Application of geometric-morphometric, hyperspectral imaging and molecular markers to the study of depth-driven differences in populations of Decapods (Crustacea)

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Keywords: Image colour calibration, image analysis, color checker, multivariate analysis, mtDNA sequencing.

Introduction

The levels of environmental light experienced by animals during their phases of behavioural activity determine the type of experienced interspecific interactions [1]. The form and colour of an organism constrains its use of ecosystem resources. At the same time, resource accessibility contributes to the construction of its form. That process occurs *via* evolution through the confrontation of individuals with important ecological tasks such as feeding, mating, displacement, and predatory evasion [2].

The squat lobster, *Munida tenuimana*, is an ecologically key crustacean decapod of the Mediterranean slope [3]. Autoecological traits in relation to behaviour and population distributions are poorly understood. A curious depth-related variation in size has been reported [4]; smaller individuals are located at 900 m, while larger individuals occur both above (400-600 m) and below (1000-1500 m) that depth. Curiously, 900-1000 m depth corresponds to the lower border of the twilight zone in the Mediterranean Sea [5].

In this work, we propose the use of geometric morphometry and hyperspectral imaging applications to ask whether distribution of sizes above and below the twilight zone is significantly associated to variation in a suite of selected morphological characters and-or colour pattern. We also coupled the morphological surveys with the analysis of sequence variation in a fragment of the mitochondrial DNA (mtDNA) region encoding for the subunit I of NADH dehydrogenase gene (ND1) to test for any potential bathymetric subdivision in the population structuring.

Materials and methods

During the PROMETEO field surveys onboard of the R/V "*García del Cid*" trawl sampling was carried out at different depths. Sample sizes (N) varied with local population abundances and were the following: >700 m, N=62; 900-1050 m, N=11; 1200 m, N=24; 1350 m, N=72; 1500 m, N=60.

All animals were photographed with a Nikon Coolpix P600 providing high resolution (13.5 real MP) TIFF 8bit image (from RAW format). Manual white balance control, exposure and metering methods, were enabled. ISO sensibility was set to 100 to avoid noise appearance. The GretaMachbeth ColorChecker 24 patch was used as reference standard. MATLAB 7.1 R14 was used to perform an image calibration based on

PLS (Partial Least Square) supervised multivariate modelling [6].

Future studies (results not yet available) will focus on:

-Shape analysis through geometric morphometric survey on 35 carapace landmarks

-Colour pattern warping on photographs

Colorimetric and spectral data were acquired through an optical system able to capture the image over a wide wavelength range (i.e. 400-975 nm) and returning data with 5 nm step, following the CIE L*a*b* colorimetric standards and spectral values. The spectral system was made with 4 components: a sample transportation plate (Spectral Scanner DV, Padova, Italy); a collimated illumination device (Fiber-lite) made by a 150 W halogen lamp (the light source); one illumination opening in optical fibre of 200 mm long and 2 mm width, using the standard illumination-optical device geometry $\beta_{45/0}$ in relation to the transportation plate (i.e. bearing the sample) and presenting a minimum light divergence; an imaging spectrograph (ImSpec V10-Specim Ltd., Oulu, Finland) coupled with a standard C-mount zoom lens and a Teli CCD monochrome camera.

Hyperspectral imaging characterization [7] of colorimetric and spectral data on a Region Of Interest (ROI) of animals from different depth groups was carried out by selecting a posterior part of the carapace. Animals were grouped with Partial Least Squares Discriminant Analysis (PLSDA; [8]) (Fig.1). Prior PLSDA analysis, the dataset was pre-processed with the 'mean centre' algorithm and divided into 75% to build the model (calibrated and validated) and 25% for the independent test set.

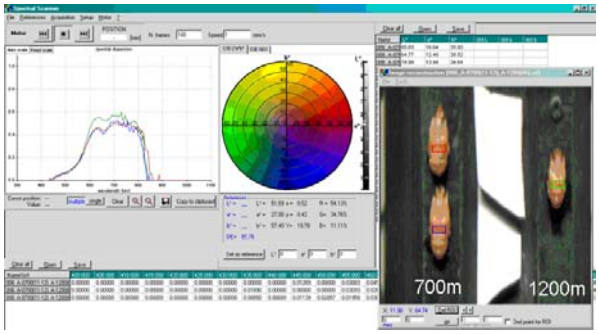

Fig. 1: Software output of the spectral and colour analysis *via* hyperspectral imaging of the ROI in *Munida*.

A 315 base pair (bp) fragment of the mtDNA ND1 gene was PCR amplified and sequenced in a subset of 96 individuals (400m N=5; 700m N=17; 900m N=6; 1050m N=6; 1200m N=19; 1350m N=19; 1500m N=20). Finally, four individuals from a far away location (Gulf of Alicante) were sequenced to test for levels of genetic variation at increasing geographical scale. SAMOVA [9] was used to test for population structuring without any *a priori* grouping of samples.

Results and Discussion

Hyperspectral imaging results on colorimetric and spectral data are reported in Tab. 1 It is possible to observe that, with respect to the probability of random assignment of an individual into a depth unit (20%), the percentage of correct classification of the independent test set, was very high for Spectral data (83.64%) and for Colour data (62.45%).

	Colour	*Spectra*
N	232	232
n° LV	2	16
% Cumulated Variance X-block	91.81	99.98
Mean Specificity (%)	62.26	90.64
Mean Sensitivity (%)	76.26	93.32
Mean RMSEC	0.41	0.33
Random Probability (%)	20	20
Mean % Corr. Class. Model	49.42	87.28
Mean % Corr. Class. Test	62.45	83.64

Tab. 2: Characteristics and principal results of the PLSDA models performed on Colour and Spectra amplings carried out at 5 depths (Y.block). N is the number of samples. n° LV is the number of latent vectors for each model. Random Probability (%) is the probability of random assignment of an individual into a depth unit.

Moreover the colour data (expressed in the CIE L*a*b* values) showed significant differences between <900 m / 900-1050 m / and >1100 m, meanwhile the three depth units 1200/1350/1500 m appeared as non-significantly different.

MtDNA data revealed eight unique haplotypes largely shared across sampled locations. Overall level of genetic divergence was low (F_{ST}= -0.05; P n.s.) suggesting extensive gene flow. However, SAMOVA showed that the most likely population structure was that with samples grouped according to the depth of origin (F_{CT} = 0.152; P < 0.05).

In this study, we showed how the combination of novel and diverse technological

tools could be efficiently used to approach problems such as behaviour and structuring of populations subjected to decreasing levels of environmental light.

Conclusions

The problem of colouration in marine invertebrates has been mostly studied in pelagic species leaving this field poorly explored for demersal species [10]. The successful application of hyperspectral imaging techniques to the study of emitted colouration and spot patterning in decapods will contribute to the understanding of constrains to their population distributions in continental margins in relation to light availability. The joint analysis of selectively neutral molecular markers will help in understanding the relative importance of population stochastic processes (i.e. larvae dispersal) over local adaptive phenomena.

Acknowledgements

This work was funded by the project PROMETEO (CICYT; CTM 2007-66316-C02-02/MAR) and by the project High-Vision (DM 19177/7303/08) from the Italian Ministry of Agricultural, Food and Forestry Politics.

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