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Ecomorphological comparisons of sagittae in *Mullus barbatus* and *M. surmuletus*

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For both *Mullus barbatus* and *M. surmuletus*, the relationship between sagitta area (O), sulcus acusticus area (S) throughout postlarval growth was characterized by their negative allometric growth. The adjusted mean S : O ratio differed between the two species. This may be associated with the difference in size and shape of the sagittae, a difference in somatic growth, and differences in food and spatial niches. The inner ear of *Mullus* cannot be considered as specialized and the interaction of stimuli detected by the sensory barbels and the inner ear together could be a compensatory mechanism that helps in the food search.

Key words: *Mullus barbatus*; *M. surmuletus*; otolith area (O); sulcus acusticus area (S); S : O ratio.

INTRODUCTION

The otolith organs of the inner ear of teleost fishes are mechanoreceptors that process acoustic and postural information (Schuijf, 1981; Fay, 1984; Gauldie, 1988; Rogers & Cox, 1988). Shape and growth rate of otoliths are species specific (Platt & Popper, 1981; Nolf, 1985; Wilson, 1985). Some authors have indicated that biological constraints (Nolf, 1985; Wilson, 1985; Bori, 1986; Secor & Dean, 1989; Reñones *et al.*, 1995), result in differences in the size of the skull and the endolymphatic sac (Gauldie & Nelson, 1990), and differences between metabolic rates linked to environmental factors (Popper & Coombs, 1982; Wilson, 1985) may influence the shape and growth rate of the otoliths.

In teleost fishes the shape of the sensory macula of the otolithic endorgans varies, as does that of the otolith through ontogenetic development. This change in the macula parallels growth of the sulcus acusticus (Platt & Popper, 1981). The conservative nature of the sulcus acusticus and the growth of the macula may reflect their function as organs of equilibrium and hearing, to maintain precise configuration with respect to other parts of the fish otic apparatus (Gauldie, 1988; Lombarte, 1992; Lombarte & Popper, 1994; Arellano *et al.*, 1995). Gauldie (1988) postulated a model of otolith function in which the saccular otolith acts as a system of levers through which sound waves are converted into shearing forces in the plane of the hair cell in the macula. Gauldie's model implies that the shape of the sagitta, measured as the ratio of

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macula to otolith area (M : O) or its approximation by sulcus acusticus area/sagitta area (S : O), is related to the frequency response and the auditory threshold of the otolith.

The comparative study of otolith morphology and its relation to environmental variables provides a method for elucidating characteristics of biological significance. The present study compares the morphological differences in the relationship between otolith area (O), sulcus acusticus area (S) and S : O ratio during ontogeny of two species of *Mullus* from the north-western Mediterranean Sea. The Mullidae differs from other percoid families in a pair of highly developed hyoid barbels (that appear early in the planktonic state) which reflects the ecological specialization of different species of Mullidae (Gosline, 1984; Lombarte & Aguirre, 1997). The species considered in this paper were *Mullus barbatus* L., and *M. surmuletus* L., which live on the continental shelf and have similar benthophagous feeding habits. However, *M. surmuletus* prefers sandy and rocky bottoms, while *M. barbatus* prefers muddy bottoms and is found in deeper waters than *M. surmuletus* (Reñones *et al.*, 1995; Sánchez *et al.*, unpubl.). The specific aim was to determine whether the S : O ratio showed a compensatory relationship with the reduction of the visual field (produced in muddy and deeper waters) in species with non-specialized hearing such as *M. barbatus* and *M. surmuletus* that also have an alternative sensory system on their barbels (chemoreceptor and mechanoreceptor).

MATERIALS AND METHODS

Specimens were sampled from the commercial trawl fishery in 1994–1995 (EC project MED92/009) in the north-western Mediterranean Sea. The specimens were identified, their total length (L_T) was measured in mm, and the right and left sagittae were removed and cleaned under a dissection microscope. The sagitta morphology description has been based on the criteria given by Schwarzhans (1980).

The outline of each sagitta and the sulcus acusticus was drawn with the aid of a camera lucida, and the resulting image was digitized (Fig. 1). The sagittae were not digitized directly because the tone was uniform and it was not possible to distinguish the sulcus acusticus area from the rest of the otolith (Lombarte, 1992). The otolith area (O) and sulcus acusticus area (S) were measured in mm^2 using a digital image processing system OPTIMAS 6.0 (Optimas Co., 1996).

The relationships between L_T *v.* O and L_T *v.* S in each side for both species, were determined by fitting a power equation $Y=aL_T{}^b$ to the data. If the slope differed significantly from 0, the independent variable changed as the fish grew. The ratio between the sulcus acusticus area and the sagitta area (S : O) was calculated. A Student's *t*-test was used to compare the slopes of the morphometric relationships between them and with the hypothetical value of isometry. For the relationships L_T *v.* O and L_T *v.* S a slope equal to 2 represents isometry. The S : O ratio between *Mullus* species was compared by means of two-way analysis of covariance (ANCOVA: Statistica 5.1, Stat. Soft Inc., 1998), with species and sides as factors and L_T as covariant. A Student's *t*-test was used to compare the adjusted means of the S : O ratio between *M. barbatus* and *M. surmuletus* and with the S : O values available in the literature. In all cases 95% confidence levels were calculated.

RESULTS

A total of 72 otoliths from 45 specimens of *Mullus barbatus,* ranging from 56 to 220 mm L_T and 102 otoliths from 58 specimens of *M. surmuletus*, ranging from 55 to 290 mm L_T were used.

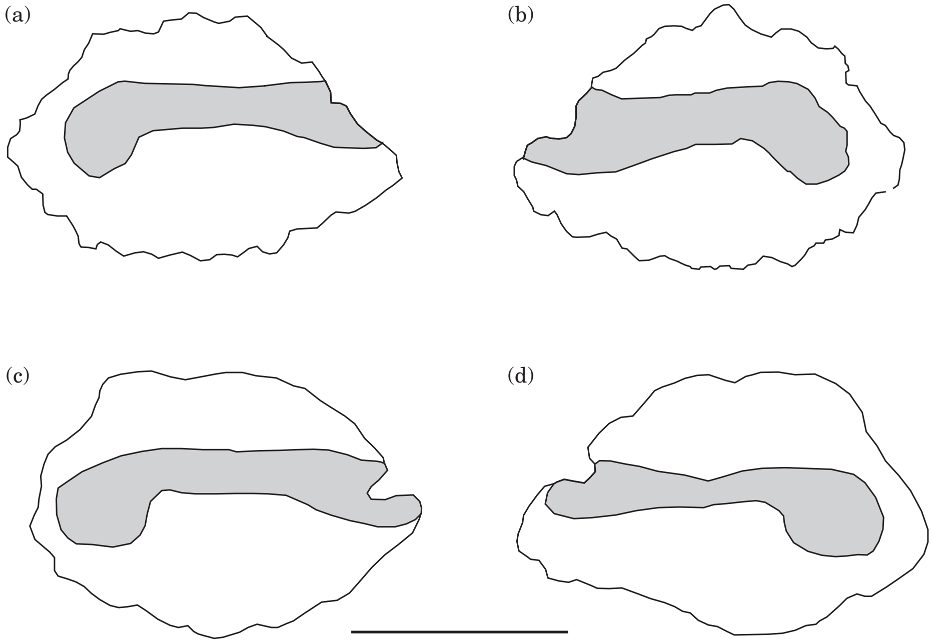

FIG. 1. Medial views of the saccular otolith (sagitta) of: *Mullus barbatus* (185 mm L_T) left (a) and right (b), and *M. surmuletus* (185 mm L_T) left (c) and right (d). Sulcus acusticus area is shown in grey. Bar = 10 mm.

GROSS MORPHOLOGY

The sagittae of *M. barbatus* and *M. surmuletus* are generalized perciform otoliths, elliptic, their caudal area is higher than the anterior (Fig. 1), and in the anterior part of the otolith a well-defined difference in the rostrum and antirostrum delimits the excisura ostial. However, the sagittae of *M. barbatus* are higher, have irregular margins and are dome-shaped, while those of sagittae of *M. surmuletus* are longer, have comparatively smooth margins and are medio-laterally flattened. The sulcus acusticus of both species is of the homosulcoid type (Schwarzhans, 1980). They do not have a well-developed ostial colliculum, and their caudal colliculum is curved ventrally.

GROWTH OF THE OTOLITH (O) AND SULCUS ACUSTICUS AREA (S)

The relationship between otolith area (O) and L_T (Table I) showed no significant differences between the left and right side for both *M. barbatus* ($t = -0{\cdot}0295$, d.f. = 69 and $P < 0{\cdot}05$) and *M. surmuletus* ($t = -1{\cdot}2567$, d.f. = 87 and $P < 0{\cdot}05$). The growth relationship was $O = 0{\cdot}0097 L_T^{1{\cdot}2119}$ ($r^2 = 0{\cdot}9574$, $n = 72$) in *M. barbatus* and $O = 0{\cdot}0159\ L_T^{1{\cdot}1147}$ ($r^2 = 0{\cdot}9750$, $n = 91$) in *M. surmuletus* [Fig. 2(a)]. The growth was characterized by a negative allometric relationship through ontogenetic development of *M. barbatus* ($t = -25{\cdot}1406$, d.f. = 72 and $P < 0{\cdot}05$) and *M. surmuletus* ($t = -32{\cdot}9108$, d.f. = 89 and $P < 0{\cdot}05$). Comparison of the slopes (b), showed that the relative size of otolith area (O) was not significantly different between species ($t = 2{\cdot}8605$, d.f. = 161 and $P < 0{\cdot}05$).

TABLE I. *Mullus barbatus* and *M. surmuletus*. Regression and allometric parameter (*t*) for the relationship between L_T against otolith area (O), and sulcus acusticus area (S) for left (L) and right (R) sides

Species	Regression	*a*	*b*	*n*	r^2	*t*	*P*
M. barbatus	O_L *v.* L_T	0·0097	1·2126	42	0·9667	− 22·1803	0·00
	O_R *v.* L_T	0·0098	1·2108	31	0·9534	− 15·9113	0·00
	S_L *v.* L_T	0·0034	1·1355	42	0·9122	− 15·5486	0·00
	S_R *v.* L_T	0·0019	1·2520	28	0·9263	− 10·8092	0·00
M. surmuletus	O_L *v.* L_T	0·0140	1·1393	54	0·9535	− 24·6288	0·00
	O_R *v.* L_T	0·0191	1·0781	37	0·9475	− 21·7958	0·00
	S_L *v.* L_T	0·0023	1·2442	49	0·9304	− 15·2379	0·00
	S_R *v.* L_T	0·0016	1·3107	33	0·9145	− 9·5869	0·00

In all cases 95% confidence levels were calculated.

The growth of the sulcus acusticus area (S) relative to L_T (Table I) showed no significant differences between the left and right side for both *M. barbatus* ($t = -1{\cdot}3124$, d.f.=66 and $P < 0{\cdot}05$) and *M. surmuletus* ($t = -0{\cdot}7613$, d.f.=78 and $P < 0{\cdot}05$). In *M. barbatus*, the growth relationship was $S = 0{\cdot}0027 L_T^{1{\cdot}1836}$ ($r^2 = 0{\cdot}9165$, $n = 70$) and $S = 0{\cdot}0020 L_T^{1{\cdot}2640}$ ($r^2 = 0{\cdot}9245$, $n = 82$) in *M. surmuletus* [Fig. 2(b)]. The growth was characterized by a negative allometric relationship through ontogenetic development of *M. barbatus* ($t = -18{\cdot}8545$, d.f.=68 and $P < 0{\cdot}05$) and *M. surmuletus* ($t = -18{\cdot}2630$, d.f.=80 and $P < 0{\cdot}05$). Comparison of the slopes (*b*) showed no significant differences between species ($t = -1{\cdot}3592$, d.f.=148 and $P < 0{\cdot}05$) on the relative size of sulcus acusticus area (S).

RELATIONSHIPS BETWEEN S : O RATIO AND TOTAL FISH LENGTH L_T

To determine if the S : O ratio of the two species changed through ontogenetic development, the slope values were tested against 0. The regression slope showed no significant differences for *M. barbatus* ($t = -0{\cdot}5170$, d.f.=74 and $P = 0{\cdot}6066$), whereas, the *M. surmuletus* slope was significantly different ($t = 2{\cdot}1692$, d.f.=82 and $P = 0{\cdot}0329$). The two-way ANCOVA showed that the S : O ratio was significantly different between species, but not between right-hand and left-hand sides (Table II). In *M. barbatus*, the adjusted mean S : O ratio was 0·2347 ± 0·0209 and in *M. surmuletus* it was 0·2754 ± 0·0358 (Fig. 3).

DISCUSSION

MORPHOLOGICAL CHARACTERS OF THE SAGITTAE

For both species, the power function $Y = aL_T^{\,b}$ is the most appropriate descriptor of the relationship between total length L_T, sagitta area (O) and sulcus acusticus area (S). Regression models showed that there were no significant differences between left and right side for both species [Fig. 2(a) and (b)], as others have found in teleost fishes with axial symmetry (Nolf, 1985; Secor &

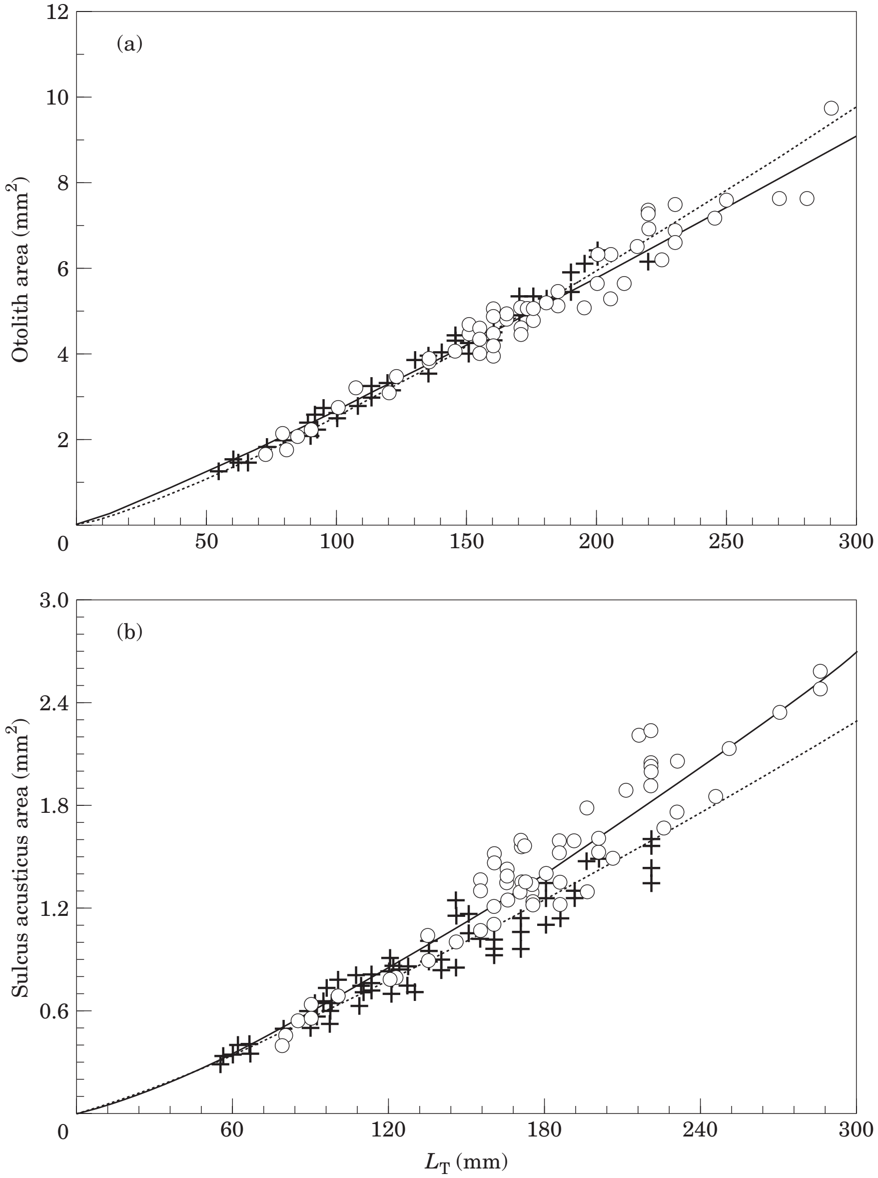

FIG. 2. Relationship between (a) otolith area and (b) sulcus acusticus area against total length of the fish. The figures show *M. barbatus* observed (+) and predicted data (- -), *M. surmuletus* observed (○), and predicted data (——).

Dean, 1989; Lombarte, 1992; Arellano *et al.*, 1995; Reñones *et al.*, 1995). Differences have been reported between otolith sides (Nolf, 1985; Bori, 1986) only in species with strong axial asymmetry.

Global models (both sides), the growth of sagitta area (O) and sulcus acusticus area (S) were negatively allometric with respect to total fish length in both species (Table I), indicating that the relative otolith area decreases with ontogenetic

TABLE II. Covariance analysis, summary of all effects

Effect	d.f. effect	MS effect	d.f. error	MS error	*F*	*P*
Species	1	0·0376	139	0·0010	36·7119	0·0000
Side	1	0·0016	139	0·0010	1·5641	0·2131
Species × side	1	0·0000	139	0·0010	0·0824	0·7743

MS effect, Ratio of the between-groups variance; MS error, the error variance.

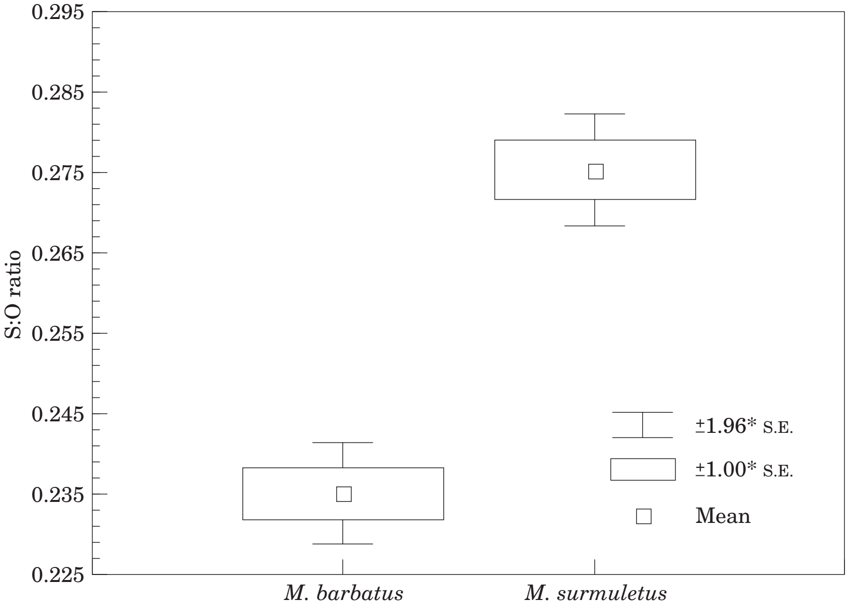

FIG. 3. Box plot for S : O ratio.

development, as in other teleosts (Wilson, 1985; Gauldie, 1988; Lombarte, 1992; Arellano *et al.*, 1995).

The regression slope between L_T and S : O ratio, was not significantly different from 0 in *M. barbatus*, meaning that there is a proportional growth between otolith area and sulcus acusticus area. The isometric relationships (O *v.* S) indicate that the sulcus acusticus area is not affected by the decrement in relative size of the sagitta with respect to body growth. The same results were found in *Hoplostethus atlanticus* Collett, *H. mediterraneus* Cuvier, *Trachurus declivis* (Jenyns) (Gauldie, 1988) and *Pomatoschistus lozanoi* (de Buen) (Arellano *et al.*, 1995). However, in *M. surmuletus*, the regression slope between L_T and S : O ratio was significantly different from 0 (P>0·05) indicating that the S : O ratio increases with body size. These changes have been associated with the reduction of the visual field as a compensatory response in *Merluccius capensis* Castelnau, *M. paradoxus* Franca (Lombarte, 1992) and *Pomatoschistus minutus* (Pallas) (Arellano *et al.*, 1995).

MORPHOLOGICAL SIGNIFICANCE

In *M. barbatus*, as in *P. lozanoi* (Arellano *et al.*, 1995), the skull and the endolymphatic sac restrict the ventral sagitta growth (Gauldie & Nelson, 1990). Although the relative sizes of the endolymphatic sac and the skull were not measured, the cephalic length L_C of *M. surmuletus* is bigger than that of *M. barbatus* in specimens of the same L_T. The sagittae of *M. barbatus* are rounded and dome-shaped, while those of *M. surmuletus* are long relative to their height and are medio-laterally flattened.

The difference in otolith size patterns may be associated with differences in the somatic growth of the two species. *Mullus barbatus* have smaller otoliths and higher somatic growth rate (k=0·380) than *M. surmuletus* (k=0·268) (Sánchez *et al.*, unpubl.). A similar relationship was observed in *Morone saxatilis* (Walbaum) (Secor & Dean, 1989), where groups with lower growth rates have proportionately bigger otoliths. A similar effect has been observed in other sensory systems of the genus *Mullus*. Lombarte & Aguirre (1997) found that the barbel surface in *M. surmuletus* was bigger than that in *M. barbatus* for the same L_T.

The shape of the otolith itself is highly species-specific with a strong similarity between otoliths from individual fishes; even when there are differences between individuals. This implies some genetically programmed control over otolith shape (Nolf, 1985; Gauldie, 1988).

Some authors postulate that the interspecific variation in the ultrastructure of the inner ear may reflect differences in auditory stimuli processing. For the same stimuli, the variation in otolith movement pattern between species could be associated with its morphology, the topography of the sensorial tissues (Platt & Popper, 1981; Popper & Coombs, 1982; Lombarte & Popper, 1994), and with the difference in oriented patterns of the hair cells that codify these movements (Platt & Popper, 1981; Schuijf, 1981; Fay, 1984).

Although behavioural flexibility of organisms frequently causes problems in correlative ecomorphology, in the genus *Mullus* the difference between S : O ratio may be associated with the difference in their food, spatial niches and depth, as Lombarte (1992) and Arellano *et al.* (1995) argue, on the assumption that within genera, the otoliths in the different species have the same basic evolutionary design and variation is related to differencies in species' ecology.

The S : O ratios in *Mullus* are relatively low compared with large pelagic [e.g. *Thunnus albacares* (Bonnaterre) and *Thyrsites atun* (Euphrasen)] and demersal fishes (*Gadus morhua* L.), and higher than benthic and shallow-water fishes [e.g. *Genypterus blacodes* (Forster), *Kathetostoma giganteum* Haast, *Rhombosolea plebeia* (Richardson), *Parika scaber* (Forster), *Pomatoschistus minutus* and *P. lozanoi*]. The S : O ratio in *M. barbatus* is similar to that in *Cheilodactylus kumu* (Cuvier), a benthic species with some swimming mobility that has a well-developed external taste bud system as in *Mullus* (Table III).

Additionally, Rogers & Cox (1988) found that fishes are most sensitive to sound where the ambient noise is high, as in reefs. These results are supported by our data: *M. surmuletus* is associated with rocky reefs and has a higher S : O ratio (0·2743 ± 0·0372) than *M. barbatus* (0·2407 ± 0·0288), which inhabits muddy bottoms.

TABLE III. *Mullus barbatus* (t*Mb*) and *M. surmuletus* (t*Ms*) *t*-test comparison between its macula : otolith (M : O) ratio ± S.D. and between the M : O ratio calculated by different authors

Ref.	Species		M/O	S.D.	*n*	t*Mb*	t*Ms*
1	*Thunnus albacares* (Bonnaterre, 1788)	S	0·540		2	− 14·545	− 7·391
	Thyrsites atun (Euphrasen, 1791)	S	0·426		1	− 9·114	− 4·207
	Gadus morhua Linnaeus, 1758*	S	0·362		3	− 6·065	− 2·419
	Arripis trutta (Forster, 1801)	S	0·233		1	0·081 NS	1·184 NS
	Cheilodactylus macropterus (Forster, 1801)	S	0·229		1	0·272 NS	1·296 NS
	Pseudophycis bacchus (Forster, 1801)	S	0·222		1	0·605 NS	1·492 NS
	Chelidonichthys kumu (Cuvier, 1829)	S	0·216		1	0·891 NS	1·659 NS
	Hoplostethus mediterraneus Cuvier, 1829	S	0·202		10	1·558 NS	2·050
	Trachurus declivis (Jenyns, 1841)	S	0·202		18	1·558 NS	2·050
	Hoplostethus atlanticus Collett, 1889	S	0·195		26	1·891 NS	2·246
	Tautoga onitis (Linnaeus, 1758)*	S	0·195		3	1·891 NS	2·246
	Perca fluviatilis Linnaeus, 1758*	S	0·175		4	2·844	2·804
	Seriolella brama (Günther, 1860)	S	0·173		1	2·939	2·860
	Genypterus blacodes (Forster, 1801)	S	0·171		1	3·035	2·916
	Kathetostoma giganteum Haast, 1873	S	0·149		1	4·083	3·531
	Rhombosolea plebeia (Richardson, 1843)	S	0·129		3	5·036	4·089
	Parika scaber (Forster, 1801)	A	1·112		3	− 41·796	− 23·369
	Rhombosolea plebeia (Richardson, 1843)	A	0·446		3	− 10·067	− 4·765
2	*Merluccius capensis* Castelnau, 1861*	S	0·281	0·024	239	− 16·383	− 1·398 NS
	Merluccius paradoxus Franca, 1960*	S	0·312	0·028	225	− 25·679	− 8·846
3	*Pomatoschistus minutus* (Pallas, 1770)*	S	0·084	0·016	224	58·135	49·790
	Pomatoschistus lozanoi (de Buen, 1923)*	S	0·092	0·015	224	55·624	47·934
4	*Mullus barbatus* Linnaeus, 1758	S	0·234	0·020	79	0·000 NS	9·286
	Mullus surmuletus Linnaeus, 1758	S	0·275	0·035	94	− 9·286	0·000 NS

1, Gauldie, 1988; 2, Lombarte, 1992; 3, Arellano *et al.*, 1995; 4, present study.

*M : O calculated from sulcus acusticus : otolith ratio (S : O) for the sagitta and astericus. NS, No significant difference between species ($P \geqslant = 0{\cdot}05$).

Both species of *Mullus* feed on benthic species, with low mobility and either cryptic, or buried in the substratum: thus visual detection of prey is very difficult. It is possible that both species are guided to prey by sound. Their highly developed hyoid barbels are then used to locate precisely cryptic or buried prey (Gosline, 1984; Lombarte & Aguirre, 1997).

The inner ear of genus *Mullus* cannot be considered as specialized anatomically. However, the interaction of stimuli detected by the sensory barbels and the inner ear could together compensate for lack of anatomical specialization and help the search for food.

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