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Biometry of the sagittal otoliths for three demersal fish species from the Eastern Adriatic Sea (Montenegro)

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The otolith morphology, biometry and otolith size and fish length relationship of three demersal fish species: red mullet Mullus barbatus*, common pandora* Pagellus erythrinus *and hake* Merluccius merluccius *were examined. Based on otoliths relative sizes* P. erythrinus *and M. merluccius have Large otoliths, while* M. barbatus *has Small sagittal otoliths. The relationships between otolith variables and fish somatic growth were described by a linear function. All relationships were statistically highly significant (p<0.001). Otolith length (OL) was found to be the variable most strongly related to fish size, with 61.2 – 95.3 % of the variability, while otolith weight had the lowest coefficient of determination (r^2). No differences were found neither between left and right otoliths, nor between male and female otoliths in these relationships. For all species studied the relationships of otolith length, height and weight against total fish length were all found to have positive allometry, indicating that the accretion of otoliths is relatively faster than increase in fish body size. The shape indices of form factor (F_F), roundness (R_D), aspect ratio (A_R), circularity (C), rectangularity (R) and ellipticity (E) were calculated and the relationships between otolith length (OL) and shape indices were determined.*

Key words: somatic growth; sagittal otolith; shape and size of otolith; hake; red mullet; common pandora

INTRODUCTION

Marine demersal resources are represented by fish and other marine organisms that live or feed on or near the sea bottom. The European hake (*Merluccius merluccius*) and red mullet (*Mullus barbatus*), along with deep-water pink shrimp (*Parapenaeus longirostris*), represent main target species both in Montenegrin demersal fishery (IKICA *et al*., 2018; JOKSIMOVIĆ *et al*., 2019) and in the rest of Adriatic (UNEP-MAP-RAC/SPA 2015). Common Pandora (*Pagellus erythrinus*) is not the main target species in Montenegrin fisheries, but is one of the "main commercial species" in Montenegro, meaning that it is counted in the group of species representing 90% in weight of total landing in the country (GFCM, 2018). All three species studied fall into this category, due to their importance to the Montenegrin fisheries in terms of quantities and economic value, and are subject to sampling in the frame of the Annual Montenegro fishery data collection programme (DCF-DCRF) according to the GFCM DCRF methodology. The total landing of European hake in Montenegro increases sligthly each year with 47 tons in 2018 (20 tons in 2016, 36 tons in 2017). The situation is similar with red mullet, with a total catch of 42 tons in 2018 (15 tons in 2016, 36 tons in 2017), while the landing of common pandora was estimated at 5 tons in 2018 (Monstat 2019). Despite the high commercial importance of these species in Montenegro, they were not a research subject in previous studies. Available data refer to the length-weight relationships (JOKSIMOVIĆ, 1999; JOKSIMOVIĆ *et al*., 2007; DULČIĆ & KRALJEVIĆ, 1996; DULČIĆ & GLAMUZINA, 2006), growth parameters estimation (JOKSIMOVIĆ, 2001; JOKSIMOVIĆ *et al*., 2007; 2008; CARBONARA *et al*., 2018) and populations characteristics (JOKSIMOVIĆ, 2000; MANDIĆ *et al*., 2008; JOKSIMOVIĆ *et al*., 2004; PEŠIĆ *et al.,* 2011; PICCINETTI *et al.*, 2021; ZORICA *et al.,* 2020; VRGOČ *et al*, 2004; VRGOČ *et al.,* 2005).

The otoliths are one of the main biomineral structures of the internal ear in fish, and play a role in balance and hearing. They serve as a permanent record of the life history of individual (BELCHIER *et al*., 2004). The sagittas are the largest pair of otoliths in most bony fishes (PAXTON, 2000). The size, shape and otolith characteristics vary between species, and the otolith morphology can differ between populations of the same species in different locations (REICHENBACHER *et al*., 2009; OZPICAK *et al*., 2018). The morphology of otolith is influenced by ontogenetic development (CAPPOCIONI *et al*., 2011), size (HÜSSY, 2008, LOMBARTE & CRUZ 2007), sexual maturation (MÉRIGOT *et al*., 2007), sex (BOLLES & BEGG, 2000) as well as diet (MILLE *et al*., 2016). Environmental factors, such as water temperature, can also produce otolith growth variation, and therefore shape variability (CARDINALE *et al*., 2004; LOMBARTE & CRUZ, 2007)

Otolith morphology is considered to be an efficient tool for fish species determination and differentiation between different fish stocks or populations when comparative genetic data are not available (ZHUANG *et al*., 2014; BOSTANCI *et al*., 2015), in ecomophological studies (AGUIRRE & LOMBARTE, 1999; CAPPOCIONI *et al*., 2011, CARDINALE *et al*., 2004), and they can serve as an index for environmental studies (DEHGHANI *et al*., 2015). With the development of image analysis system, the studies of otolith morphology have become increasingly more important in numerous ichthyological studies. Only two studies regarding the morphology and shape contour of fish species from Adriatic Sea have been conducted so far, and were related to pelagic (ZORICA *et al*., 2010) and juvenile fish species (FERRI *et al*., 2018). However, until now little information was available on demersal fish species otoliths from Montenegrin waters (South-Eastern Adriatic), and the aim of this study was to analyse the relationship of morphometric measurements of three demersal fish species and their otolith shape variations in order to determine the possible patterns between body size and otolith morphology. The other purpose of this study was to estimate the biometric relationship between fish body measurements and otolith measurements for the selected species and determine which measurements are intercorrelated.

MATERIAL AND METHODS

Study area

Eastern Adriatic is characterized by a relatively narrow continental shelf and a steep slope, reaching maximum depth of 1,233 m (TEŠIĆ, 1962). The total length of Montenegrin coast is 294 km, 112 km of which represents the coast of Boka Kotorska Bay and 182 km face the open sea. A large part of Montenegrin coastline consists of precipitous rocky cliffs intersected with beaches that become more prevalent towards the south, culminating in a long stretch of sandy beach (Velika plaža in Ulcinj, 13 km in length, the longest Montenegrin beach) extending to the Albanian border at the mouth of the Bojana River. The total surface area of internal waters is 362 km^2, while the surface of territorial waters is 2,098.9 km^2 (Fig. 1).

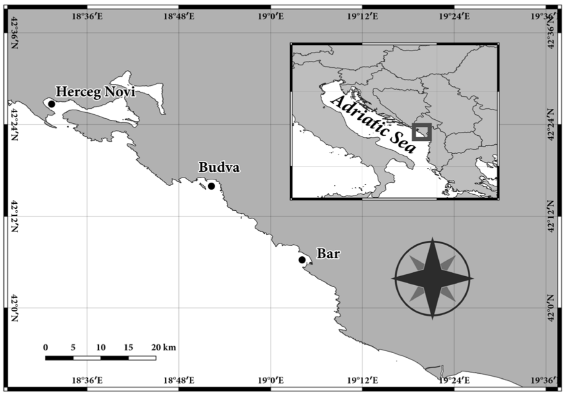

Fig. 1. Map of the study area

Sample collection

Otoliths of three demersal fish species, *M. barbatus, P. erythrinus* and *M. merluccius*, originating from the Montenegrin waters, were examined. The data was collected within the frame of Data Collection Reference Framework (DCRF) programme (GFCM, 2018) performed in Montenegro since 2017 through support by Ministry of Agriculture and Rural Development (DCF-DCRF, 2020). Within the DCRF, several fishing fleet segments are sampled on a quarterly basis, among them segments using demersal trawl nets, fixed nets, i.e. gillnets and trammel-nets (DCF-DCRF, 2020). Samples were collected during direct on-board observation. Total catch of each species was weighted, and subsamples collected for further laboratory analysis. Length of individuals was measured to the nearest 0.1 cm, and weight of individuals measured to the nearest 0.01 g. Specimens were dissected to determine their sex and remove the otoliths. Sagittal otoliths were extracted under a NIKON stereomicroscope, under 50× magnification, and washed in distilled water. After drying the otoliths with filter paper, each otolith was placed on a microscopic plate, with the convex side facing up and measured to the nearest 0.001 mm under Carl Zeiss Axio Scope A1 microscope and computer-connected camera system. Four morphometric characteristics were measured: the otolith length (OL, mm), otolith width (OW, mm), otolith area (A, mm^2) and otolith perimeter (P, mm). These measurements allowed the six shape indices to be calculated: form factor (F_F), roundness (R_D), aspect ratio (A_R), circularity (C), rectangularity (R), and ellipticity (E) (TUSET *et al*., 2003; LORD *et al*., 2012). The F_F is the inverse ratio of the squared perimeter of a circle of the same surface; R_D is the ratio between the actual area and the area of the circle of the same length while A_R is the ratio between the otolith length and otolith width (ZORICA *et al*., 2010). The otolith weight (OW) was recorded by Mettler Toledo XPE206 analytic balance to the nearest 0.01 mg. For all measurements, descriptive statistics (mean, standard error and minimal and maximal values) were calculated. The relationships between otolith measurements (length, height and weight) and fish body lengths were estimated using the linear regression model applied the method of least squares (SOKAL & ROHLF, 1981). All otolith shape indices were first analysed for normality with Kolmogorov-Smirnov and Shapiro-Wilk tests. Analysis of variance (ANOVA) was used to compare indices between analysed species. Tukey HSD post-hoc test was used to explore differences between multiple group means in cases when ANOVA showed statistically significant differences. Moreover, relationships between shape indices and sagittal otolith length were evaluated. The strength of each relationship was evaluated from the determination coefficient (r^2). The otolith relative size value of each species was calculated using the index $O_R = 1000 \times OL \times TL^{-2}$. According to O_R, otoliths were grouped into four categories: very small ($O_R <$ 0.10), small (0.10–0.32), medium (0.33–065) and large (>0.65) (LOMBARTE & CRUZ, 2007). The statistical procedure was completed using SPSS Statistics 20 and STATISTICA 7.0.

RESULTS

The sagittal otoliths of *M. merluccius* (35.71% female, 64.28% male), *M. barbatus* (70% female and 30% male) and *P. erythrinus* (68.08% female and 46.87% male) were evaluated. Table 1 shows a number of individuals studied and their length and weight distribution. There were no statistically significant differences found between left and right otoliths

Table 1. Sample size (N), total length (TL) and weight (W) range of three demersal fish species used in this study

Species	Common name	N	TL range (cm)	W range (gr)
Merluccius merluccius	Hake	56	14.5-32.3	17.35-216.32
Mullus barbatus	Red mullet	50	11.5-20.5	16.25-99.24
Pagellus erythrinus	Common pandora	47	14.0-25.7	30.37-196.52

Table 2. Descriptive statistics of morphometric characteristics of sagittal otoliths of three demersal fish species form Montenegro. Shown are mean value and standard error for maximum otolith length – OL, otolith width – OH, otolith weight – OW, area – A and perimeter – P

Species	OL (mm)	OH (mm)	OW (gr)	A (mm^2)	P (mm)
Merluccius merluccius	12.05±1.89	5.13 ± 0.76	0.071 ± 0.028	42.36 ± 12.51	30.48± 4.53
Mullus barbatus	3.07±0.36	2.27 ± 0.27	0.033 ± 0.001	4.78 ± 0.98	9.34± 1.21
Pagellus erythrinus	7.88±1.27	5.76 ± 0.88	0.089 ± 0.042	29.46 ± 1.93	22.50± 3.59

Table 3. Regression parameters of the relationship between otolith dimensions and the total body length of three demersal fish species from Montenegro. TL-total body length, OL-otolith length, OH-otolith height, OW-otolith weight, r^2-correlation coefficient, p-level of significance

Species	Relationship	Regression equation	r^2	p
	OL/TL	OL=1.1406+0.0457TL	0.9531	$P<0.001^{***}$
Merluccius merluccius	OH/TL	OH=0.8022+0.0181TL	0.9298	$P<0.001^{***}$
	OW/TL	OW=-0.0902+0.0007TL	0.9084	$P<0.001^{***}$
	OL/TL	OL=1.133+0.0126TL	0.7099	$P<0.001^{***}$
Mullus barbatus	OH/TL	OH=0.827+0.0094TL	0.6823	$P<0.001^{***}$
	OW/TL	OW=-0.0284+0.0003TL	0.6121	$P<0.001^{***}$
	OL/TL	OL=0.604+0.039TL	0.8846	$P<0.001^{***}$
Pagellus erythrinus	OH/TL	OH=0.851+0.026TL	0.8393	$P<0.001^{***}$
	OW/TL	OW=-0.146+0.001TL	0.8429	$P<0.001^{***}$

(p>0.05), or between otoliths of males and females (p>0.05). All otoliths morphometric measurements for the species studied are given in Table 2. Otoliths of *M. barbatus* had the smallest values of all observed parameters, while the otoliths of *M. merluccius* were the largest and with the highest otolith extent area (Table 2). The otolith relative size (O_R) ranged between 0.21-0.84. According to O_R size categories defined in the methodology, *M. barbatus* are considered to have Small otoliths, while *M. merluccius* and *P. erythrinus* have Large otoliths (Table 4). The otolith length, weight and height were linearly related to total fish length for the studied species. Their regression parameters were highly significant (p<0.001). The best fit for all three fish species were for the TL-OL relationships, but some differences were also noticed. Namely, all otolith–fish body biometric relationships (i.e. TL-OL, TL-OH, TL-OW) had high values for *M. merluccius* and *P. erythrinus.* The lowest values of coefficient of determination for all examined biometric relationships were found for *M. barbatus* (Table 3).

According to the mean values of six examined shape indices the otoliths of *M. merluccius* had the smallest values of F_F and R_D while the values of A_R, C and E were the highest. *P. erythrinus* has the highest value of F_F indicating flattest edges compared to other species, while *M. merluccius* has the most uneven edges. The otoliths of *M. merluccius* were the most elongated while the otolith of *M. barbatus* was the most rounded among the examined demersal fish spe-

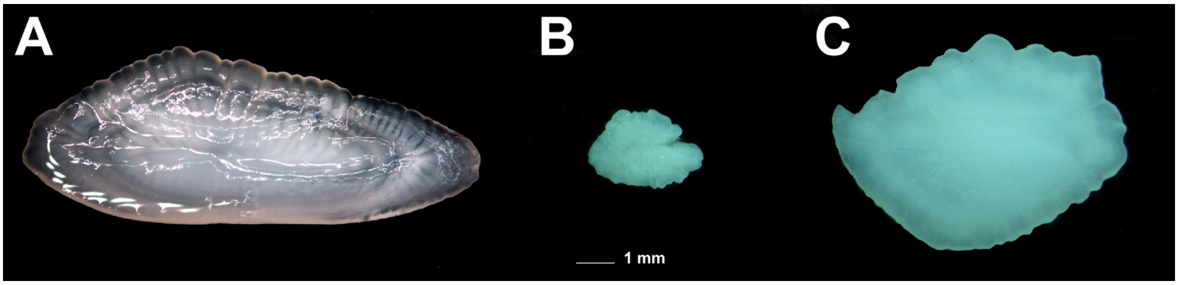

Fig. 2. Images of the saggita of three demersal fish species: A- M. merluccius; B - P. erythrinus C - M.barbatus

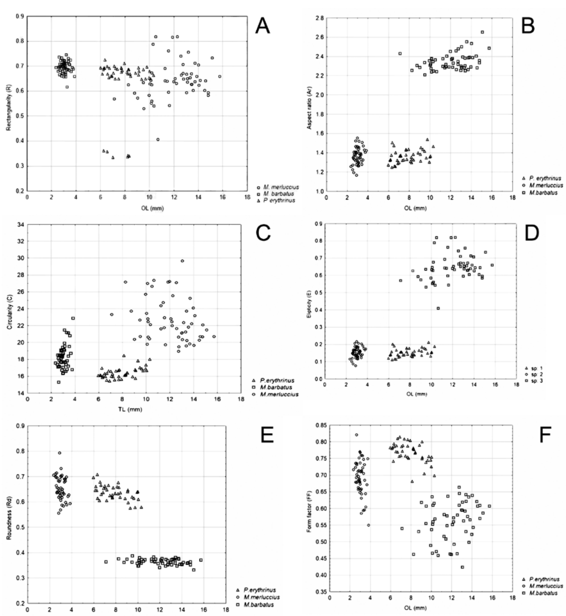

Fig. 3. Form factor (A), Roundness (B), Aspect ratio (C), Circularity (D), Rectangularity (E) and Elipticity (F) vs. maximal otolith length of tree demersal fish species

cies (Fig. 2). ANOVA showed that there were significant differences among shape indices of all analysed demersal fishes ($p<0.05$).

The values of shape indices showed correlation with otolith lengths for studied species, but the correlation values differed for different shape indices. Namely, when otoliths length increased, values of R_D and C decreased, while F_F, A_R, R and E increased for the *M. merluccius* and *P. erythrinus*. For *M. barbatus*, when otolith length increased, F_F, Rd and R decreased, while A_R, C and E increased (Table 4, Fig. 3).

DISCUSSION

This study contributes to the understanding the morphological features of saggital otoliths of demersal fish species from Eastern Adriatic Sea. The present study analysed relationships between otolith length, height and weight and fish length using a linear function. The results obtained suggest there are no statistically significant differences between left and right otoliths for the studied fish species. When considering application of these results we have to emphasize that all future analyses would be standardized by using measurements from one selected otolith, left or right. The lack of statistically significant differences between left and right sagitta indicates that otoliths on either body side are equally usable for fish-size estimations (BATTAGLIA *et al*., 2010; MEHANNA *et al*., 2016; PARK *et al*., 2018; YILMAZ *et al*., 2015). Moreover, results of this study indicate there are no differences in relationships between otolith biometry and fish size between sexes. Contrary to these results, differences in otolith variables were observed between sexes for some species (VALLISNERI *et al*., 2008; BOSTANCI *et al*., 2012; KONTAS & BOSTANCI, 2015).

There are many studies that determine the relationships between otolith biometry and fish sizes (PAXTON, 2000; ZORICA *et al*., 2010; JAWAD *et al*., 2012, DEHGHANI *et al*., 2015). For most species, the relationship between otolith length and fish length can be described by a simple linear regression (HARVEY, 2000). In present study, otolith length showed the strongest and otolith weight the weakest relationship to fish length in all three species. These results are consistent with literature data for demersal fish species from genera Symphodus and Coris (ŠKELJO & FERRI, 2012). Contrary to this, results of the study of pelagic fish species indicate that the otolith weight could be a more accurate indicator of somatic growth (ZORICA *et al*., 2010).

Otolith development occurs under dual regulation: genetic conditions regulate the form of the otolith, while environmental conditions, mainly temperature, regulate the quantity of material deposited during the formation of the otolith (LOMBARTE & LLEONART, 1993). In order to describe in detail the shape of the otoliths, six shape descriptors from scientific literature were used (TUSET *et al.,* 2003; LORD *et al*., 2012). In addition to the shape analysis, otolith relative sizes were also estimated. Variation in size and shape of sagittal otoliths has long been used as taxonomic features (NAFPAKTITIS & PAXTON, 1969). The variation in size of sagittal otoliths is significant and apparently correlated with taxonomic group, habitat, depth and luminescence (PAXTON, 2000; LOMBARTE & CRUZ, 2007). Also, environmental abiotic factors, such as temperature and salinity, are also known to influence otolith shape (LOMBARTE & LLEONART, 1993), and spatial variations in otolith shape are often interpreted as resulting from habitat differentiation (MORAT *et al*., 2012). However, such variation in abiotic factors is generally related to differences in prey categories available to an individual predator, such that geographical variations in diet composition could also generate geographical variations in otolith shape (VIGNON, 2012). The current study results show that shape indices have a different relationship pattern with maximum otolith length. More specifically, $R_{D,}$ A_R and E show similar patterns in relations to maximum otolith length for all species, while $F_{F,}$ C and R show a different morphological pattern. Namely, when otolith lengths increased, the values of F_F and R of *M. barbatus* decreased, while for *M. merluccius* and *P. erythrinus* these values increased. On the other hand, when C for *M. barbatus* increased, the values for *M. merluccius* and *P. erythrinus* decreased (Table 5, Fig. 3). Contrary to these results, the shape indices of the pelagic fish species show a similar pattern in relationship with maximum otolith length (ZORICA *et al*., 2010). The 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 (ZORICA *et al*., 2010). Obviously, difference in otolith shape reflects the characteristics of the individual habitat. GAULIDE & CRAMPTON (2002) suggest that there is evident separation between most pelagic fish species and most deep-living species. LOMBARTE & CRUZ (2007) found a relationship between otolith size composition, habitat and depth. The epipelagic community was characterized by species with very small otolith sizes, while in the demersal communities, the proportion of species with large sagittas increased with depth (LOMBARTE & CRUZ, 2007). CAPPOCCIONI *et al*. (2011) reported the occurrence of changes in the morphology of the European eel (*Anguilla anguilla*) otoliths as a function of growth and the different habitat ecology (a river and two brackish water lagoons). Also, the shape outline of otoliths changes during ontogenesis, along with the increase in animal size (CAPPOCCIONI e*t al*., 2011).

There is a clear relationship between taxonomic groups and otolith relative size (NOLF, 1985). Also, numerous endogenous factors can influence the otolith relative size, such as phylogeny (GAEMERS 1984; NOLF 1985), adaptive aspects related with the inner ear functions (PAXTON 2000; PARMENTIER *et al*., 2001), or specialization in acoustic communication (LOMBARTE & CRUZ, 2007). In this study two species from order Perciformes (*M. barbatus* and *P.erythrinus*) and *M. merlucius* from Order Gadiformes were studied. The results of otolith relative size analysis show that sagittas of *M. merluccius* and *P. erythrinus* can be classified as Large (O_R = 0.73 and O_R = 0.82 respectively) while sagittal otoliths of *M. barbatus* could be considered Small (O_R=0.21). Some taxonomic group like Gadiformes tend to have medium or large sagittae, while Perciformes, with their great ecological and morphological variability, exhibit a wide range of otolith sizes (LOMBARTE & CRUZ, 2007). These results also agreed with previously reported large-sized otoliths for *P. erythrinus* and small for *M. barbatus* (LOMBARTE & CRUZ,

Table 4. Descriptive statistics of six shape indices (F_F-form factor, R_D-roundness, A_R-aspect ratio, C-circularity, R – rectangularity, E-elipticity) of three demersal fish species from Montenegro. Shown is otolith relative size- O_R, size category in relation to O_R (S-small, L-large) also (C_o)

Species	F_F	R_D	A_R	C	R	E	O_R	C_0
Merluccius merluccius	0.56±0.06	0.36±0.01	2.35±0.09	22.63±2.54	0.64±0.08	0.40±0.02	0.73	L
Mullus barbatus	0.69±0.06	0.65±0.05	1.35±0.10	18.43±1.89	0.68±0.02	0.15±0.04	0.21	S
Pagellus erythrinus	0.71±0.13	0.59±0.11	1.36±0.06	18.63± 1.12	0.63± 0.11	0.15±0.02	0.84	L

Table 5. Coefficient of correlation (r) between the maximum length of fish otolith and shape indices (F_F-form factor, R_D-roundness, A_R-aspect ratio, C-circularity, R – rectangularity, E-elipticity) of three demersal fish species from Montenegro

Species	F_F	R_D	A_R	C	R	E
Merluccius merllucius	0.2804*	-0.2275	0.3590**	-0.2692*	0.236	0.356*
Mullus barbatus	-0.4577**	-0.5020***	0.3527*	0.4694**	-0.3945**	0.3403*
Pagellus erythrinus	0.0372	-0.0244	0.3012*	-0.0744	0.0556	0.3098*

2007). However, it should be emphasized that the values of the relative size of otoliths of *P. erythrinus* and *M. barbatus* from Adriatic Sea were higher than in the research of LOMBARTE & CRUZ (2007) (O_R = 0.69 and O_R = 0.16, respectively). On the other hand, large otoliths of *M. merluccius* are in contrast with reported medium otoliths (O_R=0.53) for hake from north-western Mediterranean Sea (Balearic Sea) (LOMBARTE & CRUZ, 2007). The same authors suggested that the importance of acoustic communications is correlated with a moderate to large otolith size in a benthic environment in order to compensate for the reduction of light with depth. Closely related species or populations from temperate or shallower waters have relatively larger otoliths than those from colder or deeper water (GAULDIE 1993; TORRES *et al.*, 2000).

In summary, this study demonstrated the variation in the size and shape of sagittal otoliths of demersal fish and confirmed its potential as a powerful tool for fish identifications and fish size estimation, important factors in fish stock monitoring and management. In addition, different patterns of otolith shape descriptors imply that morphology variations were correlated with ecological and environmental variations in depth and substrate type more than phylogenetics relationships. It is suggested that future studies should concentrate on the relationship between otolith shape and size with environmental factors.

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Odnos dužine i maksimalnog obujma za 24 riblje vrste u sjevernom Egejskom moru (istočno Sredozemno more)

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SAŽETAK

Poznavanje morfoloških odnosa, a posebno onih koji se tiču maksimalnog obujma tijela ribe (G) s ukupnom dužinom (TL) potrebito je za određivanje selektivnosti alata, a posebno za tehničke mjere za izbjegavanje hvatanja nedoraslih jedinki. Ova studija se odnosi na 24 morske vrste koje se iskorištavaju u priobalnoj ribolovnoj floti u Egejskom moru (istočno Sredozemno more), za 6 od kojih se TL-G odnosi prvi put spominju u Sredozemnom moru i susjednim morima.

Uzorci su prikupljani sezonski, od travnja 2016. do veljače 2017. Koeficijenti linearne regresije obujma tijela u tri položaja tijela (G_{eye}, posteriorno od oka; G_{head} na stražnjem kraju operkuluma; G_{max} na maksimalnoj visini tijela), s ukupnom duljinom procijenjene su za svaku vrstu i za skupine oblikovane kada su G_{eye}, G_{head} i G_{max} ucrtani u odnosu na ukupnu duljinu za sve vrste zajedno.

Utvrđene su statistički značajne razlike između tri skupine (ANCOVA, P<0,001). Usporedba odnosa ukupne duljine i obujma tijela za 18 vrsta koje su prethodno istraživane u različitim geografskim područjima Sredozemlja i susjednih mora, pokazala je razlike uglavnom s rezultatima iz portugalskih voda za određene populacije vrsta. Na temelju dobivenih jednadžbi izračunat je maksimalni obujam (G_{max}) koji odgovara minimalnoj referentnoj veličini očuvanja (MCRS) i ukupnoj dužini pri zrelosti (Lm) za svaku vrstu. Identificirane veličine oka koje odgovaraju G_{max} vrijednostima bile su dosta veće od minimalne zakonske veličine oka za mreže stajačice i unutarnju ploču troslojnih mreža, što ukazuje da relevantni trenutačni propisi o ribarstvu ne mogu ispuniti zahtjeve za održivo iskorištavanje ribljih resursa.

Ključne riječi: morfologija ribe; upravljanje ribarstvom; mreža stajačica; duljina pri zrelosti (L_m); Minimalna referentna veličina očuvanja (MCRS); trostruka mreža stajačica