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Morphometric parameters of the sagitta otolith among four carangids species in the Persian Gulf

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Abstract

Otoliths are considered as very useful structures of fishes for various studies. This investigation aimed to compare the morphometric parameters between the right and left otoliths of male and female specimens of four species of the fishes belonging to the family Carangidae from the northern Persian Gulf. The morphometric parameters such as weight, length, width, area and perimeter of sagittal otoliths were measured and compared between males and females. The relationships between otolith morphometric parameters (length, width, weight) and fork length were also examined. According to the results from independent sample t-test, significant differences were found in length, width, and area of otoliths between males and females of *Carangoides malabaricus*, whereas no significant differences were found for three other species. The otolith weight of *Carangoides coeruleopinnatus* and *Megalaspis cordyla* were significantly different between left and right otoliths (p<0.05). Likewise, significant differences were found in the otolith area of the right and left otoliths of *Carangoides. coeruleopinnatus* (p<0.05). Furthermore, no significant differences were found in the shape indices including form factor (FF), roundness (RD), aspect ratio (AR), circularity (C), rectangularity (R) and elipticity (E) between the right and left otoliths. The results suggest that the length and weight of sagittal otoliths are likely suitable indicators for fish fork length in all studied species.

Keywords: Sagitta, Carangidae, Shape indices, Morphometric, Persian Gulf

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Introduction

Various morphometric parameters have been used to identify intraspecific and interspecific differences of fishes (Ihssen *et al*., 1981). Different hard parts of fishes are used in morphometric studies including otoliths, scales and other skeletal structures (Begg and Waldman, 1999). Otoliths have been used to study the morphometric parameters of fishes in several previous studies (Lombarte and Lleonart, 1993; DeVries *et al*., 2002; See *et al*., 2016).

The family Carangidae is one of the major families of bony fishes, with a worldwide distribution, and about 140 species belonging to 32 genera (Abdussamad *et al*., 2013). This family consists of 21 genera and 50 species in the Persian Gulf and Oman Sea (Valinassab, 2013).

Otoliths are calcium carbonate and crystallized structures inside the inner ear of fishes which are used as indirect tools for studying the fish populations and assessing the relationship between the fishes and their environment (Lord *et al*., 2011; Zengin *et al*., 2015).

Due to some specific characters such as size, specificity of morphological features, ease of access, chemical composition, microstructure, ontogenic phase in which they are formed, and dependency of these characteristics on the variations in environmental factors are one of the most useful anatomic structures of fishes for various studies (Tuset *et al*., 2008). Otoliths may be used in various fields of biological studies such as: age determination, fish growth and population dynamics (Vignon and Morat, 2010; Wells *et al*., 2013). Biological functions of otoliths enable the inner ear to mediate the senses of hearing and balance (Popper *et al*., 2005). Otoliths also have important roles in various fields of scientific researches including: analysis of otolith chemical properties to distinguish fish life histories; analysis of diet for cetacean, birds, pinnipeds and piscivorous fishes and archaeological studies (See *et al*., 2016).

Teleost fish have three pairs of otoliths including the lapillus, sagitta and asteriscus. The sagittae is the largest otolith in most teleosts and show variable morphological and topographical properties among the species. Since otoliths are hard structures, they are resistant against some degree of dissolution, and the characteristic morphology of the sagitta can be used for species identification. Moreover, their resistance makes it possible to use them in the identification of prey in stomach contents of fishes, birds or mammals (Rivera Felix *et al*., 2013). The life history properties of otoliths allow accurate estimates of age and growth on both the daily and yearly scale (Zengin *et al*., 2015).

In morphological studies, data on otolith shapes were found to be very valuable for identification purposes. In some cases, the morphological features of otoliths have been used for discrimination of populations and other intraspecific features. In particular, it is found that variation of sagittal otoliths shape may be attributed to the genetic, ontogenetic and ecological factors. Accordingly, otoliths shape analysis is widely used in identification of different groups such as

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mature and juvenile specimens, populations, spawning aggregations and genders (Tyagun *et al*., 2013). Since otoliths are species-specific and barely variable with growth, the analysis of otolith shape is considered to be a useful tool to determine the stock identity (Farias *et al*., 2009).

Otoliths structure and development are influenced by external environmental conditions as well as the physiological condition of fish. These characteristics can vary among populations; therefore otoliths may show properties that are stock specific. Variation in otolith shape is frequently used for discriminating between the fish stocks (Agüera and Brophy, 2011; Zengin *et al*., 2015; See *et al*., 2016). In addition, the methods that are developed based on otoliths properties are more reliable than the ones that use external morphometric traits, because they are not affected by short-term variations in fish physiological state or by standard tissue preservation techniques, and their appearance and shape often differ geographically (Farias *et al*., 2009).

In recent years, methods for the determination of intraspecific and interspecific differences in metric characteristics of otoliths have been developed and tested (Tyagun *et al*., 2013).

This study aimed to compare morphometric parameters between right and left otoliths of male and female fishes among four species of Carangids from the northern Persian Gulf.

Materials and methods

Sampling sites

Sampling was undertaken quarterly from August 2015 to July 2016 at three sites in the northern parts of Persian Gulf in south of Iran (Fig. 1). Trawling is the dominant fishing method in the study area and it has been used as the sampling approach exclusively in the present study. A total of 138 specimens of the studied species including *Megalaspis cordyla* (Linnaeus, 1758) [n=32], *Carangoides coeruleopinnatus* (Rüppell, 1830) [n=40], *Carangoides chrysophrys* (Cuvier, 1833) [n=34], and *Carangoides malabaricus* (Bloch and Schneider, 1801) [n = 32] were collected in each season from these three sites.

GPS was used to determine the geographic position of sampling sites. Geographical coordinates of sampling locations and landing sites are as followings: Sub-region A from Kish Island (54˚02′ E) to Farur Island (54˚29′ E), sub-region B from Farur Island to west of Qeshm Island (55˚16′ E) and the sub-region C from Qeshm to Hengam Islands (55˚50′ E).

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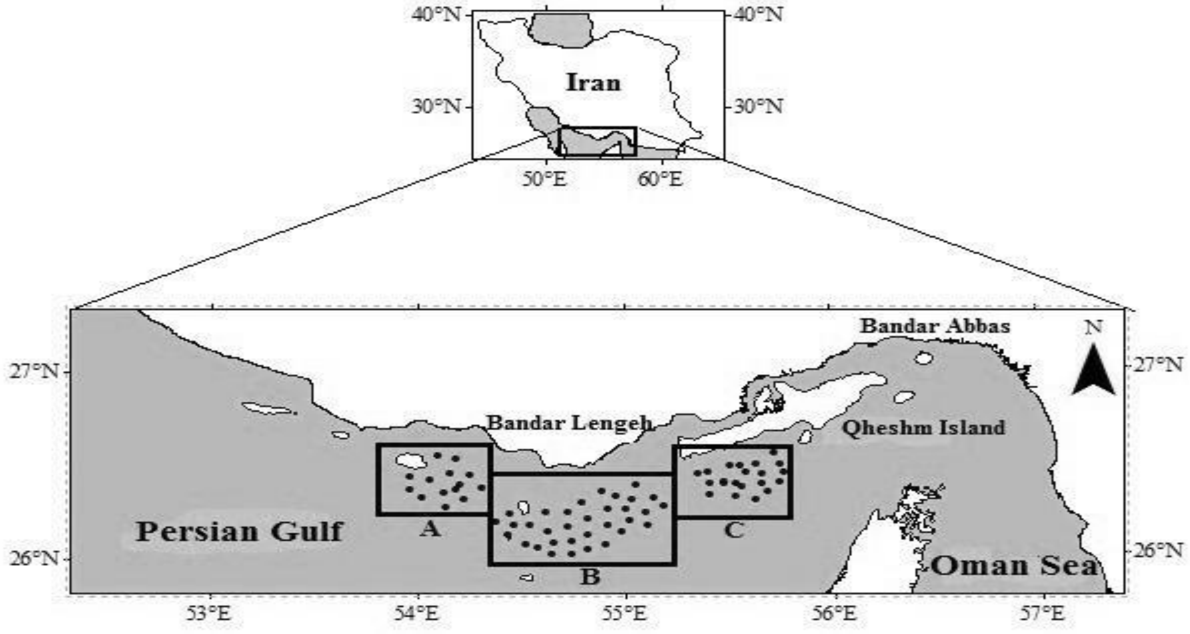

Figure 1: The map of sampling sites in the Northern Persian Gulf.

Morphometry

First, fork length (FL) was measured to the nearest±1 mm and body weight to the nearest±1 g for all specimens. Sex and maturity of the sampled individuals were determined by macroscopic examination of gonads.

The otolith extraction protocol is based on several steps (Trojette *et al*., 2015). Sagittal otoliths were removed and cleaned with ethanol 70%. Left and right sagitta were dried and preserved in an Eppendorf tube. Then, the otolith weight was measured using digital balance to the nearest 0.0001 g.

Sagittal otoliths were placed on microscope slides for observation and photography as the following: the rostrum to the left and sulcus acusticus upward (Fig. 2). Both left and right otoliths were photographed on a black background using a stereoscopic binocular microscope (ZTX-3E). Digital otolith images were taken by a CCD and saved to a PC with a digital resolution 150 dpi (10 Mega pixels) in ".jpg" and ".tft" format. The tft format is used by the camera related program (KEview) which can be used to get the angle of the pictures we need to rotate each to align in a unique form.

In order to compare the left and right saggita, the morphometric parameters including otolith length (OL, mm), otolith width (OW, mm), area (A, mm^2), perimeter (P, mm) and otolith weight (WO, g) were recorded using Dist Morphometric software. Area (A) is the total number of white pixels in the binary otolith image; perimeter (P) is the number of pixels in a 1 pixel wide outline enclosing the white area. Otolith length and otolith width are measured as the major axis and minor axis, respectively (Agüera and Brophy, 2011).

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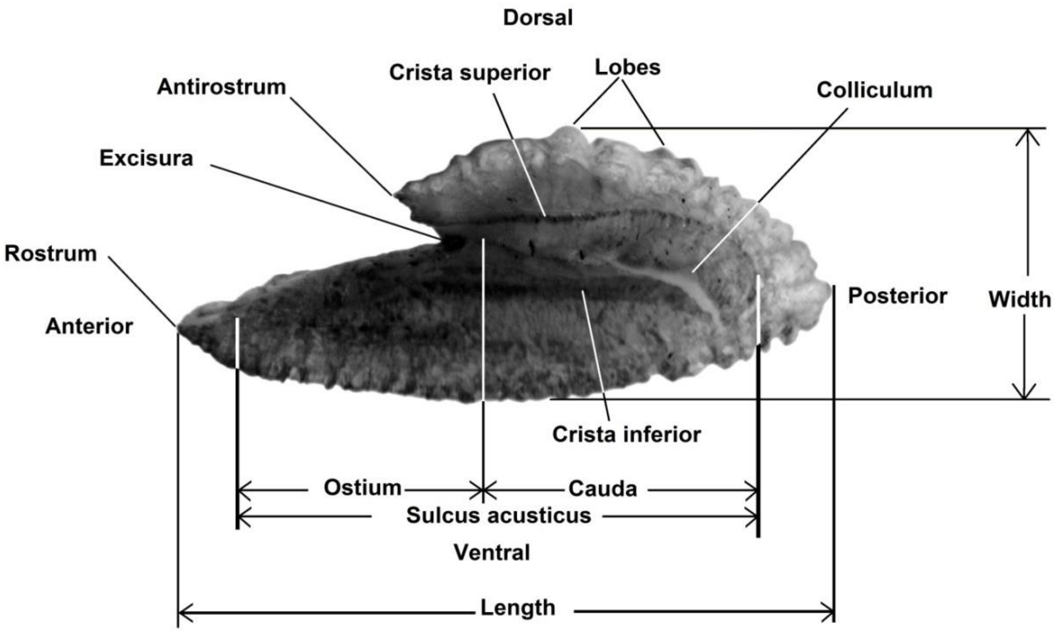

Figure 2: Proximal surface of sagittal otolith of *Carangoides chrysophrys.*

These measurements allowed the calculation of six shape indices: form factor (FF), roundness (RD), aspect ratio (AR), circularity (C), rectangularity (R), and ellipticity (E) (Tuset *et al*., 2003; Ponton, 2006) (Table 1).

Table 1: Size parameters and size based shape indices with calculation formulas.

Size Parameters	Size Based Shape Indices
Area (A)	Circularity (C)=P^2/A
Perimeter (P)	Rectangularity (R)=A/(OL*OW)
Otolith Length (OL)	Form-Factor (FF)=(4πA)/P^2
Otolith Width (OW)	Roundness (RD)=(4A)/(πOL^2)
	Ellipticity (E)=(OL-OW)/(OL+OW)
	Aspect Ratio (AR)=OL/OW

Determining the form factor as a way to estimate irregularity of surface area, roundness and circularity provides information on the similarity of various features with regard to a perfect circle; rectangularity refer to the variations in length and width with respect to the area, and elipticity specifies whether the changes in the axes are proportional (Tuset *et al*., 2003).

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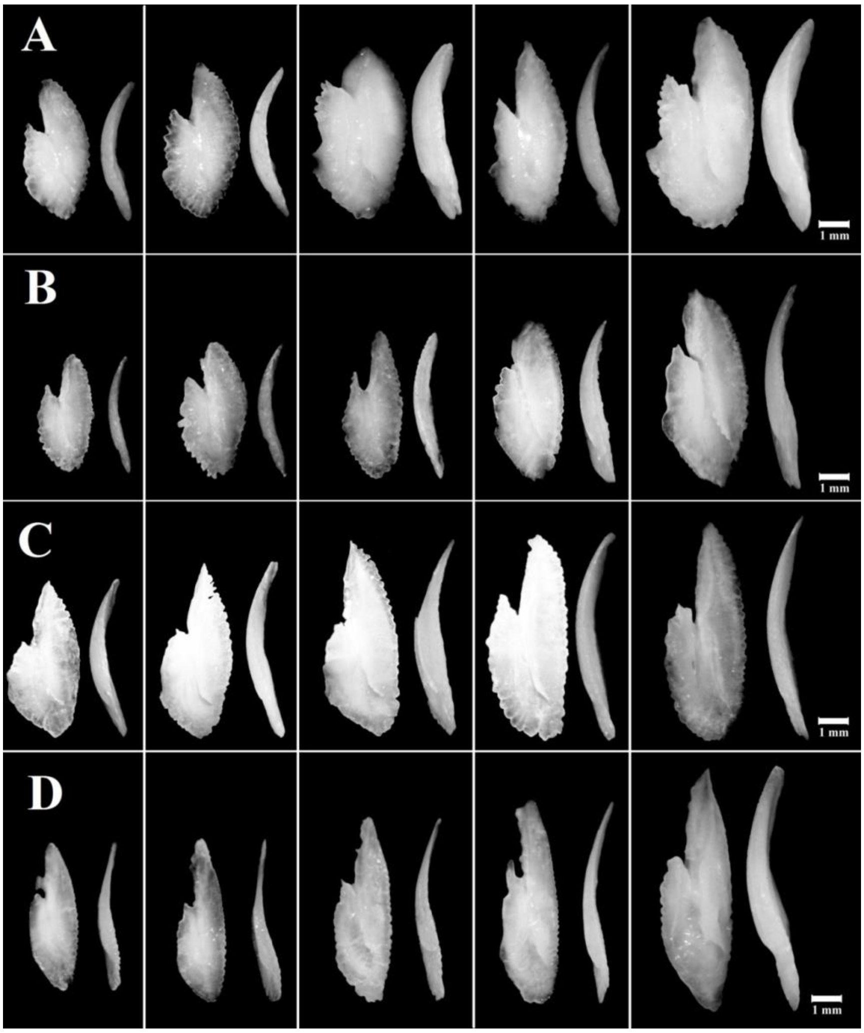

Figure 3: Dorsal and medial views of left sagitta from each carangid species using a dissection microscope (× 10). A) *Carangoides malabaricus,* B) *Carangoides chrysophrys,* C) *Carangoides coeruleopinnatus* and D) *Megalaspis cordyla.*

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Data Analysis

Statistical analyses were performed using SPSS V20.0 software. Normal distribution of the data on morphometric parameters from each location was checked according to the Kolmogorov-Smirnov test using Lilliefors modification. If the data had normal distribution, then, the statistical differences among the species and locations were tested using independent sample t-test. Otherwise, if the data was not normally distributed and none of the transformation methods provided a guarantee of a normal distribution,, the significant difference was tested by using non-parametric Mann-Whitney U test. Differences between left and right otoliths were tested using a paired t-test. Mann-Whitney U test was applied in order to determine differences between male and female otoliths.

Results

Table 2 presents descriptive statistics (mean, standard deviation, standard error, minimum and maximum values), and results from paired sample t-test for left and right sagitta of *C. caeruleopinnatus, C. chrysophrys*, *C. malabaricus* and *M. cordyla*. No significant differences were found in the otolith length, width, weight, perimeter and area between left and right otoliths of two species *C. crysophrys* and *C. malabaricus* (p>0.05). The otolith weight of *C. coeruleopinnatus* was significantly higher than that of *M. cordyla* (p<0.05). Likewise, a significant difference was found in the otolith area between right and left otoliths of *C. coeruleopinnatus* (p<0.05).

Table 2: Summary of descriptive statistics and paired t-test results for left and right Sagitta otoliths of *C. caeruleopinnatus, C. chrysophrys, C. malabaricus* and *M. cordyla.*

Otolith variables	Mean		SE		SD		Min.		Max.	
	L	R	L	R	L	R	L	R	L	R
Carangoides caeruleopinnatus										
Weight (g) p<0.05*	0.0176	0.0177	0.001	0.001	0.0063	0.0063	0.0051	0.0049	0.0259	0.0257
Length (mm) p>0.05	6.2572	6.206	0.0982	0.0986	0.6209	0.6234	4.82	4.72	7.33	7.23
Width (mm) p>0.05	2.550	2.559	0.0338	0.0339	0.2146	0.2143	2.11	2.09	2.94	2.99
Area (mm^2) p<0.05*	10.307	10.221	0.2529	0.2492	1.5993	1.5762	6.53	6.44	13.43	13.38
Perimeter (mm) p>0.05	17.473	17.367	0.3175	0.3245	2.0083	2.0526	12.97	13.05	21.92	22.62
Carangoides chrysophrys										
Weight (g) p>0.05	0.0091	0.0093	0.0007	0.0007	0.0041	0.0041	0.0047	0.0042	0.0203	0.0205
Length (mm) p>0.05	5.4606	5.4785	0.1264	0.1279	0.7372	0.7458	3.75	3.74	6.61	6.53
Width (mm) p>0.05	2.3609	2.3476	0.049	0.047	0.2899	0.2741	1.84	1.82	2.93	2.85
Area (mm^2) p>0.05	8.57	8.56	0.337	0.345	1.9648	2.0158	4.46	4.44	12.67	12.38
Perimeter (mm) p>0.05	15.8338	15.7812	0.3629	0.3664	2.1159	2.1365	11.43	11.43	19.95	20.43
Carangoides malabaricus										
Weight (g) p>0.05	0.0132	0.0130	0.0009	0.0009	0.0051	0.0050	0.0059	0.0061	0.0260	0.0249
Length (mm) p>0.05	5.7106	5.6569	0.1144	0.1195	0.647	0.676	4.57	4.42	6.89	6.78
Width (mm) p>0.05	2.8316	2.8558	0.0596	0.0636	0.3373	0.3598	2.26	2.25	3.62	3.73
Area (mm^2) p>0.05	10.5125	10.4756	0.4097	0.4364	2.3176	2.4684	6.76	6.91	16.05	16.09
Perimeter (mm) p>0.05	16.8594	16.7797	0.3504	0.3697	1.9822	2.0913	13.53	12.82	21.76	21.15
Megalaspis cordyla										
Weight (g) p<0.05*Length	0.0052	0.0051	0.0003	0.0003	0.0019	0.0018	0.0027	0.0029	0.0092	0.0094
(mm) p>0.05	6.3928	6.3356	0.1474	0.1399	0.8337	0.7914	4.69	5.01	7.86	7.83
Width (mm) p>0.05	1.9366	1.9484	0.0373	0.0384	0.2111	0.2172	1.61	1.62	2.39	2.46
Area (mm^2) p>0.05	8.2244	8.1291	0.3325	0.3372	1.8808	1.9074	5.27	5.18	12.07	12.57
Perimeter (mm) p>0.05	17.2291	17.3297	0.4132	0.4326	2.3193	2.4469	13.06	13.23	21.54	21.99

R, Right Sagitta; L, Left Sagitta, SE, Standard error; SD, Standard deviation, Min., Minimum; Max., Maximum. *p<0.05

The results from statistical comparison of each morphometric parameter for both left and right sagitta between male and female samples of *C. caeruleopinnatus, C. chrysophrys, C. malabaricus* and *M. cordyla* are summarized in Table 3. Significant differences were detected in length, width, and area of the left otoliths and width of the right otoliths between males and females of *C. malabaricus* (p<0.05). However, no significant differences were found between males and females of *C. chrysophrys*, *C. aeruleopinnatus*, and *M. cordyla* (p<0.05). The highest mean weight was detected in left otolith of males *C. coeruleopinnatus* (0.0183±0.0012 g) and the lowest mean weight was found in right otolith of males *M. cordyla* (0.0045±0.0014 g).

Table 3: Statistical comparisons for left and right sagittal otoliths of male and female *Carangoides caeruleopinnatus, C. chrysophrys, C. malabaricus* and *Megalaspis cordyla*.

Species		Sex	Mean weight sagitta	*p* values: Weight (g)	Length (mm)	Width (mm)	Area (mm^2)	Perimeter (mm)
Carangoides caeruleopinnatus	L	F	0.0171	0.722	0.937	0.633	0.630	0.176
		M	0.0183					
	R	F	0.0169	0.848	0.845	0.870	0.733	0.300
		M	0.0181					
Carangoides chrysophrys	L	F	0.0082	0.264	0.999	0.631	0.964	0.784
		M	0.0097					
	R	F	0.0082	0.292	0.924	0.734	0.989	0.908
		M	0.0096					
Carangoides malabaricus	L	F	0.0121	0.072	0.012*	0.042*	0.028*	0.082
		M	0.0156					
	R	F	0.0125	0.086	0.195	0.044*	0.083	0.345
		M	0.0153					
Megalaspis cordyla	L	F	0.0057	0.134	0.122	0.375	0.288	0.200
		M	0.0047					
	R	F	0.0056	0.093	0.120	0.284	0.166	0.119
		M	0.0045					

R, Right Sagitta; L, Left Sagitta, SE, Standard error; SD, Standard deviation, Min., Minimum; Max., Maximum.*p<0.05.

The otolith shape indices were calculated for each species using the data obtained from the measured values of the left and right sagittal otoliths. The indices were as followings: form factor (FF), roundness (RD), aspect ratio (AR), circularity (C), rectangularity (R) and ellipticity (E) (Table 4). No significant differences were found in shape indices between the right and left otoliths (p>0.05, Table 4).

The relationships of otolith weight, length and width with fork length were determined, using left otolith values for all individuals. The best fit for the FL-WO relationship was recorded for male species of *M. cordyla* (r^2=0.940), and the second best r^2 (0.918) belongs to the female species of *M. cordyla*. While the lowest value of the coefficient of determination was established for the FL-OW *C. chrysophrys* (r^2 =0.410) (Table 5).

Fig. 4 shows the increasing relationship of left otolith length (OL) with circularity (C) and ellipticity as a linear relationship, while the relationship between OL and rectangularity (R) being determined as a nonlinear relationship. As fish otolith length (OL) increased, the values of form factor (FF), roundness (RD) and aspect ratio were generally decreased. The rectangularity relationship with OL for *C. coeruleopinnatus* and *C. chrysophrys* were increasing and for *M. cordyla* and *C. malabaricus* decreasd as OL increased.

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Table 4: Summary of descriptive statistics of shape indices for Sagitta otoliths of *Carangoides ceruleopinnatus, C. chrysophrys, C. malabaricus* and *Megalaspis cordyla*

Species	Shape indices	n	Side	Mean	SE	SD	Min	Max	Sig.	
C. caeruleopinnatus	Form factor	40	Left	0.4277	0.0086	0.0544	0.3237	0.5276	>0.05	0.679
			Right	0.4296	0.0086	0.0544	0.3286	0.5498		
	Roundness	40	Left	0.3366	0.0055	0.0354	0.2753	0.4189	>0.05	0.416
			Right	0.3387	0.0054	0.0342	0.2926	0.4247		
	Aspect ratio	40	Left	0.4101	0.0064	0.0408	0.3273	0.4909	>0.05	0.069
			Right	0.4148	0.0062	0.0389	0.3576	0.5038		
	Ellipticity	40	Left	0.4195	0.0065	0.0409	0.3415	0.5068	>0.05	0.067
			Right	0.4146	0.0061	0.0383	0.3300	0.4732		
	Circularity	40	Left	29.8431	0.5973	3.7774	23.8202	38.8196	>0.05	0.655
			Right	29.7138	0.5955	3.7661	22.8574	38.2410		
	Rectangularity	40	Left	0.6436	0.0036	0.0225	0.6121	0.6956	>0.05	0.577
			Right	0.6413	0.0036	0.0226	0.5882	0.6963		
C. chrysophrys	Form factor	34	Left	0.4272	0.0065	0.0382	0.3516	0.5005	>0.05	0.605
			Right	0.4296	0.0068	0.0397	0.3622	0.5022		
	Roundness	34	Left	0.3648	0.0064	0.0376	0.2676	0.4374	>0.05	0.271
			Right	0.3617	0.0062	0.0364	0.2679	0.4407		
	Aspect ratio	34	Left	0.4358	0.0083	0.0481	0.3239	0.5356	>0.05	0.258
			Right	0.4322	0.0079	0.0463	0.3285	0.5459		
	Ellipticity	34	Left	0.3944	0.0081	0.0471	0.3024	0.5107	>0.05	0.263
			Right	0.3979	0.0078	0.0453	0.2938	0.5054		
	Circularity	34	Left	29.6537	0.4694	2.7371	25.1062	35.7418	>0.05	0.653
			Right	29.5025	0.4768	2.7802	25.0244	34.6923		
	Rectangularity	34	Left	0.6584	0.0044	0.0259	0.6069	0.7202	>0.05	0.990
			Right	0.6583	0.0046	0.0266	0.6138	0.7169		
C. malabaricus	Form factor	32	Left	0.4606	0.0051	0.0291	0.4067	0.5315	>0.05	0.592
			Right	0.4636	0.0067	0.0377	0.4141	0.5779		
	Roundness	32	Left	0.4068	0.0055	0.0309	0.3427	0.5036	>0.05	0.262
			Right	0.4126	0.0051	0.0289	0.3649	0.4653		
	Aspect ratio	32	Left	0.4965	0.0055	0.0310	0.4371	0.4371	>0.05	0.081
			Right	0.5053	0.0053	0.0298	0.4514	0.4514		
	Ellipticity	32	Left	0.3365	0.0049	0.275	0.2559	0.3917	>0.05	0.074
			Right	0.3290	0.0047	0.263	0.2816	0.3782		
	Circularity	32	Left	27.3858	0.3014	1.7053	23.6433	30.8988	>0.05	0.701
			Right	27.2692	0.3633	2.0552	21.7458	30.3430		
	Rectangularity	32	Left	0.6432	0.0032	0.0179	0.6021	0. 6706	>0.05	0.616
			Right	0.6411	0.0032	0.0180	0.6076	0.6726		
M. cordyla	Form factor	32	Left	0.3465	0.0048	0.0272	0.2985	0.3991	>0.05	0.241
			Right	0.3389	0.0051	0.0286	0.2731	0.3891		
	Roundness	32	Left	0.2555	0.0047	0.0264	0.1671	0.3051	>0.05	0.974
			Right	0.2556	0.0035	0.0196	0.2176	0.3069		
	Aspect ratio	32	Left	0.3048	0.0045	0.0256	0.2511	0.3556	>0.05	0.235
			Right	0.3087	0.0035	0.0198	0.2625	0.3473		
	Ellipticity	32	Left	0.5334	0.0053	0.0302	0.4754	0.6000	>0.05	0.218
			Right	0.5286	0.0041	0.0232	0.4844	0.5841		
	Circularity	32	Left	36.4887	0.5111	2.8914	31.4884	42.0981	>0.05	0.225
			Right	37.3485	0.5785	3.2727	32.2939	46.0081		
	Rectangularity	32	Left	0.6581	0.0068	0.0387	0.5249	0.7257	>0.05	0.187
			Right	0.6506	0.0058	0.0326	0.5942	0.7423		

Abbreviations: SE=Standard error; SD=Standard deviation; Min=Minimum; Max=Maximum; Sig=Significant differences.

Table 5: Regression equations between fork length and left otolith variables for all individuals, females and males, all regressions statistically significant at *p*<0.05.

Species	Relationship	Equations	r^2
C. caeruleopinnatus	FL – WO	WO=0.0001FL-0.0234	0.903
	FL-OL	OL=0.0099FL+3.139	0.535
	FL-OW	OW=0.0036FL+1.400	0.612
	FL - WO(Female)	WO=0.0001FL-0.023	0.917
	FL-OL (Female)	OL=0.0097FL+3.211	0.700
	FL-OW (Female)	OW=0.0039FL+1.330	0.695
	FL - WO(Male)	WO=0.0001FL-0.023	0.894
	FL-OL (Male)	OL=0.0100FL+3.044	0.558
	FL-OW (Male)	OW=0.0033FL+1.482	0.541

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Table 5 continued:

	FL – WO	WO=0.00009FL-0.0097	0.793
	FL-OL	OL=0.015FL+2.061	0.783
	FL-OW	OW=0.004FL+1.393	0.410
	FL - WO(Female)	WO=0.00006FL-0.005	0.722
C. chrysophrys	FL-OL (Female)	OL=0.017FL+1.732	0.856
	FL-OW (Female)	OW=0.002FL+1.796	0.767
	FL - WO(Male)	WO=0.000096FL-0.012	0.848
	FL-OL (Male)	OL=0.014FL+2.159	0.759
	FL-OW (Male)	OW=0.005FL+1.181	0.511
	FL – WO	WO=0.0001FL-0.0182	0.858
	FL-OL	OL=0.0173FL+1.860	0.820
	FL-OW	OW=0.0091FL+0.779	0.856
	FL - WO(Female)	WO=0.0001FL-0.020	0.902
C. malabaricus	FL-OL (Female)	OL=0.0159FL+2.101	0.781
	FL-OW (Female)	OW=0.0087FL+0.871	0.814
	FL -WO(Male)	WO= 0.00013FL-0.01	0.743
	FL-OL (Male)	OL=0.0171FL+1.976	0.820
	FL-OW (Male)	OW=0.0097FL+0.667	0.872
	FL – WO	WO=0.00003FL-0.0049	0.913
	FL-OL	OL=0.0112FL+2.568	0.684
	FL-OW	OW=0.0029FL+0.961	0.696
	FL - WO(Female)	WO=0.000033FL-0.006	0.918
M. cordyla	FL-OL (Female)	OL=0.0122FL+2.258	0. 734
	FL-OW (Female)	OW=0.0031FL+0.851	0.755
	FL - WO(Male)	WO=0.000025FL-0.003	0.940
	FL-OL (Male)	OL=0.0090FL+3.199	0.566
	FL-OW (Male)	OW=0.0025FL+1.071	0.597

Abbreviations: WO, otolith weight (g); OL, otolith length (mm); OW, otolith width (mm); FL, fork length (mm); r^2, coefficient of determination of *C. ceruleopinnatus, C. chrysophrys, C. malabaricus and M. cordyla.*

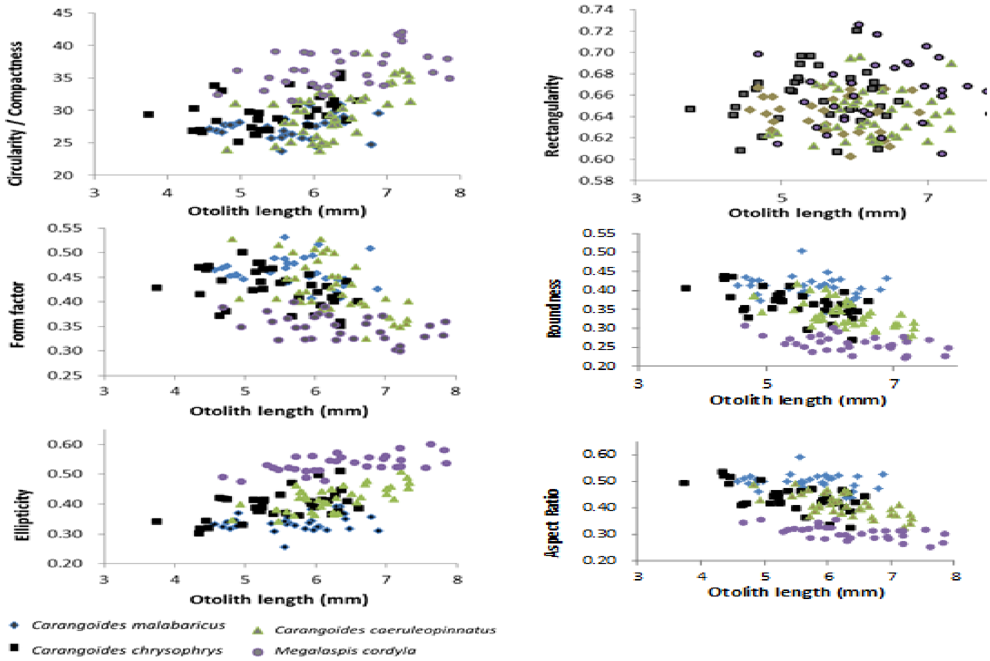

Figure 4: Shape indices: circularity, rectangularity, form factor, roundness, ellipticity and aspect ratio vs sagitta otolith's length of four Carangid species: *Carangoides caeruleopinnatus, C. chrysophrys, M. cordyla* and *Megalaspis malabaricus.*

Discussion

Sagitta otolith has been used as a taxonomic tool for identifying fishes due to their large size and degree of interspecific differences (Rivera Felix *et al*., 2013). This is because their form, weight, growth, consistency and chemical composition have a distinctive degree of interspecific variation; and they are easily accessible structures as well (Zorica *et al*., 2010).

A total of 138 fish specimens comprising of *M. cordyla*, *C. coeruleopinnatus*, *C. chrysophrys*, and *C. malabaricus* species were tested in the present study. Both right and left otoliths were extracted and analyzed according to their sex.

Several previous studies have examined the morphometric parameters and their relationship with fish body sizes. The comparisons of otolith morphometric parameters between left and right otoliths were performed frequently in many previous studies (Rivera Felix *et al*., 2013; Kontas *et al*., 2015). Otolith shape indices have been used in several studies for differentiation of fish species (Stransky and MacLellan, 2005; Zorica *et al*., 2010; Sadighzadeh *et al*., 2012) and populations (Tuset *et al*., 2003; Merigot *et al*., 2007; Duarte-Neto *et al*., 2008; Canas *et al*., 2012; Legua *et al*., 2013), as well as for the comparison of shapes of the left and right otoliths in the specimens of the same stock (Morat *et al*., 2008; Lord *et al*., 2011).

Significant differences were only detected in length, width, and area of otoliths between males and females of *C. malabaricus*. Such results were also reported by Yilmaz *et al*. (2014). However, no significant differences were found between males and females of *C. chrysophrys*, *C. aeruleopinnatus*, and *M. cordyla*. In a study from the coast of Valencia (Spain), females' otoliths length and width were significantly larger than males of *Mullus surmuletus*, *Synaptura lusitanica*, and *Uranoscopus scaber*, while there were no significant differences between males and females of *Scorpaena scrofa* (Jaramillo *et al*., 2014). This result may be due to the fact that these species are sexually dimorphic in size. Although, females were growing at a lower rate, they were larger than males (Jaramillo *et al*., 2014). In other words, it is possible that the growth rate of males and females of the same species to be different as the sex-dependent differences in morphometric parameters were reported previously (Thompson *et al*., 1999). However, it should be determined that whether such differences actually exist between male and female fish or it might be due to the sampling design. Interestingly, in the study conducted by Kontas *et al*. (2015) on *Barbus tauricus*, it was found that differences of otolith variables from family Cyprinidae might be different not only for left and right otoliths but also for males and females of the same species.

Comparison of possible differences between the left and right otoliths of fishes was a major aspect of studies conducted on fish otoliths. Valinassab *et al*. (2012) in a study on 10 species of clupeids did not find any significant differences in morphometric parameters between left and right otoliths. Similar results were reported by Megalofonou (2006) on *Thunnus thynnus* from the Mediterranean Sea coasts of Greece and Italy. Rivera Felix *et al*. (2013) also did not find any significant differences in sagittal otolith length and width between the left and right otoliths of their males and females samples. In the present study no significant differences were found in otolith length, width, weight, perimeter and areas of left and right otoliths of the two species *C. crysophrys* and *C. malabaricus.* However, in our study the otolith weight of *C. coeruleopinnatus* and *M. cordyla* and the otolith area of *C. coeruleopinnatus* were significantly different between the left and right otoliths (p<0.05). Similar finding were reported by Kontas *et al*. (2015). Based on the lack of statistically significant differences in the left and right otoliths parameters, all other statistical analysis can be performed using only the amounts of left otoliths parameters in order to avoid redundant analysis (See *et al*., 2016).

Moreover, no significant differences were found in the shape indices including form factor (FF), roundness (RD), aspect ratio (AR), circularity (C), rectangularity (R) and elipticity (E) between the right and left otoliths. Bostanci *et al*. (2016) also found no significant differences between the right and left otoliths, except their ellipticity. The results of the present study showed that there were increasing relationships between otolith length with circularity and elipticity as a linear relationship and a non-linear relationship between the otolith length and rectangularity. Zorica *et al*. (2010) revealed that three shape indices including form factor, roundness and aspect ratio were evaluated for five pelagic fish species from the Adriatic Sea (Croatia). The findings of their study showed that the shape indices differed significantly in those species; however they indicated a similar pattern with maximal otolith length. That is, the aspect ratio was in proportion to the maximal otolith length, while form factor and roundness were inversely proportional to it. This similar pattern in otolith shape indices might be due to the fact that all studied pelagic fish species have similar ecological traits and occupy the same ecological niche. This pattern might have been very likely the same with the carangids species of the present study.

Significant relationships were found between morphometric parameters of otolith (length, width, weight) and fork length for all the fish species in our study. The results suggest that the length and weight of otoliths are suitable indicators for the fish fork length. Cruz-Agüero *et al*. (2016) studied the relationships between fish length and otolith length for 14 species of Gerreidae, in coastal waters of Mexico. They found a linear relationship between fish length and otolith length (12 of 14 species).

Similar results were also previously reported by other studies (Megalofonou, 2006; Valinassab *et al*., 2012). In a study conducted by Skeljo and Ferri (2012) the otolith length showed the strongest relationship with the total length and weight of five species of Labridae from the eastern Adriatic.

Based on the results obtained, it was revealed that the shape variability of otolith needs further research in order to confirm the role of otolith morphometric measurements in fish identification.

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