Otolith growth allometry measurements in the European eel

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Abstract – *The paper presents an application and a comparison of two mathematical approaches analyzing the shape of the otolith of Mediterranean population of the European eel: the wavelet transform and Elliptic Fourier. The shape variability and its relation between the otolith growth was investigated. Partial Least Squared found an allometric and continuous growth in the relationship of otolith size and shape by comparing with a regression model between the predicted otolith sizes obtained from the EFA and WL approaches, and the observed ones. Elliptic Fourier method applied to European eels otolith shape analysis has obtained better performances then the Wavelet transform analysis using the Partial Least Square regression.*

Keywords - *European eel, Otolith, Wavelet transform, Elliptic Fourier analysis, PLS*

I. INTRODUCTION

The analysis of otolith morphology represents an efficient tool for the discrimination of fish stocks, populations, and species when genetic data are not available for comparison [1][2][3][4]. The saccular otolith (*sagitta*) is characterized by a high morphological diversification that not only reflect genetic variability, but also environmental changes. Endogenous and exogenous factors determine both otoliths overall shape and growth patterns [5]. So they are good phenotypic markers that may be more applicable for studying short-term, environmentally induced variation; perhaps more applicable for fisheries management, as opposed to genetic variation and endangered species management [6].

No studies for European eel (*Anguilla anguilla* Linnaeus, 1748) focus on the relationship between otoliths growth patterns and morphology. *A. anguilla* is a catadromouse species that constitute a single, randomly mating population [7] and animals live in all types of European and North African freshwater habitats. Changes during the growth in the otolith shape are analyzed in relation to juvenile-adult transitions (i.e. from the entry of individuals in inland waters systems up to the following reproductive migration).

In this study we evaluated if the relation between otolith growth and shape is allometric. We targeted on shape variability of the *sagittae* otolith during growth in a Mediterranean population. In order to do so, we compared two morphological analytic approaches: wavelet transform (WL, [8]) and Elliptic Fourier analysis (EFA, [9]).

II. MATERIALS AND METHODS

The sampling site was the Caprolace lagoon, situated within the Circeo National Park, (central Italy; 12°58'14.02; 41°21'7.08). 400 sedentary and downstream migrant animals were collected during 2007 with fyke nets. Fishes were sacrificed to extract the otholits from the cranium. A subsample of 150 right *sagittae* was selected for the shape analysis representing all total length size classes of eels sampled. Otoliths were photographed and measured with an approximation of 0.01mm. The images were all coded in 16-bit RGB scale and captured at high resolution. Image processing for automatic extraction of otholits outline was performed by the image analysis software Age&Shape (V.1.0, Infaimon Software, Spain); 512 points equidistant to each other were chosen on the otholit contour, starting from the rostrum as input signal for the calculation of wavelets. Level 7 of wavelet transform was selected given the sensibility of the analysis for that coefficient in the resolution of the entire otolith shape.

Elliptic Fourier analysis (EFA) consists in decomposing a curve into a sum of harmonically related ellipses [10][11][12][13]. The correct number of harmonics was calculated using the method proposed by Crampton [9]. The Fourier series was truncated for k equals to 15, the level at which the average cumulative power is 99.99% of the average total power. According to Rohlf & Archie [14], the elliptic Fourier coefficients were normalized to be invariant of size, location, rotation, and starting position (which was always approximately the tip of the

umbo). Cartesian Coordinates were considered. All further analyses were performed using the software Matlab 7.1 (The Math Works, Natick, USA).

The wavelet transform (WL) compares the signal to a finite length analysing the function called wavelet in a set of increasing scales that are obtained by dilating the wavelet. Choosing the appropriate wavelet shape and setting, a scaling parameter allows the wavelet transform to detect singularities of different sizes in the analysed signal. The successive convolution of the radius with the wavelet and blurring filters produces a complete representation (discrete wavelet transform). Using this wavelet, the fast changing points of an otolith shape appear as large values of the wavelet transform [8].

Partial Least Square analysis (PLS, [15]) was used to regress otoliths predicted lengths obtained from both EFA and wavelets approaches, against the observed size of each otolith in order to investigate the occurrence of allometry in the relationship of size and shape. PLS allow constructing predictive models when the factors are many and highly collinear. The X-block (EFA or WL coefficients) values were pre-processed by an autoscaling. Each model was validated using a full-cross validation ('Venetian blind' algorithm). The sample was randomly subdivided in two groups: a calibration set (75% individuals), used to develop the calibration model, and a prediction set made by the other 25% individuals that were used to test the model. The PLS analysis provides, the percentage of correct classification and the loadings of each species on each latent vector (LV)

In order to observe a particular trend of growth trajectory in eel otholits a clustering procedure based on k-means was used to to obtain the best number of k-clusters ([16] and modified by [17]).

III. RESULTS AND DISCUSSION

Two PLS models have been obtained from both datasets, the first is based on EFA coefficients and the second on Wavelets at level 7. PLS models characteristics and efficiency are reported in Table 1. Test results in the EFA case show percentage of correct classification of 97% while the second analysis performed with WL obtained 83.7% of correct classification. RMSAC and RMSECV are lower in the EFA model.

	EFA		WL7	
	Model	**Test**	**Model**	**Test**
% Corr. Class.	98.1	97.0	89.6	83.7
Latent vectors	15	15	15	15
RMSE	0.13	0.19	0.13	0.38
RMSEC	0.13		0.29	
RMSECV	0.34		0.39	

Tab. 1. PLS characteristics

Length values predicted by PLS models were regressed on observed otolith length for both EFA and WL7 shape descriptions (Fig. 2). In both cases a significant high correlation was found; EFA model showed an R^2=0.98, while WL7 for R^2=0.88. PLS model generated values much efficiently related with measured lengths. Moreover, observing the outlines of the extreme length predicted otoliths (Fig. 2) with both methods it is possible to notice shape changes during growth: *sagittae* become more elongated with an extension of the *rostrum* and the *postrostrum.*

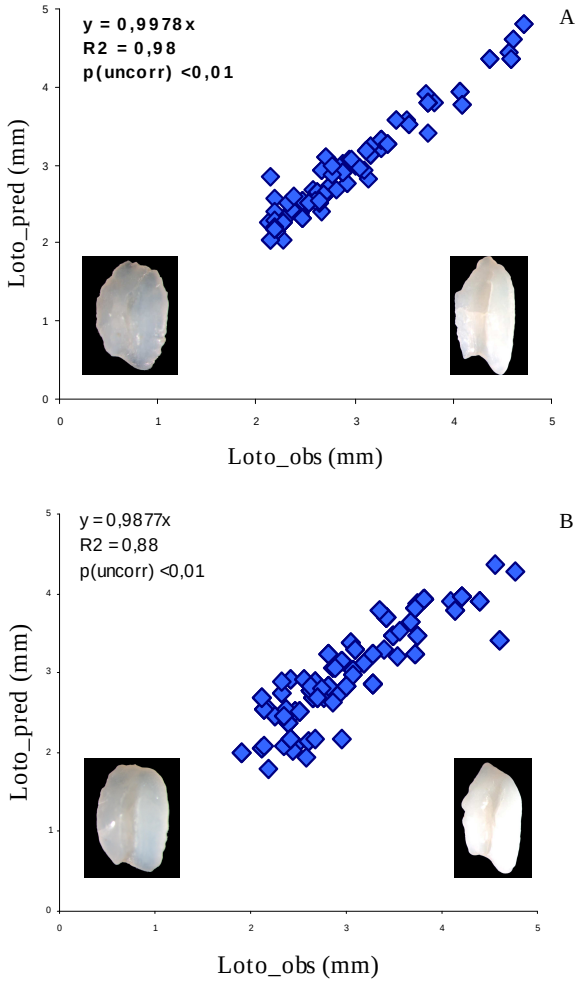

Fig. 2. Regressions between observed and predicted lengths by the PLS models from EFA (A) and WL7 (B) shape analysis approaches. On the bottom of each graph (in relation to y axis) are reported the extreme configurations of that shape variation.

K-means validation test reported that otolith growth of Caprolace eels, calculated between the otolith size and shape, is allometric and the trend of variation is continuous and not "step-shaped".

IV. CONCLUSIONS

From the methodological point of view Elliptic Fourier method applied to European eels otolith shape analysis has obtained better performances using the Partial Least Square regression between observed vs. predicted otolith length. Further studies are needed to verify and implement these results applying lower wavelet coefficients in order to be able to describe outlines at a higher resolution.

Many studies described a good linear correlation between fish length and the caudal otolith radius [18][19][20] which reflect its whole size. Our results confirm this evidence for eel population of Caprolace lagoon. Animals otolith growth showed an allometric and continuous trend. Therefore in this case it seems not possible to identify different typologies of otolith shape as a tool for indirect ageing as suggested by Doering e Ludwig (1990)[21].

REFERENCES

[1] Lombarte A., Castellon A. (1991). Interspecific and intraspecific otolith variability in the genus Merluccius as determined by image analysis. Canadian journal of zoology 69(9): 2442-2449.

[2]Castonguay, M., Simard, P.,Gagnon, P. (1991). Usefulness of Fourier Analysis of Otolith Shape for Atlantic Mackerel (*Scomber scombrus*) Stock Discrimination. Can. J. Fish. Aquat. Sci. 48(2): 296–302

[3]Campana, S.E. and Casselman, J.M. (1993). Stock Discrimination Using Otolith Shape Analysis. Can. J. Fish. Aquat. Sci. 50(5): 1062-1083.

[4] Cardinale, M., Doering-Arjes, P., Kastowsky, M., and Mosegaard, H. (2004). Effects of sex, stock, and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths. Can. J. Fish. Aquat. Sci. 61(2): 158–167.

[5] Lombarte, A., Torres, G.J. and Morales-Nin, B. (2003). Specific Merluccius otolith growth patterns related to phylogenetics and environmental factors. J. Mar. Biol. Ass. U.K. 83, 277-281.

[6] Begg, G.A., Friedland, K.D., and Pearce, J.B. (1999)., Stock identification and its role in stock assessment and fisheries management: an overview. Fisheries Research. 43(1-3): 1-8.

[7] Dannewitz, J., Maes, G-E., Johansson, L, Wickström, H, Volckaert, F.,A., M. and T. Järvi. (2005). Panmixia in the European eel: a matter of time* Proc. R. Soc. London Ser. B. 272:1129-1137.

[8] Parisi-Baradad, V., Lombarte, A., Garcia-Ladona, E., Cabestany, J., Piera, J., and Chic, O. (2005). Otolith shape contour analysis using affine transformation invariant wavelet transforms and curvature scale space representation. Marine and Freshwater Research 56:795–804.

[9] Crampton, J.S. (1995). Elliptic Fourier shape analysis of fossil bivalves: some practical considerations. Lethaia 28:179-186.

[10] Lestrel, P. E. (1997). Fourier descriptors and their applications in biology. Cambridge Univ. Press: 466 p.

[11] Loy, A., Busilacchi, S., Costa., C., Ferlin, L., Cataudella, S. (2000). Comparing geometric morphometrics and outlines fitting methods to monitor fish shape variability of Diplodus puntazzo (Teleostea , Sparidae). Aquacultural Engineering 21(4): 271-283.

[12]Costa, C., Aguzzi, J., Menesatti, P., Antonucci, F., Rimatori, V., Mattoccia, M. (2008). Shape analysis of different populations of clams in relation to their geographical structure. Journal of zoology, 276: 71-80.

[13]Menesatti, P., Costa, C., Paglia, G., Pallottino, F., D'Andrea, S., Rimatori, V., Aguzzi, J. (2008). Shape-based methodology for multivariate discrimination among Italian hazelnut cultivars. Biosystem Engineering, 101(4): 417-424.

[14] Rohlf, F. J. & Archie, J. W., (1984). A comparison of Fourier methods for the description of wing shape in mosquitoes (Diptera: Culicidae). Syst. Zool. 3: 302-317.

[15] Wold, S., Sjöström, M., Eriksson, L. (1999). Partial least square projections to latent structures (PLS) in chemistry. In: The Encyclopedia of Computational Chemistry. Wiley, New York, pp. 2006–2021.

[16] Ray, S., Turi, R.H., (1999). Determination of number of clusters in *k*-means clustering and application in colour image segmentation. In: Proceedings of the 4th Inter- national Conference on Advances in Pattern Recognition and Digital Techniques (ICAPRDT'99), Calcutta, India, pp. 137–143.

[17] Costa, C., Menesatti, P., Paglia, G., Pallottino, F., Aguzzi, J., Rimatori, V., Russo, G., Recupero, S., Reforgiato Recupero, G. (2009). Discrimination of Tarocco sweet orange [Citrus sinensis (L.) Osbeck] varieties using opto-electronic elliptic Fourier based analysis of fruit shape. Accepted by postharvest biology and technonology.

[18] Rossi, R., Villani, P., (1980). A biological analysis of eel catches, Anguilla anguilla L., from the lagoons of Lesina and Varano, Italy. J. Fish. Biol. 16, 413±423

[19] Ricc, F. (1990) Back-calculation of fish length: a critical review. J Fish Biol 36:883–902

[20] Ricc, F. (1995) The analysis of otolith data—a mathematician's perspective (what, precisely, is your model?). In: Secor DH, Dean JM, Campana SE (eds) Recent developments in fish otolith research. The Belle W. Baruch Library in Marine Science No 19. University of South Carolina Press, Columbia, SC, p. 81–94.

[21] Doering, P. and Ludwig J. (1990). Shape Analysis of Otoliths - a Tool for Indirect Ageing of Eel, *Anguilla anguilla* (L.)? Internationale Revue der gesamten Hydrobiologie und Hydrographie 75 (6): 737-743.