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# Differentiating Morphology of *Scylla serrata* Molt Stages Using Image Analysis

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**Abstract:** Segregation of mud crabs by molting stage before stocking in ponds will reduce cannibalism. A wide yellowish band along the dactyl in the swimming leg recedes as molting progresses from pre-, inter- to post-molt stage therefore banding may be used to classify molt stage by eye. However, this process may be subjective. In this study, single pixel and whole image protocols to evaluate color patterns in crabs at different molt stages were tested for their usefulness as an image analysis tool to standardize molt stage classification. Thirty-four juvenile mud crab dactyls were photographed. Red, Green and Blue (RGB) values were obtained from twenty equidistant pixels along the dactyl edge of each crab using GNU Image Manipulation Program (GIMP) version 2.8. ImageJ for 64-bit Windows version 1.8 was used to determine the mean RGB color profile. Color profiles were analyzed using one-way ANOVA and Principal Component Analysis (PCA). No visible clusters were observed in the PCA plot. ANOVA results show no significant difference in the RGB values. The single pixel and whole image method were both unable to differentiate between molt stages. Alternative approaches and programs in the image analysis protocols are needed to develop a standardized method of molt stage detection of mud crabs using image analysis.

**Key Words:** *Scylla serrata, image analysis, single pixel, whole image*

## 1. INTRODUCTION

Crab aquaculture is a high-value industry due to the large demand of the mud crab *Scylla serrata* (Keenan et al, 1998). *S. serrata*, can be found in the Indo-Pacific region, residing in littoral zones of mud flats of mangrove forests (Keenan and Blackshaw, 1998). Segregation to molt stage of crabs may reduce the losses due to asynchronous molting in ponds since newly-molted mud crabs are vulnerable to cannibalism (Quinitio et al, 2010). Currently, molting can be determined by eye through examination of a yellowish band along the edge of the dactyl in the swimming leg. This recedes as the molting progresses to pre-, inter- and post-molt stages (Quinitio et al, 2015).

Image processing has been used for

identifying physiological and developmental stages of cell cultures (Carpenter et al, 2006), plants (Florindo et al, 2016), fishes (Li and Hong, 2014), food quality assessment (Du and Sun, 2004) and remote sensing applications (Blaschke, 2010). The two programs used, GNU Image Manipulation Program (GIMP) and ImageJ among other available programs, were chosen due to previous experience and its ease of use. GIMP is a freeware that specializes in image retouching, composition and authoring (Jensen, 1986). This was used to look into multiple single pixel red, green, blue (RGB) values in one image in this study. ImageJ (GNU Image Manipulation Development Team, n.d.) is a specialized program developed by the National Institute of Health (NIH) that is used for biological studies focused on imaging such as in cell studies, microstructural analysis and immunostaining (Schneider et al, 2012). This study used a Color Profile plugin that gave the mean RGB value of each image.

In this study, two image analysis approaches were explored to standardize molt staging of juvenile mud crabs. Inconclusive results will allow for more exploratory studies using more advanced software.

## 2. METHODOLOGY

### *2.1. Sample Collection and Molt Staging*

Thirty-four juvenile mud crabs of about 2 – 3 cm in size were provided by CDO Foodsphere, Inc. from Iba, Zambales. The surface of the dactyl from each crab was examined using a Nikon SMZ800 stereoscopic microscope with Nikon DS-Fi1 camera head. The dactyl was laid flat on the stage plate with lens 22 cm directly above the sample with a light source above and below the stage. The light attached to the microscope had no changeable setting. 10x magnification was set with the region of interest was adjusted to setting #2. Classification according to molt stage was done following the guidelines of Joana Huervana during sample collection (Joana Huervana, personal communication, April 29, 2016). Soft-shelled crabs were immediately assigned as post-molt. Pre-molt crabs have a thick band, and banding recedes as the stages progress from pre- to inter-and post-molt (Fig. 1). One picture per crab dactyl was used for image analysis.

### *2.2. Image Processing*

Pictures of crab dactyl per molt stage were edited using GIMP. The images were cleaned up by improving the brightness and contrast using GIMP. The background was also removed by cropping the dactyl portion, placing it on a white background.

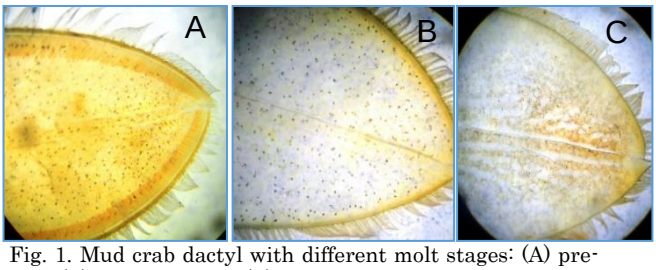

Fig. 1. Mud crab dactyl with different molt stages: (A) pre-molt, (B) inter-molt and (C) post-molt.

#### *2.2.1. Single Pixel Color Profiling*

GIMP version 2.8 was used to quantify RGB values of twenty equidistant pixels, ten along the top and ten along the bottom dactyl edge. Values from 680 points from the 34 dactyls were used for statistical analysis.

#### *2.2.2. Whole Dactyl Image Color Profiling*

The Color Profiler plugin developed by Prodanov (Prodanov, 2004) for ImageJ version 1.8 (for 64-bit Windows) was used to obtain the mean RGB values for the whole image from the 34 dactyls. These values were obtained from all pixels on the cropped dactyl image.

### *2.3. Statistical Analysis*

The values from both GIMP and ImageJ methods were analyzed using Principal Component Analysis and One-Way ANOVA ($p<0.01$) implemented in Dell™ STATISTICA™.

## 3. RESULTS AND DISCUSSION

Results from the single pixel method show strong overlaps. The approach show no variation in image values among the 680 points obtained from all dactyls (Fig.2).

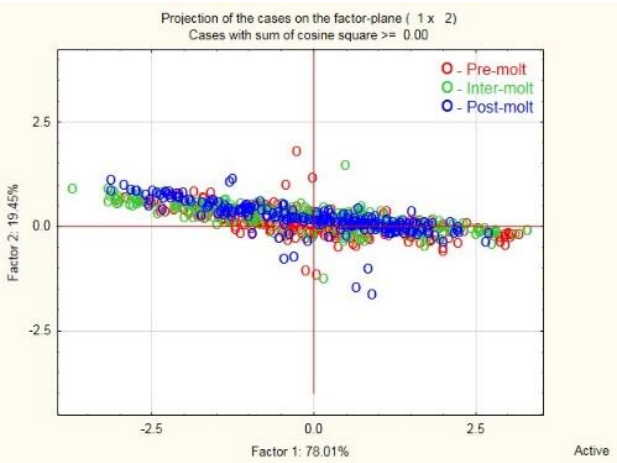


Fig. 2. PCA of RGB values from the single pixel method per crab molt stage.

Seventy-eight percent of variation was explained by two factors. No clean clusters were identified in the PCA. One-way ANOVA (Fig. 3) also showed no significant difference among the RGB values in the three molt stages.

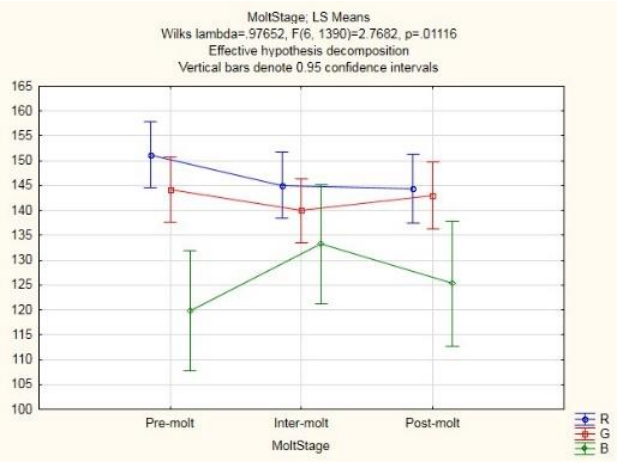


Fig. 3. Graph of mean RGB values from the single pixel method per molt stage using one-way ANOVA.

Similar results to that obtained using the single pixel method was observed in the whole dactyl image method for the PCA (Fig. 4). There were less points as the whole image was used for the RGB values. One-way ANOVA (Fig. 5) of the ImageJ method also showed no significant difference among the RGB values.

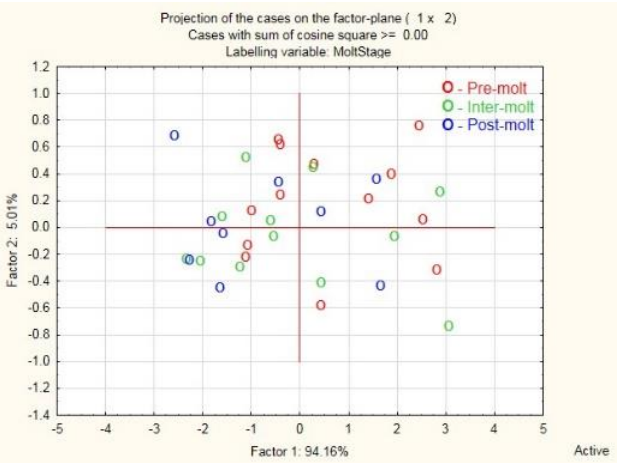


Fig. 4. PCA of RGB values from the whole dactyl image method per crab molt stage.

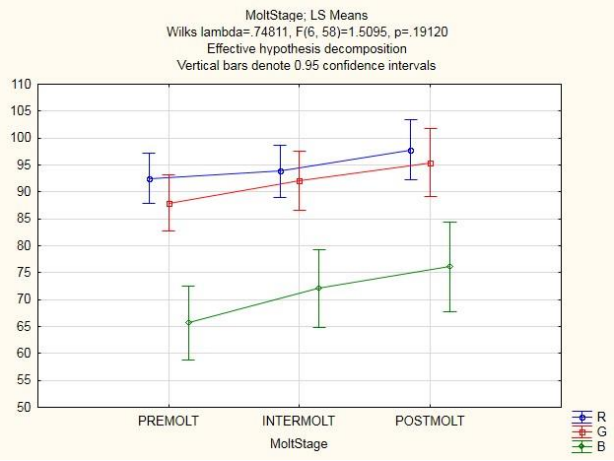


Fig. 5. Graph of mean RGB values from the whole dactyl image method per molt stage using one-way ANOVA.

Both approaches were unable to differentiate molt stages based on the obtained RGB profiles. To proceed with this, a more advanced method between individual pixel and whole area analyses focusing on depth and width of the dactyl band will be used rather than color. Better image processing software and methodology is needed to improve on obtaining distinctive image values to establish molt stages. Other computer programs and customized image analysis will be done.

## 4. CONCLUSIONS

The whole image and single pixel approaches were not effective. Both methods showed no clustering and no significant difference between the molt stages. Improvement of the image analysis methods is needed to detect distinctive image values and establish uniqueness of molt stages. More features may be examined in order to have a more definitive diagnostic technique for identification of molt stages in crabs. Looking into a more specific region of interest will be done in the succeeding experiments. Molecular methods such as qPCR may be employed rather than segregating mud crabs by eye.

## 5. ACKNOWLEDGMENTS

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