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Diet and internal physiological changes of female orange mud crabs, *Scylla olivacea* (Herbst, 1796) in different ovarian maturation stages

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ABSTRACT

Recently, there has been a growing interest in the ovarian maturation of mud crabs, genus *Scylla*. Studies regarding the factors that affect ovarian maturation in mud crabs, however, are still lacking. This study, therefore, evaluates the relationship between diet and internal physiological changes of female orange mud crabs, *Scylla olivacea*. Sixty female adult *S. olivacea* were sampled from Setiu Wetland, Malaysia. Foreguts were sampled to study fullness and content. The hepatopancreas was sampled to study digestive enzyme activity, biochemical composition, and histology. Ovaries were sampled to study ovarian biochemical composition and histology. Foregut fullness data suggest that there is an increase in feeding with advancing ovarian maturation in mud crabs. Data for foregut contents indicated that when the ovary was in Stage 3 of maturation the diet was animal-based. The activity of amylase, cellulase, lipase, and trypsin during Stages 1 and 4 of ovarian maturation were less than during Stage 3 of ovarian maturation. Biochemical composition (protein and lipid) was greater during Stage 3 of ovarian maturation. Histological analysis of the hepatopancreas indicated an increase in hepatopancreas tubules, B and R cells during Stage 3 as compared with Stage 1 of ovarian maturation. Histological analysis of the ovary indicated increases in oocyte diameter due to concentrated large yolk globules. Based on these results, it is concluded that vitellogenesis mainly occurred during Stage 3 of ovarian maturation, based on evidence of increased feeding with the diet being predominantly animal-based. Likewise, digestive enzyme activities, proteins, lipids, B cells, and R cells were all greater during Stage 3 of ovarian maturation.

1. Introduction

Mud crabs in the genus *Scylla* are an economically important species, and are widely cultured in many countries for meat quality and desirable flavor (Holme et al., 2009; Azra and Ikhwanuddin, 2016; Zeng et al., 2016; Fazhan et al., 2017a). Increasing interest in the culturing of Portunid crabs has led to the over-harvesting of stocks from the wild (Fazhan et al., 2017b; Azra et al., 2018). Thus, the development of female broodstock is important to increase the quantity of breeding females in hatcheries, and to improve the larval production in crab hatcheries (Wu et al., 2010; Chang et al., 2017; Waiho et al., 2018). The study of stages of ovarian maturation could provide baseline information for further reproductive biology studies on mud crabs (Quinitio et al., 2007).

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Recently, there has been a growing interest in the biological factors related to mud crab reproductive biology, and considerable attention has been given to factors such as natural diet, digestive enzymes, biochemical compositions, and enzyme activities in the hepatopancreas, which are closely inter-related, and important for producing the egg yolk.

In general, the natural diet of mud crabs can be divided into four basic components, including mollusks, crustaceans, fish, and plant material (Mohapatra et al., 2005; La Sara et al., 2007; Funde et al., 2009; Nayak et al., 2014). These natural diets are important for ovarian maturation, because the diet contains carbohydrates, proteins, lipids, and carotenoids, which are metabolized by mud crabs into egg yolk constituents. A previous study also indicated that these nutrients were stored in the hepatopancreas for a period of time prior to being utilized by the ovary for egg yolk development (Ravichandran et al., 2009). These nutrients also have a role in the reproduction of decapod crustaceans (Franceschini-Vicentini et al., 2009), based on evidence of lipid and protein mobilization from the hepatopancreas and transfer to the ovary (Fatima et al., 2013). A single egg yolk precursor (vitellogenin) has been reported to be contained in the hepatopancreas before being transported into the ovary as vittelin (Wilder et al., 2010).

Digestive enzymes are protein molecules that catabolize polymeric macromolecules into smaller components that are subsequently metabolized (Rothman et al., 2002). Carbohydrate sources such as starch, cellulose, and chitin are digested as a result of α-amylase, cellulase, and chitinase actions (Hutcheson et al., 2011). Organic compounds such as lipids and proteins are digested by lipase and trypsin (Kuster et al., 2001; Lason and Ogonowski, 2010). An increase in feeding to obtain more nutrients during vitellogenesis causes a simultaneous increase in the secretion of digestive enzymes by the hepatopancreas.

When these factors are considered, two priority areas were identified and the objectives in the study were developed. These were 1) to relate the natural diet composition based on contents of the foregut to the digestive enzymes in the hepatopancreas, and biochemical compositions of both the ovary and the hepatopancreas during different stages of ovarian maturation in mud crabs, and 2) to describe the histological changes of the hepatopancreas at different stages of ovarian maturation.

2. Materials and methods

2.1. Specimen collection

Sampling was conducted from September 2014 to February 2016 (18 months) in the Setiu Wetlands, in Terengganu Coastal waters, Malaysia. The goal was to sample as many wild female mud crabs as possible, without consideration of season and specific habitat. The mud crabs were sampled by using traps baited with fish. Sampling focused on mature female *S. olivacea* with a carapace width of greater than 8.0 cm (Waiho et al., 2016). Mature females were identified using the characteristics described by Ikhwanuddin et al. (2011). The crabs were dissected to classify stages of ovarian maturation. Four stages of ovarian maturation were categorized based on descriptions from a previous study of Ghazali et al. (2017). The stages of ovarian maturation of *S. olivacea* in the present study were categorized into four stages (Stage 1: immature, Stage 2: early maturation; Stage 3: late maturation and Stage 4: complete maturation). Due to the lack of samples with Stage 2 of ovarian maturation (early maturation) which most likely occurred due to the shorter duration of this maturational stage (8 days) as previously reported by Heppi et al. (2014), further analyses only focused on comparing data collected during Stages 1, 3 and 4 of ovarian maturation. Foreguts, ovaries, and hepatopancreas were sampled for further analysis.

2.2. Foregut fullness and foregut content

All foreguts of crabs ($n = 60$) were assessed for fullness and contents were analyzed. Crabs had different stages of ovarian maturation [Stages 1 ($n = 26$), 3 ($n = 13$), and 4 ($n = 21$)]. Only recently caught crabs were examined to avoid bias in data regarding digestion processes. The mud crabs were sampled by using traps baited with fish, thus fish was excluded in foregut content analysis. Crabs were dissected within 2 h of being captured. Foreguts were collected, and foregut fullness was determined visually, as the foregut is thin-walled and translucent (Vismanathan and Raffi, 2015). Foregut fullness was scored using a subjective scale ranked from one to five (0%: empty, 25%: minimal, 50%: moderate, 75%: full and 100%: gorged). After determination of foregut fullness, foreguts were fixed in 10% formalin for gut content analysis using stereo and compound microscopes. There were several considerations in determining foregut fullness based on findings in previous studies (Vismanathan and Raffi, 2015; Williams, 1981). In brief,

I Foreguts which were estimated to have a fullness of 50% and greater (moderate, full, and gorged) were used for biochemical analyses. Hence, the total number of *S. olivacea* with a foregut fullness of 50% or greater were used in the present study as follows, Stages 1: $n = 16$, 3: $n = 8$, and 4: $n = 14$).

II All food items were listed. Only predominant food items, however, were included in determining the percentage of foregut content. Rare food items would have required a much larger sample size to get reliable estimates of foregut content percentage. Examples of rare dietary content items in the present study are nematodes and phytoplankton (2.5%).

III Inorganic compound such as sand and unidentified food items were excluded in estimates of percentage of foregut content.

IV Calculation of the percentage of foregut content was computed using the following equation:

Percentage foregut content of prey = number of stomachs with particular food group/$n \times 100$, where n = total number of crab specimens.

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