COLOUR CALIBRATION FOR QUANTITATIVE BIOLOGICAL ANALYSIS: A NOVEL AUTOMATED MULTIVARIATE APPROACH

Corrado Costa[1], Federico Pallottino[1], Claudio Angelini[2], Marco Proietti[2], Fabrizio Capoccioni[3], Jacopo Aguzzi[4], Francesca Antonucci[1], Paolo Menesatti[1]

(1) CRA-ING Agricultural Engineering Research Unit of the Agriculture Research Council,
(2) Dipartimento di Biologia Animale e dell'Uomo, Università "La Sapienza",(3) Laboratory of Experimental Ecology and Aquaculture, Dept. Biology, University of Rome "Tor Vergata", Via della Ricerca Scientifica, 00133, Rome, Italy
(4)Institut de Ciències del Mar (CSIC)

Abstract - This work proposed a novel approach to practical use of digital photography for biological purposes.

Keywords - Image color calibration, image analysis, color checker, multivariate analysis.

I. INTRODUCTION

In biology, as well as in agriculture, colour images are acquired and analysed for several different purposes e.g. studying the animal beahviour [1] [2], distinguishing among animal related groups [3] [4] and plant science [5]. The illuminant and therefore the light source present when acquiring an image is crucial in determining the quality of obtained images. Different light sources present different emission spectra dominated by diverse wavelengths that affect those reflected by the object under analysis. Thus, following Planck law, light sources can be classified on the base of their colour temperature. Therefore, the calibration of acquired pictures is compulsory in order to discriminate samples or recognise colour patterns.

In digital photography, with printing aims, a number of software are available for colour flow management from the acquisition to the print. Conversely, there are no practical standard methods of illumination and colour calibration to make pictures easily comparable for scientific purposes. In addition, the camera settings and its sensor's response to light, play a crucial role. Although some problems may occur when digital photography for objective colour quantification and pattern recognition is used inappropriately, the benefits it provides are several. Examples of advantages are: flexibility, low cost, accessibility, and the amount of information provided. Therefore this study aims to minimize the effects of illuminants and camera settings introducing a novel approach to colour image calibration based on automated multivariate analysis. This allows practical colour quantification in biological systems analysis.

II. MATERIALS AND METHODS

To diminish the colour variance among calibrated pictures the following camera characteristics and settings have to be adopted as previously reported [6]. The camera (Nikon Coolpix P600) provided high resolution (13.5 real MP) TIFF 8bit image (from RAW format) with good macro features and optical 4x NIKKOR lens. Manual white balance control, exposure and metering methods, were enabled. ISO sensibility was set to 100 to avoid noise appearance. The GretaMachbeth ColorChecker 24 colour-patch was used as reference standard while the relative software, ProfileMaker Pro 5.0 (PROM), was adopted as conventional calibration system.

MATLAB 7.1 R14 was used to perform the image calibration based on: i. second order polynomial interpolation (POLY2); ii. PLS (Partial Least Square) calibration. RGB declared values of the ColorCheker (24 patch) were used as y-block. The x-block was represented by the mean RGB value of the same 24 patch. Eight different light conditions were used to acquire pictures: 200 watt Tungsten bulbs; weakened Tungsten; flash; weakened flash; internal shadow; internal shadow slightly underexposed; external shadow; and finally, full sun (midday). These could represent unknown conditions of light colour temperature, thus the operativity of digital image acquisition.

The ColorCheker was displaced next to different biological samples on a black cardboard. For each condition, three consecutive images were acquired and the same 5 uniform Region Of Interest (ROI) belonging to biological objects (Fig. 2-A) were consequently extracted from each image. To quantify the efficiency of the different calibration systems (PLS, POLY2 and PROM) with respect to the original images (NONE) mean intra- and inter-euclidean distances were calculated. Intra-distances represent differences among mean ROI values of the same illumination condition (triple). Inter-distances represent differences among mean ROI values of different illumination condition.

The colour calibration based on the PLS model was then applied to 3 biological case studies, for which pictures were taken in non-standardized light conditions. In these case studies 2 kind of colour checker were used: GretaMachbeth ColorChecker 24 patch, IFRAO standard scale 7 patch. 1st case: colour pattern of the squat lobster Munida tenuimana (Crustacea: Decapoda) caught at different depths of Mediterranean continental margins (400-1500 m). 2nd case: dorsal colour pattern of Salamandra salamandra (Amphibia: Urodela) a quite widespread European species having several subspecies recognisable on this parameter. 3rd case: Anguilla anguilla (Teleostea: Anguillidae) lateral body colouration to observe contrast between dorsal and ventral skins along the lateral line as an evidence of the developmental transition between yellow and silver eel stage. One image per case study was calibrated.

III. RESULTS AND DISCUSSION

Table 1 shows as intra-distances among pictures taken in sequence at the same illuminant conditions are always lower then the inter-ones. Both the new approaches proposed (PLS and POLY2) allow achieving a better calibration with respect to the conventional software (PROM). The distances are lower were the colouration of the sample are more homogeneous (ROI1= Leave; ROI2=dark background).

Figure 1 presents the original and the PLS calibrated images of the three biological case studies.

Finally, the process was fast and comparable to other available methods, with potentially hundreds of images taken in a day, quickly calibrated with a custom platform such as MATLAB environment. Detailed and complex measurements of traits associated with colour can be undertaken rapidly, with measurements and calculations that would normally be painstakingly undertaken by hand, including morphometric measurements and shapes analysis, such as Fourier analysis [7].

ACKNOWLEDGEMENTS

This work was funded by the project High-Vision (DM 19177/7303/08) from the Italian Ministry of Agricultural, Food and Forestry Politics.

REFERENCES

[1] J.A. Endler, A predator's view of animal color patterns, 1978, Evol. Biol. 11:319–364.

[2] K. Summers, R. Symula, M. Clough, T.W. Cronin, Visual mate choice in poison frogs, 1999, P. Roy. Soc. Lond. B Bio. 266:2141-2145.

[3] H.C. Schaefer, M. Vences, M. Veith, Molecular phylogeny of Malagasy poison frogs, genus Mantella (Anura: Mantellidae): homoplastic evolution of colour pattern in aposematic amphibians, 2002 Org. Divers. Evol 2:97-105.

[4] C. Costa, C. Angelini, M. Scardi, P. Menesatti, C. Utzeri, Using image analysis on the ventral colour pattern in Salamandrina perspicillata (Savi, 1821) (Amphibia, Salamandridae) to discriminate among populations, 2009, Biol. J. Linn. Soc. 96:35-43.

[5] D.H. Voss, Relating colorimeter measurement of plant color to the Royal Horticultural Society Colour Chart, 1992, HortScience 27(12):1256-1260.

[6] M. Stevens, C.A. Pàrraga, I.C. Cuthill, J.C. Partridge, T.S. Troscianko, Using digital photography to study animal coloration, 2007, Biol. J. Linn. Soc. 90:211–237.

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

	Mean Intra-Distance				Mean Inter-Distance			
	NONE	PLS	POLY2	PROM	NONE	PLS	POLY2	PROM
ROI 1	2.7	1.2	1.1	2.2	24.5	6.6	4.6	17.3
ROI 2	11.0	8.3	8.3	9.9	38.6	14.8	15.0	28.7
ROI 3	11.3	6.6	6.7	8.8	43.1	14.9	15.1	27.4
ROI 4	11.9	8.5	8.0	9.2	40.9	20.4	19.2	23.9
ROI 5	7.6	3.8	3.4	6.5	25.4	8.9	7.4	19.7

Tab. 1. Mean intra- inter-Euclidean distances among RGB mean ROI values; the scale reported is 0-255.

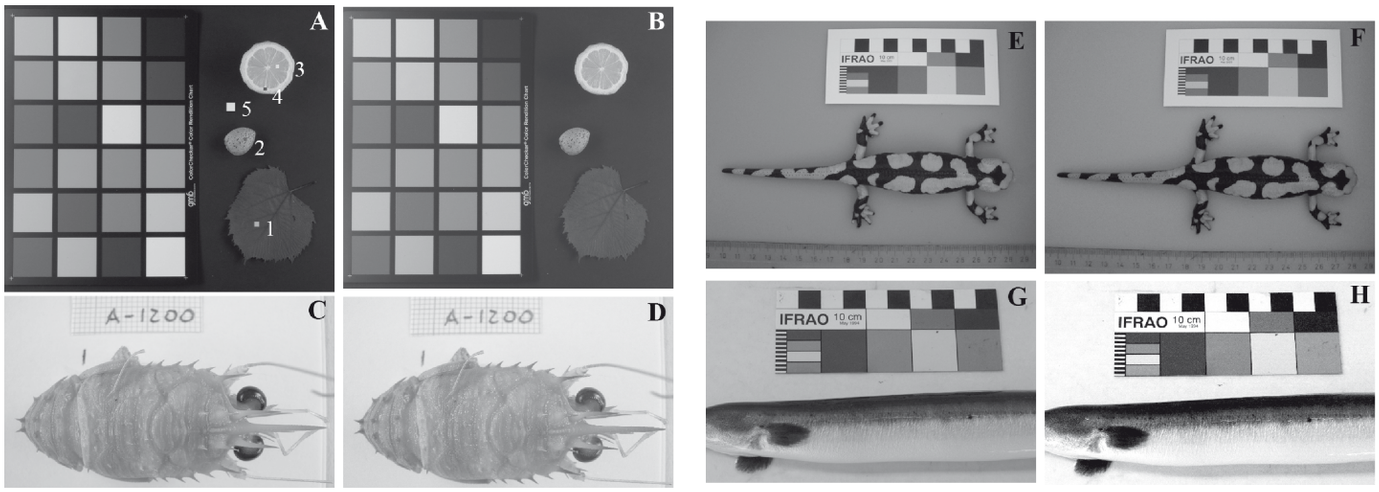

Fig. 1. A. Original image used to build the models (coloured squares represents the object ROIs reported in Table 1: 1. red ROI on a leave, 2. blue ROI on an almond, 3. green ROI on the lemon segment, 4. brown ROI on the lemon flavedo, 5. pale gray ROI on the dark background). B. Original image used 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

F. Capoccioni[1], C. Costa[2], P. Menesatti[2], A. Lombarte[3], J. Aguzzi[3], E. Ciccotti[1]
(1) University of Rome Tor Vergata, Dept. Biology, e-mail: f.capoccioni@fastwebnet.it
(2) CRA-ING Agricultural Engineering Research Unit of the Agriculture Research Council
(3) Institut de Ciències del Mar (CNIMA-CSIC)

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 catadromouse 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 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. Image processing for automatic extraction of otholits outline was performed by the image analysis software Age&Shape (Ifaimon); 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 [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 otholits 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-