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Are hake otolith macrostructures randomly deposited? Insights from an unsupervised statistical and quantitative approach applied to Mediterranean hake otoliths

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Abstract:

Individual fish age data are crucial to fish stock assessment, so their accuracy and precision are vital. The acquisition of age data most often relies on interpreting fish otoliths, a complex task in which expert subjectivity increases with the complexity of the structural patterns of the otoliths. The question arises for certain fish species (e.g. hake, *Merluccius merluccius*) whether the deposition of otolith macrostructures is meaningful for age estimation. A quantitative method based on the evaluation of otolith similarity in terms of structural patterns is presented to investigate this issue. It relies on the determination of a typology of otolith macrostructures from an unsupervised statistical analysis of the distributions of their characteristics. This typology provides a basis for analysing and comparing structural patterns of otoliths through evaluation of structural otolith similarities. Application to a set of Mediterranean hake otoliths discriminates three types of macrostructure, one likely associated with fish responses to environmental or endogenous factors, and the other two meaningful at a population or group level. Comparisons of structural patterns based on the proposed structural similarity measure over two successive years support the assumption that otolith patterns are stable over time, although male and female otoliths differ significantly in structural pattern. The results bring new evidence that hake otolith patterns are not random and may be relevant to age estimation.

Keywords: age, age estimation method, growth, *Merluccius merluccius*, otolith, quality control

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Introduction

Catch proportions at age are key inputs to stock assessment models and are routinely used for fisheries management. The time and the duration of life history events are also required information for many ecological studies. To provide information of the age structure of exploited populations, the ages of almost a million fish are estimated annually using otoliths (Campana and Thorrold, 2001). However, the interpretation of fish otoliths is far from a trivial task and data quality remains crucial. For instance, a review of age data for ICES stocks shows that data quality is modest or poor for 75% of the stocks (H. Sparholt pers. comm.). There is therefore a clear need to assess the accuracy and precision of age estimation procedures (see Campana, 2001) as well to develop quality control mechanisms (Morison *et al.*, 2005). Besides these aspects, the interpretation of otolith growth marks is often a complex task in which subjectivity increases with the complexity of the structural pattern of the otolith. Although the complexity of the interpretation depends on various parameters, it is above all species-dependent.

Improved understanding of the biological meaning of growth marks and the development of a quantitative framework aimed at defining an objective procedure for otolith interpretation are complementary solutions to reducing interpretation subjectivity, and are especially needed for complex species.

We seek to develop the quantitative framework here, for European hake (*Merluccius merluccius*). Recent advances in signal processing and pattern recognition provide appropriate tools to achieve this goal, as exemplified in other applications to otolith research (Fablet and Le Josse, 2005; Fablet *et al.*, 2007). The development of a measure of otolith similarity in terms of structural information is viewed as a first step towards extracting relevant patterns in complex arrangements. The European hake is a good candidate for such developmental work. For Atlantic hake, the precision of age estimation has recently improved as a consequence of international otolith reading workshops being convened (e.g. Anon., 2002). However, the results of recent tagging experiments (de Pontual *et al*., 2003, 2006) appear to invalidate the interpretation criteria agreed at one such workshop and described by Pineiro and Sainza (2003). The tagging work suggested age overestimation of the recovered fish and therefore an underestimation of somatic growth (de Pontual *et al*., 2006), which clearly impacts stock assessment and management advice (Bertignac and de Pontual, 2007). Uncertainties in age and growth information on Mediterranean hake are well documented (Oliver *et al.*, 1992; Morales-Nin and Aldebert, 1997) and have been discussed in several workshops (GFCM, 1982; Oliver *et al*., 1989). Knowledge on growth has been improved for the early life stages from an analysis of otolith microstructures in different areas of the Mediterranean (Arneri and Morales-Nin, 2000; Morales-Nin and Moranta, 2004; Belcari *et al*., 2006). However, to date, structural patterns of otoliths (in terms of macrostructures) have been suggested to be unusable for age estimation (Morales *et al*. 1998).

To investigate these issues, a quantitative method has been developed. From an analysis of large sets of otoliths, it performs an unsupervised extraction of a typology of otolith macrostructures and the statistical analysis of associated otolith macrostructure patterns. Here, we describe the proposed approach and demonstrate how it can be applied to discussing relevant questions regarding structural patterns of Mediterranean hake otoliths.

Material and methods

Biological material

We used a set of Mediterranean hake otoliths (sagittae) previously prepared for routine age estimation in transverse sections. The fish were collected from both commercial landings and scientific surveys, the international bottom trawl survey (MEDITS), the small pelagic survey (PELMED), and the fishing technology survey (TECPEC), carried out in the Gulf of Lions from June to November of 2002 and 2003. The size range of the fish was 8–50 cm. Sampling design requires 10 fish per 1-cm size class, but in 2003, there were very few fish from 19 to 25 cm. Total length (cm), total weight (g), sex (male, female, undetermined), and maturity were recorded for all fish. The length distribution of the processed sample set is shown by sex and sampling year in Figure 1.

Suggested approach

With a view to analysing the structural patterns of otoliths, a key step is to define a similarity measure between otoliths in terms of structural information, i.e. a numerical measure quantifying how similar two otoliths are, based on a numerical representation of structural patterns. This is viewed as a key component of the framework needed to determine objective and validated protocols for otolith interpretation. Such a similarity measure provides the basis of standard analytical tools, for instance statistical tests or classification tools, for analysing the structural patterns of otoliths. Formally, the key feature of the proposed approach is to map a sequence of macrostructures observed on a section of an otolith to a one-dimensional structural signal.

Such a representation of the structural information of otoliths is required to encode the presence of different types of macrostructure (Campana and Thorrold, 2001; Panfili *et al*., 2002). Macrostructures that are visible on otoliths may have different origins. Seasonal growth structures can be differentiated from checks (Panfili *et al*., 2002), but it needs to be stressed that checks might be associated with major ontogenetic change as well as with fish-specific responses to environmental or endogenous factors. Although the former type of checks can be used as relevant time references at a group or population level, the latter type can be regarded as structures formed at non-specific, i.e. random, times during the life of a fish[1]. As far as ageing and temporal otolith calibration are concerned, differentiating random checks from other age-related macrostructures is required to evaluate structural similarity. As a first step, the determination of such a typology of otolith macrostructures can be derived from an unsupervised statistical analysis of characteristic features of the macrostructures observed in a set of otoliths. Exploiting the resulting macrostructure typology, any sequence of otolith macrostructure can be converted to a sequence of labels, in terms of macrostructure type, before the computation of a one-dimensional structural signal and the introduction of a structural similarity measure.

Macrostructure descriptors and parameters

The transverse sections were observed with a stereomicroscope connected to a high resolution video camera and a computer. Observations were made under reflected light at constant magnification in order to avoid bias that may be induced by different scales of observation. A clearing medium (a mixture of glycerine and alcohol) was used to enhance the contrast of observations. Information acquisition consisted of specifying a radial from the nucleus to the edge of the ventral side of the otolith, following the maximum growth axis (Figure 2). Each translucent macrostructure identified and delimited by markers was automatically characterized by three criteria (Figure 3): width (W), distance from the nucleus to the middle of the zone (D), and translucency intensity (TI). The last of these is computed as a normalized measure of image intensity[2]. Width and distance to nucleus are determined in mm. During the observations, three types of translucent zone were observed: large translucent zones close to the nucleus (WTZC), large translucent zones distant from the nucleus (WTZD), and thin translucent zones or checks (TTZ) (Figure 4). Two observations per otolith were carried out by the same reader to assess the precision of interpretation. Although the operator had no previous experience of otolith analysis, interpretation was subsequently modified for just 17% of the sample. Measurements (W, D, TI) were automatically acquired on digital images using TNPC software (Fablet and Ogor, 2005).

Unsupervised typology extraction of otolith macrostructure

Let us use $\{M_i\}$ to denote the specific set of otolith macrostructures where each macrostructure M_i is characterized by its feature vector $y_{M_i} = \{W_{M_i}, D_{M_i}, TI_{M_i}\}$. The unsupervised determination of macrostructure typology is stated as the extraction of the relevant clusters (or classes) in the feature space $\{W, D, TI\}$. To this end, the distribution of the macrostructures within this feature space is modelled as a Gaussian mixture. Each macrostructure type T is assumed to be described by a Gaussian distribution $g(.|\Theta_T,\Sigma_T)$, where Θ_T is the vector of mean characteristics (W_T, D_T, TI_T) and Σ_T the covariance matrix:

$$g(y|\Theta_T, \Sigma_T) = \frac{1}{\sqrt{2\pi|\Sigma_T|^2}} \exp\left(-\frac{1}{2}(y-\Theta_T)^T \Sigma_T^{-1}(y-\Theta_T) \right),$$

The mixture model evaluates the likelihood of a macrostructure characterized by its feature vector y as follows: $p\left(y|\{\pi_{T_k}, \Theta_{T_k}, \Sigma_{T_k}\}_k\right) = \sum_{k=1}^{K} \pi_{T_k} g(y_{T_k}|\Theta_{T_k}, \Sigma_{T_k})$, where K is the number of macrostructure types and $\{\pi_{T_k}\}_k$ the prior probabilities of the different macrostructure types. Following *a priori* analysis of structural patterns of otoliths, a mixture model with three components is considered. The

[1] We subsequently refer to this second type of check as a random check, given that they cannot be associated with specific age-related fish life history events meaningful at a population or group level. By contrast, major ontogenetic checks and growth macrostructures form a macrostructure category referred to as potentially age-related macrostructures.

[2] The normalized image-intensity measure is normalized with respect to the local mean and standard deviation of image intensities along the analysed reference growth axis to remove bias that may be induced by varying lighting and otolith reflection conditions. Local mean and standard deviation of image intensities are estimated using a Gaussian window whose scale parameter is set with respect to the macrostructure width.

estimation of model parameters is carried out according to the maximum lilkelihood criterion (see Appendix).

This methodology permits the identification within a quantitative and objective framework of a typology of otolith macrostructures. Of all macrostructures types, those corresponding to thin structures with clear spatial variability are unlikely to be relevant for age estimation or associated with checks (TTZ). The other types of macrostructure are considered to be major structures (WTZC and WTZD), potentially age-related and meaningful at a population or group level. It must be stressed that they may include both actual annual growth structures and checks associated with major ontogenetic change.

Representation of the structural patterns of otoliths

Given the extracted macrostructure typology, any particular macrostructure can be assigned a macrostructure type from its descriptors. Formally, given a macrostructure *M* described by its feature vector y_M, the most likely macrostructure type T_M is derived from the estimated mixture model (see Appendix).

Applying this probabilistic rule of labelling to all macrostructures, the structural information conveyed by an otolith can be encoded by its associated sequence of macrostructure labels. Two types of representation are investigated. First, coding WTZC and WTZD labels as code 1 and TTZ labels as code 2, an otolith is described by a series of 1 and 2 according to the nature, number, and positions of the translucent zones along the radial. Such representation is compact and straightforwardly defines a categorization of otolith patterns (Figure 5). Statistical analysis of the distribution of the observed otolith pattern categories permits investigation of whether or not specific structural patterns can be identified for otolith subsets. We stress, however, that this representation of structural information does not take into account relative distances between macrostructures.

A second representation of the structural pattern of otoliths can then be introduced. Given the sequence of macrostructure labels $\{T_{M_1},....,T_{M_n}\}$ and the associated macrostructure widths and positions $\{W_{M_i}, D_{M_i}\}_i$, we define a normalized structure-based otolith signal *s* (Fablet, 2006a) as a function of the distance to the nucleus. Keeping only the main macrostructures (i.e. macrostructures labelled as WTZC and WTZD), signal *s* is set to –1 within macrostructure zones and as a sinusoidal function rescaled between –1 and 1 elsewhere (Figure 5). As this structural signal is defined as a function of the distance to the nucleus, it intrinsically encodes both the number and the relative positions of the macrostructure zones. Issues arising from differences in the number of otolith macrostructures as well as from comparing otoliths of different age or length can then be dealt with.

Analysis of structural otolith similarity

The proposed representations of the structural information conveyed by otoliths provide the basis for evaluating the similarity between otoliths in terms of structural pattern. A first approach relies on the statistical analysis of the relative frequencies of the otolith pattern categories observed for a given otolith set. These statistics permit investigation of meaningful differences among otolith subsets (for instance, otolith groups formed according to fish sex or year of catch) using standard non-parametric statistical tests (Legendre and Legendre 1998).

A second approach uses the computed structural signals to define explicitly the similarity measure between two otoliths as a function of the difference between these signals. Rather than straightforwardly exploiting the Euclidean distance, this similarity measure is defined as the minimum distance between the structural signals of two otoliths with respect to admissible growth-warping functions (Myers and Rabiner, 1981). Such a definition is aimed at coping with the variability of the structural pattern derived from individual otolith growth variability. Similar approaches are exploited in medical imaging to analyse differences among individuals, for instance for brain mapping (Hellier *et al*., 2003). Formally, the similarity measure $D(s_1,s_2)$ between two signals s_1 and s_2 is defined as follows:

$$S(s_1, s_2) = \min_{\Phi\in\Xi} \int_D \left\| s_1(D) - s_2\left(\Phi(D)\right) \right\|^2, \qquad (1)$$

where Ξ is the set of admissible warping functions, defined in our case according to regularity constraints, i.e. the set of smooth monotonous functions with bounded variations[3] (Hellier *et al.*, 2003). For any otolith set, this similarity measure is used to compute a similarity matrix that stores the similarity measures computed for all otolith pairs. From this similarity matrix, the organization of the similarities of the structural patterns of otoliths within the considered sample sets can be visualized using linear or non-linear mapping (Borg and Groenen, 1997), and statistical differences between otolith subsets can be evaluated using standard distance-based statistical tests, for instance Mantel's test (Legendre and Legendre, 1998). For this study, this numerical framework was applied to test for sex-based and cohort-related differences in structural patterns of otoliths.

Results

Macrostructure typology

A database of 628 otoliths was considered, 80% of the original available otolith set (fish length range 8–50 cm). In ~10% of the samples, sections prepared according to the routine otolith preparation protocol had not passed through the nucleus, and poorly contrasted otoliths accounted for another 10%. In both cases, precise determination of the features of the translucent zones (width, distance to the nucleus, and opacity) was not possible, so to preclude bias in the analysis, those samples were not considered. As shown in Figure 6, omitting those otoliths does not lead to any particular sampling bias.

The unsupervised extraction of the typology of otolith macrostructures derived from the Gaussian mixture model led to three standard modes for the whole dataset (Figure 7). Three macrostructure types were associated with those modes. Translucency intensity was not a discriminant feature in any case.

Of the three modes, the biggest prior was at 0.5127 and corresponded to the thinnest marks ($0.02<W<0.08$ mm) along the radial ($0.4<d<3.9$ mm): they are referred to as checks (TTZ). The mean position was $d = 1.78$ mm and $W = 0.04$ mm. The second Gaussian mode (prior 0.1781) was associated with thicker marks ($0.08<W<1.2$ mm) close to the nucleus ($0.5<d<1.6$ mm): they are referred to as large translucent zones close to the nucleus (WTZC). Their mean position was $d = 0.91$ mm, and mean width $W = 0.37$ mm. The third mode (prior 0.30919) involved rather thicker marks ($0.08<W<0.84$ mm), far from the nucleus and widely distributed ($1.16<d<3.8$ mm): they are referred to as large translucent zones distant from the nucleus (WTZD). Their mean position was $d = 2.20$ mm and their mean width $W = 0.19$ mm.

Correlation hypotheses between *W* and *D* features were tested for each mode using a Student's *t* correlation test for the estimated covariance matrix. Although there was no correlation for the TTZ mode ($p > 0.1$), correlations between the distance to the nucleus and the width of the translucent zones for the two other WTZ modes were significant ($p < 0.0001$).

Assignment of each macrostructure to each of the three types is illustrated in Figure 8.

Otolith pattern categorization

Global analysis of 628 otoliths led to the creation of 157 different otolith pattern categories based on encoding by 1 WTZC, 1 WTZD, and 2 TTZ. The number of occurrences of these categories ranged from 1 to 53 (Figure 9a). Note that just 18 categories were found nine times or more and that they included 63% of the samples (Figure 9b). In that case, one-third of the macrostructure sequence begins with a TTZ and two-thirds with a WTZ.

Statistical analysis of wide translucent macrostructures

We note empirically that the number of WTZs increased with fish size, as illustrated for samples of indeterminate sex, females, and males (Figures 10a, b and c). More precisely, for hakes of indeterminate sex and length 8–20 cm, most of the observed structural patterns (65%) did not contain any WTZ, whereas above 20 cm the observed structural patterns involved at least one WTZ (Figure 10a).

[3] Considering the set of smooth monotonous functions with bounded variations leads to an additional regularity term in the minimization defined by Equation (1). This regularity term is evaluated as the norm of the gradient of the warping function Φ. For further detail of the implementation of this minimization, the reader is referred to Hellier *et al.* (2003), a study that uses a similar formulation in the field of medical imaging.

Figures 10b and c indicate a succession of peaks corresponding to an increase of WTZ number in the structural sequences with fish length. Notably, WTZ deposition takes place on average at greater length in females than in males. The otoliths with one WTZ were mainly 17–26 cm for males and 18–32 cm for females. Those with two WTZs dominated between 27 and 33 cm for males and between 33 and 38 cm for females. The structural sequences with three WTZs formed a peak between 35 and 37 cm for males and were in the majority between 39 and 46 cm for females. Thereafter, for both sexes, otoliths with four WTZs dominated. The most complex structural sequences, with six and seven WTZs, have been found only for females, and they appeared at a relatively large size, at 42 and 49 cm, respectively (Figure 10c).

Statistical analysis of the computed structure-based otolith signals

Structural patterns of otoliths were further analysed using the similarity measure introduced in the previous section, to test for significant similarities or difference among sample subsets. More precisely, we asked two questions: does the structural pattern of otoliths remain stable over time for young fish, and do males and females from a given length range differ in their otolith pattern?

For hake of 8–18 cm, the typological analysis was similar for 2002 and 2003, with the presence of checks and translucent zones close to the nucleus not very broad (<0.4 mm) (Figures 11a and b). Analysis of the structural otolith similarity matrix between otoliths from these two years revealed three groups of patterns, but no group specific to a given year (Figure 11c). There was no significant difference based on structural similarity defined by Equation (1) (p = 0.468). Otoliths of young hake shared similar structural patterns in 2002 and 2003.

Different otolith patterns between males and females have been recorded before. The structure-based otolith similarity measure was also used to test for this quantitatively. Focusing on the length range 24–34 cm, for which there were significant numbers of otoliths, it was clear that the otolith patterns of both males and females involved the three types of macrostructure, with visually similar distributions of checks and translucent rings close to the nucleus (Figures 11d and e). However, analysis of structural otolith similarities with respect to sex led to a significant difference in terms of structural patterns (p = 0.049), illustrated by mapping the similarity matrix, which involves several clusters, some specific to just one sex (Figure 11f).

Discussion

Workshops devoted to improving European hake age determination have been conducted for at least ten years, in order to agree age-estimation criteria and to standardize methodology. They resulted in increased precision of ages (Piñeiro *et al.*, 2004), but could not address the issue of accuracy, emphasizing that the criteria needed to be validated. Chemically tagging fish as a basis of mark-recapture analysis, thought to be the best method for validating the periodicity of growth increment formation (Campana, 2001), has recently been used for Atlantic hake (de Pontual *et al.*, 2003), and the first results show that growth is underestimated as a consequence of age-overestimation (de Pontual *et al.*, 2006). Underestimation of growth during the first year of life has also been demonstrated recently by Arneri and Morales-Nin (2000), Morales-Nin and Moranta (2004), Kacher and Amara (2005), and Belcari *et al*. (2006), based on an assumption of daily deposition of micro-increments. Therefore, various studies support the hypothesis that hake is a fast-growing species. The initial results of a hake-tagging programme started in 2005 in the Gulf of Lions confirmed the fast-growing hypothesis (C. Mellon, unpublished data). The lack of a methodology for validating age estimates despite the many years of age-reading experience in Europe underscores the need for a method to be developed that reduces the subjectivity of expert interpretation and is based on determination of a typology of otolith macrostructures.

Structural patterns of otoliths

Hake otolith rings are visible at low magnification. On a seasonal scale, the accretion process alternatively produces opaque and translucent zones, which differ in their organic matter/mineral ratio, crystal size, and the width of their primary increments (Wright *et al*., 2002). Some species, such as plaice (*Pleuronectes platessa*), lay down simple patterns in their otolths, and a pair of translucent ring/opaque zones is considered to be deposited annually. For other species, such as hake, otolith interpretation is complex because of the presence of numerous macrostructures. There are many thin translucent zones that probably correspond to short environmental and/or physiological events, and the difficulties in interpreting such otoliths often increases with the size of the fish.

Classification of the rings is a major problem in European hake. Here, a systematic delimitation of all types of translucent zone, thin and large, on the main radial with markers, and a statistical treatment of a large set of radials was carried out. The unsupervised statistical analysis of the distribution of the characteristics of these macrostructures led to definition of three main macrostructure types, two corresponding to wide translucent zones, and one to thin translucent zones. The last mode is associated with a large variance in the distance to the nucleus. This mode is therefore likely to be associated mainly with specific fish responses to environmental or endogenous factors. By contrast, the spatial extent of the other two modes is more clearly defined, suggesting that they are relevant at a population level, for instance in response to seasonal variations or major ontogenetic events. To our knowledge, this is the first time that quantitative discrimination of meaningful translucent zones has been attempted for otolith checks. The application to Mediterranean hake otoliths, whose interpretation is difficult, stresses the potential of the proposed framework to be applied to other fish species with complex otolith patterns

From the extracted macrostructure typology, a probabilistic labelling scheme can be derived to assign any particular macrostructure to a macrostructure type. This scheme provides the means of investigating similarities in terms of structural information among otoliths. The number of WTZs evolves coherently with fish length, i.e. their distribution against fish length is clearly modal and the mean length associated these modes increases with the number of WTZs. In addition, an evaluation of statistical differences based on structural otolith similarity reveals no significant difference between the structural patterns of otoliths of young fish sampled in 2002 and in 2003, supporting the assumption that otolith patterns may be stable over time. We stress that the proposed similarity measure allows for gradual changes in growth patterns over time. Hence, the similarity in terms of structural information has to be considered in terms of the relative positions of the relevant translucent zones. The analysis of hake 24–34 cm long requires the detection of a significant difference between male and female otoliths in terms of structural patterns. Such a result is coherent with known differences in growth patterns of male and female hake. As otolith structures relevant at a population level can be assumed to be deposited in a similar way for males and females, it is to be expected that they will be found at a greater length for females than for males, as revealed by our analysis.

The results show that our approach allows us to differentiate, using quantitative features, random macrostructures (likely to be associated with individual physiological responses) from otolith macrostructures that are clearly not random and that can be considered at a population level. This finding contradicts that of Morales-Nin *et al.* (1998), who concluded from analysis of sulcal ring patterns of Mediterranean hake that ring formation in the otolith showed no synchronicity at a population level and was dependent mainly on physiological events. Our results therefore provide a basis for future work aimed at defining a well-founded and objective age estimation protocol for European hake. To this end, our proposed methodological framework should be applied at a broader scale, specifically to otolith subsets sampled in different seasons and years, with a view to determining the period of deposition of the macrostructures and to constructing models of otolith patterns. Also, given that our analysis was limited to fish <50 cm, there would likely be merit in applying the proposed methodology to older fish. We anticipate that the unsupervised extraction of associated otolith macrostructure typology will lead to the identification of more macrostructure types potentially meaningful for age determination. As correlations between the distance to the nucleus and the width of the translucent zones were significant for the two WTZ modes, application to otolith sets involving greater fish lengths might require consideration of other features to characterize translucent zones. In particular, it might be important to consider a relative width measure (for instance, relative to the width of neighbouring translucent zones) to account for the fact that the width of the translucent zones decreases as the fish become older.

Fast growth hypothesis

Assuming that the number of WTZs is correlated to fish age, it is of interest to compare the distribution in the number of WTZ, in terms of fish length, with the current hypotheses on hake growth. Two growth hypotheses, fast (FGH) and slow (SGH), have been given in the literature for the first year of life. A slow growth rate (1.15 cm month^{-1}) was estimated for the Gulf of Lions by Aldebert and Recasens

(1995) and Morales-Nin and Aldebert (1997), and another of between 0.7 and 1.2 cm month^{-1} for the Ligurian Sea by Orsi-Relini *et al*. (1989). Those values (Table 1) were calculated using a method based on length frequency analysis (Fishparm/Bhattacharya). Fast growth (defined as 1.2–2.5 cm month^{-1}) was estimated for the Catalan coast at >1.6 cm month^{-1} by Morales-Nin and Moranta (2004), in the Gulf of Alicante at 1.7 cm month^{-1} by Garcia-Rodriguez and Esteban (2002), and in the northern Tyrrhenian Sea also at 1.7 cm month^{-1} by Belcari *et al*. (2006). Those values of growth rate were obtained from the Fisat program for length frequency analysis.

To compare the evolution of the number of otolith macrostructures labelled as meaningful with respect to these two growth hypotheses (slow and fast), we analysed the length distribution of the fish whose otoliths were assigned numbers (from zero to seven) of WTZ otolith structures (Figure 10). These length distributions are compared with the predicted length ranges for ages 1–7 for each growth hypothesis. The outcome is that the fast growth hypothesis applies to our results better than the slow growth hypothesis, for both sexes (Figure 10). For FGH, structural sequences consist of zero and one WTZ during the 1st year, predominantly one and two WTZs during the 2nd year, and mainly two and three WTZs during the 3rd year (Figure 10a). For the 2nd and 3rd years, the appearance of an additional translucent ring seems to correspond to a 2nd half of that year of life. Conversely, there is no consistency with SGH. This analysis should be considered with respect to growth variability, which may be high for hake. Further, it is likely that not all macrostructures are seasonal structures. Among the structures identified as potentially age-related, some are likely to be associated with major ontogenetic events. Hence, more than one WTZ structure could be observed for some years of the fish life. It is not straightforward to generate a perfect match between the length distribution of a given number of observed otolith WTZs and the length range derived from FGH at a specific age. The important result regarding hake otolith interpretation is that the otolith patterns involve some population-related determining factors.

The establishment of a validated ageing protocol requires further understanding and characterization of the conditions and processes leading to formation of the WTZ macrostructures. To this end, future work should rely on experimental approaches based on both tank and field experiments.

Methodological aspects and future work

The methodological developments described in this paper are aimed at characterizing in an unsupervised and objective way the deposition of otolith macrostructures from a statistical analysis of the structural information in sets of otoliths. To this end, an unsupervised typology of otolith macrostructures has been derived. This typology then permitted definition of a similarity measure between otoliths in terms of structural information, with a view to quantifying similarities or differences among otolith subsets with respect to macrostructure patterns.

The potential of the proposed methodological framework for analysing structural patterns of otoliths has been demonstrated for a set of several hundred otoliths of Mediterranean hake, which is defined as a complex species in terms of otolith interpretation. The proposed framework could be applied similarly to other complex (in terms of age) or new species for which no validated interpretation protocol is available. Moreover, such unsupervised analysis can also be applied to collections of marked otoliths derived from mark-recapture experiments. In those cases, extraction of the macrostructure typology can also rely on a time-calibration of the macrostructures associated with tagging and recapture date(s) and should contribute to the development of validated and objective interpretation schemes.

Another area of application for the proposed methodology is the comparison, in quantitative terms, of the interpretation of otolith patterns by experts. Evaluation of intra- and inter-expert agreement in otolith interpretation serves as a basis for standardizing age estimation protocols and implementing quality assurance and control in age estimation (Morison *et al*., 2005). Such associated quantitative analysis is restricted to computing agreement rates or the coefficients of variation of age structures, because quantitative evaluation of the similarity of the interpretation of otolith macrostructures is not possible because of the absence of metrics. The proposed measure of similarity of otolith patterns can be applied to the patterns interpreted by experts with a view to quantifying interpretation differences, though such application will rely on a predetermined macrostructure typology agreed among experts. The analysis could be performed repeatedly for a single expert to evaluate potential drift in interpretation, between experts to check for consistency of interpretation protocol among experts and/or institutes, and for training to evaluate the relevance of the interpretation with respect to annotated reference collections.

The main limitation of the proposed framework lies in its need for manual detection of otolith macrostructures, i.e. selection of the positions and widths of the macrostructures. This acquisition step is time-consuming and could induce some subjectivity. However, the required degree of expertise for the task is low because it is limited to selecting the positions of the macrostructures and does not involve their interpretation in terms of growth structures or checks. This statement is supported by the low variability observed between two acquisition sessions for a reader with no previous experience in otolith interpretation. Future work will include coupling of the proposed framework with automated detection of otolith macrostructures (Fablet, 2006b) in order to reach fully automated and unsupervised characterization of otolith patterns. Additionally, we will investigate the use of complementary features of otolith macrostructures, especially in terms of oxygen and carbon isotope descriptors (e.g. Hoie *et al.*, 2004), with a view to enriching the macrostructure typology. Additional descriptors will be performed within the methodologies described here. Finally, future work will pursue the development of measures of structural otolith similarity accounting for typology and the relative positions of macrostructures and variability in otolith growth, especially for applications aimed at checking protocol coherency.

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Figure legends do not embolden lettering in any Figure

Figure 1. Length distribution of the processed sample by sex and sampling year.

Figure 2. Example of the analysis of translucent zones of hake otoliths from a transverse section of the sagitta, with a radial (▬) and markers (▮).

Figure 3. Illustration of the features used for characterizing the translucent zones (appearing dark when observed under reflected light) of hake otoliths: width (*W*), distance to nucleus (*D*), and translucency intensity (*TI*), the last computed as the mean image intensity. Italicize *W* and *D*, as in text and equations

Figure 4. Examples of the three types of translucent structures derived from the proposed unsupervised statistical analysis: a wide translucent zone close to the nucleus (WTZC), a wide translucent zone distant from the nucleus (WTZD), and a thin translucent zone or check (TTZ).

Figure 5. Representations of structural patterns of otoliths: definition of otolith pattern categories corresponding to specific sequences of labelled macrostructures in terms of macrostructure typology (bottom left), and the associated structural signal accounting for both type and relative position of the translucent macrostructures (bottom right). Make some of the lettering smaller – it is too large

Figure 6. Length distribution of the set of discarded otoliths, by sex.

Figure 7. Parameters of the Gaussian mixture model showing three modes for the distribution of the features of translucent structures. Ellipses are drawn to indicate the standard deviation of each Gaussian mode (we display the ellipses corresponding to a distance of one standard deviation to the Gaussian

centre, i.e. the ellipse accounts for 68% of the samples). Make the ellipses thinner lines and remove the colour

Figure 8. Assignment of the macrostructures of all processed otoliths to the extracted macrostructure type. Dots indicate wide translucent zones close to the nucleus (WZTC), stars thin translucent zones (TTZ) or checks, and crosses wide translucent zones distant from the nucleus (WZTD). Some of the lettering is too large

Figure 9. Relative frequencies of the otolith pattern categories derived from overall analysis of the labelled structural patterns of the processed otolith set: (a) relative frequencies for the 157 observed categories, and (b) relative frequencies of the categories met at least nine times. Move the (a) and the (b) close to their respective panels

Figure 10. Proportions of fish featuring a certain number of translucent rings (WTZ) as a function of fish length and sex. On each panel, two hypotheses of growth are represented according to the size of hake, the slow growth hypothesis (SGH) attributable to Aldebert and Recasens (1995), and the fast growth hypothesis (FGH) of Garcia-Rodriguez and Esteban (2002). Move the (a), (b) and (c) close to their respective panels PUBLISHER – REPRODUCE IN COLOUR

Figure 11. Statistical analysis of structural otolith similarity. Panels (a), (b), (d), and (e) show the results of the typological analysis of translucent otolith structures, and (c) and (f) the results of the quantitative analysis of structural otolith similarity, visualized via the Sammon algorithm (Borg and Groenen, 1997). *n* is the number of otoliths analysed, and *p* the value of the *p*-statistic in the permutation test of Mantel (Legendre and Legendre, 1998). *n* and *p* need to be italicized, some lettering sizes need to be adjusted down, SEX is mis-spelled, and I don't like the solid black line down the middle

Running headings

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Deposition of macrostructures in Mediterranean hake otoliths

Appendix

Estimation of mixture model parameters

Given the set of feature vectors $\{y_{M_i}\}_i$, estimation of the parameters of the mixture model is carried out according to the Maximum Likelihood (ML) criterion. Model parameters are determined $\{\pi_{T_k}, \Theta_{T_k}, \Sigma_{T_k}\}_k$ such that distribution $p\left(y \middle| \{\pi_{T_k}, \Theta_{T_k}, \Sigma_{T_k}\}_k\right)$ best fits the distribution of the data $\{y_{M_i}\}_i$:

$$\{\hat{\pi}_{T_k}, \hat{\Theta}_{T_k}, \hat{\Sigma}_{T_k}\}_k = \arg \max_{\{\pi_{T_k}, \Theta_{T_k}, \Sigma_{T_k}\}_k} \prod_i p\left(y_{M_i} \middle| \{\pi_{T_k}, \Theta_{T_k}, \Sigma_{T_k}\}_k\right).$$

To solve this maximization issue, we use the EM (Expectation–Maximization) algorithm. Refer to Bishop (1995) for further detail on implementation of this iterative procedure.

Assigning a macrostructure type to a given macrostructure

Assignment of a macrostructure type T_M to a given macrostructure M, characterized by the associated features y_M, is derived from maximizing the posterior classification likelihood:

$$T_M = \arg \max_{T_k \in' \{WTZC, WTZD, TTZ\}} p\left(T_M = T_k \middle| y_M, \{\hat{\pi}_{T_k}, \hat{\Theta}_{T_k}, \hat{\Sigma}_{T_k}\}\right),$$

where the posterior likelihood is evaluated as

$$p\left(T_M = T_k \middle| y_M, \{\hat{\pi}_{T_k}, \hat{\Theta}_{T_k}, \hat{\Sigma}_{T_k}\}\right) = \frac{\hat{\pi}_{T_k} g\left(y_{M_i} \middle| \{\hat{\Theta}_{T_k}, \hat{\Sigma}_{T_k}\}\right)}{\sum_l \hat{\pi}_{T_l} g\left(y_{M_i} \middle| \{\hat{\Theta}_{T_l}, \hat{\Sigma}_{T_l}\}\right)}.$$

Table 1. Von Bertalanffy growth parameters of *Merluccius merluccius* according to the slow growth hypothesis (SGH) demonstrated by Aldebert and Recasens (1995), and to the fast growth hypothesis (FGH) of Garcia-Rodriguez and Esteban (2002).

Hypothesis	**Sex**	L_∞ **(cm)**	K	t_0
SGH	Male	72.8	0.149	–0.383
	Female	100.7	0.124	–0.350
FGH	Male	93.0	0.20	–0.091
	Female	108.0	0.21	0.115

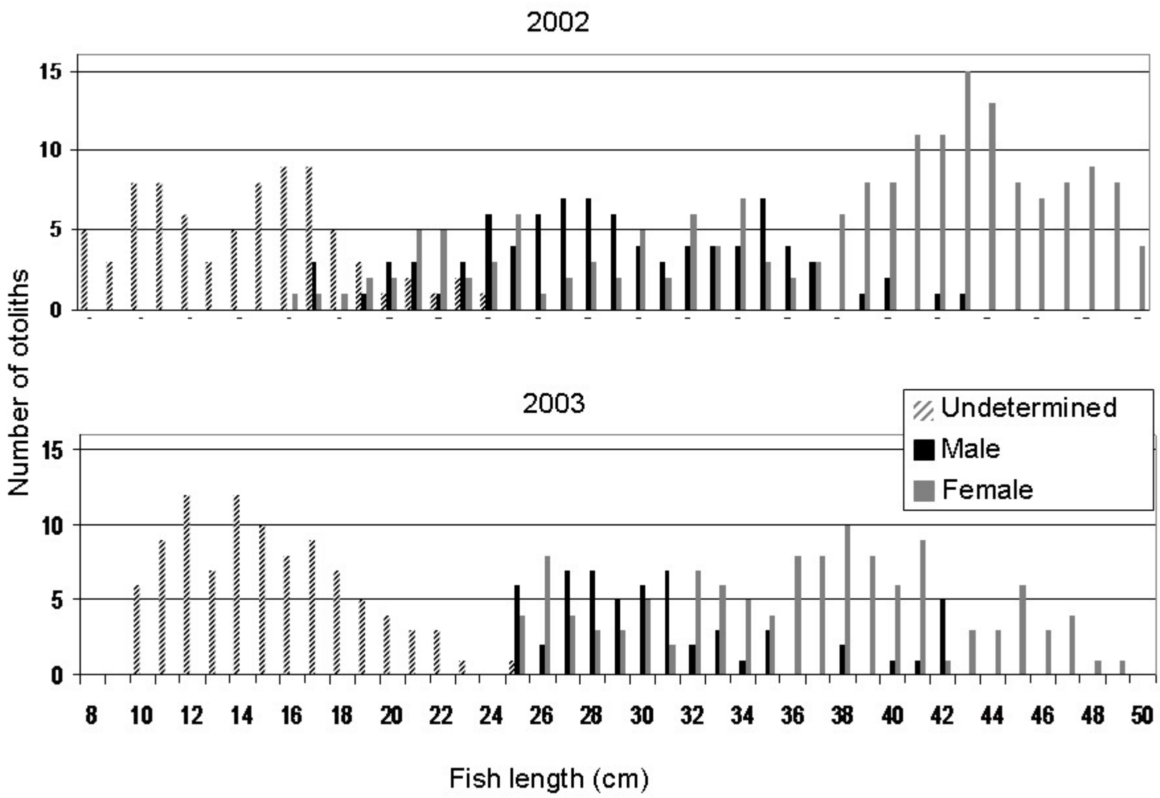

2002
2003
Number of otoliths
Fish length (cm)
Undetermined
Male
Female

Fig. 1.

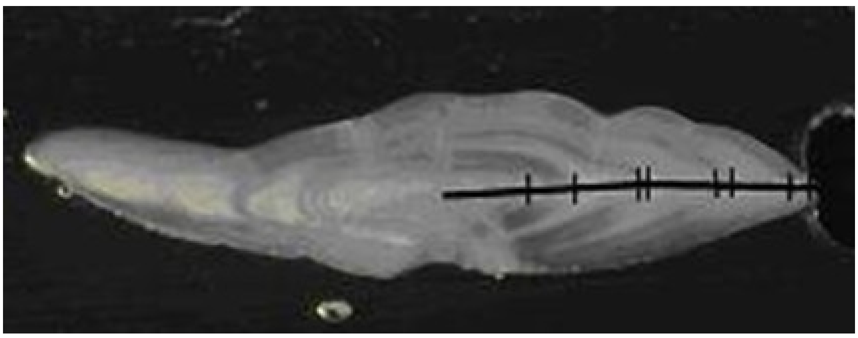
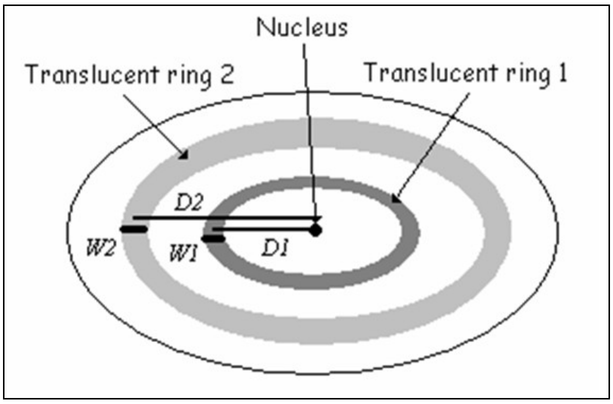
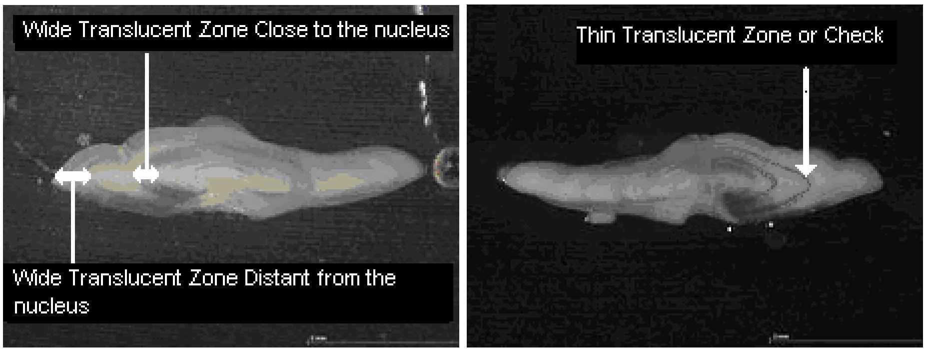


Fig.2


Nucleus
Translucent ring 2
Translucent ring 1
D2
W2
W1
D1
Fig.3.


Wide Translucent Zone Close to the nucleus
Wide Translucent Zone Distant from the nucleus
Thin Translucent Zone or Check
Fig. 4.

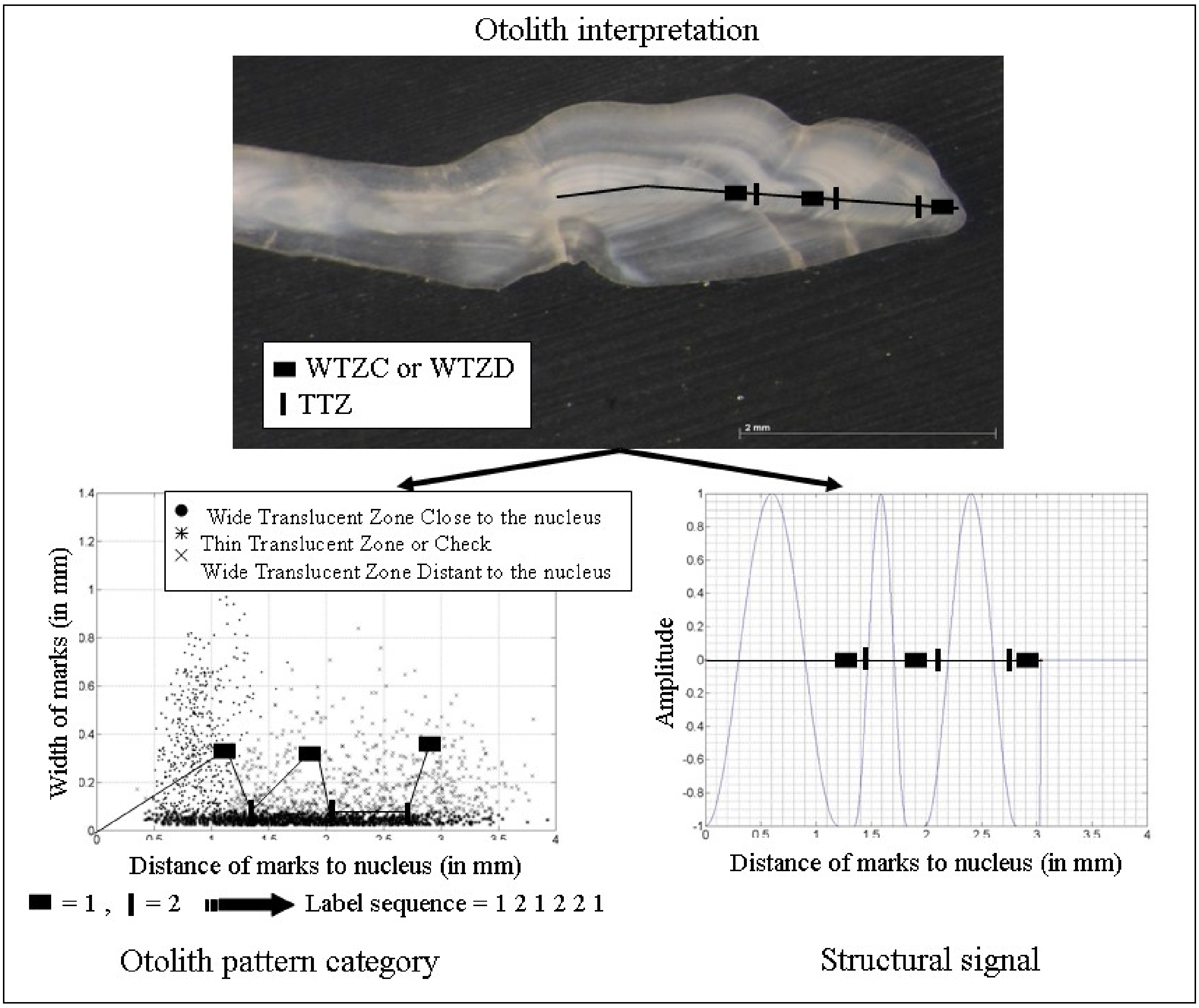

Otolith interpretation
WTZC or WTZD
TTZ
2 mm
Wide Translucent Zone Close to the nucleus
Thin Translucent Zone or Check
Wide Translucent Zone Distant to the nucleus
Width of marks (in mm)
Distance of marks to nucleus (in mm)
= 1 , = 2
Label sequence = 1 2 1 2 2 1
Amplitude
Distance of marks to nucleus (in mm)
Otolith pattern category
Structural signal

Fig. 5.

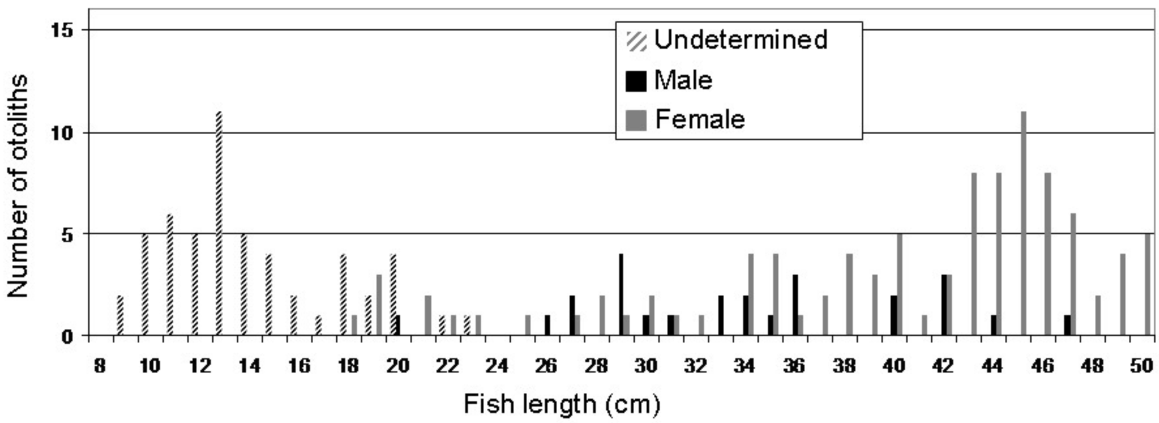

Undetermined
Male
Female
Number of otoliths
Fish length (cm)

Fig. 6.

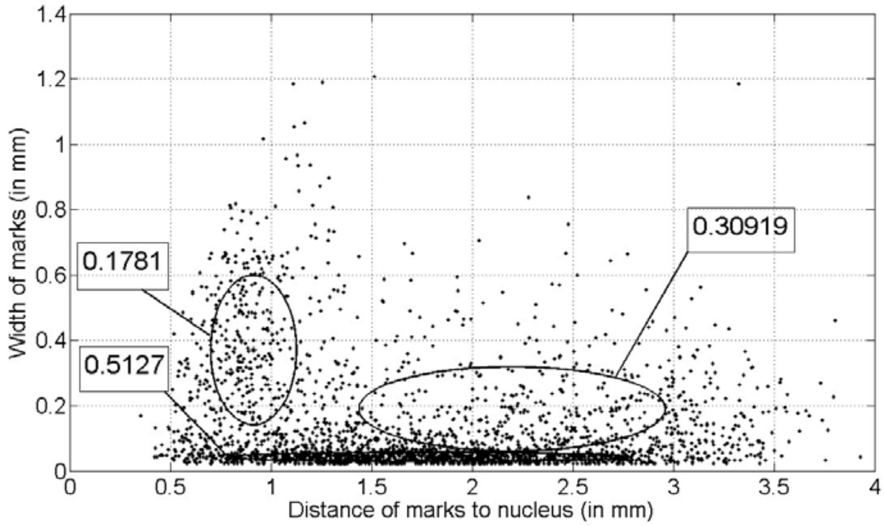

0.1781
0.5127
0.30919
Width of marks (in mm)
Distance of marks to nucleus (in mm)

Fig. 7.

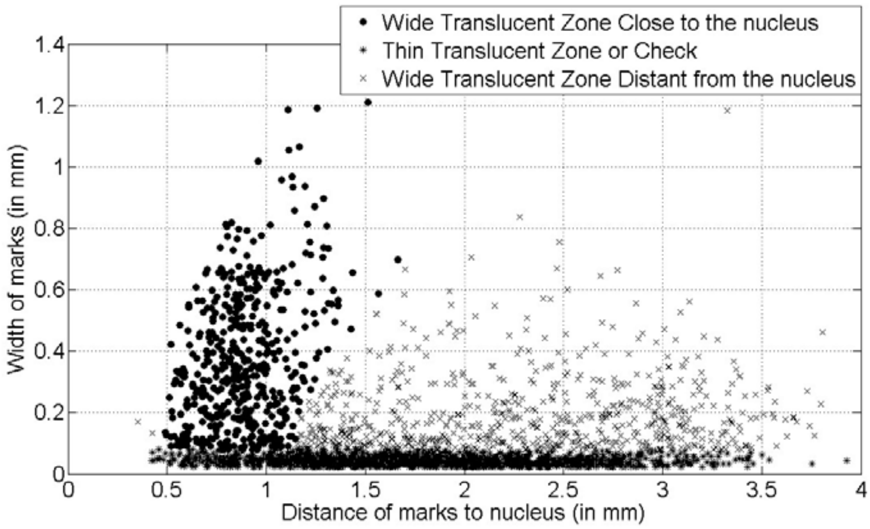

Wide Translucent Zone Close to the nucleus
Thin Translucent Zone or Check
Wide Translucent Zone Distant from the nucleus
Width of marks (in mm)
Distance of marks to nucleus (in mm)

Fig. 8.

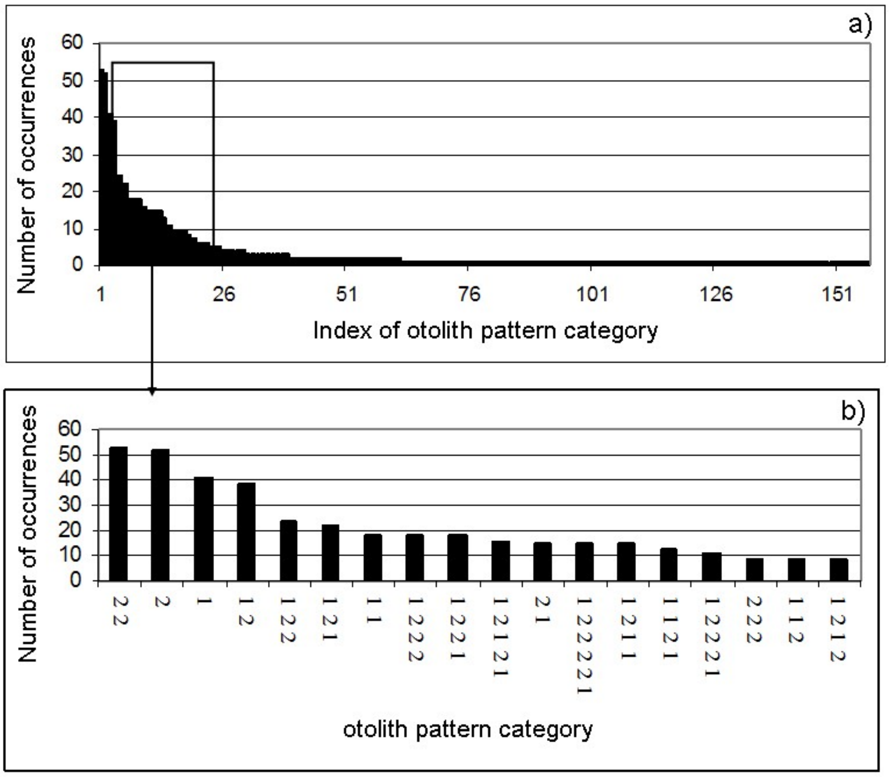

a)
Number of occurrences
60
50
40
30
20
10
0
1
26
51
76
101
126
151
Index of otolith pattern category
b)
Number of occurrences
60
50
40
30
20
10
0
2 2
2
1
1 2
1 2 2
1 2 1
1 1
1 2 2 2
1 2 2 1
1 2 1 2 1
2 1
1 2 2 2 2 1
1 2 1 1
1 1 2 1
1 2 2 2 1
2 2 2
1 1 2
1 2 1 2
otolith pattern category

Fig. 9.

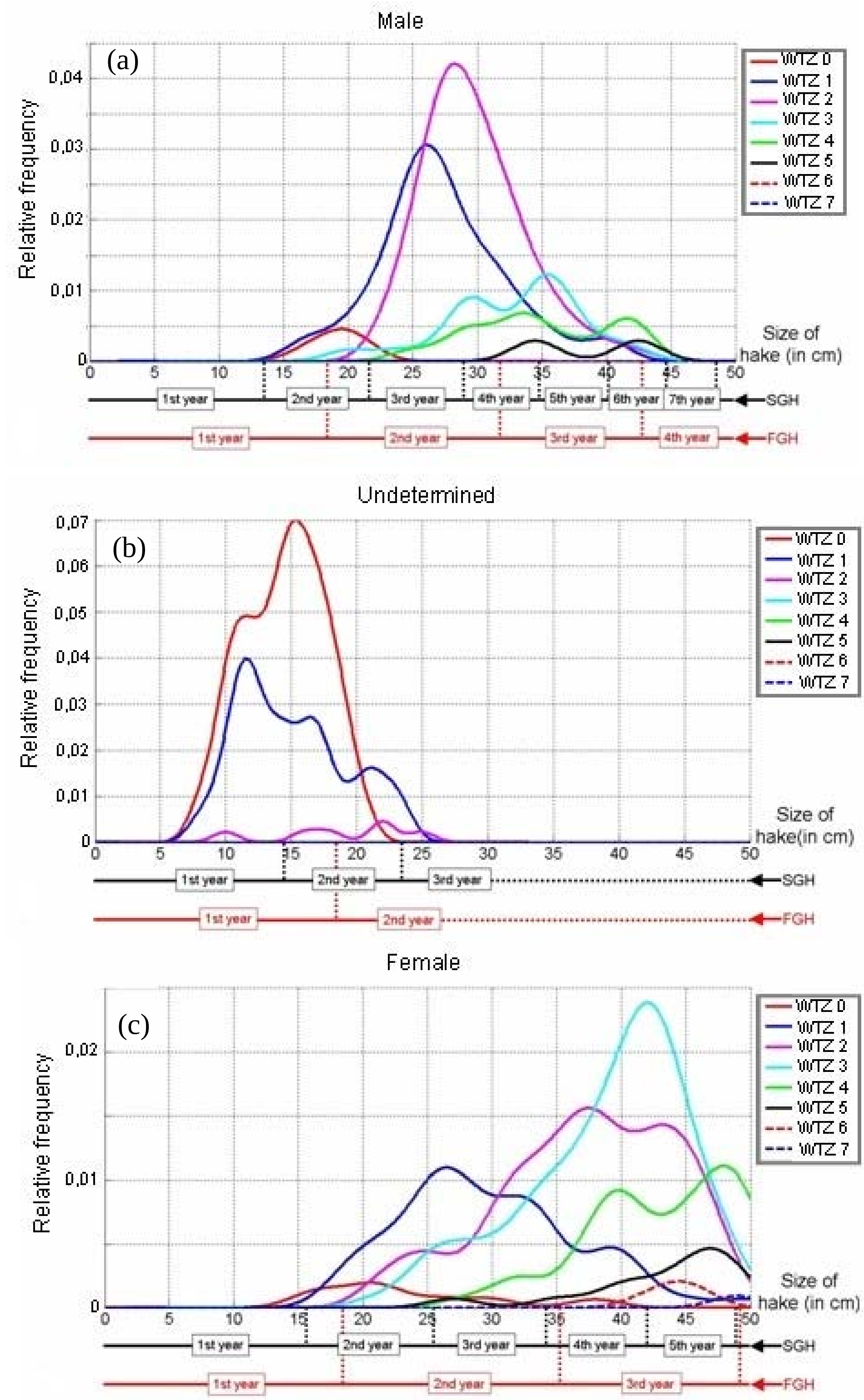

Male
(a)
Relative frequency
WTZ 0
WTZ 1
WTZ 2
WTZ 3
WTZ 4
WTZ 5
WTZ 6
WTZ 7
Size of hake (in cm)
1st year
2nd year
3rd year
4th year
5th year
6th year
7th year
SGH
FGH
Undetermined
(b)
Size of hake(in cm)
Female
(c)
Size of hake (in cm)

Fig. 10.

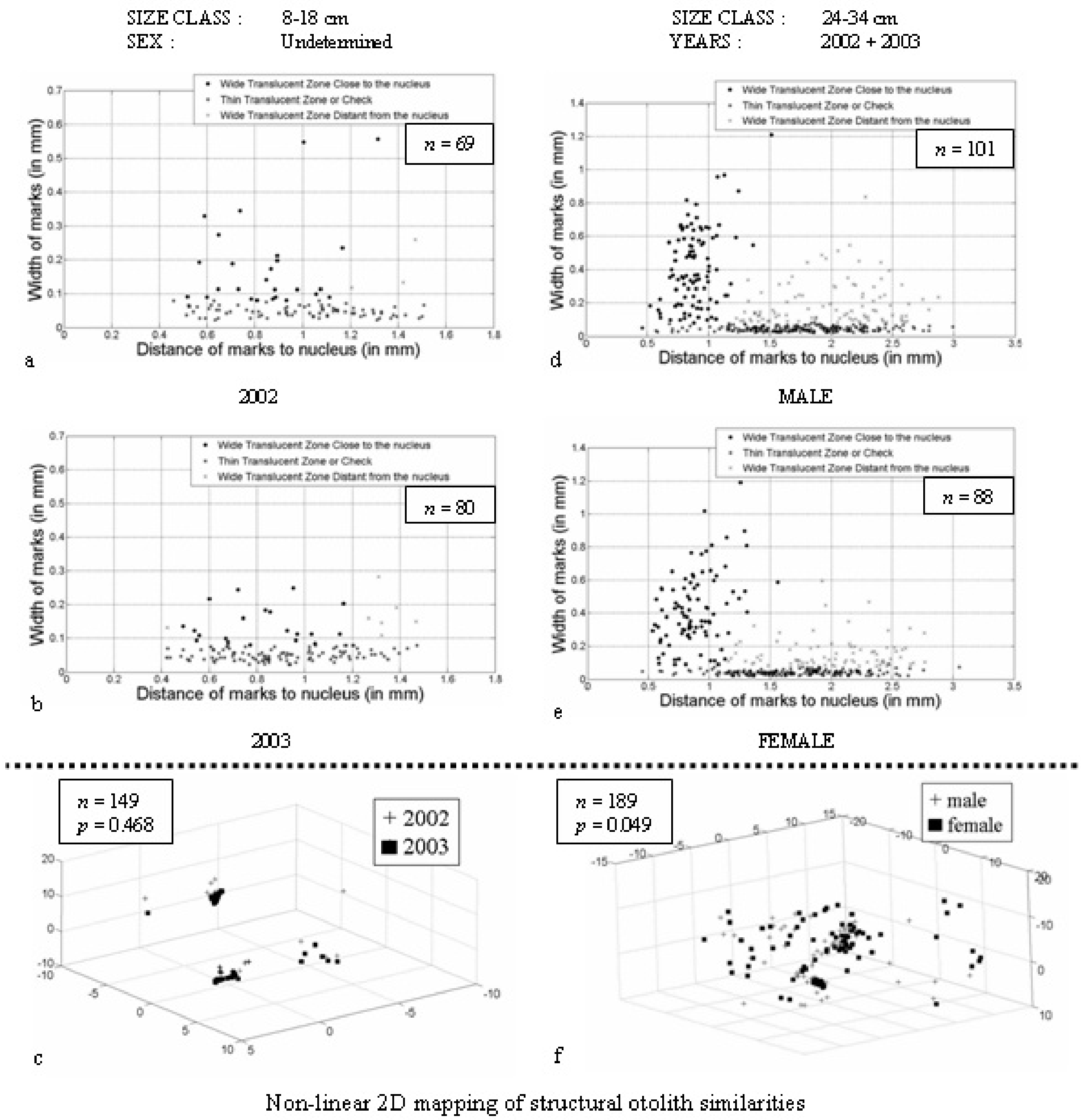

SIZE CLASS : 8-18 cm
SEX : Undetermined
SIZE CLASS : 24-34 cm
YEARS : 2002 + 2003
Wide Translucent Zone Close to the nucleus
Thin Translucent Zone or Check
Wide Translucent Zone Distant from the nucleus
n = 69
n = 101
n = 80
n = 88
Width of marks (in mm)
Distance of marks to nucleus (in mm)
a
d
2002
MALE
b
e
2003
FEMALE
n = 149
p = 0.468
+ 2002
■ 2003
n = 189
p = 0.049
+ male
■ female
c
f
Non-linear 2D mapping of structural otolith similarities

Fig. 11.