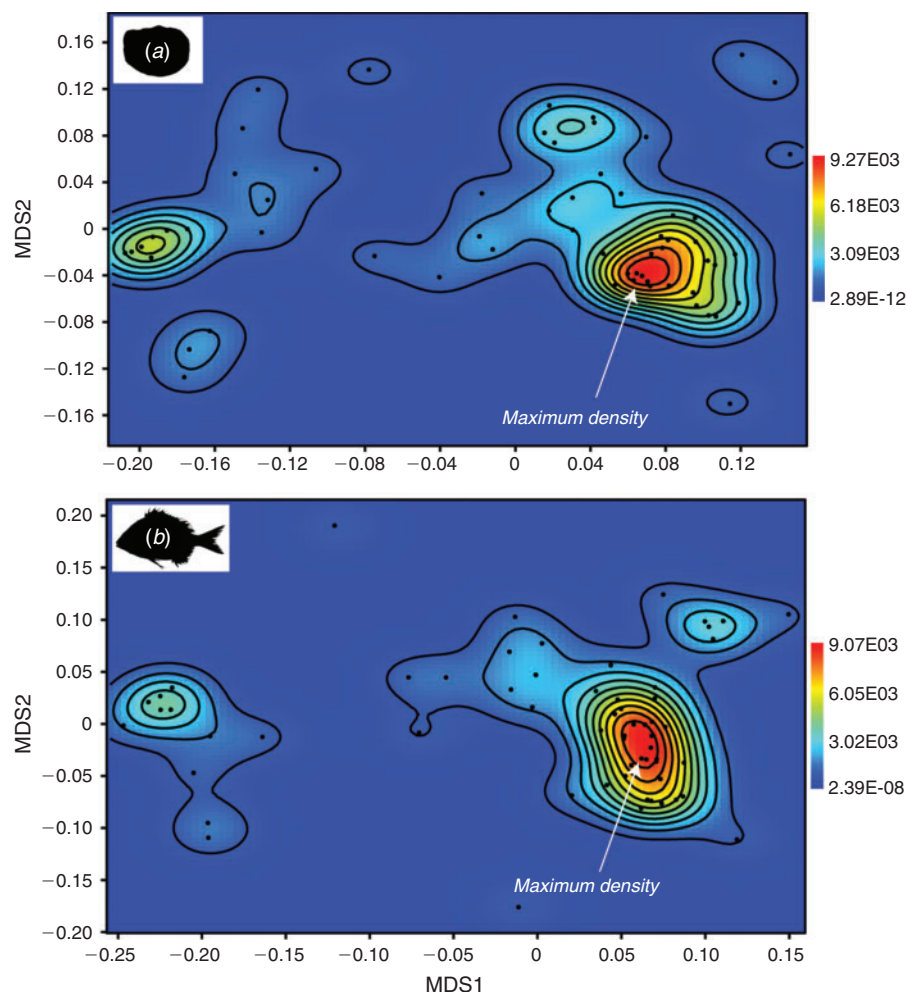


Fig. 5. Representation of the Kernel density of morphospaces obtained from the three first warps for (a) otolith and (b) body shape. Arrow indicates the spatial location of maximum density. MDS1 and MDS2 are the axes obtained using non-metric multidimensional scaling.

asymmetry (Fig. 4b). This morphological homogeneity was partially disrupted in the morphological analysis of the otoliths because of variability in the opening and type of the sulcus acusticus. Many Perciformes were grouped on an equal basis as a consequence of the analogy between body and otolith morphology, and only a small number of species (e.g. sciaenids) were grouped according to other criteria. Although the otolith morphospace was clearly more heterogeneous, the density graphs showed similar patterns: a large group of Perciformes and two small sets primarily composed of flatfish and mullets, with highly differentiated body and otolith morphologies in both cases (Fig. 5). Thus, the Mantel test ($r = 0.529$; $P < 0.0001$) revealed a significant correlation between the morphological distances for body shapes and those for otolith shapes, indicating similarity in the spatial variability of most of the species studied. This was due to a close correspondence between the body and otolith shapes of sparids (Sparidae, Perciformes), flatfish and mullets (Mugilidae, Perciformes), which were dominant in the fish assemblages studied.

Morphological diversity

The morphological indices were affected by the presence of extreme shapes and spatial heterogeneity of data. In the otolith morphospaces, the lobate shape of the sagittae of *Z. faber* (Fig. 6a) noticeably increased the morphological disparity ($MD = 0.0758$) in Assemblage B, whereas Assemblage A exhibited the lowest values ($MD = 0.0458$) because of a lack of unusual shapes (Table 1). Moreover, morphological richness was related to species richness; hence, the greatest value was obtained for Assemblage C ($MR = 7.31$; Table 1). Structurally, the spatial distribution of species based on otolith shape was similar for Assemblages A and C, with the same *EMI* values (0.156; Table 1), whereas the *EMI* value increased Assemblage B as a consequence of a decrease in the similarity of otolith shapes.

For the body morphoshape, the extreme shape of *C. conger* determined that Assemblage C reached the highest MD value ($MD = 0.168$; Table 1). MR was also linked to species richness, providing the greatest value for Assemblage C ($MR = 7.11$;

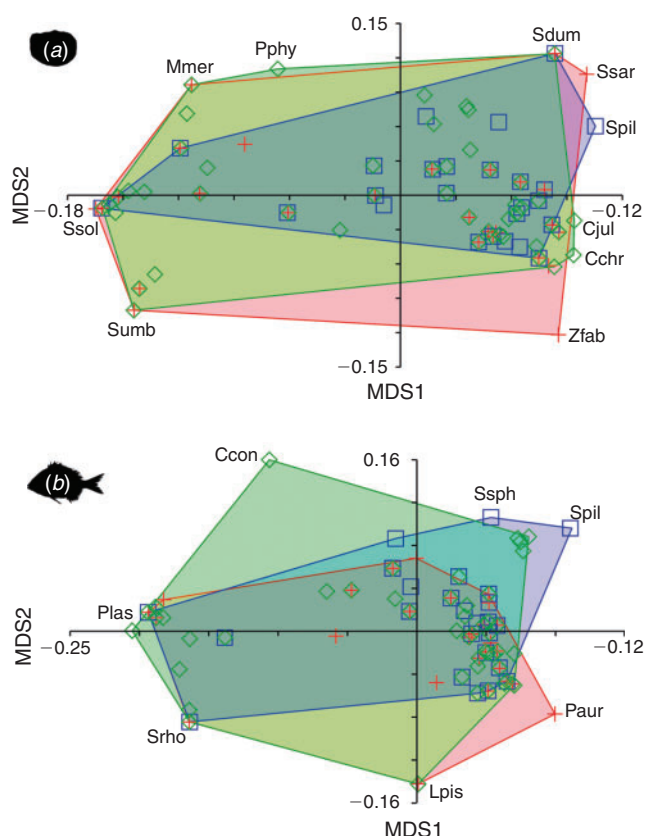


Fig. 6. Convex hull and spatial distribution of species within morphospace using (a) otolith and (b) body shape for three coastal fish assemblages of the western Mediterranean: rocky-sandy (squares), sandy (crosses) and artificial reef-sandy (diamonds). Species abbreviations are given for peripheral species of the convex hull. Cchr, *Chromis chromis*; Ccon, *Conger conger*; Cjul, *Coris julis*; Lpis, *Lophius piscatorius*; Mmer, *Merluccius merluccius*; Paur, *Pagrus auriga*; Plas, *Pegusa lascaris*; Pphy, *Phycis phycis*; Sdum, *Seriola dumerilii*; Spil, *Sardina pilchardus*; Ssar, *Sarda sarda*; Ssol, *Solea solea*; Ssph, *Sphyrna sphyraena*; Srho, *Scophthalmus rhombus*; Sumb, *Sciaena umbra*; Zfab, *Zeus faber*. MDS1 and MDS2 are the axes obtained using non-metric multidimensional scaling.

Table 1). Spatial distribution was higher in Assemblage B ($EMI=0.182$; Table 1).

Compared with other diversity indices (see Table 1), *MR* increased with functional diversity. In addition, the *MR* values obtained from otolith shape showed stronger relationships with functional diversity than *MR* values obtained from fish body shape. The *EMI* indices showed a similar tendency in the morphological analyses of otolith and body shapes. Moreover, larger *EMI* values corresponded to greater values of the Shannon index. Finally, the *MD* of the otoliths was related to the Shannon index, whereas the *MD* of the body shapes was linked to taxonomic diversity.

Discussion

Relevance of the sulcus acusticus as a descriptor

The results of the present study showed that features of both the otolith and sulcus acusticus were well-defined and together improved the ability to distinguish interspecific variability

within fish assemblages. Several studies have found ecomorphological patterns through the analysis of otolith shape (Volpedo and Echeverría 2003, Volpedo *et al.* 2008; Lombarte and Cruz 2007; Teimori *et al.* 2012). For example, a circular or polygonal shape with a poorly defined rostrum is typical of fish living on soft-bottom habitats (called 'Group 1'; see Volpedo and Echeverría 2003, Volpedo *et al.* 2008) an elongated shape with a variable rostrum is common for fish inhabiting hard-bottom habitats and a lanceolated or rectangular shape with a prominent rostrum and a deep V-shaped excisura is characteristic of pelagic species. Although the correspondence between otolith shape and type of habitat is not consistent in all cases, these groups were differentiated based on the aspect ratio (height : length) of the otolith, rostrum length v. otolith length and the sulcus : otolith area ratio. These features were also implicit in the warps obtained, but the major problem is that these patterns can be partially biased from a morpho-functional perspective: croakers or drums (Sciaenidae, Perciformes) and flatfish (Pleuronectiformes) belong to Group 1, but the shape of the sulcus in flatfish is completely different from that in the croakers. From a functional perspective, croakers are considered specialists in sound production (Luczkovich *et al.* 1999; Ramcharitar *et al.* 2001), whereas flatfish are not hearing specialists (Popper and Fay 1993). For this reason, the inclusion of sulcus shape provided relevant functional information. In addition, the warps showed variability in relation to the relative size of the sulcus ostium and cauda. The ostium size is correlated with an increase in the proportion of horizontally oriented sensory hair cells (Popper and Coombs 1982), which is related to the detection of directional acoustic stimuli and the location of prey (Popper and Fay 1993). Although it is unknown how the type of sulcus actually affects sound transduction, the properties of the sulcus are species specific (Nolf 1985; Gauldie 1988; Torres *et al.* 2000; Reichenbacher and Reichard 2014). Thus, the cluster analysis indicated that, for example, mullets (Mugiliformes), croakers, hakes, cod (Gadiformes) or dories (Zeiformes) were isolated because they have a special type of sulcus (Tuset *et al.* 2008). Therefore, joint analysis of otolith outline and sulcus shape is a powerful tool for the study of fish assemblages.

Interpreting the otolith morphospace

According to the concept of limiting similarity, species have a minimum distance in a one-dimensional niche space (MacArthur and Levins 1967), which should lead to regular spacing of species within morphospace (Ricklefs 2012). However, many studies have shown different levels of species packing or grouping, including the presence of empty morphospace zones (Gatz 1979; Goatley *et al.* 2010; Ricklefs 2012). The degree of species packing seems to coincide with the morphologies that are best adapted for the most efficient exploitation of resources in the ecosystem (Schoener 1974; Gatz 1979; Wainwright and Richard 1995; Ricklefs 2012).

Previous studies have found a clear correspondence between otolith morphospace and the trophic niche of fish (Lombarte *et al.* 2010; Tuset *et al.* 2015). In the present study, sparids and flatfish will be the best species adapted within these fish assemblages, forming similar packing species in the otolith and body morphospaces. However, the otolith morphospace

showed a different spatial variability for some species. Scophthalmids and soleids (flatfish) appeared separated in the otolith morphospace because their otoliths are mainly differ in the type of opening of the sulcus, which is ostial in scophthalmids and pseudo-ostial or mesial in soleids (Tuset *et al.* 2008). Scophthalmids feed on highly mobile prey and these foraging techniques require strong visual and hearing abilities, whereas soleids, with a smaller mouth, feed on slow-moving prey (Guedes and Araújo 2008). A similar pattern is seen in species such as *Trachinus radiatus* (Trachinidae, Perciformes), *Uranoscopus scaber* (Uranoscopidae, Perciformes) and *L. piscatorius*, which are ambush predators (Bagge 2004; Rizkalla and Philips 2008), burying into the sand and having similar morphological features in otolith shape to the scophthalmids.

In addition, other ecological adaptations were represented in the otolith morphospace. The sensorial macula and sulcus acusticus have been related to auditory frequency, mobility and depth distribution of fish (Gauldie 1988; Lombarte and Popper 1994; Torres *et al.* 2000; Sadighzadeh *et al.* 2014). We found that sagittae with a higher sulcus : otolith ratio were located in the periphery of the morphospace. In the case of gadiforms, they inhabit deeper waters and live close to the bottom during daytime, but move off-bottom at night to feed, emitting high-frequency sounds (Gauldie 1988). Faster-swimming pelagic species have small otoliths, but they also have a high sulcus : otolith ratio (Paxton 2000, Volpedo and Echeverría 2003). This likely allows them to get the maximum information about the environment around them, especially about predators and prey, as well as to maintain body orientation and coordination of movements during swimming (Kasumyan 2004). The largest otoliths with a high sulcus : otolith ratio were obtained in scianids, which produce low-frequency sounds during competition for feeding or during the breeding season (Horodysky *et al.* 2008).

The findings suggest that species clustered together based on otolith morphology did not share the same body shape. In fact, otolith shape grouped species with similar ecological strategies related to feeding or resting on the bottom (Hobson 2006). For example, jacks and mackerels (Carangidae and Scombridae, Perciformes), fast-swimming pelagic species, were clearly isolated based on the cluster analysis of otolith shape, whereas jacks were grouped with sciaenids and mullets based on cluster analysis of body shape. This is because, morphologically, these species share the presence of two dorsal fins. Therefore, the spatial distribution of otolith shapes seems to provide a better ecological interpretation of species than that obtained from analysis of fish shapes.

Measuring biodiversity

Taxonomically closer species share similar morphological features (for otolith, body or both) and this explains why they are strongly grouped in the morphospace. This is reflected by *MD*, which is linked to taxonomic diversity (McClain *et al.* 2004; Gerber *et al.* 2008; Farré *et al.* 2013). Body shape in fish is determined by different ecological behaviours, for example swimming, searching for food, striking and capturing prey or evading predators (Walker 2010). It is therefore a consequence of evolutionary process and the reason why the fish body morphospaces are also used to analyse the trajectory of anatomical

radiation of fossil and recent fish (e.g. Peres-Neto 2004; Friedman 2010). The spatial distribution of Anguilliformes, Clupeiformes, Lophiiformes, Mugilliformes and Pleuronectiformes clearly showed body shapes isolated and differentiated in the periphery of the morphospace, which increases the *MD* value, indicating a clear relationship with taxonomic differentiation. In contrast, the sagittal otoliths have a higher degree of dissimilarity within taxonomic groups (e.g. Nolf 1985; Volpedo and Echeverría 2000; Campana 2004; Tuset *et al.* 2008), which is why the *MD* did not show a relationship with average taxonomic distinctness. The morphological convergence in some characteristics (e.g. ostium : cauda ratio and height : length ratio) led to clustering of Perciformes and Pleuronectiformes, Anguilliformes and Gadiformes or Pleuronectiformes and Lophiiformes and a decrease in *MD*.

Sagittal otolith shape is markedly species specific (e.g. Gaemers 1983; Lombarte *et al.* 1991; L'Abée-Lund and Jensen 1993; Tuset *et al.* 2003; Sadighzadeh *et al.* 2012). Its variability is a phenotypic indicator that produces higher morphological distances between closely related species compared with body shape. It influences the spatial heterogeneity and diversity of shapes within morphospace and estimation of *EMI* and otolith *MR*. This morphological differentiation is also reflected in *FD*, reinforcing the functional character of the otolith. Furthermore, as opposed to body shape, otolith morphological indices are not strongly influenced by extreme (or peripheral) morphologies, and only some species or groups present unusual shape, such as Gadiformes, Gasterosteiformes, Stephanoberyciformes, Tetraodontiformes or Zeiformes (e.g. Nolf 1985; Tuset *et al.* 2008; Deng *et al.* 2013).

Understanding the origin and maintenance of biodiversity is a core challenge in ecology, evolution and conservation science (Gaston 2000). In recent years, *FD* has been considered key in biodiversity studies because it explains the roles that organisms play within ecosystems. The functional traits used to define this diversity are related to food strategies, trophic position, size, locomotion, mobility, lifestyle, activity or distribution in habitat (e.g. Petchey and Gaston 2006; Somerfield *et al.* 2008; Villéger *et al.* 2010). Many of these ecological factors are also related to otolith and sulcus shape and size (e.g. Gauldie and Crampton 2002; Volpedo and Echeverría 2003; Lombarte and Cruz 2007; Volpedo *et al.* 2008; Lombarte *et al.* 2010; Sadighzadeh *et al.* 2014). In this context, the present study clearly demonstrated that otolith shape has an important functional character, hence its strong relationship with *FD*. Otolith shape makes it possible to easily compare recent and fossil fish assemblages or to measure the diversity of fish assemblages as an alternative in cases where ecological information may be absent or scarce.

Acknowledgements

The authors are very grateful to all the people who helped in the development of the AFORO database, especially colleagues of Instituto de Ciencias del Mar (ICM-CSIC) in Barcelona. The present study was financed by the projects 'ESCAL 1' (Ref. PCC30004/99) and 'ESCAL 2' (Ref. 02P30015) of the Direcció General de Fisheries of the Government of Catalonia and 'CONFLICT' (Ref. CGL2008-00047), 'AFORO3D' (Ref. CTM2010.1970) and 'ANTROMARE' (Ref. CTM2009.12214) of the Spanish National Research. M. Farré acknowledges a Ph.D. scholarship from the Government of Andorra (ATC-010-AND; 2012-14).

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