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Blue whiting otoliths pair's symmetry side effect

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Abstract
Otoliths are paired calcified structures located in the inner ear of the teleost fishes. Fisheries scientists have been using the otoliths for a variety of applications as fish ageing, species identification and also species interaction. The symmetry between left and right otoliths is considered species dependent. The application of analysis techniques on otoliths images has been increasing in recent years. The diagnosis on otoliths symmetry as a species characteristic is currently being done. It is expected that the otolith surface (distal *versus* proximal) used to collect images should have no influence on the symmetry results. The goal of this study is to investigate if the otolith position (distal *versus* proximal) when analyse images has influence in the diagnostic of the symmetry shape between the left and right otoliths. The results showed that the otolith face orientation should be taken into account during the otoliths images processing for symmetry shape analysis. On blue whiting the otolith images should always be obtained from the concave side, because for symmetry studies the position real matter.

Keywords: Otoliths image analysis, morphometry analysis, fish otoliths, marine science application, elliptic Fourier analysis

1. Introduction

Otoliths are constituted by three pairs of calcified structures, the *sagittae*, *lapilli* and *asterisci*, located in the inner ear of the fishes, involved in hearing and balance systems [1]. The *sagittae* otoliths are characterized by high morphological variability between species [2]. The environment and endogenous factors influence both overall otolith shape and growth patterns [3, 4]. Otoliths have been used for a variety of purposes, as important tools in taxonomy, for species identification, for determining fish age, to investigate changes in marine populations and to infer the fish diet in marine food webs.

The use of otoliths for age determination has long been recognized by fisheries biologists [5] and broadly applied to assess the stock abundance. Traditionally the otoliths age classification has been based on a direct observation through stereo-microscope. In recent years, the application of image analysis on otoliths facilitated the construction of image databases. These otoliths image databases allow to save the age classification, which provides an effective tool on the training of new age readers and also to use on international calibration exchanges.

The otoliths images are also used in studies to distinguish between populations of the same species. Taking into account that symmetry is species dependent, thus it is expected that the otolith surface (distal *versus* proximal) used to collect images should have no influence on the results. Previous works studying otoliths asymmetry have been conducted, in round and flatfishes [6] and on blue whiting (*Micromesistius poutassou*) [7], but only used images obtained from the otolith distal surface

In fisheries science, currently there is an increase demand to produce even more accurate data, to extend the number of species studied, to study more deeply those species and to annually produce for assessment studies a great quantity of data to be used on complex statistical models. This data also includes the collection of otoliths to achieve species age stock structure annually. The marine institutes involved in blue whiting stock assessment on the Northeast Atlantic, sample by year around 102000 fishes and from these around 30000 otoliths were processed for age reading [8]. The aim of this study is to investigate if the otolith surface position on blue whiting could have influence in the diagnostic of the symmetry shape between the left and right otoliths of the same pair.

2. Material and Methods

2.1 Sample collection

The blue whiting samples were collected on the Portuguese southern coast (around Latitude 36.7ºN, Longitude 7.7ºW), during October of 2015 and March of 2016. Total length (cm), total weight (g) and sex of all blue whiting sampled were recorded and the otoliths were removed from each fish. After, the otoliths were washed and stored dry. Forty pairs of blue whiting otoliths were considered for this study, 17 from October and 23 from March. The left and right otoliths of the same pair were considered for the otolith shape analysis.

2.2 Otolith shape analysis

Images of the whole left and right otoliths were scanned (Epson V750) under reflected light and stored with high resolution (3200 dpi). Image processing was performed using the TNPC software (version 7) with the proximal surface facing up (concave side in the scanner) (Fig. 1 (a)) and with the distal surface facing up (convex side in the scanner) (Fig. 1 (b)).

Otolith length and width were measured and the contour of each otolith was obtained using the automatic threshold in the TNPC software. To describe otolith contours, Elliptic Fourier Analysis (EFA) was carried out. For each otolith, the first 99 elliptical Fourier harmonics (Hi) were extracted and normalised with respect to the first harmonic to be invariant to otolith size, rotation and starting point of the shape measurements. To determine the number of harmonics needed to reconstruct the otolith outline, the Fourier Power (PF) was calculated for each individual otolith k as a measure of the amount of contour rebuilt by each harmonic:

$$PF(n_k) = \sum_{HI=1}^{n_k} \frac{A_{HI}^2+B_{HI}^2+C_{HI}^2+D_{HI}^2}{2}$$

where A_{HI}, B_{HI}, C_{HI} and D_{HI} are the parameters of the HIth harmonic and n_k is the total number of harmonics included. The value of n_k was chosen such that $PF(n_k)$ explains 99.99% of variance in contour coordinates or, in other words, such that shape is reconstructed at 99.99%. The PF was calculated for each individual and the maximal number of harmonic was kept to the shape analysis. Consequently, only the first 34 harmonics were included in the statistical analysis.

2.3 Statistical Analysis

A Principal Component Analysis (PCA) was carried out on the Elliptic Fourier Descriptors (EFD) matrix of the sample (EFDs as columns and individual otoliths as rows) and a sub-set of the resulting principal components were selected as otolith shape descriptors according to the broken stick model. This allowed the number of variables used to describe otolith shape variability to be decreased while ensuring that the main sources of shape variation were kept, and to avoid co-linearity effect between shape descriptors [9]. The statistical analysis followed the procedure used and described by [6]. The following shape comparisons were performed: (i) left otolith using images from the distal and proximal surface; (ii) the right otolith using images from the distal and proximal surface; (iii) images from the proximal surface of right and left otoliths; and (iv) images from the distal surface of right and left otoliths.

All plots and statistical analyses were performed using the statistical environment R [10].

3. Results and Discussion

The length of the fishes collected in October 2015 ranged between 15cm and 17cm and weighted between 15g and 40g (Figure 2). The fishes from the March 2016 sample were larger (between 27cm and 30.7cm) and heavier (from 85g to 175g) than those sampled during October 2015.

In the October 2015 sample, the blue whiting were constituted by 7 females, 2 indeterminate and 8 males (Figure 3). In the March 2016, sample consisted of only females and males, 15 and 8, respectively with no indeterminate observed.

The samples used comprised of two groups, one constituting the smallest fishes and the other the biggest fishes, and from both sexes. The presence of fishes from both sexes and different sizes allowed studying left and right otoliths symmetry across different blue whiting length ranges.

The otoliths shape comparisons based on the EFD are shown in Figure 4. Differences in the symmetry were observed in: the left otolith images from distal and proximal position (Figure 4 (i)); the right otolith images from distal and proximal position (Figure 4 (ii)); the left and right otoliths images taken from the proximal position (Figure 4 (iii)); the images taken from left and right otoliths collected from the distal surface were symmetric (Figure 4 (iv)).

The result obtained on this study emphasizes the need to carefully evaluate the differences between the otoliths side, when obtaining images, before performing morphometry on those structures. In the majority of otoliths morphometric studies, the images were recorded with the *sulcus acusticus* facing up to the camera, from the distal face [e.g.] [1, 11, 12, 7]. Notwithstanding, in some works the face were images were captured was not mentioned [e.g.] [13, 14]. On some otoliths shape analysis studies although the image from left and the right otolith were recorded, just one of the otoliths was used to perform the analysis. As an example for cod, is assumed a shape symmetry between the left and the right otolith of the same pair, thus in such cases were the chosen otolith (left or right) was damaged or crystalline, the other otolith (right or left) is used in its place [15, 16]. Although, for cod, the images were captured with the otolith positioning with the proximal side facing up to the camera [15] and also with the distal side facing up [1]. To our knowledge the effect of the otolith position was never been evaluated in other species. According to this study results, this effect should be studied for other species otherwise the images must be obtaining always with the otolith on the same position. The standardization of the otolith side on image along the different works for the same species will avoid some degree of uncertainty between the results and allow a better comparison and interpretation of the results between them.

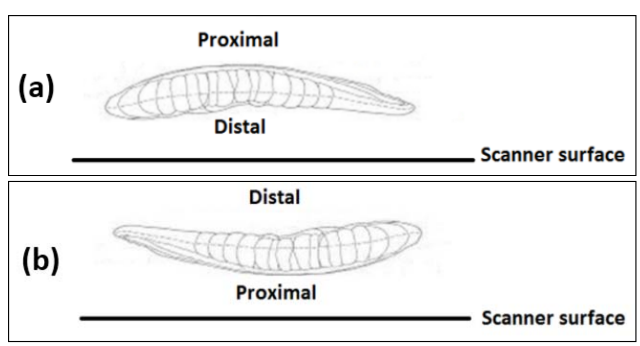

Fig 1: A lateral image of the right otolith, showing the otolith position in the scanner surface: (a) the distal surface (concave side); (b) the proximal surface (convex side).

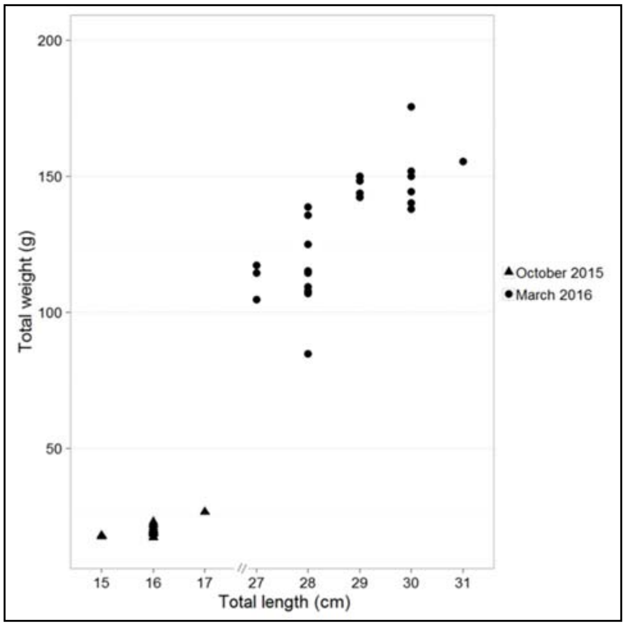

Fig 2: Total length (cm) and total weight (g) of the blue whiting samples collected during October 2015 (n=17) and March 2016 (n=23).

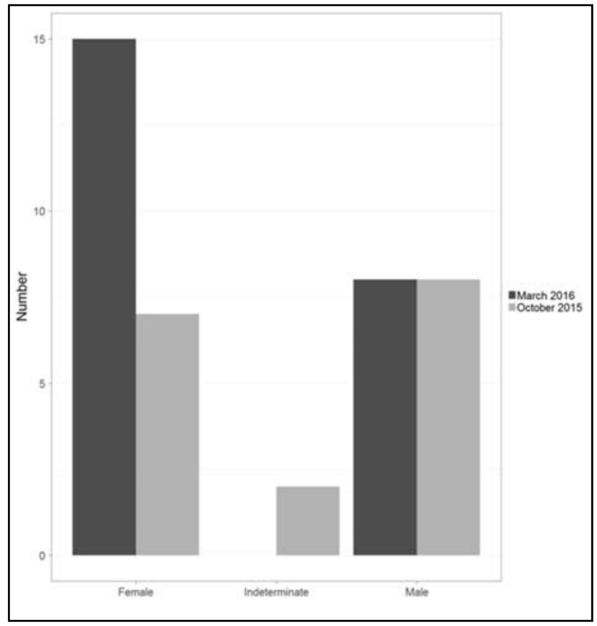

Fig 3: The number of females (n=22), indeterminate (n=2) and males (n=16) in the blue whiting samples from March 2016 and October 2015.

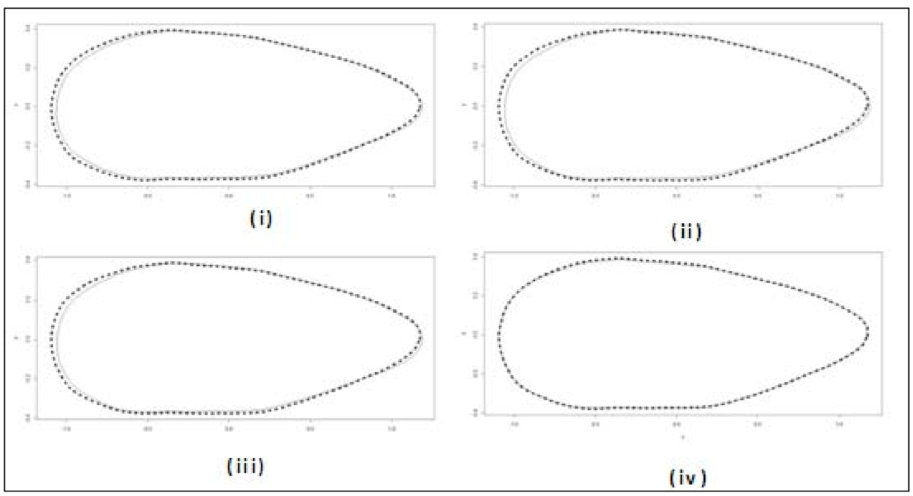

Fig 4: Mean otolith outline shapes formed with reverse Fourier transform of the outline using the first 34 harmonics showing the overlap and variations between: (i) left from the proximal surface (dark grey dash line) and left from the distal surface (grey solid line); (ii) right from the proximal surface (dark grey dash line) and right from the distal surface (grey solid line); (iii) right from the proximal surface (dark grey dash line) and left from the proximal surface (grey solid line); (iv) right from the distal surface (dark grey dash line) and left from the distal surface (grey solid line).

4. Conclusions

The results obtained, in the comparison of left and right otoliths shapes from the same otolith pair, showed that the otolith face orientation should be taken into account during the otoliths images processing for symmetry shape analysis. The expected symmetry, between the left and the right otoliths, according to the previous studies [6] on blue whiting was only observed using the images taken from the distal surface. In conclusion, for blue whiting, the otolith images should always be obtained from the concave side, because for symmetry studies the position real matter.

In the future, a larger scale blue whiting sample will be used to study if on this species the geographical area, age group and sex could have a significant effect on the otolith's morphometry.

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