Presented at the DLSU Research Congress 2017
De La Salle University, Manila, Philippines
June 20 to 22, 2017

Expressions of Molt-Inhibiting Hormone (MIH) and Extracellular Signal-regulated Kinase (ERK) during the molting stages of the giant mud crab, *Scylla serrata*

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Abstract: Survival and growth of mud crabs and any other crustaceans depend on molting. Molting is influenced by several hormones, two of which are Molt-Inhibiting Hormone {MIH} and a molt promoting hormone, the Extracellular Signal-regulated Kinase (ERK). Controlling and synchronizing the molting of the crabs would be a big boost to the mud crab industry, especially in the production of soft shell crabs. The best way to control molting would be to stock juvenile crabs of the same molting stage together. In this study, hormone expressions were analyzed using quantitative Real-Time Polymerase Chain Reaction (1RT-PCR). Molt stages of 36 juvenile *S. serrata* were established based on MIH and ERK expression levels that was analyzed using Principal Component Analysis (PCA). One group of six individuals had MIH value equal to zero and presumed to be in the PR stage. The rest of the crabs had values for MIH and were presumed to be in the PM-IM stage. The value of ERK for the PM-IM stage was higher at 24.79 than in the PR stage at 21.67. Based on the data gathered, morphological markers may now be identified to standardize hormone expression. This would be the first study concerning the comparison of hormones during molting.

Key Words: soft-shell; molting; hormone

1. INTRODUCTION

Over the past decade, the Philippines has constantly increased its production of mud crabs. Through the years, its demand in the market has increased, especially the soft-shelled mud crabs. These crabs have undergone recent molting activity, which discards their hard shells and leaves their body unprotected and vulnerable. These type of crabs

command a higher economic value than regular crabs.

All crustaceans undergo what is known as molting. It is a process by which they shed their old shells to be replaced by new ones. This allows them to grow in size and weight. One disadvantage of molting, however, is it leaves them vulnerable to predators, even to others of the same species. Cannibalism is a common occurrence during this stage. To prevent cannibalism and ensure higher survival, it would be advisable to stock juvenile crabs at the same molting stage in the same ponds. However, there is no current practice of this method in the field.

Molting is governed by molt inhibiting and promoting hormones. Molt-inhibiting hormones and the presence of Extracellular Signal-regulated Kinase act as a balance of the molting cycle of crustaceans. If MIH is activated, the ERK level is decreased and vice versa. There are three stages of molting: post-molt (PM), inter-molt (IM), and pre-molt (PR). MIH is one of the hormones responsible for slowing down and regulating the crab's ability to molt. This hormone is commonly found in the eyestalks. ERK, on the other hand, is activated once MIH levels are reduced. ERK is one of the starting point for crustaceans to undergo the molting process.

Identification of molt stages can be done visually using a non-evasive method by using the dactyl of the swimming legs of crabs. This method was suggested by Joana Huervana of SEAFDEC-AQD. However, this could be subjective.

This study is an attempt to standardize the molt stages by grouping images of the dactyls at known molting stages based on the MIH and ERK hormone.

2. METHODOLOGY

A. Animals

A total of 36 juvenile *Scylla serrata* were obtained from the province of Iba, Zambales, Philippines. Tissues including the gills and eyestalks were extracted from each crabs. These were submerged in RNAlater and stored on -80°C until RNA extraction.

B. RNA Extraction and quantitative Real-Time PCR

Tissues stored at -80°C were used for RNA extraction using TRIzol reagent following the manufacturer's protocol. The quality and concentration of RNA was measured through spectrophotometry at an absorbance of 260 nm.

KAPASYBR FAST One-Step qRT-PCR kits were used for this experiment according to its protocol. Primers and qRT-PCR settings for both MIH and ERK were synthesized and derived using Huang's and Ma's protocols respectively [2,3].

C. Statistical Analysis

Principal Component Analysis (PCA) was performed through STATISTICA using the data gathered from qRT-PCR.

D. Classification of Molt Stages

Molt stages were presumptively classified based on the clusters formed in the PCA.

3. RESULTS AND DISCUSSION

Based on the PCA generated, two clusters were formed. Cluster A was presumed to be the crabs in the PR stage while cluster B was presumed to be in the PM-IM stage (Fig. 1).

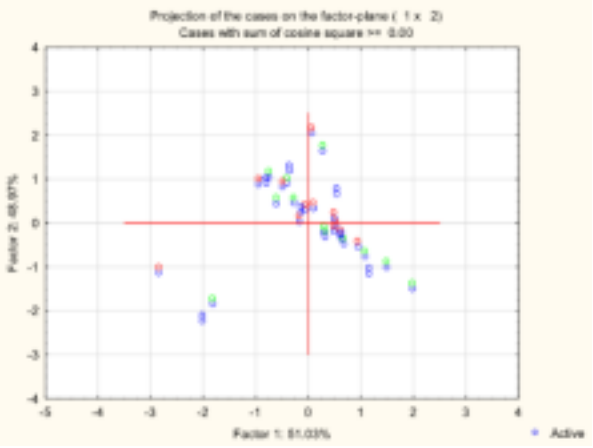

Fig. 1. PCA showing two clusters: A) PR and B) PM/IM.

Cluster A had six individuals with MIH levels equal to zero. These crabs are presumed to be in the PR stage. During the PR stage, molt inhibiting hormones tend to decrease as preparation for the removal of its shell. The rest of the individuals, Cluster B, was presumed to belong to the PM-IM stage because of their values for MIH

During the stages of PM and IM, the molt inhibiting hormone increased, which effectively stops the crabs from further molting. In order for the crabs to produce hard bodies shells, it needs to a higher level of MIH than ERK during the PM and IM stage (Table 1). This transition of MIH from higher to lower value can be seen during PM-IM to PR stage [2].

Table 1. Average and ratio of MIH and ERK of each molt stage.

Stage	# of crabs	MIH	ERK	MIH/ERK
PM-IM	30	25.1	24.79	1.01
PR	6	0	21.67	0

There are a lot of hormones that are active during molting. Molt promoting, such as ERK, is considered to help crabs molt. Although ERK aids during molting, it may be expressed in all three stages. A crab that is about to molt or is in the PR stage should contain a higher concentration of ERK [3]. The values gathered, however, were not what was expected as ERK has higher level in PM-IM stage than in the PR stage.

Fig. 2. Dactyls of mud crab showing the molt stage. Left: PM-IM stage; Right - IM-PR stage.

Visualization of the dactyl was observed under a microscope (Fig. 2). This method of classification relies on the intensity of the yellow coloration lining the dactyl. Staging also depends on the black dots and its distance from the lining of the dactyl. There are no known studies regarding the classification of molt stages

through this method. Based on the results gathered, morphological markers may be identified to standardize the hormone expression.

4. CONCLUSIONS

Based on the gathered data, six individuals were presumed to be in the PR stage given that their MIH value was equal to zero. The other individuals were presumed to be in the PM-IM stage due to their value of MIH. ERK was higher in the PM-IM stage compared to the PR stage, which was not what was expected. Other hormones such as ERK may be used as a comparison with MIH to further confirm the role of MIH in molt stages. Morphological markers may now be determined to standardize hormone expression. An experiment with larger sample population is now being tested to further refine the current results.

5. ACKNOWLEDGMENTS

This research was supported by the Department of Science and Technology - Philippine Council for Agriculture, Aquatic, and Natural Resources Research and Development and the Southeast Asian Fisheries Development Center - Aquaculture Department, along with CDO Foodsphere, Inc. for providing the mud crabs.

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