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Comparison of Protein Concentration among Various Organs of the Mud Crab *Scylla serrata* Sex Phenotypes

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Abstract: Mud crabs are priced in the market depending on their sex and weight. Aside from male and female, an intermediate sex phenotype exists in *Scylla serrata* species. This sex phenotype has a high market value due to their size and right combination of fat and lean meat. The literature claims that intermediate sex phenotype is either a feminized male or a late-maturing female. This study aims to establish general physiological affinity of intermediate sex phenotype to either male or female. Comparison of protein concentrations among non-reproductive tissues of male, female and intermediate *Scylla serrata* will give an indication of the possible physiological processes to which each sex phenotypes are similar. Hepatopancreas, gill and muscle tissues were dissected, flash-frozen with liquid nitrogen and homogenized using a motor driven tissue-tearer. A total of 36 fractions were quantified for protein content following a standard Bradford assay. Protein concentrations across tissues were more similar between male and intermediate sex phenotypes with a mean protein concentration of 120.98mg/ml and 130mg/ml respectively, while the female sex phenotype has a more variable protein concentration across tissues with a mean protein concentration of 173.82mg/ml. Results from the principal component analysis showed high similarity of protein content in gills across all the sex phenotypes. Muscle protein concentrations were more similar between the female and the intermediate and hepatopancreas protein concentration is more similar between the male and the intermediate. Results of this study serve as a baseline for further protein and proteomic studies as well as gene expression experiments involving *Scylla serrata* sex phenotypes.

Key words: Mud crab; *Scylla serrata*; sex phenotypes; protein



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1. INTRODUCTION

Mud crab *Scylla serrata* is regarded as an important commodity in the Asia Pacific region (Pavasovic et al., 2004) and a major source of income for local fishermen and crab farmers (Islam et al., 2010). Market value of mud crabs *Scylla serrata* depends on their sex phenotype and weight. Aside from female and male, mud crabs also have an intermediate sex phenotype, locally known as “baklang alimango”. The intermediate exhibits a mix of the female and male morphological characteristics. Both female and intermediate are preferred due to their bigger size and presence of roe. There are a few theories to explain the occurrence of the intermediate sex phenotype in *Scylla serrata* species. The Philippine Bureau of Fisheries and Aquatic Resources (BFAR) states that this sex phenotype is a late-maturing female mud crab. Possible explanation for this is the decline in the activity of Y-organ affects the timing of sexual maturity due to inhibited vitellin synthesis (Jivoff et al., 2007; Meusy, 1980). On the other hand, Kristensen et al. (2012) claims that male crabs exhibiting female characteristics are morphologically feminized by parasitic barnacles *Sacculina carcini*.

Proteins are biomolecules which play a major role in the dynamics and structure of organisms (Murray et al., 2012). Changes in the protein concentration indicate modification in the physiology of an organism (Bond et al., n.d.).

The protein levels present in the hepatopancreas, gill and muscle tissue of male, female and intermediate *Scylla serrata* will help determine physiological affiliation of intermediate to either female or male.

Results of this study will be useful for further protein and proteomic studies of *Scylla serrata* sex phenotypes. It will also help in refining analysis of voluminous transcriptome and genomic data when comparing sex phenotypes of *Scylla serrata* for studies on natural adaptation, temperature stress and possible biomarkers for breeding which are ongoing at the Practical Genomics Laboratory.

2. METHODOLOGY

Sample Preparation

Hepatopancreas, gill and muscle tissue were collected from female, male and intermediate mud crab. Body weight of the crabs used ranges from 100-200 grams; while carapace width ranges from 80-120 millimeters.

Protein extraction and quantification

Cell fractions were obtained on each tissue following the procedure in the Laboratory Manual of Basic Protocols in Cell and Molecular Biology by Glenn Oyong (2012). A total of 36 cell fractions (crude, nuclei, soluble, microsome) were processed. Protein concentration of each fraction from different organs and sex phenotypes were measured using the Bradford's Assay.

Data analyses

Principal Component Analysis was done to visualize the data structure which will help determine which among the *Scylla serrata* sex phenotypes are more similar in terms of protein concentration.

3. RESULTS AND DISCUSSION

Protein concentrations between male and intermediate sex phenotypes were more similar across all tissues with a mean protein concentration of 120.98mg/ml and 130mg/ml respectively, while the female sex phenotype has a more variable protein concentration across tissues with a mean protein concentration of 173.82mg/ml.

Whole tissue Protein Concentration

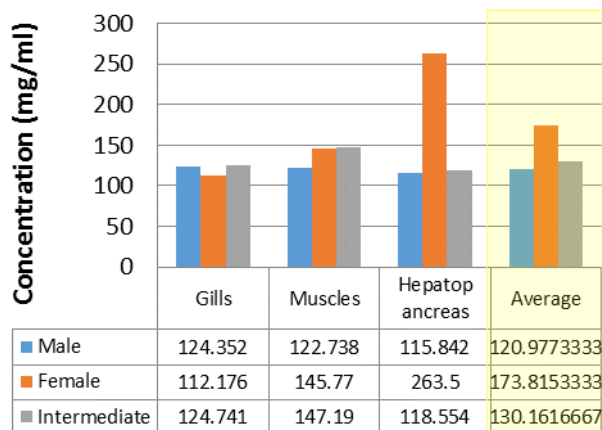


Fig. 1. Protein concentration of gill, muscle and hepatopancreas from male, female and intermediate mud crab.

Results from the principal component analysis showed that protein concentration from gills of all sex phenotypes grouped together. This indicates gill proteins from different fractions are more similar across all sex phenotype compared to muscle and hepatopancreas tissues. Protein concentrations from muscle tissues were more similar between females and intermediates. Male and intermediate are more similar in terms of hepatopancreas protein concentration.

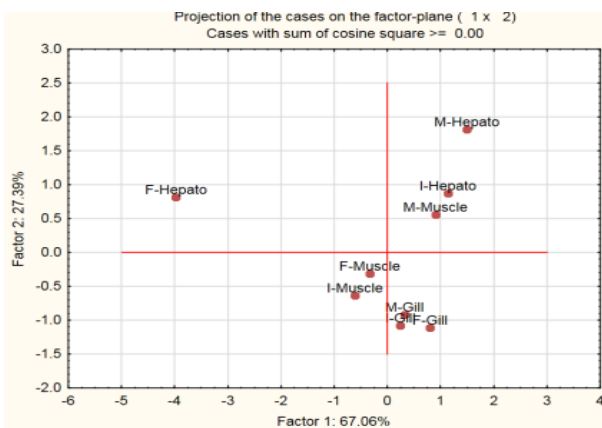


Fig. 2. Principal component analysis of the protein concentration from gills, muscles and hepatopancreas of male, female and intermediate mud crabs.

Results from the muscle and hepatopancreas tissues are important for biomarker identification among the sex phenotypes. DNA regions associated with proteins of muscle and hepatopancreas of *Scylla serrata* will be a good source for mining biomarkers that will distinguish each sex phenotype from one another. This is useful for current efforts involving breeding of the intermediate mud crab and its characters. Gill tissues, on the other hand, is an ideal tissue sample to use for studies involving natural adaptation to stress in which sex phenotype is not a major consideration.

4. CONCLUSIONS

This study established that protein concentration of hepatopancreas and muscle of *Scylla serrata* vary depending on sex phenotype, and protein concentration of gills is similar across all sex phenotypes. These observations can serve as baseline information for further protein and proteomic studies involving *Scylla serrata* sex phenotypes. This may also be useful for applications in biomarker identification and gene expression study of *Scylla serrata*. It is recommended to do protein profile of the various organs using a larger sample size of *Scylla serrata* through SDS-PAGE and identify specific proteins using a more advanced protein analysis technology. Another recommendation is to confirm if the physiological similarity and differences among sex phenotypes observed in this experiment is the same for different sites, as well as different sizes and species of *Scylla*.

5. ACKNOWLEDGMENTS

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