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Relationship between body and otolith morphological characteristics of sabre squirrelfish (*Sargocentron spiniferum*) from the southern Red Sea: difference between right and left otoliths

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Abstract: Otolith morphology analysis is one of the main tools used for fish or fish stock identification. Moreover, otolith shape can also be used in fish dietary studies (stomach content) for the identification of prey fishes and their size according to the relationship between fish and otolith sizes. In the present study, the relationship between fish length and otolith morphological dimensions was investigated for the sabre squirrelfish, *Sargocentron spiniferum* (Forsskål, 1775) (family: Holocentridae). Samples of 185 fish were collected from the coast of the Red Sea, Egypt. To analyze the relationship between fish and otolith, otolith morphometric measurements (length, width, area, perimeter, weight, sulcus, and ostium) and shape factors (aspect ratio, compactness, form factor, rectangularity, roundness, ellipticity, squareness) describing outline shape were extracted using image analysis. Generalized linear models were applied for the relationship between body length and each otolith morphology feature. From the relationships between the total length of fish and fourteen morphology features, only otolith length, caudal length, and squareness were significantly correlated with fish size. Our results provide more information for the relationship between fish length and otolith morphometric features.

Keywords: Fish size; otolith shape; head side; *Sargocentron spiniferum*; Red Sea; Asymmetry.

1. Introduction

The sabre squirrelfish *Sargocentron spiniferum* (Forsskål, 1775) is a member of the family Holocentridae, which is mainly distributed in the Indo-Pacific from the Red Sea and East Africa to the Hawaiian Islands and Ducie Islands extending south to Australia. They can be diagnosed by several morphological criteria: head and body red, scale edges silvery white, spinous dorsal crimson in color, other fins orange-yellow and vertically oblong fin crimson spot on preopercle behind eye [1]. This solitary species that inhabits a variety of reef zones from reef flats to lagoon and seaward reefs to a depth of at least 120 m in New Caledonia, north to southern Japan and the Ogasawara Islands [1-3]. This fish occurs under ledges during the day [3], and when smaller in size, this fish inhabits shallow, protected areas. It is a nocturnal fish that feeds on crabs, shrimp, and small fishes [4]. The trophic level was estimated at 3.8 in Moorea island (French Polynesia [5]). Only one growth study has previously been carried out in the Egyptian Red Sea with the oldest specimen at seven years old [6]. Moreover, the maximum length and weight data are known (total length of 45.8 cm and total weight of 2 600 g) and showed that this species is the largest species of squirrelfish and the highest-bodied *Sargocentron* species. In 2014, among 200 tons of total world catches of Sabre squirrelfish (*Sargocentron spiniferum*), the catches from the Northern and Central Red Sea were in the order of 55 tons, which identifies this geographical area as the largest fishery for this species (source FishStat database). Its level of exploitation appeared to be controlled with the level of catches close to the maximum sustainable yield (MSY) which identified a hypothetical equilibrium state between the exploited population and the fishing activity [7]. This species is commercially exploited mainly by small-scale fishing in the Red Sea.

Many researchers have used the clear and distinct growth increments of calcified structures to study the age and growth of fishes [8, 9]. Among calcified structures, otoliths which are located in right and left inner ears of fishes, are the most used in the study of fish biology, ecology, and fisheries science [10-12]. Each fish present three pairs of otoliths. The sagittae, the largest of the three pairs of otoliths, are widely used to describe age and growth in fish [9]. In one ageing study on *Sargocentron spiniferum,* the whole sagittal otolith was used to determine the growth. Fish use their otoliths for balance and hearing, sensing small changes in the direction and pressure of water. In fisheries science, otoliths are also used to estimate movement, varied habitat, population dynamics, and trophic level for fish species [12-20].

Otolith morphology is used to recognise fish species in taxonomic, phylogenetic, paleoichthyological and dietary studies [21-24]. Moreover, to follow the growth of fish from the otolith, there must be a proportional relationship between the growth of the fish and the otolith. This kind of relationship can even be used to predict the age directly without observing growth increments [25]. One recent study showed, however, that directional asymmetry between right and left otoliths within individuals could affect the results from the otolith shape analysis as tool to identify the stocks [26]. Consequently, the aim of the present work was to estimate the relationship between fish size and otolith outline features according to the location of the otolith, i.e., left versus right inner ear for *S. spiniferum* from the Egyptian waters of the Red Sea to answer two questions :

Is there a morphological difference between the right and left otoliths for this species?

Is it possible to use all the size parameters of the otolith equally to correlate fish growth with otolith growth for this species?

Is it possible to associate the otolith shape (i.e. shape indices) with the body length for this species?

2. Materials and Methods

Fish species were randomly collected from the commercial catch of the hook and line fishery at Shalateen fishing ground from the southern Red Sea, which is located 520 km south of Hurghada (Figure 1), Egypt, between March 2018 and February 2019.

Figure 1. Egyptian Red Sea map showing the studied Shalateen area.

In the laboratory, total fish length (TL) was measured to the nearest 0.1 cm, then the sex was determined from macroscopic observation. The total length of the species ranged between 17.7 and 45.8 cm. Sagittal otoliths (370 paired left and right otoliths) were extracted from the inner ear of 185 S. spiniferum, cleaned and dried. Otolith weight (OW) for each head side was measured using a digital balance AS220 k/1 to the nearest 0.0001 g. Otolith images were captured using a Euromex CMEX-10 PRO camera with a stereomicroscope. The following otolith measurements: length (OL, mm), width (OH, mm), area (OA, mm2), perimeter (OP, mm), sulcus length (SU), ostium length (OS) were taken using image processing systems (Image J analysis software, [27]; detailed descriptions is in Figure 2).

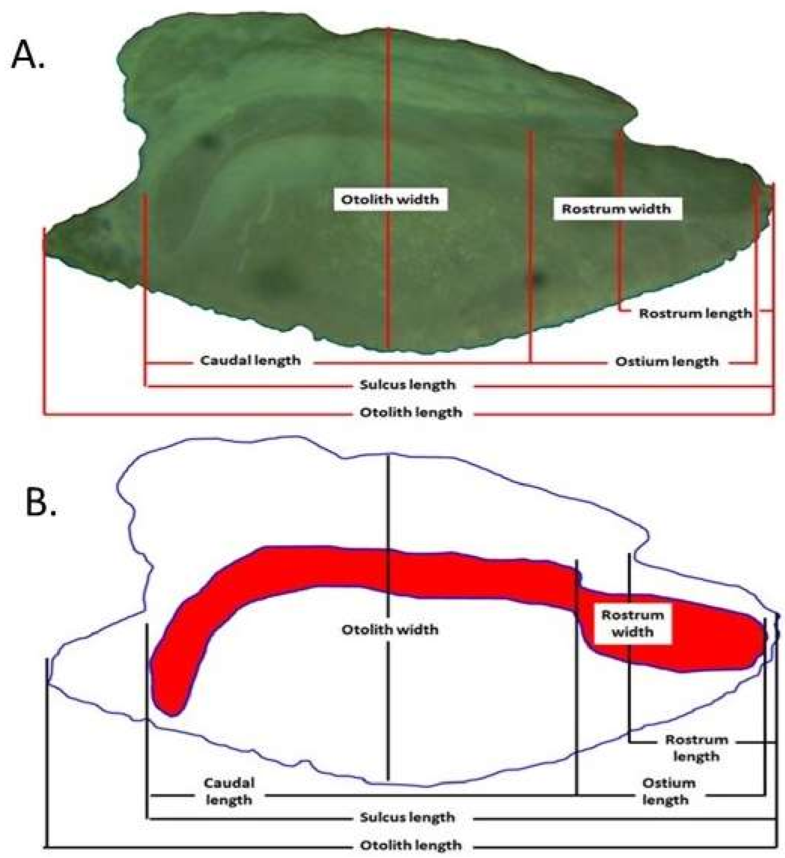

Figure 2. Otolith image **(A.)** and scheme (B.) of *Sargocentron spiniferum* illustrating various features of the otolith measurements. The red area showed the *sulcus acusticus*.

From these size parameters, the following shape indices were calculated: form factor (FF), aspect ratio (AR), circularity (CI) rectangularity (RE), roundness (RO), ellipticity (EL), compactness (C), and squareness (SQ) [28-33]. The formulae of these shape index factors (FF, C, AR, CI, RO, RE, EL, and SQ) are presented in Table 1.

Table 1. Size dimension parameters and shape indices of otolith used to describe *S. spiniferum* otolith characteristics.

Size parameters	Shape indices	
Length : OL	Circularity :	$CI = \frac{OP}{OA^2}$
Width : OH	Ellipticity :	$EL = \frac{OL - OH}{OL + OH}$
Perimeter : OP	Roundness :	$RO = \frac{4\ OA}{\pi\ OL^2}$
Area: OA	Aspect ratio :	$AR = \frac{OL}{OH}$
Weight: OW	Rectangularity :	$RE = \frac{OA}{OL\ .OH}$
Sulcus : OSL	Form-factor :	$FF = \frac{4\ \pi\ OA}{OP^2}$
Ostium : OOs	Compactness :	$CO = \frac{OP^2}{OA}$
Cauda : OCu	Squareness :	$SQ = \frac{OA}{OL.OW}$

All analyses were performed on the base-10 logarithm of the body and otoliths data to apply the generalized linear model. Subsequently, the relationship of fish length with each otolith feature (size parameters or shape indices) was modelled according to the side (S):

$$\text{TL} \sim \text{Otolith feature} + \text{Side} + \text{Otolith feature:Side} \quad (1)$$

The normality and the homoscedasticity of the residuals were assessed by visual inspection of diagnostic plots. The significance of explanatory variables was tested by likelihood ratio tests between nested models while respecting marginality of the effects (type 2 tests [34]) that are supposed to follow a χ^2 distribution under the null hypothesis while correcting for multiple comparisons using a Bonferroni procedure. Statistical analyses were performed in the R statistical environment [34] stats package [35].

3. Results

A general pattern of *S. spiniferum* sagittae can be recognized in adult individuals: the otolith shape of *S. spiniferum* is ovate with sinuate margins, and is elongated, reflecting slower growth of the dorso-ventral axis compared to the antero-posterior axis. The dorso-ventral axis has the highest growth, with with a much higher distance than that observed on antero-posterior axis with the rounded posterior area and the lobed anterior area. The *sulcus acousticus* is ostial with the heterosulcus and ostium formed by a short, funnel-like ostium that opens to the anterior margin, and closed, tubular cauda at least two times larger than the ostium (Figure 2). Descriptive statistics for left and right otoliths of *S. spiniferum* are given in Table S1.

The total length of the species ranged between 17.7 and 45.8 cm. Analysis of the relationships between fish length and fourteen otolith shape descriptors using a generalized linear model showed that there is a significant relationship between eight otolith parameters (ostium length, caudal length, otolith area, otolith perimeter, compactness, form factor, circularity, and squareness) with the total length of fish. Only the relationship of body length with otolith length, caudal length and squareness ($P<0.05$) was significant for right and left otolith (Table 2).

Table 2. Generalized linear models for the relationship between fish length and each otolith variable for *S. spiniferum* from Table 0. The relationship between TL and each otolith descriptor was showed according to the significance of side effect, OL, otolith length; OH, otolith width; SU, sulcus; OS, ostium; OA, otolith area; OP, otolith perimeter, FF, form factor; AR, aspect ratio; C, compactness; RE, rectangularity, RO, roundness; EL, ellipticity; C, circularity y; SQ, squareness.

Otolith descriptor	TL	Side effect	Relationship between TL and otolith descriptor	R^2
OL	0.514	0.010	Left side TL = 0.719+ 0.065OL	0.665
			Right side TL = 0.636+ 0.072OL	0.852
OH	0.356	0.148	TL = 0.896+ 0.078OH	0.736
SU	0.821	0.396	TL = 0.904+ 0.057SU	0.777
OS	0.013	0.687	TL = 1.116+ 0.087OS	0.586
CA	< 0.001	< 0.001	Left side TL = 1.117+ 0.069CA	0.384
			Right side TL = 1.047+ 0.007CA	0.806
OA	< 0.001	0.532	TL = 1.095+ 0.006OA	0.851
OP	0.011	0.990	TL = 0.689+ 0.025OP	0.813
AR	0.977	0.868	TL = 1.063+ 0.675AR	0.138
CO	0.034	0.746	TL = 1.331+ 0.010C	0.037
FF	0.009	0.388	TL = 1.656- 0.833FF	0.043
RE	0.332	0.659	TL = 1.807- 0.427RE	0.166
RO	0.547	0.294	TL = 1.734- 0.327RO	0.111

EL	0.601	0.120	TL = 1.686- 0.872EL	0.132
CI	< 0.001	0.849	TL = 1.331+ 0.010CI	0.037
SQ	< 0.001	< 0.001	Left side TL = 1.734- 0.328SQ	0.111
			Right side TL = 1.785-0.396SQ	0.138

The correlation between fish total length and otolith growth showed that with an increase in the total length, the following factors of otolith size also increased: ostium length, caudal length, otolith area, otolith perimeter (Figure 3). The relationship between body length and the aspect ratio was, however, close to 1, confirming that the otolith of *S. spiniferum* was oval. Among the shape indices, only four showed a significant relationship with total length. If the circularity and compactness increased with the body size, this trend was reversed for the other shape indices (Squareness and Form Factor, Figure 3).

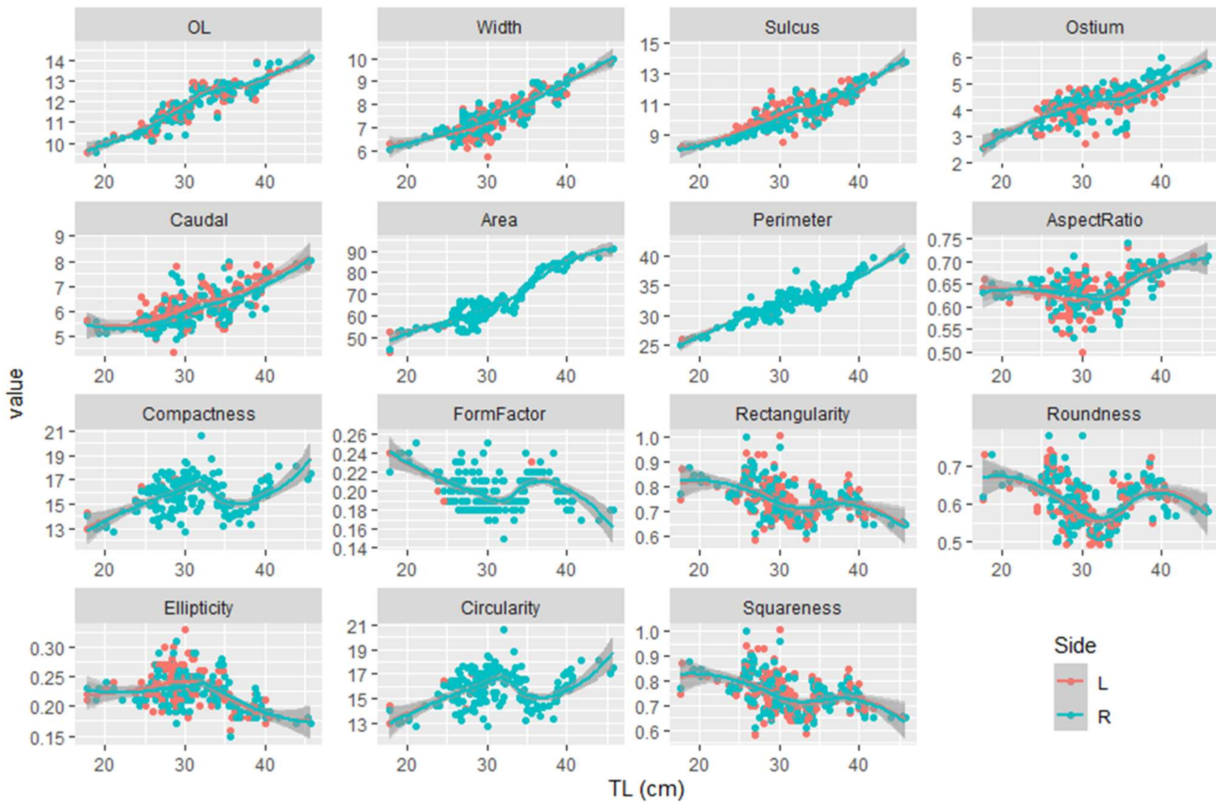

Figure 3. Relationships of body length with the otolith morphological structures (red points = left otolith, and green points = right otolith) according to the side of *S. spiniferum* captured from Shalateen, Egypt, Red Sea.

4. Discussion

The sabre squirrelfish *Sargocentron spiniferum* (Forsskål, 1775) contributes to important fisheries, especially the small artisanal fisheries at Shalateen fishing ground, Red Sea, Egypt. Basic data on the biology and dynamics of the species are essential for successful stock assessment and consequently in fisheries management. The observed fish length and shape of the otolith in this study should encourage more research to verify the essential role of otolith morphometric measurements in fish stock identification. If the relationship between fish and otolith growth has been observed for *S. spiniferum* as in many other fish species, it should be noted that simple descriptors such as otolith length and width are not usable for this species but it is necessary to use more integrative factors of otolith size such perimeter or area. In the same way, the descriptors (ostium length, caudal length) of the *sulcus acusticus* (a depression situated in the proximal surface of the sagitta and it allows the connection between the sensorial macula and the otolith), are also usable to describe the relationship between body and otolith size. The morphology of the *sulcus acusticus* could be linked to ecomorphologic (functional and environmental) and phylogenetic aspects [36]. Previous studies have focused mainly on the relationship between only otolith length and fish length [11, 37-41]. For *Sargocentron rubrum*, sampling was done in the southeastern Mediterranean Sea (Arsuz coast) and the relationship between body size and otolith parameters (length and width) were estimated [32]. The authors observed correlations between otolith length and width in relation to fish length but no side effect on these relationships. In our study, the results of generalized linear models showed that the relationship between otolith parameters and fish length can be affected by the choice of the otolith (right and left otoliths). Our study on *S. spiniferum* from the southern Red Sea showed in particular that the relationship between otolith and fish length was side-dependent. These difference could be explained by either the difference in species (*S. rubrum vs S. spiniferum*) or the difference in environment (Red Sea *vs* Mediterranean Sea). The recent review analyzing 91 published studies on marine fish stock identification based on otolith shape analysis, identified the significant bilateral asymmetry for *Liza ramada*, *Boops boops*, *Diplodus annularis*, *Diplodus puntazzo*, *Clupea harengus* and *Scomberomorus niphonius*. Conversely, symmetry between left and right otolith shapes was observed for other fish species as *Gadus morhua*, *Synechogobius ommaturus*, *Coryphaena hippurus*, *Xiphias gladius*, *Scomber scombrus*, and *Lutjanus kasmira* [42]. Within a species, the level of asymmetry between left and right otoliths may vary greatly between several habitats [43]. For *S. spiniferum* from the southern Red Sea, if the relationship between fish size and otolith size is used, it is necessary to identify the used side to avoid introducing a bias in the results. Moreover, otolith asymmetry could be considered as a sensitive indicator of fish health that directly affects the fish performance because otoliths are essential to balance and hearing [44, 45]. Based on the present data, the relationships between TL and AR, CO, and CI were determined as linear, while the relationships among TL and FF, RE, RO, EL, and SQ were determined as nonlinear. The shape of otolith from different geographical areas is, however, influenced by both environmental parameters (e.g salinity, temperature) and biotic parameters, for example prey availability, and depends on individual genotype [14, 46-48]. Consequently, an interaction of environmental and genetic fluctuation generates the morphological variance in otolith shape that may allow the differentiation of stock units but also requires the shape to be tested locally before using shape data from another geographical area. However, the factors that affects the shapes are not fully understood and have not yet been investigated deeply [49]. A recent and on-going work showed that the ontogenetic trajectory of otolith shape could be impacted by the environmental disturbance during the early life stage [50]. The relationships between fish size and otolith shape indices demonstrate the high variability in fish length and morphometric parameters, indicating that the otolith of *S. spiniferum* is oval. The results of this study on the fish size and otolith morphometric parameters are useful for further research on verifying the role of otoliths in identification, discrimination and taxonomic classification of fish. Finally, the estimation of the generalized linear models in the present work may be good tool to study the relationship between fish and otolith morphometric features, which are used for fish population dynamics, stomach contents analyses of piscivorous predators, paleontological composition, and yield estimates.

Supplementary Materials: Table S1. Summary of descriptive statistics and paired t-test results for left and right otoliths of S. spiniferum (n=185).

Author Contributions: Conceptualization, methodology Y.A. A. O., S. M. E. M., A. S. M. and S. F. M.; formal analysis, Y.A. A. O. and K. M.; writing—original draft preparation, Y.A. A. O.; writing—review and editing, K.M.

Funding: no specific funds for this study

Institutional Review Board Statement: Ethical review and approval were waived for this study, because the fish were obtained from fisheries and were already dead when the otoliths were extracted.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We would especially thank K. M. MacKenzie for this valuable help in editing this manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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