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Ecological aspects of anchovy : *Engraulis encrasicolus* (Engraulidae. Teleostei) in the moroccan atlantic cost

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ABSTRACT: Anchovy is a small fatty fish. But it is mostly unsaturated fats. beneficial for the heart and the vessels. It contains the famous omega 3, whose protective effects on the cardiovascular system are widely recognized. The Moroccan sea, for centuries, contains a coveted natural treasure. The Moroccan anchovy that reflects the tradition, history and authenticity of fishing in Morocco. If Morocco has become a major exporter of semi-preserved anchovies, it is because the Moroccans were able to extract from their contrasting seas quality productions recognized by their originality and Moroccan identity and have produced canned with industrial know-how, recognized as a guarantee of quality. This know-how, which guarantees the authenticity and typicality of Morocco, is today a heritage that Morocco continues to preserve and enhance. Recognized for a long time by consumers, fresh Moroccan anchovies, with salt or oil, vinegar or spices, make their authenticity a motto of quality, identity and originality.

KEYWORDS: *Engraulis encrasicolus*, Anchovy, Reproductive biology, Moroccan, quality.

1 INTRODUCTION

Anchovies and sardines often represent the most abundant species in the productive upwelling regions of the oceans (Checkley et al.. 2009). The total biomass of fishes in those upwelling systems tends to be dominated by one species of sardine (or Sardinella) and one species of anchovy, and frequently only one of the two is dominant at any particular time (Cury et al.. 2000).

Estimating the age of fish is one of the most important elements in studying the dynamics of their populations. It is the basis of calculations leading to knowledge of growth, mortality, recruitment and other basic parameters of their populations.

The age of many fish species can be determined from discontinuities occurring in their skeletal structures. These discontinuities can result either from changes (such as temperature) in the environment where the fish is found, or from changes (such as reproduction) in fish physiology. However, many fish live in such a uniform environment that there are no discontinuities in their skeletal structures and the age determination of these fish must be indirect; it can often even be impossible. The methods used for fish with skeletal discontinuities will be described in the first part of this section and the methods available for fish with no skeletal discontinuities will be described in the second part. The third part is devoted to growth rates and the fourth part describes methods to obtain compositions in age groups from age-length keys.

2 BIOLOGY OF THE COMMON ANCHOVY

The European anchovy (*Engraulis encrasicolus*) is a small pelagic fish resource, distributed along the eastern Atlantic coastline, and in the Mediterranean, Black and Azov seas (Whitehead et al.. 1988.)

2.1 SYSTEMATIC

Order	Family	Genius	Species
Clupéiformes	Engraulidés	Engraulis	*E. encrasicolus* (Linné. 1758)

2.2 GEOGRAPHICAL DISTRIBUTION

The anchovy (*Engraulis encrasicolus*) is the only anchovy species in the Bay of Biscay, while there are at least eight in the world (Whitehead et al.. 1988). European anchovy is distributed in the North-East Atlantic, from Morocco to the North Sea and in the Mediterranean (Figure 1).

Coastal pelagic species, descending in winter between 100 and 180 m deep; sometimes captured up to 400 m. Anchovy is a species distributed over a large area of distribution. It is widespread throughout the eastern Atlantic from the shores of the Norway north of Bergen (62° N) to South Africa (23° S). It is also found in the Baltic Sea, the English Channel, North Sea. This species is also present throughout the Mediterranean Basin including the Black Sea and the Sea of Azov.

In Morocco, detections of anchovies from the Mediterranean are limited to traces in the eastern part, between Saïdia and Nador and at the Bay of Betouya. In the Atlantic North, the anchovy shows a discontinuous distribution, with generally low densities, the strata the more important in terms of surface area and density is between Assilah and Rabat, off Casablanca and off south of El Jadida. At the level of the Central Atlantic, the distribution of anchovies is very extensive on the coastal strip, the concentration maximums are often at the level of Agadir, between Tantan and Foum Agouitir and between Laayoune and Boujdor. In the southern zone, the distribution of anchovy is generally low and is limited to the area between Dakhla and Lagouira, especially of Cap Barbas (INRH. 2014).

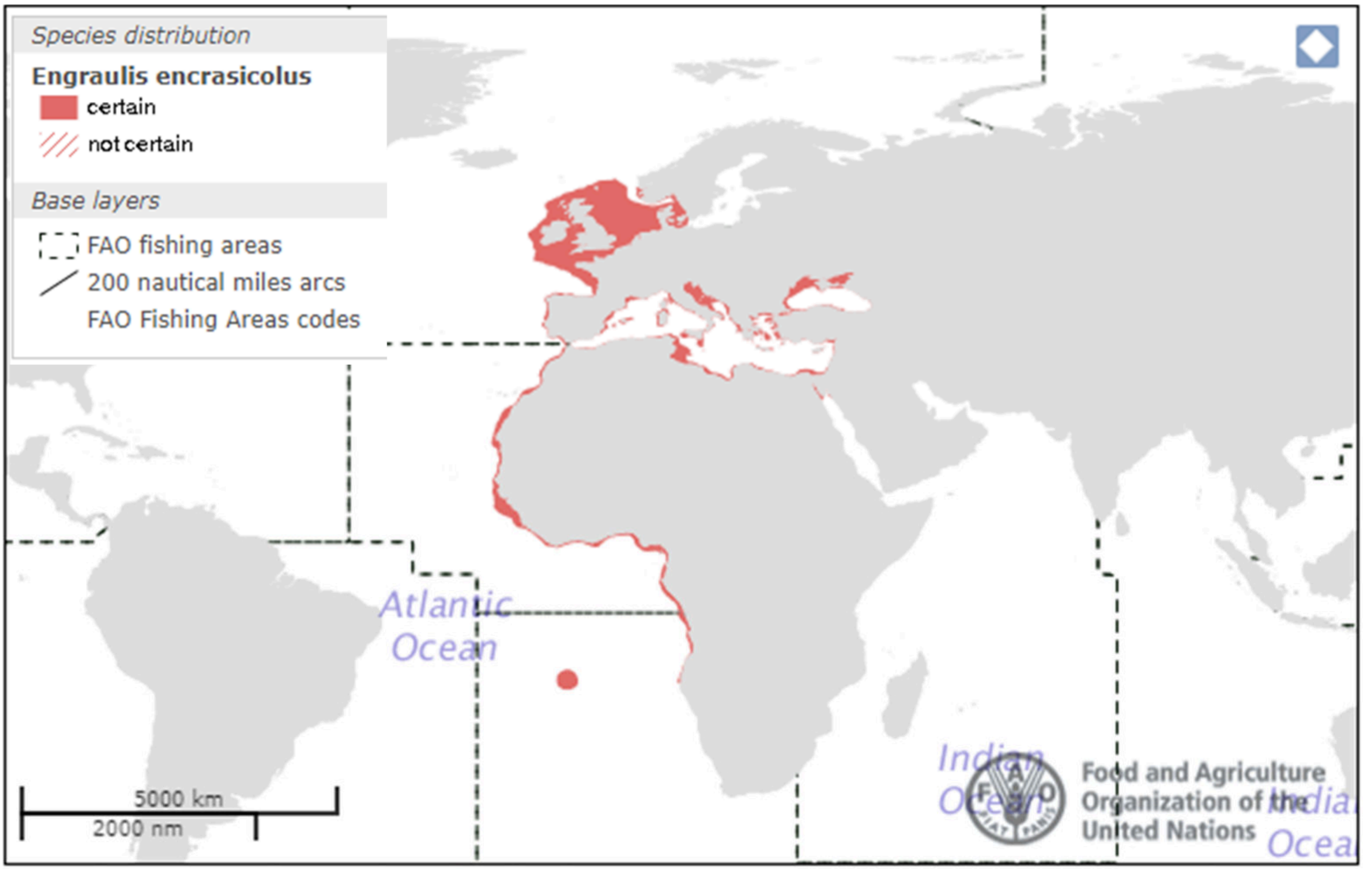

Fig. 1. Launch the Aquatic Species Distribution map viewer (FAO. 2014)

2.3 PRODUCTION

Anchovy fishing in Morocco is known as sporadic and random fishing. Catches vary from one year to the next, in particular because of the geographical distribution and behavior of schools that are not always accessible to seiners and the environmental variability that determines the abundance of fish, this species. The anchovy catch reported in 2014 is around 18 thousand tones, 35% of which is at the zone level North, 63% in the central zone and 1% in the Mediterranean area. A very small catch is landed in zone C (Figure.2). The anchovy stock between Cape Spartel and Cape Bojdour is considered fully exploited. However, this diagnosis of full exploitation should be considered with caution given the rather large fluctuations observed in acoustic abundance indices for this short-lived species whose abundance depends on variations in recruitment (INRH. 2012).

The stock of anchovy shows a slight improvement in 2014 compared to 2013, all remaining in a state of overexploitation (INRH. 2014).

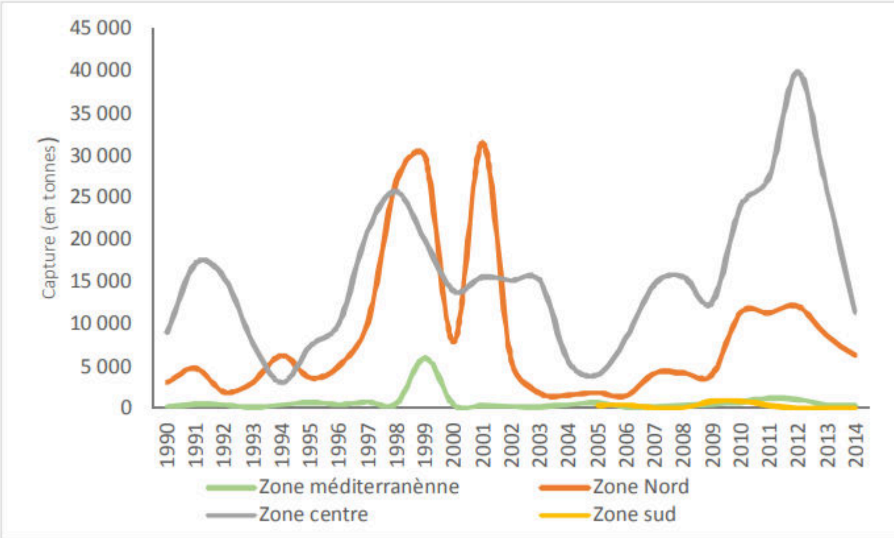

Fig. 2. Geographical distribution of anchovy

2.4 HABITAT

Anchovy is a gregarious pelagic fish that lives and moves in schools, whose way of life is more related to the quality of water bodies than to particular probes or latitudes (Whitehead et al.. 1988). It lives in coastal waters up to 150 m deep, and its affinity for slightly desalinated waters makes it appear regularly in the plumes of rivers (especially in front of the Gironde) or the brackish water lagoons. Migrations of anchovy from the Bay of Biscay are very little known. In fact, the fragility of the anchovy makes any tagging operation impossible, and no monitoring of the movement of the fish makes it possible to establish a clear migratory pattern. Nevertheless, research carried out in recent years makes it possible to establish distribution hypotheses according to their biological stages:

- During the nesting period (April - August), anchovy is attracted by mixing zones of saline waters or different temperatures which constitute highly productive environments (Motos et al.. 1996). This is the case of desalinated water plumes induced by rivers and certain areas where particular hydrological phenomena occur (upwellings, upwelling).
- The laying is followed by a period (August to November) of strong growth (75% of annual growth). Anchovies then occupy the plateau from the coast to the 100 to 120 m probes in the northern Bay of Biscay.
- From the egg-laying areas, the eggs and larvae drift with the currents. Larvae remaining on the continental shelf benefit from better growth and a higher survival rate (Allain et al.. 2003). Since the circulation of water bodies varies from year to year depending on weather conditions, the abundance of recruitment (year-old fish) will strongly depend on the climatic conditions during this period (Borja et al.. 1998).

2.5 DIET

Adult anchovy feeds mainly on zooplankton, especially copepods and crustacean larvae, as well as pelagic fish eggs and fry (Plouvenez & Champalbert. 1999).

2.6 GROWTH

The growth of anchovy is very fast the first year and then slows down. An anchovy born in spring measures between 8 and 11 cm in its first winter. Longevity reaches 5 years but the majority of individuals do not exceed 3 years.

2.7 REPRODUCTION

Anchovy spawns over a period of several weeks (about 30 eggs spawning in the season, every 3 to 4 days), in waters with temperatures between 14°C and 19°C (Motos et al.. 1996). Anchovy attains sexual maturity at the end of its first spring and its laying is from April to August; the oldest fish beginning in April, followed by the youngest in May. The spawning of the spawning season is an asset for the survival of eggs and larvae which are thus more likely to develop in a favorable environment.

3 AGE STUDY TECHNIQUES IN ANCHOVY

3.1 OTOLITHOMÉTRIE

3.1.1 GENERAL INFORMATION ABOUT OTOLITHS

The age of the individuals can be determined from the otoliths (from the Greek oto: the ear and lithos: the stone) which are mineralized concretions located in the membranous labyrinth of the inner ear of teleost fishes. There are three pairs of otoliths, the lapilli, the sagittae and the astericii, contained in each otic capsule on either side of the brain, and participating in the mechano-receptive function of hearing and equilibration (Baillon. 1991).

The pair of sagitta, already present at hatching, is the one that is best suited for reading age since it is the largest (Reibish. 1899). It is ellipsoidal in shape, laterally compressed, with a convex (outer) distal surface and a concave (internal) proximal surface carved out of a groove called the sulcus acusticus (Figure 3). The anterior part consists of two advancements: the longest corresponds to the rostrum, the shortest to the antiroster.

In the case of anchovy in the Bay of Biscay, the ventral edge is ornamented with more or less numerous notches depending on the individuals and their age. It is from the initial crystals secreted by the inner ear, the primordium, that otoliths begin their growth (Campana & Neilson. 1985). Each otolith develops by successive and centrifugal apposition of layers of aragonite, resulting from the crystallization of calcium carbonate on a reticulate protein matrix consisting of otoline (Morales-Nin. 1987). Otolin represents only 0.2 to 10% of the weight of the otolith (Degens et al.. 1969), so they are structures essentially made up of calcium, present in the form of aragonite (Gauldie. 1997), exceptionally in the form of vaterite (Gauldie. 1997) or calcite (Morales-Nin 1985) and showing trace elements such as iron, copper, sodium, silicon or magnesium. Aragonite has the advantage of being a metabolically inert crystal (Mugiya. 1974), which gives it its conservative properties: unlike other mineralized tissues of the body that can undergo a metabolic turnover, aragonite is not modified by the variations of the metabolism and keeps in memory all the biological events of the life of the animal (Lecomte-Finiger. 1999).

The annual cycle of the individuals is thus recorded in the form of alternately opaque and translucent rings, corresponding respectively to the period of rapid growth of the fish from April to September when aragonite is deposited, followed by a period of very slow growth, without aragonite deposit during winter (Campana. 2001). It is therefore these concentric layers which make it possible to determine the age of the individuals, since in the temperate zones a clear layer and a dark layer are deposited each year.

Otoliths have been known for a long time as indicators of age (Baillon. 1991), but also of growth (Campana. 1992), of belonging to a species and even more recently for the discrimination of stocks (Campana & Casselman. 1993). The otoliths collected will therefore be used to determine the age classes, and then the comparison of their shape may, if necessary, allow us to Discriminate different populations. These results will then be integrated into the PELGAS database for further studies.

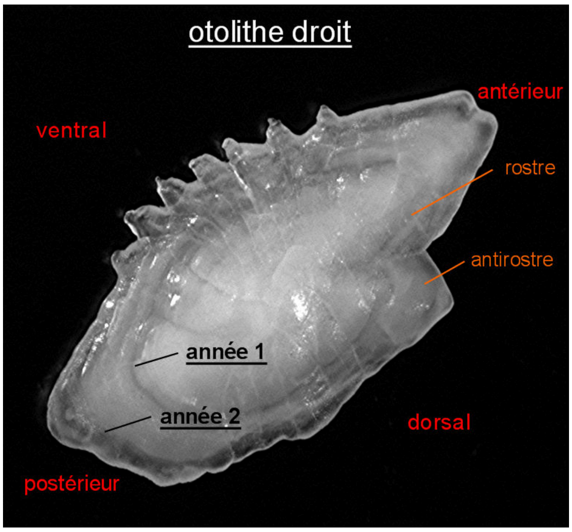

Fig. 3. Location of growth rings on the otolith.

3.1.2 SAMPLING SAGITTAE

The pairs of sagittae were extracted using fine forceps after cutting the skull. They were then cleaned under a binocular loupe and rinsed with distilled water, then dried before being kept in tubes bearing all the references of the fish. In the rest of the report, the term otoliths will refer to sagittae.

3.1.3 OTOLITHS DIGITIZATION

The otoliths were digitized using a binocular loupe (Leica WILD M8. x18 magnification) equipped with a video camera (SONY XC-77CE) connected to a PC computer. The acquisition of images and their processing is performed using an image analysis software (VISILOG 6.3. Noésis company). The otoliths are placed separately under the magnifying glass: they are placed flat above a black base on their external face, so that the internal face can be scanned. Episcopal fiber optic lighting allowed the intensity and direction of the luminous flux to be adjusted. For each otolith a series of 3 images was recorded:

- The first photograph is made under a weak light to be able to visualize the rings of growth (figure 4a).
- The second is carried out under conditions of overexposure of the otolith to light to obtain maximum contrast with the black background (Figure 4b).
- The last image is made from the second photograph after binarization of the image by the software (Figure 4c).

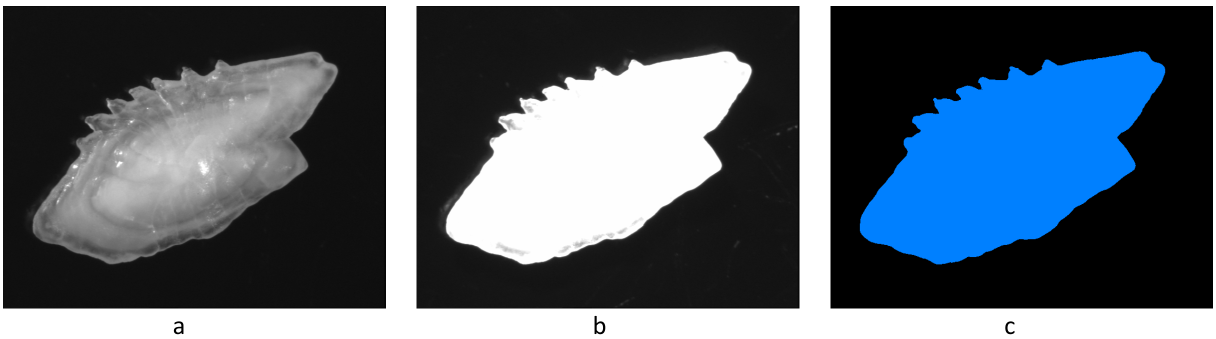

Fig. 4. Photographs of otoliths. (a) normal light. (b) overexposure (c) binarized.

3.1.4 ESTIMATION OF AGE AND GROWTH MORPHOLOGY OF OTOLITHS

The age is estimated by counting the number of rings on the first photograph: an opaque zone and a translucent zone consecutive correspond to a year of growth. A ring is accepted in the count if it is completely around the otolith, otherwise it will be considered a false ring. Only the past years for each individual are taken into account: even if the anchovy has resumed growth, it will belong to the year-class corresponding to the year of the last visible translucent rin.

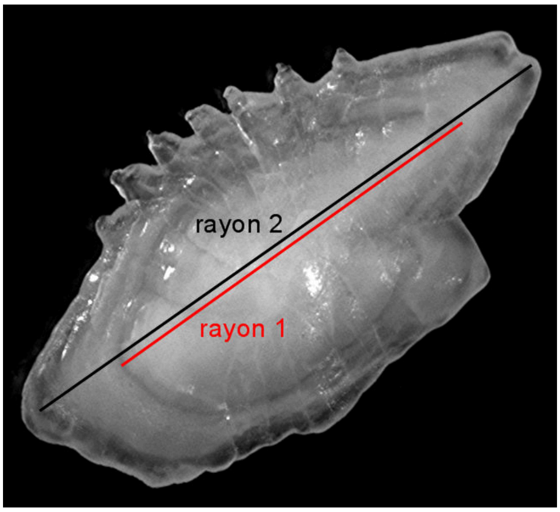

Fig. 5. Measure of annual diameters

This image also measures the size of the annual diameters as well as the total length of the otolith if it is in a period of resumption of growth (Figure 5).

Measurements are made in pixels on right and left otoliths since previous studies have shown that asymmetry may appear in other species (Takabayashi & Ohmura-Iwasaki.2003).

3.1.5 MEASURING GROWTH

The growth rate (K) of individuals in each trawl can be calculated from a data table with the age and length of each fish using FISHPARM software (Prager et al.. 1989). In fact. the growth of individuals can be described by the model of Von Bertalanffy (Von Bertalanffy. 1938. 1957) which defines the length of fish according to age. according to the equation:

$$L_t = L_\infty \left(1-e^{-K(t-to)}\right)$$

Or:

Lt is the length of the individual at time "t".

L_∞ represents this same length for an infinite time.

K defines the growth coefficient.

to corresponds to the theoretical age for which the length is equal to zero.

K values are associated with a confidence interval to compare them.

3.1.6 ANALYSIS OF THE SHAPE OF THE OTOLITH

3.1.6.1 FOURIER ANALYSIS

The shape of an object can be described to various degrees of precision by using the decomposition of its contour by Fourier series. The contour is defined by a periodic function expressing itself in a sum of terms of a trigonometric series based on sine and cosine. This series consists of compounds called harmonic whose coefficients can serve as descriptive variables for the shape of the object. This system thus makes it possible to roughly describe the contour of the object by low frequency harmonics, and the addition of increasing order harmonics increases the accuracy.

Fourier analysis can be applied to an outline in different ways: in this study, the elliptical Fourier transform will be used since it is the most powerful method in the field of taxonomic description (Rolf & Archie 1984. Ferson et al.. 1985. Crampton 1995).

3.1.6.2 THE ELLIPTIC FOURIER TRANSFORMATION

The principle of this method is based on the calculation of the closed contour of an object, which can be represented by two series x (t) and y (t) corresponding to the projections of the contour on the abscissa axis and the axis of the ordinates of any reference. The projections are a function of the distance (t) measured along the contour, from an arbitrary point. For the projection on the two axes of the series x (t) and y (t), the Fourier transformation is calculated as follows (Kuhl & Giardina. 1982):

$$x(t) = (A_0 / 2) + \sum_{n=1}^{N} (A_n \cos n\omega t + B_n \sin n\omega t) \qquad (1)$$

$$y(t) = (C_0 / 2) + \sum_{n=1}^{N} (C_n \cos n\omega t + D_n \sin n\omega t) \qquad (2)$$

For the function x (t) corresponding to the projection of the contour on the abscissa axis, the two Fourier coefficients were calculated as follows:

$$A_n = (T / 2\pi^2 n^2) + \sum_{p=1}^{K} (\Delta x_p / \Delta t_p) . [\cos (2\pi n t_p /T) - \cos (2\pi n t_{p-1} /T)] \qquad (3)$$

$$B_n = (T / 2\pi^2 n^2) + \sum_{p=1}^{K} (\Delta x_p / \Delta t_p) . [\sin (2\pi n t_p /T) - \sin (2\pi n t_{p-1} /T)] \qquad (4)$$

Where :

t : is the distance of the arc measured along the contour from an arbitrary starting point.

T: is the period of the functions x (t) and y (t) which makes it possible to define the wavelength (ω) like : $\omega=2\pi/T$;

n represents the number of harmonics;

N is the total number of harmonics used to approximate x (t);

K is equal to the number of points p defining the contour;

Δx_p represents the displacement on the x-axis of the contour between the points p-1 and p.

Δt_p is the length of the linear segment between points p-1 and p;

tp is the cumulative sum of segment lengths Δt_p ;

The pair (A_0. C_0) indicate the coordinates of the center of gravity of the object.

The coefficients C_n and D_n for the projection y (t) are calculated in the same way. From a closed contour, four coefficients per harmonic can be calculated. A_n and B_n for the projection x (t) on the abscissa axis. C_n and D_n for the projection y (t) on the ordinate axis. The principle of the method is shown schematically in Figure 6.

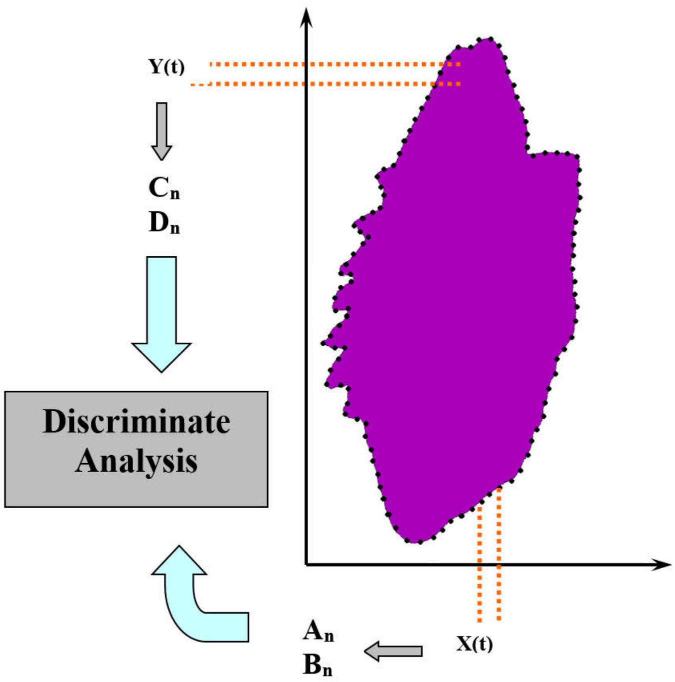

Fig. 6. Principle of the description of a closed contour by the elliptic Fourier descriptors

From the photographs of each otolith, the computer processing was realized thanks to the software Shape version 1.2 (Iwata & Ukai. 2002) which uses invariance and standardization procedures suggested by Kuhl and Giardina (1982). It calculates the Fourier coefficients so as to make them independent of the position of the otolith, its size, its orientation as well as the position of the starting point of the contour.

In addition, the inverse transformation of the Fourier descriptors makes it possible to visually and progressively determine the quality of the approximation of the real contour by the calculated contour by recalculating the coordinates of the k points of the contour from the Fourier coefficients An, Bn, Cn, Dn, for a given number of harmonics, and thus makes it possible to determine the number of harmonics necessary for the description of the contour of the otolith. In addition, the Fourier power (PF) has been calculated to determine the number of harmonics sufficiently coherent for the best reconstruction of otoliths (Crampton. 1995):

$PFn = (An2 + Bn2 + Cn2 + Dn2) / 2$

With An, Bn, Cn, Dn the Fourier coefficients at the nth harmonic.

By graphically representing the average cumulative Fourier power as a function of the number of harmonics, obtained from a sub-sampling of 30 otoliths taken at random from all the individuals, the information gain for the description of the contour at each harmonic has been determined. A threshold of 99.99% of the total average cumulative Fourier power was chosen to decide the number of harmonics sufficient to describe the contour of the otoliths of this subsample. This same number was then considered to analyze all the otoliths in this study.

3.1.7 STATISTICAL ANALYSIS

Discriminant analysis, multivariate analysis of ordination under stress, was made from the Fourier coefficients: it makes it possible to test, using a certain number of quantitative variables (index of age, first ray, trawl, coordinate, Fourier coefficient), the membership of individuals to groups defined a priori (samples from different sites) and the validity of these groups.

Unlike unconstrained ordination methods (principal component analysis, for example), where the objects are arranged according to their main axes of variation, the discriminant analysis aims to find the linear combinations of the descriptors that maximize the difference between known groups, while minimizing the variability within each group.

The relative contribution of the descriptors to the final discrimination is evaluated by the coefficients of the standardized discriminant functions. In addition, the discriminant analysis makes it possible to reclassify individuals a posteriori on the data used for the discrimination. This reclassification is then compared to the initial ranking of the predefined groups.

The quality of the discrimination of the different groups is measured by the value of the Wilks lambda which is the ratio of the intragroup variance and the total variance. It varies between 0 and 1, a low Wilks lambda value indicating good discrimination.

Cohen's kappa coefficient makes it possible to determine, based on the percentage of correctly reclassified individuals, the proportion of truly reclassified individuals that is not due to chance. This index varies between 0 and 1, with 0 indicating that the results obtained by the discriminant analysis can be explained by chance alone, and 1 indicating 100% correct reclassification. The significance of this coefficient is evaluated by a Z test (normal law): if Zcalculated is superior to Zthoric, it is significant (Titus et al.. 1984).

Since discriminant analysis is robust enough to support a normality gap (Legendre & Legendre 1984), the conditions to perform this analysis are the independence of observations and a number of independent quantitative variables less than the number of observations.

It should be noted that in all discriminant analyzes, the first harmonic has been removed since it corresponds to an identical perfect ellipse for each otolith.

4 ELLIPTICAL FOURIER DESCRIPTORS

4.1 FOURIER POWER

The cumulative Fourier power, calculated from the first 30 harmonics, reaches the value of 99.99% at the 22nd harmonic: the contour can, therefore, be approached fairly accurately by the first 22 harmonics of the analysis of Fourier.

These 22 harmonics thus provide a data set of 88 elements per otolith (22 harmonics x 4 coefficients). However, since the software used for the discriminant analysis (Statgraphics Plus) can process only 70 elements per otolith, it was necessary to reduce the number of harmonic for the description of the contours: only the first 17 were preserved. The power at the 17th harmonic is 99.975% which remains acceptable to describe the contour of otoliths satisfactorily (Figure 7).

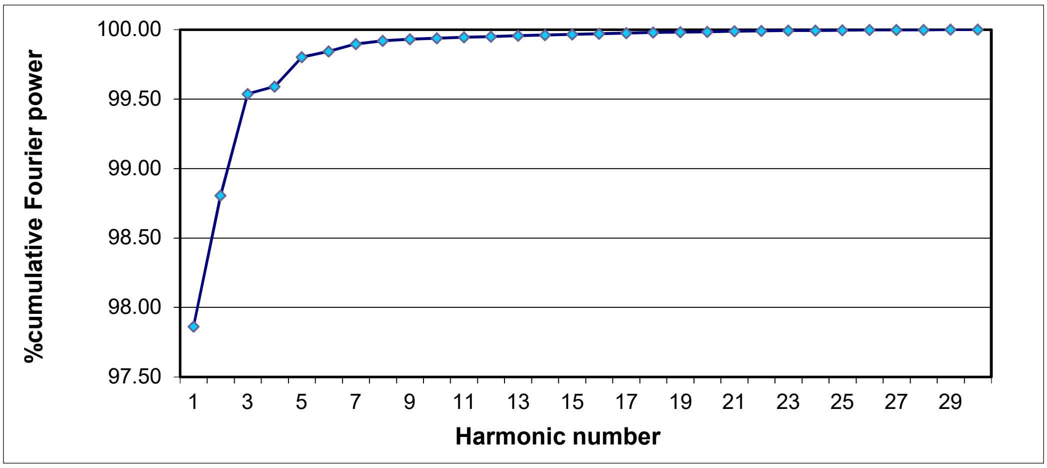

Fig. 7. Cumulative percentage of the average Fourier power as a function of the number of harmonics describing the otolith contour

4.2 DISCRIMINATE ANALYSIS

The data set of this study respects the conditions of application of the discriminate analysis since the objects are independent (all the otoliths are different), and the number of objects (700 otoliths) is greater than the number of descriptors (4 Fourier coefficients x 16 harmonic). The 16 harmonics correspond to the 17 necessary harmonics calculated by the Fourier power, minus the first harmonic which represents the perfect ellipse.

To carry out the discriminate analyzes, the individuals were divided into several classes according to geographical criteria (depth = 3 classes, longitude = 3 classes, latitude = 5 classes, trawl = 7 classes), morphometric (radius1 = 5 classes), asymmetrical and age (age = 3 classes, side-age = 6 classes divided as follows: otolite right of 1an = class1, right-2ans = class2, right-3years = class 3, left-1an = class4, left-2years = class5 , left-3years = class6.)

The Wilks coefficients obtained for each discriminant analysis are quite high and indicate very poor discrimination (Table I). The lowest coefficient is 0.234 and is considered high in comparison with results obtained on other species. The maximum is reached with depth discrimination where Wilks' lambda is 0.717. The reclassification percentages are also very low and do not exceed 76.32% of highly reclassified individuals. Cohen's Kappa coefficients indicate that for discrimination of age, first ray, trawl and right-left-age differentiation, correctly reclassified individuals not being randomly accounted for 51.31%, respectively 75%, 38.13% and 51.01% which is relatively low.

Table 1. Summary of the results of multiple discriminant analyzes performed between otoliths.

	Lambda Wilks	p-value	correctly classified observation (%)	Kappa	$Z_{calculated}$	$Z_{théoritical\ p<0.001}$
Depth	0.717	0.000	53.480	51.07	30.65	3.090
Longitude	0.680	0.000	57.950	45.80	27.48	3.090
Latitude	0.560	0.000	52.980	45.37	27.23	3.090
Age	0.522	0.000	76.320	51.310	30.790	3.090
Radius 1	0.472	0.000	56.620	35.750	21.450	3.090
Trawl	0.341	0.000	47.020	38.130	22.880	3.090
Side-age	0.234	0.000	63.580	51.010	30.610	3.090

5 DISCRIMINATION BY FORM ANALYSIS

The results of the discriminant analyzes carried out using the Fourier coefficients show that it is difficult to discriminate groups of anchovies from the shape of their outline. Associated reclassification percentages may appear relatively low when compared to studies of other teleost species. Some authors consider that only classification values higher than 75% are acceptable (Friedland & Reddin 1994). However, grades of 56 to 81% have been reported for the identification of Melanogrammus aeglefinus stock in George Bank in Canada (Begg & Brown. 2000). The results obtained are therefore considered acceptable for this species of anchovy *Engraulis encrasicolus*.

The best discrimination has a Wilks lamda equal to 0.234 and concerns the differentiation of otoliths according to their age and their side (right or left). However, if we look at the coefficient of the analysis applying to age alone, we see that the lambda increases to 0.522 which indicates a less good discrimination: it is therefore very likely that it is the variable "right This strongly influences the analysis, especially since it has been proved that the bilateral asymmetry of otolith pairs in European anchovy larvae may be a function of the environment (Somarakis, 1997). This age discrimination may be due to the fact that during growth, the crenellations present on the ventral edge of the otoliths disappear as the aragonite settles in the hollow of the ornamentations. However, all otoliths do not necessarily present these ornamentations, and the number of crenulations is itself very variable and does not seem to be correlated to groups of individuals, which makes differentiation difficult. Nor is it possible that this differentiation is related to the growth phenomenon discussed in the previous section: perhaps the growth rate influences the shape of the otolith.

Based on these considerations, it can be said that there is a fairly good discrimination between trawls, even if the lambda is only 0.341 and the Kappa coefficient estimates that only 38.13% of the individuals are really well reclassified without intervention of chance. This form discrimination by trawl can certainly be explained by differences in environmental parameters, since it has been shown that the shape of the otolith can be very dependent on the surrounding environment (Campana & Casselman, 1993). It is therefore possible within a species that individuals present identical forms of otolith if they live in the same conditions, and yet be genetically very different. In the same way, it can thus be said that individuals with dissimilar otolith forms do not necessarily mean that they belong to different groups. In addition, anchovy being a migrant pelagic species, it is very likely that the regular change of environment tends to standardize the shape of otoliths. One could also assume that during these migrations, anchovies only follow the movements of water bodies that are favorable to them, and thus change their geographical location but remain permanently in the same environmental conditions. This would then result in the formation of otoliths of the same shape, without meaning that they are genetically close.

However, the absence of stock discrimination can come from the fact that the spatial scale of the Bay of Biscay is too small to visualize a difference within a migratory species. Trawl discrimination cannot therefore be used as a reliable basis for defining several populations in the Bay of Biscay.

Since this work has only been carried out on 350 individuals and on a very small geographical scale, it is difficult to formulate an hypothesis concerning the distribution of anchovies. In view of these results, it can be said that there is a slight temporal differentiation within the population, since there is discrimination according to age as shown by the work of Gonzales Salas (2005), who could, however, be related to growth rate, but no geographical differentiation can be visualized from this study.

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