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Preliminary data on the study of otolith morphology of five pelagic fish species from the Adriatic Sea (Croatia)

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*The otolith (*sagitta*) measurements of five pelagic fish species -* Engraulis encrasicolus *(Linnaeus, 1758);* Sardina pilchardus *(Walbaum, 1792);* Scomber scombrus *Linnaeus, 1758;* Scomber japonicus *Houttuyn, 1780 and* Belone belone *(Linnaeus, 1761) – were obtained by image analysis of catch samples retrieved from the Croatian part of the Adriatic Sea. Three dimensionless shape descriptors of form factor (F_F), roundness (R_D) and aspect ratio (A_R) were analysed and revealed statistically significant differences between the otoliths of the stated pelagic fish species. Maximum otolith length (LO) and otolith weight (WO) were linearly correlated to total fish length (TL) for each studied fish species; they were increasing proportionally during fish growth.*

Key words: otolith morphology, small pelagic fish, Adriatic Sea

INTRODUCTION

In teleosts, the inner ear is a complicated structure of canals, sacs and ducts filled with endolymph. Each otic sac contains the otoliths of a calcareous structure named *sagitta*, *lapillus* or *asteriscus* (BOND, 1996). The otoliths differ in size and shape. In most species *sagitta* otolith is the biggest one and mostly used in during the ageing of fish. Even though the otoliths inside the inner ear have similar functions, their form, size, weight, growth, consistency and chemical composition vary considerably among species (LABEELUND, 1988; GAULDIE, 1994; KARLOURIGA, 2000; YOSHINAGA *et al*., 2000). Nevertheless, otoliths are generally considered as taxonomical and biological archives as they reflect growth and development of species. Due to that, they are routinely used for characterization of events in the fish life history, age and growth estimations, stock discrimination of exploited fish populations as well as permitting the study of food webs from partially digested remains (COTTRELL *et al*., 1996). As digital technology developed, fishery biologists started using shape analysis in order to identify stocks by means of morphometric characters of fish or otoliths (BOLLES & BEGG, 2000; TORRES *et al*., 2000; BEGG *et al*., 2001; MONTEIRO *et al*., 2005; TUSET *et al*., 2006; PONTON, 2006; ZORICA *et al*., 2007) and to study geographical variations in fish populations (GAEMERS, 1984; NOLF, 1985; CASTONGUAY *et al*., 1991; CAMPANA & CASSELMAN, 1993; PARMENTIER *et al*., 2001; GAULDIE & CRAMPTON, 2002; TUSET *et al*., 2003).

The purpose of this study was to analyse pelagic fish variations in otoliths shape and their morphometric measurements in species in order to derive some morphometric otolith regularities that could be used as taxonomic features. Additionally, the purpose of this study was to determine whether otolith length and otolith weight would be a more powerful indicator of differences in somatic growth. Otoliths of five pelagic fish species originating from the Adriatic Sea – *Engraulis encrasicolus*, *Sardina pilchardus*, *Scomber scombrus*, *Scomber japonicus* and *Belone belone*, were examined.

MATERIALS AND METHODS

The otoliths of 30 specimens of each of five pelagic species – *E. encrasicolus* (TL: 12.5 – 16.5 cm), *S. pilchardus* (TL: 12.5 – 17.5 cm), *S. scombrus* (TL: 19.5 – 37.1 cm), *S. japonicus* (TL: 20.2 – 31.5 cm) and *B. belone* (TL: 23.3 – 66.2 cm), were analysed. Fish were caught by purse seine and beach seine from January to December 2005 in the Croatian part of the Adriatic Sea (Fig. 1).

Fig. 1 - Map of the study area

Total lengths (TL) were measured to the nearest mm, while wet weights of the collected specimens were weighed to the nearest 0.01 g. The *sagitta* otoliths were removed by opening the otic bulla, washed in freshwater, dried and stored in plastic tubes prior to analyses. The weight of the undamaged and cleaned otoliths was measured on Mettler analytical balance (to the nearest 0.01 mg). Morphological measurements were obtained by digitising the images of each otolith using a camera coupled to an OLYMPUS binocular microscope and PC. Image analysis and processing was carried out using OLYMPUS DP-software. For morphometric analysis, the following measurements were recorded: maximum otolith length (LO, mm), otolith width (W, mm), area (A, mm^2), perimeter (P, mm) and weight (WO, mg). In order to describe the shape of the otolith, three dimensionless shape factors were calculated according to PONTON (2006):

$$F_F = 4 \cdot \pi \cdot A \cdot P^{-2}, \; R_D = 4 \cdot A \cdot (\pi \cdot LO^2)^{-1}, \; A_R = LO \cdot W^{-1},$$

where F_F is a form factor, R_D roundness, A_R aspect ratio, A otolith area (mm^2), P otolith perimeter (mm), LO otolith length (mm) and W otolith width (mm). The form factor (F_F) is the inverse ratio of the squared perimeter of an object to the squared perimeter of a circle of the same surface. The smaller it is, the lacier is the outline of the otolith. The roundness (R_D) is the ratio between the actual area and the area of a circle of the same length. Namely, this factor is larger if and when the shape of otolith is more circular. The aspect ratio (A_R) is the ratio between the otolith length and otolith width. This factor expresses the shape tendency of the otolih; the more elongated the otolith of the fish, the larger the aspect ratio is. All otolith shape indices were first examined for normality with Kolmogorov – Smirnov (K-S) test. If this criterion was satisfied, analysis of variance (ANOVA) was then used to determine is there was a statistically significant difference between analysed species according to all three shape factors. Conversions among total length of the fish and otolith length as well as weight of otolith were accomplished with the linear regression model by the method of least squares (SOKAL & ROHLF, 1981). For the statistical analysis StatSoft 5.5 was used.

RESULTS

Pelagic fish specimens (n=150) representing five different species belonging to four families were analysed. The otolith images of five examined species are presented in Fig. 2.

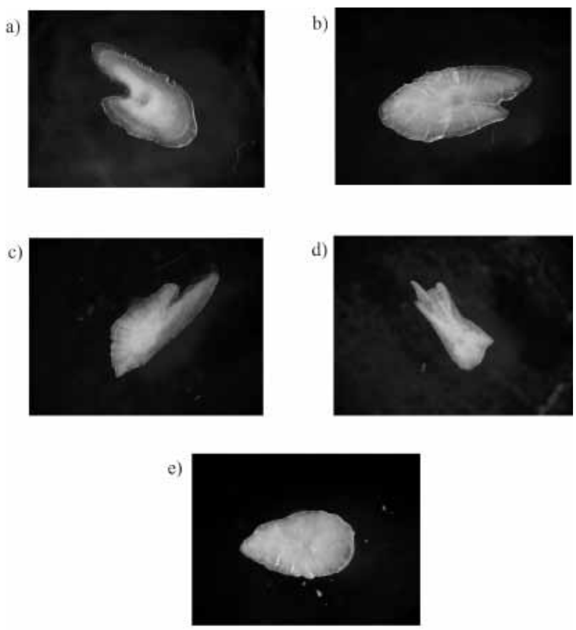

Fig. 2. Images of the sagitta of five pelagic species: (a) E. encrasicolus, *(b)* S. pilchardus, *(c)* S. scombrus, *(d)* S. japonicus *and (e)* B. belone

The morphometric measurements and three examined shape factors of otolith varied significantly among species (Table 1). The values of individual maximum otolith length (LO) were between 2.26 mm (*S. pilchardus*) and 5.74 mm (*S. japonicus*). Otoliths of *S. japonicus* were the largest, reaching up to 5.74 mm, starting from 2.81 mm. Their otoliths also had the highest perimeter value (from 5.72 to 17.34 mm). Otoliths of *S. pilchardus* had the smallest values of all observed parameters. Their individual lengths ranged from 2.26 mm to 3.44 mm, with the average otolith weight of 1.37±0.077 mg. Overall individual weight of otolith (WO) ranged from 0.76 (*S. pilchardus*) to 13.31 mg (*B. belone*). The heaviest were the otoliths of *B. belone* with an average otolith weight of 4.61±0.564 mg; they were the widest and with the highest otolith extent area. According to the mean values of three examined shape factors the otoliths of *S. japonicus* had the smallest form factor ($0.302 < F_F < 0.481$) and factor of roundness ($0.279 < R_D < 0.469$), while the otoliths of *B. belone* had the largest values of those two shape factors ($0.340 < F_F < 0.639$; $0.324 < R_D < 0.626$). Furthermore, the otoliths of *S. japonicus* showed the highest values for aspect ratio ($1.371 < A_R < 3.622$), while the lowest values for this shape factor were noted for *S. scombrus* ($0.685 < A_R < 2.785$). Hence, the lacier and least circular were the otoliths of *S. japonicus,* especially in comparison to *B. belone*, while the otoliths of *S. scombrus* were the least elongated among analysed pelagic fish species.

The Kolmogorov-Smirnov (K-S) test carried out on shape factor data sets for each examined small pelagic fish species confirmed a normal distribution ($P>0.05$; non significant), while ANOVA showed that there were significant differences among shape indices of all analysed pelagic species (Wilks' Lambda = 0.0041, df=10, $P<0.05$).

Nevertheless, as fish otolith length was increasing, the values of form factor (F_F) and roundness (R_D) were generally decreasing. The values of aspect ratio (A_R) were proportionally increasing with otolith enlargement for all five pelagic fish species (Fig. 3). Coefficients of correlation (r) between examined shape factors and maximal otolith length are presented in Table 2. They are high and significant.

Table 1. Mean amount and standard error of otoliths morphometric measurements (maximum otolith length - LO, otolith width - W, area - A, perimeter – P, otolith weight – WO) and three shape descriptors (form factor - F_F, roundness - R_D, aspect ratio - A_R) of five pelagic fish species caught in the eastern part of the Adriatic Sea

Species	LO (mm)	W (mm)	WO (mg)	A (mm^2)	P (mm)	F_F	R_D	A_R
E. encrasicolus	3.29±0.045	1.48±0.020	2.49±0.087	3.48±0.079	10.03±0.162	0.44±0.006	0.41±0.005	2.22±0.029
S. pilchardus	2.80±0.052	1.40±0.031	1.37±0.077	2.70±0.096	8.96±0.184	0.42±0.007	0.44±0.005	2.00±0.023
S. scombrus	3.42±0.104	1.43±0.027	2.10±0.153	3.61±0.151	10.28±0.300	0.43±0.012	0.40±0.014	1.44±0.085
S. japonicus	4.17±0.131	1.49±0.073	3.45±0.215	4.91±0.264	12.58±0.424	0.38±0.008	0.36±0.007	2.36±0.115
B. belone	3.62±0.170	2.03±0.097	4.61±0.564	5.54±0.534	11.09±0.499	0.54±0.012	0.52±0.006	1.76±0.040

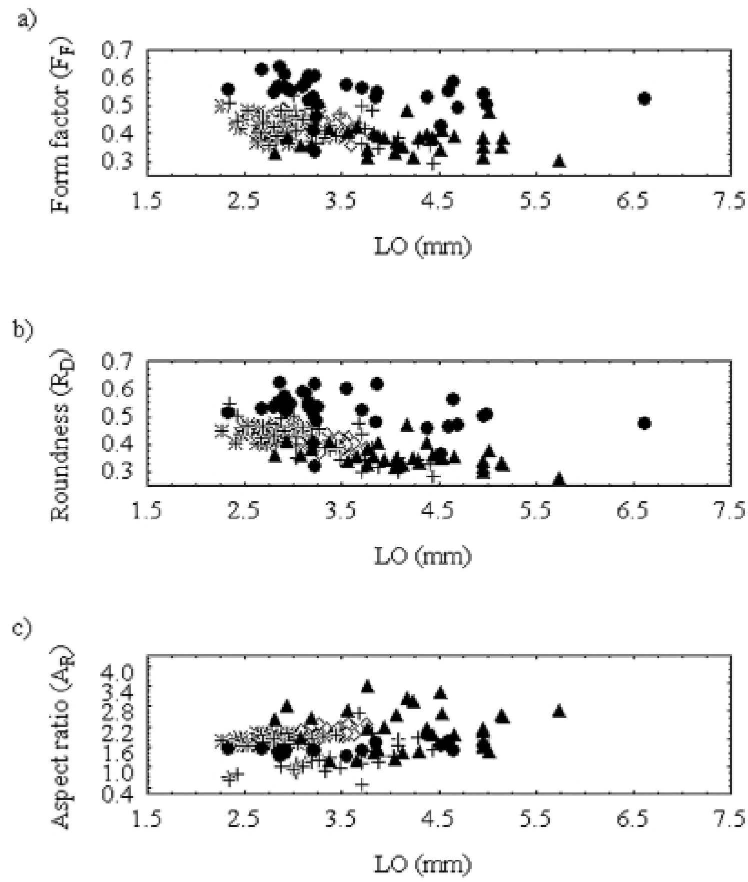

Fig. 3. Form factor - F_F (a), roundness - R_D (b) and aspect ratio - A_R (c) vs. maximal otolith length (LO) of five pelagic species: E. encrasicolus *(rhomb),* S. pilchardus *(star),* S. scombrus *(plus),* S. japonicus *(triangle) and* B. belone *(circle)*

Otolith growth rate was described by a linear regression model with two equations: LO=aTL + b and WO=aTL + b, where TL is the total length of the fish (cm), LO is the maximal otolith length (mm), WO is the weight of the otolith (mg), *a* is the angular coefficient characterizing the growth rate of the otolith and *b* is a constant for the observed species. Maximum otolith length and otolith weight were linearly related to total fish length for the studied fish species. Their regression parameters were highly significant (P<0.050) (Table 3).

The best fit for the TL - LO relationship was recorded for *E. encrasicolus* (r^2=0.751), then for

Table 2. Coefficient of correlation (r) between the maximum length of fish otolith and shape factors (F_F- form factor, R_D- roundness and A_R-aspect ratio)

Species	Shape factor		
	F_F	R_D	A_R
E. encrasicolus	-0.744*	-0.758*	0.685*
S. pilchardus	-0.622*	-0.513	0.138
S. scombrus	-0.844*	-0.908*	0.816*
S. japonicus	-0.361	-0.694*	0.224
B. belone	-0.436	-0.557	0.778*

Table 3. Relationship between the total fish length– maximal otolith length (TL – LO) and total fish length– otolith weight (TL – WO) and coefficients of determination (r^2) for five pelagic fish species caught in the Croatian part of the Adriatic Sea

Relationship	TL - LO		TL - WO	
Species	Equation	r^2	Equation	r^2
E. encrasicolus	LO= 0.14TL+1.22	0.751	WO=0.24TL-1.01	0.651
S. pilchardus	LO= 0.12TL+1.03	0.583	WO=0.24TL-2.31	0.812
S. scombrus	LO= 0.09TL+0.84	0.715	WO=0.14TL-1.83	0.738
S. japonicus	LO= 0.16TL+0.12	0.747	WO=0.33TL-4.90	0.941
B. belone	LO= 0.06TL+1.33	0.656	WO=0.25TL-5.85	0.904

S. japonicus (r^2=0.747), while the lowest value of the coefficient of determination was established for *S. pilchardus* (r^2=0.583). Moreover, the values of otolith weight for the specimens of *S. japonicus* and *B. belone* showed the best linear correlation to total length of the fish (r^2=0.941 and 0.904, respectively). The lowest values of the coefficient of determination for the TL - WO relationship was noted for *E. encrasicolus* (r^2=0.651).

DISCUSSION

The *sagitta* otoliths are the most widely used in comparative taxonomy studies because their form, weight, growth, consistency and chemical composition have a distinctive degree of interspecific variation; they are easily accessible structures as well (NOLF, 1985). Our results suggest that differences in otolith geometric measures are detectable in all five pelagic fish species. Even though there are many approaches to defining the otolith shape, we used three standard shape descriptors from the scientific literature (ROSIN, 2005): form factor (F_F), roundness (R_D) and aspect ratio (A_R). The results of this study show that the shape indices differed significantly in analysed species even though they indicate a similar pattern with maximal otolith length (Table 2 and Fig. 3). Namely, the aspect ratio (A_R) was in proportion to the maximal otolith length, while form factor (F_F) and roundness (R_D) were inversely proportional to it (Table 2). This similarity in otolith shape descriptors may come from the fact that all studied pelagic fish species have similar ecological traits and occupy the same ecological niche. According to PARMENTIER *et al.* (2001), fish occupying the same ecological niche show resemblances in otolith shape; pelagic fish species are known as fast swimmers and the shape of their otolith could be an element contributing to making the neurocranium lighter in order to reduce energy expenditure during swimming. On the contrary, in bentic, commensal and parasitic species, the swimming constraint is obviously weaker and does not act as a restricting factor on the otolith development. This is reinforced by their thicker otoliths. GAULIDE & CRAMPTON (2002) reported that there is evident separation of the shape component between most pelagic fish species and most deep-living species.

Results of this study indicate that otolith weight would be a more accurate indicator of somatic growth (Table 3). That could be explained by the fact that otolith weight is most sensitive to variations in growth rate and most closely related to changes in fish metabolism (BOEHLERT, 1985; REZNIK *et al.*, 1989; SECOR & DEAN, 1989; PAWSON, 1990; FLECHER, 1991). Moreover, otolith length measurements are compromised by high variability in otolith shape.

Concerning the findings in this study, it is evident that otolith shape variability encourages further research on verifying of the role of otolith geometric measurements in fish identification.

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Preliminarni podaci o morfologiji otolita pet pelagičnih vrsta riba iz Jadranskog mora (Hrvatska)

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SAŽETAK

U radu se iznose rezultati morfometrijske analize otolita brgljuna *Engraulis encrasicolus* (Linnaeus, 1758), srdele *Sardina pilchardus* (Walbaum, 1792), skuše *Scomber scombrus* Linnaeus, 1758, lokarde *Scomber japonicus* Houttuyn, 1780 i iglice *Belone belone* (Linnaeus, 1761). Za svaku je istraživanu vrstu analizirano po 30 jedinki koje potječu iz lovina ostvarenih na području istočnog dijela Jadrana tijekom razdoblja siječanj - prosinac 2005. godine. Na temelju analize triju faktora oblika otolita (F_F, R_D, A_R) je uočeno da postoji statistički značajna razlika između otolita promatranih pelagičnih vrsta riba. Značajna linearna korelacija je utvrđena između najveće dužine otolita (LO) i ukupne dužine tijela ribe (LT) kao i mase otolita (WO) i ukupne dužine tijela ribe (LT) - s porastom ukupne dužine tijela analizirane pelagične vrste se proporcionalno povećavala najveća dužina otolita i masa otolita.

Ključne riječi: morfologija otolita, pelagične vrste riba, Jadransko more