



## The uses of Otolith shape in discrimination of the sand sole (*Solea lascaris*, Risso 1810) population.

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### Abstract

In this study three samples of the sand sole (*Solea lascaris*, Risso 1810) otoliths were compared for the shape (on the length standardized Wavelet/Fourier coefficients), for weight and for five morphometric characters (mean.radii, otolith.area, otolith.length, otolith.perimeter, otolith.width). In order to verify if these three groups of the sand sole are coming from three different regions (ESSAOUIRA, IFNI and AGADIR) belongs to the same population. The result show significant difference between the three groups in the same way for morphometric and shape analysis of the otoliths, for weight comparison, the otoliths of ESSAOUIRA is heavier than IFNI and AGADIR. These results are supported by the discriminant linear analysis that shows a classification percentage of 80% on Fourier standardized coefficient, and by the principal component analysis (PCA) which expresses 75.75% and 21.42% for the first and second axes respectively on the morphometric variables. The shape and morphometric analysis was carried out with shape R package which is software package that runs on the R platform and is specifically designed to study otolith shape variation among fish populations. All these difference existing between the three groups can be related to the environmental specificity and to the population adaptation for each environment.

## 1. Introduction

The Sand sole (*Solea lascaris* Risso 1810), as all teleost, has calcium carbonate structure named otoliths. In each fish ear chamber, there are three otoliths: sagittae, lapilli and asterisci. The sagittae is the more important structure generally used for fisheries research because of the valuable information's provided by this structure and the ease of its manipulation.

Sagittae otolith has been used generally for age determination, stock discrimination and life history description [1]. The morphology of otoliths as other scientific methods (morphometric, tagging markers, meristic, genetics, parasites...) has been widely used for stock discrimination of pelagic and benthic fish populations [2-8]. Unlike other scientific methods, the otolith shape analysis is not expensive and provides strong results for an estimate close to the reality of the existing stocks. This information will be the basis of the implementation of rational fishery strategy management concerning this species.

*Solea lascaris* is one of the main flatfish Targeted by small-scale fisheries vessel in the Central Atlantic coast of Morocco. Despite of the economic importance of this flatfish, there is almost no scientific research on this species in this area, so there is no strategy for fishery management of this species in this area. However, some researches on biometry and diet of *S. lascaris* has been carried out recently [9,10], which have shown the existence of more than one population that cohabits in this area.

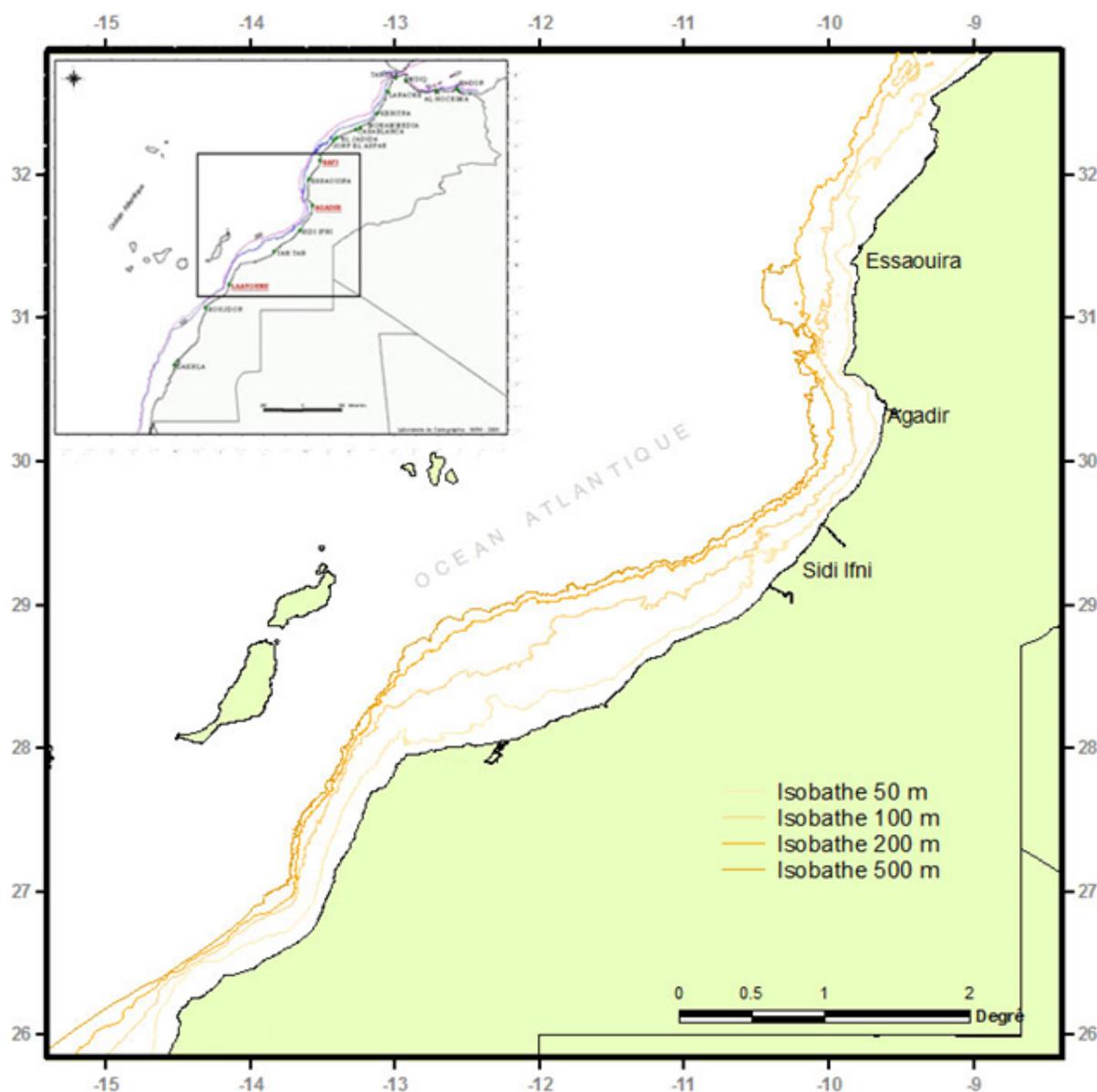
The researches made in other regions on this species concern the biometrics [11-13], the taxonomy [14], the diet [15,16] the reproduction [17,18] and the [19]. However, this study concern the analysis of the morphometric characters and shape of the otoliths, which carried out to justify if these three groups of the sand sole are coming from three different regions (ESSAOUIRA, IFNI and AGADIR) belongs to the same population.

## 2. Material and Methods

### 2.1. Sampling:

The individuals of *Solea lascaris*, were sampled from commercial captures in three main harbours of Moroccan Central Atlantic coast between 28°26' and 33°30' (Figure 1), most commonly caught by small-scale fisheries vessel with gill nets at 10 to 70 m depth. The whole individuals, which are 90 fishes, were measured for length and weight. Two different lengths were taking, standard and total nearest 1 mm. The weight was measured as the total body weight at 0.01g precision. The sagittae otoliths were extracted after dissection process, cleaned with distilled water, and then dried and stored in Eppendorf 1ml tube.

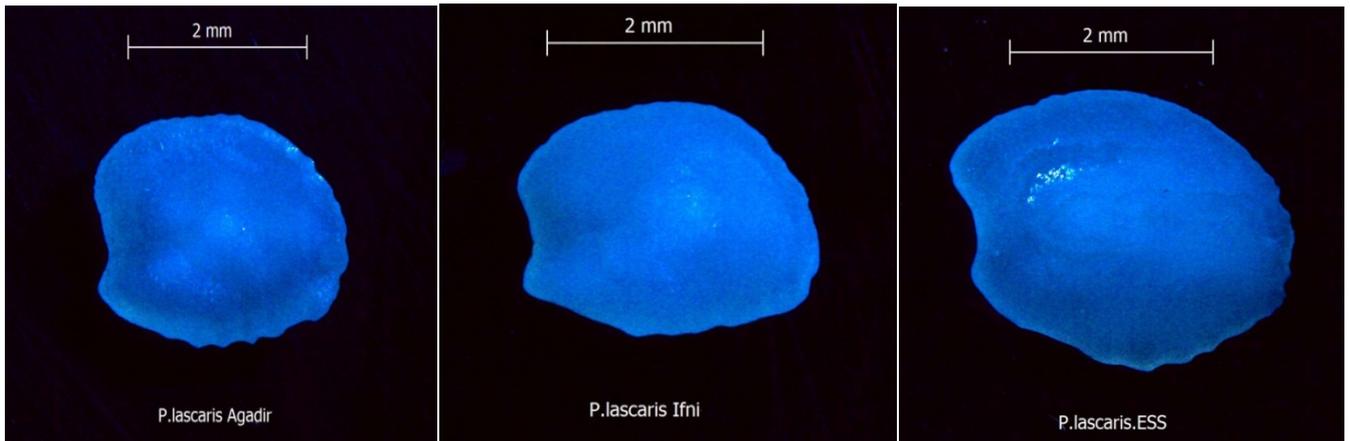
Left and right otoliths were weighed on a RADWAG 2009 (AS 110/C/2) analytical balance with 0.0001 g precision, and placed under a stereomicroscope with a camera Leica attached for image digitizing [5].



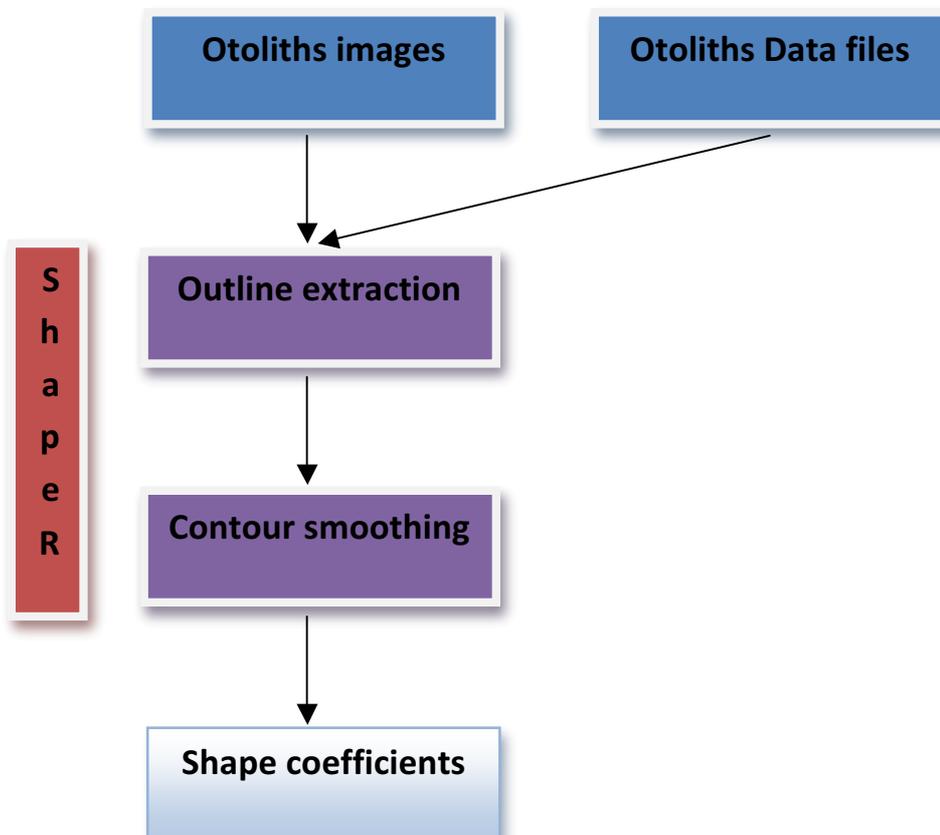
**Figure 1:** Map showing sampling station, the main port of the Central Atlantic coast.

### 2.2. Image analysis:

All data and images were prepared to be analysed by the shape R package in the software R [20]. This package elaborates by [21] uses together the data and the images to elaborate otolith shape analysis (Figure 2). Shape R package can extract a huge number of images contour. These packages make also the Fourier and Wavelet transformation and at the end visualize the mean shape (Figure 3).



**Figure 2:** The sand sole otolith from the three stations (AGADIR, IFNI and ESSAOUIRA)



**Figure 3:** Schematization of image processing with Shape R.

### 2.3. Statistical analysis:

The otoliths shape variation was assessed by the Canonical Analysis of Principal Coordinates (CAP) [22] on the length standardized Wavelet/Fourier coefficients with smoothed and unsmoothed outlines. The distance variation between groups was tested with ANOVA. Yet the estimation of the classification rate was evaluated with the Linear Discriminant Analysis [21]. All the otoliths shape measurements were adjusted with the total length to eliminate the allometric effect on the coefficient measurements [23],[24][25].

For the analysis of the otolith morphometric variables (mean.radii, otolith.area, otolith.length, otolith.perimeter, otolith.width) collected from shape data in shapeR package, the principal component analysis (PCA) and MANOVA test were conducted between the three populations.

For all the statistical analysis we use the software R and the packages related to each analysis.

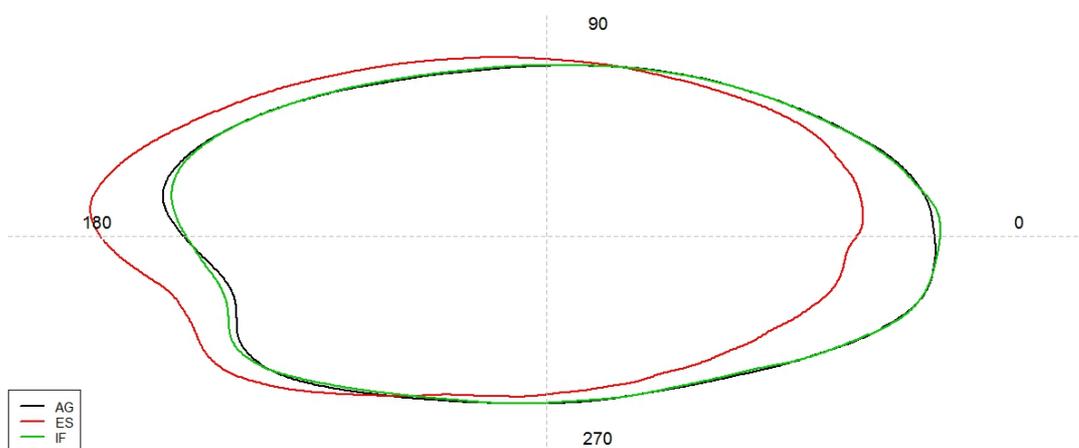
#### 2.4. Otolith weight:

In this part we search if the difference between right and left otolith weights can lead us to differentiate between the three populations. In each station we measured the difference between left and right otolith then we compared results for the tree station.

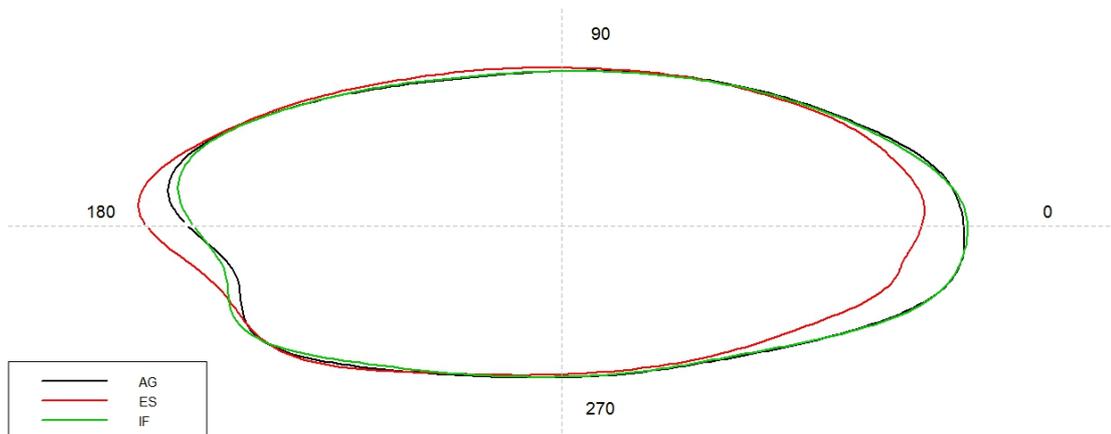
The left and right otolith weight comparison of all specimens was assessed with paired t-test. Before that, the distribution of the both left and right otolith was carried out with Shapiro-Wilk test. And the results show those two parts did not follow a normal distribution. And Kendall's rank test show 0.89 of correlation between the left and right otolith. Otherwise, ANOVA test was applied to investigate the relation between groups (stations) and the difference (left otolith weight ( $W_l$ )-right otolith weight ( $W_r$ )) between left and right otolith weight. The Tukey POST HOC TEST was conducted to show which group is different from the others.

### 3. Results

The mean shape of Wavelet and Fourier contours coefficient of three populations (Agadir, Ifni, and Essaouira) in the Atlantic Centre of Morocco, show a great similarity between Ifni and Agadir. In the other hand, these two populations show a high difference with the population of Essaouira (Figure4,5).



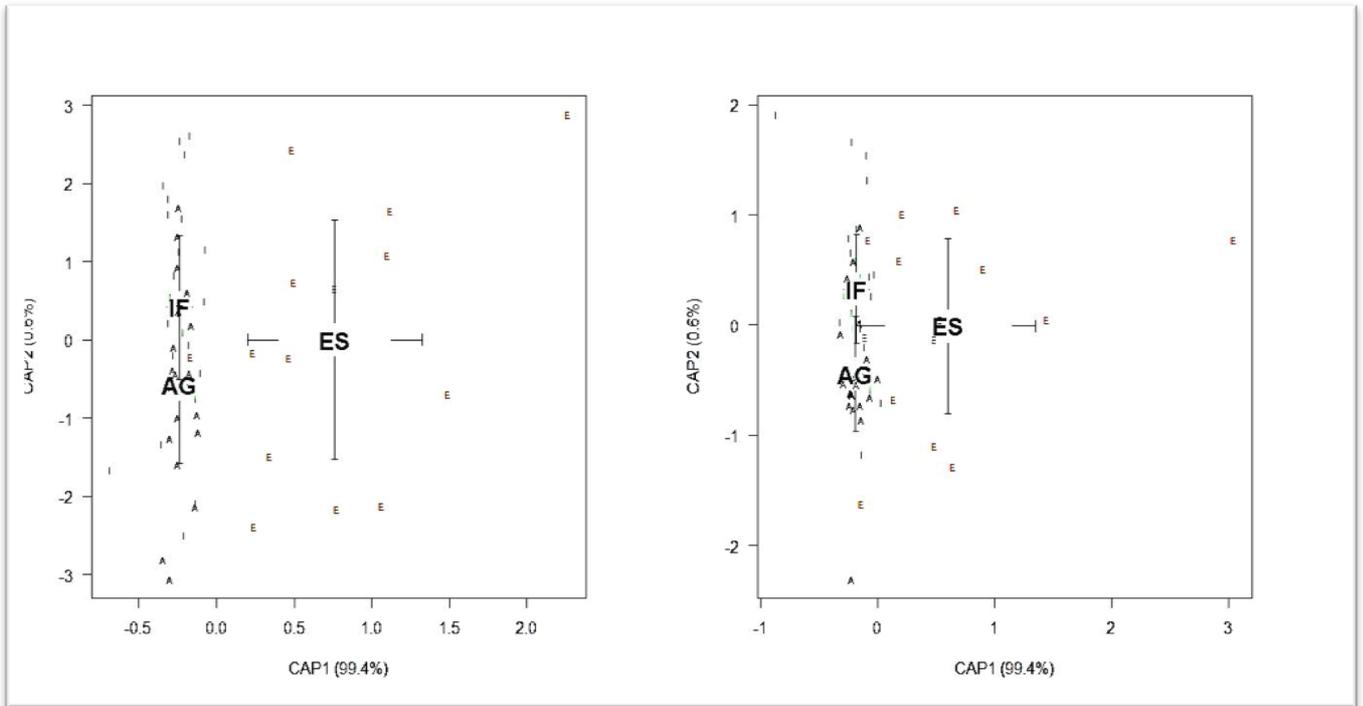
**Figure 4:** The mean shape of Wavelet contours coefficient of three populations (Agadir, Ifni, and Essaouira).



**Figure 5:** The mean shape of Fourier contours coefficient of three populations (Agadir, Ifni, and Essaouira).

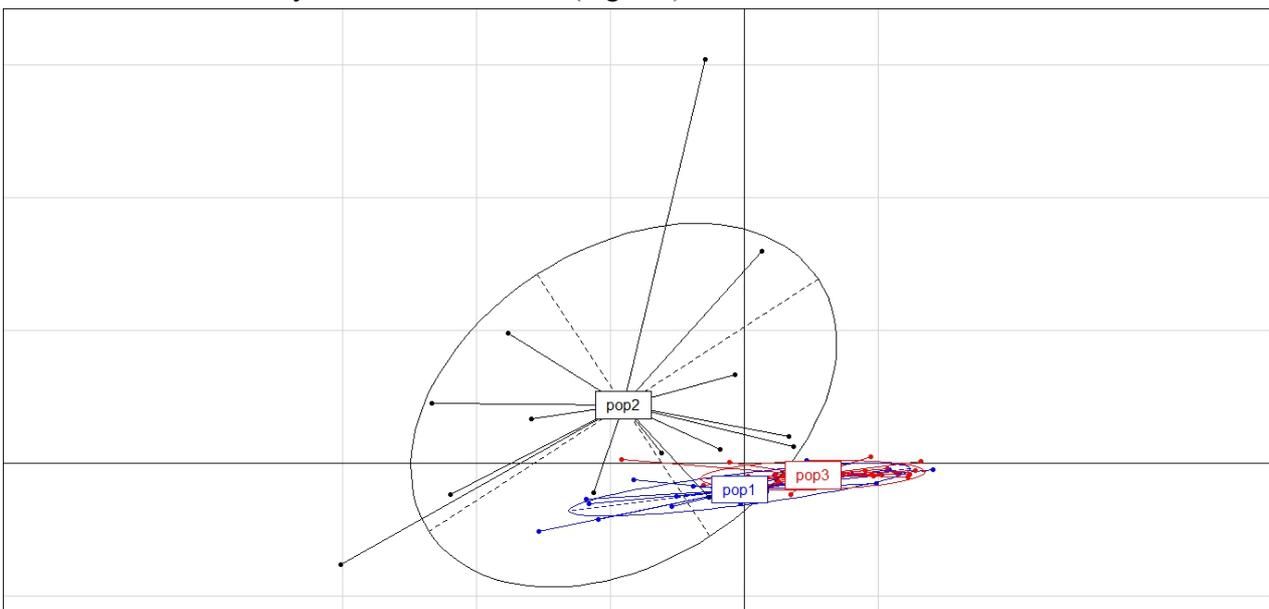
The result of the ANOVA test shows a significant difference at 0.1% between otolith shape of the three groups (Agadir, Ifni, and Essaouira).

The Canonical Analysis of Principal Coordinates (CAP) shows the same result as the mean shape Wavelet and Fourier contours coefficient (Figure 6).



**Figure 6:** Otolith shape of samples from three sand sole populations in the Atlantic Centre of Morocco using Canonical analysis of Principal Coordinates with the Wavelet coefficients.

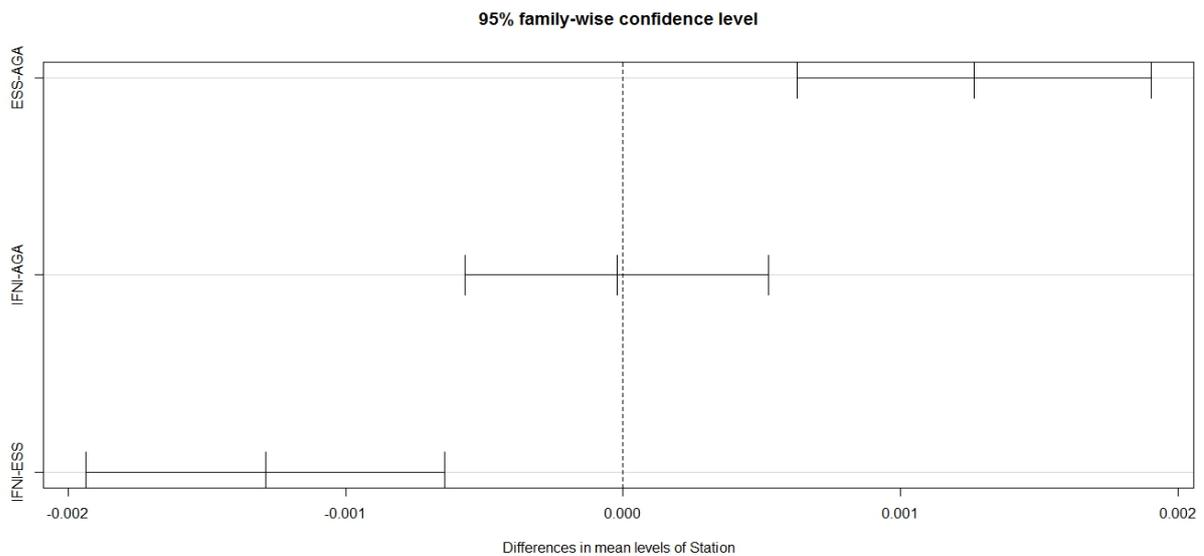
The Essaouira sample is dissimilar to the other samples according to the first axis (CAP1). Generally Ifni and Agadir show no difference between the two groups according to the first axis. In all analysis of Wavelet and Fourier coefficient, the first axis (CAP1) explains 99.4% of the variation between the populations, and the second axis indicates only 0.6% of the variation (Figure7).



**Figure 7:** Otolith morphometric data of sample from three sand sole populations in the Atlantic Centre of Morocco using PCA on otoliths morphometric variable (mean.radii, otolith.area, otolith.length, otolith.perimeter, otolith.width)

The linear discriminant analysis (LDA) based on Fourier standardized coefficient in the three locations (ESSAOUIRA, AGADIR, and IFNI) show 80% of score rate of the classifier and 0.0031 of standard deviation using bootstrap estimation. The otoliths morphometric show the same result of Wavelet and Fourier coefficient analysis. CPA otoliths morphometric results show 75.75% and 21.42% for the first and second axis respectively of the projected inertia (Figure7). According to MANOVA test, it can be seen that the four otoliths morphometric variables are highly significantly different among the three populations at 0.1% of probability. Total length is ranged between 175-365 mm, total weight also ranged between 48-437 g and mean of the left and right otolith is respectively (0.01037, 0.009201g). The paired t-test show significant difference between the left and right otoliths weight ( $t = -9.932$ ,  $P < 0.001$ ).

ANOVA test shows a significant difference of the percentage of the difference between the right and left otolith weight in the tree stations ( $p < 0.001$ ). The post hoc test shows that there is no significant difference between IFNI and AGADIR ( $p = 0.995 > 0.05$ ). However, there is significant difference between ESSAOUIRA and IFNI ( $p = 0.00003$ ) and between ESSAOUIRA and AGADIR ( $p = 0.000032$ ) (Figure8)



**Figure 8:** The difference between the three populations using otoliths weight ( $W_l - W_r$ ).

#### 4. Discussion

The FOURIER and WAVELET elliptical analysis of otoliths shape show a significant difference between the three populations, the same result was found for otolith morphometric analysis. These results agree with those concerning biometric study of *S. lascaris* in the same area [9]. In fact, this intergroup variability is maybe associated with genetic and environmental factors [26-28] such as temperature, salinity, depth, and food pattern. Diet of the sand sole was analysed in this area [10] and revealed different food behaviour between the three populations. Moreover, the temperature and salinity vary from area to another, especially in the ESSAOUIRA station, which present low-temperature values [29]. Furthermore, other factors may affect otolith shape variability between groups, the sexual maturity effect, which could modify the outline contour of otoliths [30], and the growth rate that is affected by exogenous and endogenous [30-32].

The Major roles of *S. lascaris* otoliths organs, as most of the flatfish, are the hearing and gravity perception [33,34]. Also the three areas show different substrate's nature, in ESSAOUIRA area, mud is the dominant substrate, muddy sand dominates AGADIR shelf and shelly sand in IFNI [35]. Thus, we suggest that probably other factors, as bottom substrate and predation strategy may involve into discrimination of the three populations.

Even if the distance between the three stations does not exceed 200km, they present high environmental variation. *S. lascaris* as all living marine organisms, adapt with its environment and create specific mode of life that probably affect its morphology, physiology and obviously the shape of its otoliths [36].

The left otoliths were heavier than the right one for *S. lascaris*. The same results were found by [37] for the same species and for most of the right eyed soleidea class. The left and right otoliths weight difference ( $W_l - W_r$ ) show significant dissimilarity between the tree stations. The otolith of ESSAOUIRA population is the heavier,

and IFNI and AGADIR shows the same mean otolith weight. Moreover, this result may have relation with diet of the three populations. It was found [10] that the AGADIR and IFNI sand sole prefer Bivalvia, which have low mobility, while those of ESSAOUIRA prefer polychaeta and decapoda. In fact [38,39] find that the weight of the otolith may involve in the sensitivity of the inner ear of the fish. In this direction we can suggest that the feeding behaviour probably affect the weight of otolith.

In this context we conclude that the adaptive behaviour to environmental factors requires common characteristics for each population of *Solea lascaris* that develop speciation in there morphological, physiological and behaviour characteristics.

Despite of the importance uses of otolith shape to discriminate fish population, it's important to notice that Mechanism describes otolith shape is not clearly identified.

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