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Paradox of otolith shape indices: routine but overestimated use

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Abstract: The identification of fish species using otolith shape has been common in many fields of the marine science. Different analytical processes can be applied for the morphological discrimination, but reviewing the literature we have found conceptual and statistical limitations in the use of shape indices and wavelets (contour analysis), being specially worrying in the first case due to their widespread routine use. In the present study, 42 species were classified using otolith shape indices and wavelets and applying traditional and machine learning classifiers and performance measures (accuracy, Cohen's kappa statistic, sensitivity and precision). Our results were conclusive, wavelets were a more adequate option for the classification of species than shape indices, independently of classifiers and performance measures considered. The artificial neural network and support vector machine provided the highest values for all performance measures using wavelets. In all cases, the measures of sensitivity and precision pointed out a higher confusion between some otolith patterns using shape indices. Therefore, we strongly discourage the routine use of shape indices for the identification of species.

Keywords: otolith, shape, fish species, classification, methodological comparison

41 Introduction

42 The otoliths or ‘ear bones’ of fishes are calcareous structures located in the inner ear of vertebrates,
43 and they act as sound detectors as well as organs of equilibrium (Popper and Coombs 1982; Popper
44 and Fay 2011; Schulz-Mirbach and Ladich 2016). Although there are three pairs (i.e., *sagittae*, *lapilli*
45 and *asterisci*), the *sagittae* otolith (henceforth referred to as otolith) shows a higher interspecific
46 variability in marine species (Gaemers 1984; Nolf 1985; Smale et al. 1995; Campana 2004; Tuset et
47 al. 2008; Lind and Chang 2012). Its shape was the focus of study in the first half of the 19th century by
48 George Cuvier (Koken 1884; Campbell 1929), but it acquired a new analytical dimension with the
49 development of digital revolution in the 1980s. In that period, the term ‘*otolithometry*’, coined by
50 Gaertner (1982), was used to quantify interspecific and intraspecific variability using otolith biometry
51 and its relationship with physiological, genetic, ecological and evolutionary characteristics (Bird et al.
52 1986; Lombarte and Castellón 1991; Lombarte et al. 2010; Vignon and Morat 2010). The area and
53 perimeter were examined in addition to the initial linear measurements, such as length and width
54 (Gauldie 1988; Lombarte 1992; L’Abee-Lund 1988), allowing the calculation of classical shape
55 descriptors based on the measure combinations (e.g., ellipticity, circularity and rectangularity),
56 commonly known as ‘shape indices’. These measurements became relevant and were additionally
57 applied with the contour analysis (Fourier descriptors) for the identification of fish stocks (Smith 1992;
58 Cadrin and Friedland 1999; Bergenius et al. 2005; Burke et al. 2008) and separately for taxonomic,
59 ecomorphological and ontogenetic studies (Tuset et al. 2003; Volpedo and Echeverría 2003; Zhuang et
60 al. 2014; Pavlov 2016; Lin and Al-Abdulkader 2019). In turn, shape indices have been used in
61 investigations of trophic (de Carvalho et al. 2019; Qamar et al. 2019; Assis et al. 2020) and
62 phylogenetic nature (Zhuang et al. 2014; Teimori et al. 2019). However, they have some analytical
63 problems that may disrupt the accuracy of the classifiers. First, most shape indices are strongly
64 intercorrelated, leading to multicollinearity problems. Although the application of principal component

analysis (PCA) and redundancy elimination (e.g., stepwise procedure or VIF analysis) are plausible alternatives (Naes and Mevik 2001; Burke et al. 2008; Zhuang et al. 2014), many studies ignore this problem, which may overestimate the predictive power of their models (Mérigot et al. 2007; Gorini and Burrati 2017; Lin and Al-Abdulkader 2019). When PCA is performed, the first component may reach up to 99% of the total variance (Pavlov 2016), which is why the selection of more components would not avoid partial multicollinearity. Second, many automated image analyses turn the otoliths in relation to maximum Feret's length (Tuset et al. 2003; De la Cruz-Agüero et al. 2012; Zischke et al. 2016), which may distort the measures for uncommon shapes in fish taxa such as Beryciformes, Caproiformes, Gobiiformes, Gasterosteiformes, Pleuronectiformes, Tetraodontiformes and Zeiformes (Smale et al. 1995; Lombarte et al. 2006; Tuset et al. 2008).

The methods of contour analysis provide more complex knowledge on the otolith shape variability. Although elliptical Fourier analysis (EFA) is the most common method (Bird et al. 1986; Castonguay et al. 1991; Campana and Casselman 1993), it only gives a global approximation of outline variability since each harmonic coefficient by itself has no morphological meaning and cannot discriminate local singularities (Rohlf and Archie 1984; Parisi-Baradad et al. 2005; Reig-Bolaño et al. 2010). For that reason, new approaches such as wavelets, curvature scale space, bending energy, fractal dimension, short-time Fourier transform, mean distances, and geometric morphology have been developed (Monteiro et al. 2005; Parisi-Baradad et al. 2005, 2010; Piera et al. 2005; Youssef et al. 2016). Among them, multiscale wavelet analysis has been demonstrated to be an excellent method for underlining morphological singularities and their position along contour (Parisi-Baradad et al. 2005, 2010; Lombarte and Tuset 2015), becoming a powerful method to detect inter- and intraspecific differences (Sadighzadeh et al. 2014; Tuset et al. 2015, 2020; Lombarte et al. 2018). The AFORO database (<http://aforo.cmima.csic.es/>; Lombarte et al. 2006), the world's largest marine fish otolith database consisting of more than 2000 species and 7000 images, operates with this technique and has the option of attaching images for the online classification of species based on the *K*-nearest neighbours (KNN)

learning algorithm (Parisi-Baradad et al. 2010). The conceptual development and mathematical analysis of wavelets involved learning from the analysis of points (coefficients) (Sadighzadeh et al. 2012) to all-signal processing by means of principal component analysis demonstrating the real power of this technique (Abaad et al. 2016; Tuset et al. 2016, 2018; Costa et al. 2018).

Instead of the extended opinion, we support the hypothesis that classical shape indices lose much information about the real otolith shape, which is why they are not recommended for works related to the identification of fish species. To clarify this specific question, this paper aims to (i) test the degree of multicollinearity between shape indices and to select the most adequate for posterior analyses, (ii) apply different parametric (linear discriminant analysis) and nonparametric algorithms (*K*-nearest neighbours, random forest, support vector machine and neuronal networks) to find the best solution for each morphological method (shape indices *versus* wavelet), (iii) examine the use of performance measures as a tool in the determination of the suitability of models, and (iv) conclude on the suitability of shape indices for the identification of species.

Material and methods

Otolith test sets

Otolith images and morphological information were extracted from the AFORO database. The main reason for this selection was to have a good representation of the morphological patterns described by Tuset et al. (2008) (Table 1, Fig. 1) and to avoid one specific fish assemblage affecting the final purpose of the study. Thus, the set was composed of 420 otoliths of 42 fishes belonging to 37 families and 25 orders (Table 1). The total number of otoliths by species varied between 5 and 12, although the most common was >9 otoliths by species. Total length (*TL* in mm) was mostly the reference fish size, but in some fish taxa, the preanal (*PL* in mm; e.g., Trachyrincidae) and furcal length (*FL* in mm; e.g.,

Scombridae) were used. In those specimens where the reference length was other (standard or furcal), it was transformed using models provided by Fishbase (Froese and Pauly 2019) or by the relationship between the otolith length and fish length given by Giménez et al. (2016). The set was characterized by the presence of juveniles and adults, which is why the intraspecific morphological variability was guaranteed.

Shape indices

The AFORO database provided morphometry data on otolith length (L in mm), width (W in mm), area (A in mm²) and perimeter (P in mm) (Table S1, see Supplementary material). Estimating the shape indices was initially necessary to remove the effect of the individual size of each specimen. For that, all measurements were normalized taking into account the allometric relationships, *otolith measure* = $a(\text{fish size})^b$ (Lombarte and Lleonart 1993). For each species, otolith measures (y) were transformed into z according to $z = y(x_0 x^{-1})^b$, where x is the fish size of each individual, x_0 is the mean fish size for each species, and b is the slope of the allometric relationship between fish size and each otolith measure.

The shape indices estimated from the standardized morphometric data were as follows (Russ 1990; Tuset et al. 2003; Volpedo and Echevarría 2003; Assis et al. 2020): aspect ratio ($AR = WL^{-1}$), circularity ($CIR = P^2 A^{-1}$), edge complexity ($EC = P(2\sqrt{\pi A})^{-1}$), ellipticity ($ELL = (L-W)/(L+W)$), formfactor ($FF = (4\pi A)P^{-2}$), rectangularity ($REC = A(LW)^{-1}$) and roundness ($RO = (4A)(\pi L^2)^{-1}$) (Table S2, see Supplementary material). The aspect ratio expresses the tendency in the shape (circular or elongated). Roundness and circularity provide information on the similarity of various features to a perfect circle, taking a minimum value of 1 and 4π (12.57), respectively. Edge complexity describes irregularities on otolith edges. Ellipticity indicates whether the changes in the axes are proportional. Formfactor is a means to estimate the surface area irregularity, taking values of 1.0 when it is a perfect circle and <1.0 when it is irregular. Finally, rectangularity describes the variations of length and width with respect to

the area, with 1.0 being a perfect square. Similar to other biological indices (e.g., functional indices; Dumay et al. 2004), allometry was eliminated by standardizing the original otolith measurements by computing the indices (Zischke et al. 2016; Tuset et al. 2018).

Wavelets

The wavelet transformed (*WT*) provides a 2ⁿ multiscale decomposition (Mallat 1991) based on normalized equally spaced radii length. In particular, we considered the wavelet function at the 5th scale because several studies have demonstrated that this scale better describes the specific characteristics of otolith contour (Parisi-Baradad et al. 2010; Sadighzadeh et al. 2012; Tuset et al. 2016, 2018, 2020; Lombarte et al. 2018). A total of 512 equidistant Polar coordinates for each otolith were extracted using the *rostrum* (see otolith terminology in Tuset et al. 2008) as the origin. Wavelets were directly obtained from the AFORO database (Fig. 2). A principal component analysis (PCA) based on the variance–covariance matrix was performed to reduce the dimensionality of the 512 data points obtained for each individual without a significant loss of information. Significant eigenvectors were identified by plotting the percentage of total variation explained by the eigenvectors *vs.* the proportion of variance expected under the ‘broken-stick model’ (Gauldie and Crampton 2002). Given that interspecific differences might be attributed to allometry, linear correlations were tested between $\log_{10}$ -*L* and the principal components. The effect of otolith length was removed using the residuals of the common slopes of the linear regressions of each component on otolith length, building a new PCA matrix.

Statistical methods

A Spearman correlation (ρ) was conducted to examine the pairwise association between shape indices. When the correlation coefficients were ≥ 0.6 , they were considered problematic by collinearity between them. For that, the variance inflation factor (VIF) was used to check the collinearity between

the shape indices, where VIF scores greater than 10 were eliminated as predictors (Quinn and Keough 2002). This VIF factor was estimated with the *vifstep* function of the *usdm* package (Naimi et al. 2014) for R (R Core Team 2020). To avoid the loss of information of some indices, a PCA analysis was also performed with all indices. Only the first component was significant and explained 99.6% of the variance (Fig. S1, see Supplementary material), which was why this option was discarded after analyses.

Four classifiers were compared. a) Linear discriminant analysis (LDA) is the most common parametric method used in the literature (Stransky et al. 2008a, b; Zhang et al. 2013; Teimori et al. 2019). b) *K*-nearest neighbour (KNN) determines the classification of objects finding the *k* closest neighbours of a query element inside the database by comparing one by one in the feature space and with a distance metric defined in this space (Marti-Puig and Reig-Bolaños 2016); the advantage of KNN is its higher tolerance in the data structure (Hastie et al. 2009). c) Random forest (RF) involves a nonlinear ensemble or forest of many tree classifiers in which class assignment occurs using an average of the forest of trees (Breiman 2001; Jones and Checkley 2017). It balances errors in data sets where classes are imbalanced and can train with a relatively small number of samples to obtain good results. It is a very common method in the classification of species/stocks based on otolith microchemistry (Mercier et al. 2011; Régnier et al. 2017). d) Support vector machine (SVM) with linear kernel is a maximum margin classifier that maps the original feature space to higher dimensions. It has the ability to deal with high-dimensional datasets and its flexibility in modelling diverse data sources (Ben-Hur et al. 2008). It has been used for automatic fish ageing (Fablet et al. 2005; Bermejo et al. 2007) and identification of species using shape and microchemistry (Castro 2007). e) Artificial neural networks (ANNs) are systems based on a network architecture, where the smallest unit is the neuron. Thus, the network is composed of three neuron layers: input layers groups: input layer (morphological variables), hidden layers (nodules from $i = 1 \dots n$) and output layer (species). We used a multi-layer perceptron (MLP) architecture and a back-propagation gradient algorithm to calibrate it

(Yousef et al. 2016; Ciaburro and Venkateswaran 2017). The ANN has also been used in other otolith applications, such as ageing (Roberston and Morison 1999; Moen et al. 2018) and microchemistry (Hanson et al. 2004; Mercier et al. 2011).

The classifications were performed using the caret package in R (Kuhn 2008) for R. Leave-one-out cross-validation (LOOCV) was the strategy selected; in this case, all observations are used for both training and validation, where each one is being used once for validation. Thus, all observations are tested against the rest, so we know how each particular element is classified. This strategy is recommended by small datasets by species (Marti-Puig et al. 2015, 2020), as occurs in our dataset. The validation method was repeated 1000 times for each analysis. The models were built using scaled and centered predictor variables. The optimal hyperparameters of KNN (k), RF (mtry), SVM (σ and C) and ANN (hidden units) were defined during preliminary tuning (Fig. S2, see Supplementary material). The additional R packages used for each model were MASS (Venables and Ripley 2002) of LDA, caret (Kuhn 2008) for KNN, randomForest (Liaw and Wiener 2002) for RF, kernlab (Karatzoglou et al. 2004) for SVM and RSNNS (Bergmeir and Benitez 2012) for ANN.

The performance of these models was evaluated and compared using several criteria: accuracy, Cohen's kappa (κ) statistic, sensitivity and precision. The accuracy was derived from the confusion matrix following the following formulae: $(TP+TN)/(TP+FP+TN+FN)$, where TP indicates otoliths correctly classified, TN denotes otoliths correctly non-assigned, FP reveals otoliths assigned to other species, and FN gives otoliths wrong classified by the model to other species. The kappa statistic estimates the improvement over chance of the percent corrected classification rates (Titus et al. 1984). The sensitivity establishes the proportion of otoliths correctly classified in relation to classified by the algorithm as true (TP/FN). The precision indicates the proportion of otoliths correctly classified by the model (TP/FP) (Altman and Bland 1994; Powers 2011).

Results

The shape indices presented statistically significant non-zero correlations ($p < 0.05$), except for the rectangularity with aspect ratio (Spearman's $\rho = -0.01$) and ellipticity ($\rho = 0.01$) (Fig. 3). Only these three indices showed correlations < 0.6 with the remaining indices. The VIF analysis evidenced the high collinearity between the shape indices indicating a multicollinearity effect ($VIF > 10$; Table S3), being the best option to avoid this problem the selection of rectangularity, ellipticity and formfactor ($VIF < 2$; Table S3, see Supplementary material). Therefore, only three indices (rectangularity, ellipticity and formfactor) were used for the classifiers. In the wavelet analysis, the first 17 components of the PCA analysis accounted for higher variance than expected by chance alone (96.4%), reaching the first three components $\sim 56\%$ of the variance (Table S4, see Supplementary material).

The comparison of the accuracy and kappa index between classifiers and otolith analysis methods (shape indices *versus* wavelet function at 5th scale) demonstrated that a) ANN and RF presented lower accuracy for the shape indices (accuracy and kappa indices $< 70\%$), b) the use of wavelets always provided better results ($> 80\%$ for both) than shape indices, and c) SVM was the best method for shape indices (79.6% of accuracy and Cohen's κ indicates that a classification efficiency of 79.3% better than would have occurred by chance alone) and for wavelets (96.7% and 96.6, respectively), although ANN also gave excellent results for wavelets (96.7%) (Table 2). However, the analysis of the distribution of the value of sensitivity and precision for each species did not confirm the efficiency described, especially for the shape indices, there were species with values sensitivity $< 10\%$ (Fig. 4a) and precision $< 50\%$ (Fig. 4b). In particular, ANN showed problems of reliability in $\sim 50\%$ of species (Table S5), whereas KNN, LDA and RF were $\sim 36\%$ (Tables S6-S8, see Supplementary material) and SVM $\sim 36\%$ (Table S9, see Supplementary material). The efficiency problem of these classifiers with otolith shapes not excessively rare is highlighted. In contrast, the identification of species using the wavelet function

only induced some mistakes for RF (~14%; Table S8, see Supplementary material) and one low sensitivity in *S. kaupii* for KNN (Table S6, see Supplementary material). The ANN, LDA and SVM classifiers maintained high values for the performance measures (Tables S5, S7 and S9, respectively; see Supplementary material), but their distributions were less efficient when using LDA (Figs. 4a and b).

Discussion

The present study compared the efficiency in the identification of fish species using classical otolith shape indices and contour analysis, represented by the wavelet transformed function. Our findings were conclusive. a) Wavelet analysis is a better method than shape indices for shape analysis, independent of the type of classifier. b) The correct analysis of wavelets is essential for obtaining satisfactory results. The wavelet transform is a signal processing technique for why few points (or coefficients) cannot rightly define the otolith shape, and a drastic reduction, as occurs in the shapeR package, is advised against (Libungan and Pálsson 2015). A clear example of this effect can be illustrated using the study performed by Lin and Al-Abdulkader (2019), who compared otolithometry, shape indices and wavelets for the identification of species and families. The wavelet function (only represented by 26 coefficients) obtained the worst results in almost all cases. c) The accuracy cannot be the unique criterion for the justification and selection of analytical variables and classifiers. We strongly recommend that any study should not accept as valid an unbalanced model where the prediction of one or some species may be low, despite the high accuracy of the whole model. Therefore, it is necessary to reinforce the complementary use of performance measures. Finally, d) the accuracy of models depends not only on the number of species analysed but also on the variability of shapes and the ability of the method to discriminate them.

Limitations of shape indices

Our findings demonstrated that shape indices are not the good methodology assuring the correct classification of all fish species. This is mainly because the ideal geometric form of the shape indices does not necessarily imply the real description of the object. Thus, otoliths clearly distinctive from the naked eye can have similar values of shape indices, as occurred between *Arctozenus risso* (otolith with trapezoidal shape) and *Engraulis encrasicolus* (otolith with elliptic shape) (Fig. 5a, Table S2, see Supplementary material). However, the strong multicollinearity between the indices limited the number of variables in analytical procedures, thus avoiding a better accuracy of models. Considering the LDA classifier, a low sensitivity occurred in 45.2% of species, whereas Lin and Al-Abdulkader (2019) provided 37.5% sensitivity using all shape indices. This means that multicollinearity would overfit the accuracy and performance measures, but in otoliths with less heterogeneous morphologies, the problem would not be solved. This is clearly exemplified in the present study, where otoliths with uncommon shapes, such as trilobulated (*Macroramphosus scolopax*), pentalobulated (*Zeus faber*), hour-glass (*Capros aper*), sagittiform (*Gadella maraldi*), or tall (*Argyropelecus hemigymnus*), were correctly classified, despite presenting higher intraspecific variability. In contrast, more common morphologies, such as elliptic (*Alosa fallax*, *Engraulis encrasicolus*, *Lithognathus mormyrus* and *Vinciguerrria nimbaria*), rectangular (*Chelon aurata*), cuneiform (*Labrus mereula*), or oval (*Mullus surmmuletus*), revealed a lower sensitivity and precision. Nevertheless, the low number of shape indices and the number of specimens considered also influence in the model accuracy.

The otolith shape depends on the ecological, evolutionary and phylogenetic characteristics of species (Škeljo and Ferri 2012; Zhuang et al. 2014; Tuset et al. 2015, 2016; Mille et al. 2016; Lombarte et al. 2018; Teimori et al. 2019), which are not always differentiated by the shape indices. For instance, otolith shape changes can be more or less prominent and localize in sections or the whole contour during ontogenetic development (Monteiro et al. 2005; Tuset et al. 2008; Biolé et al. 2019). When the

shifts are small, the shape indices may remain constant, which occurs, for example, in *Synphobranchius kaupii* (Fig. 5b; present study) and other fish species (Tuset et al. 2003, 2008; Adelir-Alves et al. 2018; Avigliano et al. 2018). Even if the shifts are noticeable, the interspecific differences may not significantly increase the model confusion. In addition, models with few numerous and heterogeneous variables conditioned the discriminatory ability of species. All these particulars arise in the present study, where only three shape indices were included in the models presenting two of them (rectangularity and formfactor) a low dispersion, why the combination of these variables was insufficient for building a model with a high sensitivity and precision. Given the problems that may appear, why do the shape indices provide good results in some studies? Usually, these studies compare few species with clear variations in otolith shapes. For that reason, to infer the idea that shape indices can be used to identify species from the comparison of the number of species reduced, usually belonging to the same family, is simply a fallacy. Any automated taxon identification (ATI) using bigdata (e.g., AFORO database) can never apply classical shape indices for the identification of species, and they are only useful tool in specific cases. Although alternative shape indices have been proposed with success (Tuset et al. 2006), this line of research has not been fully explored. Therefore, it seems more logical to continue working with contour analysis, particularly with the wavelet function (Sadighzadeh et al. 2012), which provides sufficient accuracy considering the magnitude and limitations of the AFORO database. Moreover, the possibility of delimiting specific sections of otoliths opens very interesting analytical options (Tuset et al. 2015).

Learning algorithms

The major limitation of classical classifiers in the identification of species and stocks is the violation of normality. It can be a serious problem when it is caused by outliers (Zuur et al. 2009), which results in intra- and interspecific variability. Although parametric methods can be more powerful, machine-algorithm methods are a valid alternative, providing excellent results in the identification of species

(Parisi-Baradad et al. 2010; Zischke et al. 2016; Youssef et al. 2016) and stocks (Smoliński et al. 2020). In this context, the present study is the first comparing different models using the shape indices and wavelet function. In the case particular of shape indices, the SVM algorithm achieved the highest accuracy, but KNN provided the best results for the performance measures. Given that the increment of accuracy in both models is relatively small (~6%), it seems more reasonable to consider the KNN model as the best-performing algorithm. In this context, it was highlighted that both ANN and RF attained the worst accuracies, which suggests an effect of a low number of variables (three) considered. In contrast, the use of wavelets increased noticeably (~15-37%) the success of classification and performance measures, with SVM and ANN being the best options. Nevertheless, all models provided good accuracies and have been used with successful KNN (Parisi-Baradad et al. 2010), LDA and SVM (Zischke et al. 2016), and ANN (Youssef et al. 2016). However, SVM and ANN are complex processes that significantly increase the lead time of forecasting. This leads to problems that are more pragmatic than mathematical: speed up or be accurate. When the study is limited in the number of species and numerous in specimens, the processing time of complex learning algorithms is assumable. However, when scientists using online classification are looking for a faster solution, KNN continues to be an acceptable option. In any case, these findings stress the importance of the model choice and the characteristics of the otolith data set on the analysis. For instance, SVM seems to be the algorithm more useful for the identification of stocks using the otolith shape (Smoliński et al. 2020), and RF outperforms ANN using otolith microchemistry (Mercier et al. 2011). In contrast, DNN (deep neural network, a model of ANN using a large number of hidden layers) would have considerable potential for ageing (Moen et al. 2018; Moore et al. 2019). Consequently, the generalized application of more complex learning algorithms in all fields of otoliths seems unstoppable, including the identification of species from bigdata and hence the parametric models living on borrowed time.

Conclusions

The choice of the criteria at different levels of mathematical procedures makes the standardization of a methodology in this field complex, including issues such as how to solve the number of components in the PCA (Jolliffe 2002) and the effects of small rotations and variations in the starting point of contour analysis on the model efficiency (Marti-Puig and Reig-Bolaño 2016; Marti-Puig et al. 2020). It is likely that the future includes deep learning classifiers based on multiple images as occurs in ageing (Moen et al. 2018), which could be positive when considering the *sulcus acusticus* and training and test sets. However, this requires a large amount of information that is not available and likely cannot be never available for many rare or few common species, but that is the purpose of the AFORO database. In addition, our target is provided as the best method and model for the classification of species for researchers in different fields, and KNN is currently a faster solution but revisable in the future. We do not reject the use of the classical shape indices, but whether the assertion on their goodness in relation to the contour analysis, especially when the multicollinearity effect is not considered, and few species are being compared (Lord et al. 2012). Thus, their routine use loses its essence and usefulness, showing their visibility in models with performance measures. Each year, hundreds of scientists extract thousands of otoliths for different purposes, creating large amounts of data and images. This big data information requires a deeper computation and accuracy than classical shape indices can provide.

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Legends

Fig. 1. Left *sagittae* otolith of each studied species, indicating the anatomical type. aa, triangular, *Synchiropus phaeton*; ba, square, *Gobius cruentatus*; bb, square, *Odondebuenia balearica*; ca, rectangular, *Pomatomus saltatrix*; cb, rectangular, *Scomber colias*; cc, rectangular, *Lestidiops jayakari*; da, trapezoidal, *Arctozenus risso*; ea, pentagonal, *Epigonus telescopus*; eb, pentagonal, *Glossanodon leioglossus*; fa, hexagonal, *Evermannella balbo*; ga, discoidal, *Microchirus boscanion*; gb, discoidal, *Synaphobranchus kaupii*; ha, oval, *Diaphus holti*; hb, oval, *Mullus surmuletus*; ia, inverse oval, *Trachyrincus scabrus*; ja, elliptic, *Cataetx alleni*; jb, elliptic, *Cepola macrophthalma*; jc, elliptic, *Dissostichus eleginoides*; jd, elliptic, *Stromateus fiatola*; je, elliptic, *Engraulis encrasicolus*; jf, elliptic, *Alosa fallax*; jg, elliptic, *Lithognathus mormyrus*; ka, fusiform, *Serranus cabrilla*; la, oblong, *Phycis physis*; lb, oblong, *Chelon auratus*; ma, lanceolated, *Trachurus picturatus*; na, inverse lanceolated, *Merluccius hubbsi*; oa, spindle-shaped, *Sphyreana sphyraena*; pa, inverse spindle-shaped, *Micromesistius poutassou*; qa, pyriform, *Vinciguerria nimbaria*; ra, inverse pyriform, *Mora moro*; sa, bullet-shaped, *Argyrosomus regius*; ta, tall, *Argyropelecus hemigymnus*; ua, hour glass trilobulated, *Macroramphosus scolopax*; va, hour glass, *Capros aper*; vb, hour glass, *Lagocephalus lagocephalus*; wa, pentalobulated, *Zeus faber*; xa, semicircular, *Lophius piscatorius*; ya, irregular, *Hoplostethus mediterraneus*; za, cuneiform, *Labrus merula*; *a, kidney-shaped, *Merluccius paradoxus*; +a, sagitiform, *Gadella maraldi*.

Fig. 2. Illustration of the variability of 5th wavelets in different otolith patterns. R, *rostrum*.

Fig. 3. Matrix correlation plot between otolith shape indices showing histogram, kernel density overlays, correlations and level of statistical significance (*). *AR*, aspect ratio; *CIR*, circularity; *EC*, edge complexity; *ELL*, ellipticity; *FF*, formfactor; *REC*, rectangularity; *RO*, roundness.

616 **Fig. 4.** Violin plots for sensitivity (*a*) and precision (*b*) of different algorithms of classification applied to fish otoliths for
617 the identification of species using shape indices (_s) and wavelets (_w). ANN, artificial neuronal networks; LDA, linear
618 discriminant analysis; KNN, *K*-nearest neighbours; RF, random forest; SVM, support vector machine.

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620 **Fig. 5.** Examples where otolith shape indices are not representative of the inter- (*a*) and intra-specific (*b*) variability.

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Table 1. Taxonomic groups (Nelson et al. 2016), species, number of specimens by species and description of the general shape of otolith, including the most remarkable traits in otolith contour according to Tuset et al (2008).

Order	Family	species	Acronym	Number	General shape	local traits of the contour
Anguilliformes	Synphobranchidae	<i>Synphobranchus kaupii</i>	<i>Skau</i>	12	Discoidal to square	margin with small irregularities
Clupeiformes	Engraulidae	<i>Engraulis encrasicolus</i>	<i>Eecn</i>	11	Elliptic	ventral margin serrated
	Clupeidae	<i>Alosa fallax</i>	<i>Afal</i>	7	Elliptic	antero-dorsal margin with deep indentations
Argentiniformes	Argentinidae	<i>Glossanodon leiglossus</i>	<i>Glei</i>	11	Pentagonal	prolongated rostrum
Stomiiformes	Phosichthyidae	<i>Vinciguerrria nimbaria</i>	<i>Vnim</i>	8	Elliptic to pyriform	rostrum well developed
	Sternptychidae	<i>Argyropelecus hemigymnus</i>	<i>Ahem</i>	9	Tall	entire margins
Aulopiformes	Paralepididae	<i>Arctozenus risso</i>	<i>Aris</i>	12	Trapezoidal	postrostrum developed with excisura caudalis
		<i>Lestidiops jayakari</i>	<i>Ljay</i>	8	Rectangular	deep excisura ostii with anteroventral projection
	Evermannellidae	<i>Evermannella balbo</i>	<i>Ebal</i>	5	Hexagonal	two conspicuous needle shape ventral projections
Myctophiformes	Myctophidae	<i>Diaphus holti</i>	<i>Dhol</i>	11	Oval	conspicuous serrated ventral margin
Zeiformes	Zeidae	<i>Zeus faber</i>	<i>Zfab</i>	10	Trilobulated to pentalobulated	colliculum extensively protruding
Gadiformes	Trachyrincidae	<i>Trachyrincus scabrus</i>	<i>Tsca</i>	12	Elliptic to inverse oval	lobed shaped anterodorsal prominence
	Gadidae	<i>Micromesistius poutassou</i>	<i>Mpou</i>	12	Inverse spindle-shaped	lobed to entire margins
		<i>Phycis phycis</i>	<i>Pphy</i>	10	Oblong	lobed to entire margins
	Merlucciidae	<i>Merluccius hubbsi</i>	<i>Mhub</i>	12	Inverse lanceolated	indentations in dorso-posterior margin
		<i>Merluccius paradoxus</i>	<i>Mpar</i>	9	Oblong to kidney-shaped	lobulated margins
	Moridae	<i>Mora moro</i>	<i>Mmor</i>	12	Inverse pyriform	anterodorsal prominence
	Moridae	<i>Gadella maraldi</i>	<i>Gmar</i>	9	Sagittiform	dorsal dome and elongated postrostrum
Trachichthyiformes	Trachichthyidae	<i>Hoplostethus mediterraneus</i>	<i>Hmed</i>	11	Irregular	deep indentations in dorsal margin
Ophidiiformes	Bythitidae	<i>Cataetyx alleni</i>	<i>Call</i>	9	Elliptic	entire margins
Gobiiformes	Gobiidae	<i>Gobius cruentatus</i>	<i>Gcru</i>	11	Square to rectangular	postero-dorsal development
		<i>Odondebuenia balearica</i>	<i>Obal</i>	9	Rectangular	entire margins
Mugiliformes	Mugilidae	<i>Chelon auratus</i>	<i>Caur</i>	12	Rectangular to oblong	deep and large indentations in the ventral margin
Istiophoriformes	Sphyraenidae	<i>Sphyraena sphyraena</i>	<i>Ssph</i>	9	Spindle-shaped	notch in excisura ostii
Carangiformes	Carangidae	<i>Trachurus picturatus</i>	<i>Tpic</i>	12	Lanceolated	crenated to lobulated margins

Pleuronectidiformes	Soleidae	<i>Microchirus boscanion</i>	<i>Mbos</i>	7	Discoidal to elliptic	entire margins
Syngnathiformes	Macroramphosidae	<i>Macroramphosus scolopax</i>	<i>Msco</i>	9	Hour-glass trilobulated	pointed triangular-shape postrostrum
Callionymiformes	Callionymidae	<i>Synchiropus phaeton</i>	<i>Spha</i>	8	Triangular	dorsal margin lobed with indentations
Scombriformes	Scombridae	<i>Scomber colias</i>	<i>Scol</i>	12	Rectangular	dorsal concavity
	Stromateidae	<i>Stromateus fiatola</i>	<i>Sfia</i>	8	Elliptic	notch in excisura ostii
Labriformes	Labridae	<i>Labrus merula</i>	<i>Lmer</i>	10	Cuneiform	deep angled notch in excisura ostii
Perciformes	Serranidae	<i>Serranus cabrilla</i>	<i>Scab</i>	10	Fusiform	prominent rostrum and excisura ostii
	Pomatomidae	<i>Pomatomus saltatrix</i>	<i>Psal</i>	12	Rectangular	deep and irregular indentations in ventral margin
	Epigonidae	<i>Epigonus telescopus</i>	<i>Etel</i>	9	Pentagonal to rhomboidal	indentations in dorsal margin
	Mullidae	<i>Mullus surmuletus</i>	<i>Msur</i>	12	Oval	margins crenate to irregular
	Cepolidae	<i>Cepola macrophthalma</i>	<i>Cmac</i>	9	Elliptic	moderate peaked rostrum
	Notothenidae	<i>Dissostichus eleginoides</i>	<i>Dele</i>	8	Elliptic	excisura ostii, irregular margins
Acanthuriformes	Sciaenidae	<i>Argyrosomus regius</i>	<i>Areg</i>	9	Bullet-shaped	entire margins
Spariformes	Sparidae	<i>Lithognathus mormyrus</i>	<i>Lmor</i>	12	Elliptic to oblong	postrostrum pointed with irregularities
Caproiformes	Caproidae	<i>Capros aper</i>	<i>Cape</i>	12	Hour glass	deep excisurae, ventral margin irregular
Lophiiformes	Lophiidae	<i>Lophius piscatorius</i>	<i>Lpis</i>	11	Semicircular	irregular and deeply lobulated
Tetraodontiformes	Tetraodontidae	<i>Lagocephalus lagocephalus</i>	<i>Llag</i>	9	Hour-glass	developed colliculum

Table 2. Accuracy and kappa index obtained by different classifiers using shape indices and wavelet function at 5th scale for fish otoliths.

Method	Classifiers				
Shape Indices	LDA	KNN	RF	SVM	ANN
Accuracy	0.724	0.736	0.676	0.796	0.607
95% CI	0.678-0.766	0.691-0.777	0.629-0.721	0.756-0.835	0.559-0.654
Kappa	0.717	0.729	0.668	0.793	0.597
Wavelet					
Accuracy	0.914	0.886	0.826	0.967	0.967
95% CI	0.883-0.934	0.852-0.915	0.787-0.861	0.945-0.982	0.945-0.982
Kappa	0.912	0.883	0.822	0.966	0.966

Note: ANN, artificial neuronal networks; CI, confidence interval; LDA, linear discriminant analysis; KNN, *K*-nearest neighbour; RF, random forest; SVM, support vector machine.