

Fig. 1. A. Original image used to build the models (coloured squares represents the object ROIs reported in Table 1: 1. red ROI on a leaf, 2. blue ROI on an almond, 3. green ROI on the lemon segment, 4. brown ROI on the lemon flavedo, 5. pale gray ROI on the dark background). B. Original image used to build the models after PLS calibration. C. Original image of *Munida tenuimana*. D. PLS calibrated image of *Munida tenuimana*. E. Original image of *Salamandra salamandra*. F. PLS calibrated image of *Salamandra salamandra*. G. Original image of *Anguilla anguilla*. H. PLS calibrated image of *Anguilla anguilla*.

OTOLITH GROWTH ALLOMETRY MEASUREMENTS IN THE EUROPEAN EEL

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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]. 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 [2]. 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 [3].

No studies for European eel (*Anguilla anguilla* Linnaeus, 1748) focus on the relationship between otoliths growth patterns and morphology. *A. anguilla* is a catadromous species that constitute a single, randomly mating population [4] 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) and Elliptic Fourier analysis (EFA).

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 otoliths 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. Image processing for automatic extraction of otoliths outline was performed by the image analysis software Age&Shape (Ifaimon); 512 points equidistant to each other were chosen on the otolith contour, starting from the rostrum as input signal for the calculation of wavelets. Level 7 of wavelet trans-

form 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 [5]. The correct number of harmonics was calculated using the method proposed by Crampton [6]. 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 [7], 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. 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, [9]) was used to regress otoliths predicted lengths, obtained from both EFA and wavelets approaches, against the observed sizes of each otolith in order to investigate the occurrence of allometry in this relationship. 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 otoliths a clustering procedure based on k-means was used to obtain the best number of k-clusters [10].

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. Test results in the EFA case show a percentage of correct classification of 97% while the second analy-

sis performed with WL obtained 83.7% of correct classification. RMSAC and RM-SECV are lower in the EFA model.

Length values predicted by PLS models were regressed on observed otolith length for both EFA and WL7 shape descriptions. PLS model generated values much efficiently related with measured lengths. In both cases a significant high correlation was found (puncorr.<0.01); EFA model showed an R2=0.98, while WL7 for R2=0.88.

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 [11] 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)[12].

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POTENTIAL APPLICATIONS OF AUTOMATED VIDEO-IMAGE ANALYSIS IN THE PELAGIC AND DEMERSAL ENVIRONMENT INCLUDING THE DEEP-SEA

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I. INTRODUCTION

The identification of species, the estimation of their biomasses and associated behavioural rhythms is acquiring increasing importance for fishery management and biodiversity estimation in deep-water continental margin areas and the deep-sea [1].

In the past two decades, the number of submarine video-stations has progressively along with socio-economic interest ocean exploration. In this context, expandable Submarine Stations at different depths such as JAMSTEC's Real-Time Deep-Sea Floor Permanent Observatory of Sagami Bay (1100 m) and SARTI-UPC's western Mediterranean OBSEA (20 m) were installed to measure several submarine parameters, including videos [2].

Accordingly, we have elaborated a novel morphometry-based protocol for automated video-image analysis of data from the JAMSTEC and UPC-SARTI cameras. Our approach accomplishes species identification with Fourier Descriptors and Standard K-Nearest Neighbours analyses on their outlines, and performs animal movement tracking (by frame subtraction), both in the demersal and in the pelagic realm.

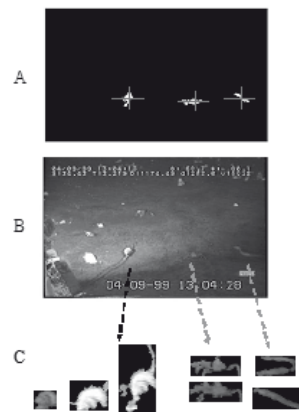
II. MATERIALS AND METHODS

For Sagami Bay we analysed one week of footage (09-04-2009 to 16-04-1999), from the infrared 3CCD video-camera.

For OBSEA, ten minutes of footage were obtained at midday (23-06-2009), from the OceanCam OPT-06 video camera.

Video-image analysis for both cameras followed the same procedure: A) selection of the frame to be analyzed; B) definition of a region of interest; C) identification of displacing objects in consecutive frames by Area thresholding (within the circle (which circle?)); D) grey-level scale thresholding; E) display of original greyscale image with object identified in overlay representation for comparison Figure 1 illustrates the identification of unknown biological objects by a trained operator in Sagami footage. Object selection (Figure 1A); class attribution (Figure 1B); the saving of newly classified objects as single images for their later individual processing by Fourier Descriptors analysis (Figure 1C).

(left) Fig. 1. The object selection by Expert Supervision



(below) Fig. 2. Automated counting of fishes in the OBSEA footage. Two consecutive frames at 30 s distance (A, B) are reported as well as a time series of individuals per unit of time as an example of biomass counting applications

