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Histological Study on the Development of Digestive System in Zoeal Stages of Mud Crab (*Scylla olivacea*)

Pattanee Jantrarotai , Nopparat Srakaew and Anong Sawanyatiputi

ABSTRACT

Histological study of the digestive system was conducted in the zoeal stages (zoea 1- zoea 5) of mud crabs (*Scylla olivacea*). The results showed that the digestive system of zoeae could be divided into three parts; foregut, midgut and hindgut. For the foregut, the gastric mill was not present in the cardiac stomach. The gland filter, anterior midgut caeca and posterior midgut caeca were not developed in the first and the early second zoeal stage. However, in the third zoea stage, these structures were developed and progressively increased in the later zoeal stage. The midgut gland or hepatopancreas was developed throughout the zoeal stages (zoea 1- zoea 5). This result showed the complexity of hepatopancreas , the more branched and larger size of this structure were found in the later zoeal stage.

Key words: *Scylla olivacea* , mud crab, histological study, digestive system, zoea

INTRODUCTION

Mud crabs (*Scylla olivacea*) are a member of Family Portunidae. They are valuably important to socio-economic of Australia, Japan, Taiwan, Indonesia, Philippines and Thailand (Keenan, 1999; Ruscoe *et al*., 2004). In Thailand, this species is commonly found along the coast of the Gulf of Thailand and Andaman Sea. At present, the population of mud crabs decrease drastically due to their habitat destruction (mangrove destruction), water pollution, and over-exploitation. Concerning on the decline of mud crab population, attempts have been made to conserve wild crab stocks by increasing the population through aquaculture practices. In aquaculture, the concentrations were made on increasing the production of crab seeds in zoeal stage. However, high mortality usually occurs during the rearing from the second to the third zoeal stage (zoea 2 – zoea 3) (Jantrarotai *et al*., 2002). Different causes of high mortality were speculated, one of which was on the use of larval feed and feeding management. According to the study of Jantrarotai *et al.* (2004) in *Scylla olivacea*, it was reported that live rotifers are suitable for the early zoeae which agreed with the study of Li (1990 cited by Li *et al*., 1999) in *Scylla serrata*. The latter suggested that the incomplete development of digestive system could effect the utilizing of diets. This may cause high mortality of zoeae. Therefore, the study on the development of digestive system in zoeal stages of *Scylla olivacea* by using histotechnique would provide a basic information to modify the diet and improve the feeding of larval crabs.

MATERIALS AND METHODS

Preparation of the specimen

Mud crab zoeae were reared after hatching at Ranong Coastal Aquaculture Station,

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Ranong Province. The zoeae were classified into five stages (zoea 1-zoea 5) as described by Ong (1964).

Preparation for histological studies

The zoeae in each stage (zoea1-zoea5) were fixed in Davidson's fixative (Moore *et al*., 1953 cited by Arkarajamorn, 1991) for 1 day and then washed several times in distilled water to eliminate the fixative. These zoeal samples were further processed by dehydrating in a series 50, 70, 80, 95, and 100% ethanol. To maintain the delicate tissue structures, zoeae were immersed twice (10 min each) in a mixture of 100% ethanol and xylene at the ratio of 1:1 v/v. The zoeae were then cleared in xylene, infiltrated with melted paraplast and embedded. The embedded zoeae were then serially cross- and long-sectioned using rotary microtome. The sections (5.0 μm thick) were affixed to a glass slide and dried on a slide warmer. The sections were stained with Harris's hematoxylin and eosin (H&E) (Luna, 1968) and covered with a glass cover-slip attached with permount-mounting medium. The specimens were examined and photographed by using a digital camera under a compound microscope.

RESULTS AND DISCUSSION

The digestive system of mud crabs (*Scylla olivacea*) in zoeal stage (zoea1-zoea5) could be divided into three parts; foregut, midgut, and hindgut. The photographs of the major zoeal digestive structure are illustrated in Figures 1 - 9. The foregut consisted of mouth, esophagus, and stomach which was divided into cardiac stomach, and pyloric stomach (Figure 1). The mouth was found located anteriorly on the ventral surface of its body. The esophagus was a communicating channel between mouth and cardiac stomach. Below the esophageal epithelium, there is a cluster of glandular tissues called esophageal tegumental gland. The function of these glands probably synthesize and produce mucus to lubricate the passage of ingested materials through the gut (Younge, 1924 cited by Icely and Nott, 1992). The cardiac stomach of zoeae was saclike chamber that receives food from the esophagus. The size of cardiac stomach was related to the zoeal stage; the more advance the zoeae, the larger size of this structure. The gastric mill or masticatory structure was not found in the cardiac stomach of *Scylla olivacea* which contrast to *Scylla serrata* of which its stomach contain this organ (Li 1990 cited by Li *et al*., 1999).

The pyloric stomach was lined by the simple columnar epithelium. The gland filter also called the gastric sieve was present in the ventral portion of the pyloric stomach. The gland filter was separated into two halves by the intrusion of the interampullary ridge from the stomach floor (Figures 2, 3). Each half of the gland filter was an oblong, flattened and comprised a row of setae which served as the filter to retain the rough food particles for enzymatic action before entering the intestine. However, this structure was not found

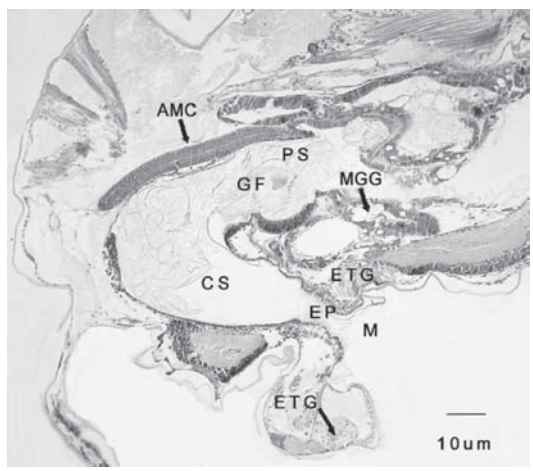

Figure 1 Photomicrograph of a long section of fifth zoeal stage (anterior region). AMC: anterior midgut caeca, CS :cardiac stomach, EP: esophagus, ETG : esophageal tegumental gland, GF: gland filter, M : mouth, PS: pyloric stomach.

in the first and the early second zoeal stage. For the third zoeae, this structure was more developed.

The midgut comprised intestine, anterior midgut caeca, posterior midgut caeca and midgut gland or hepatopancreas (Figure 4). The intestine was a tube and it was approximately circular in cross- section and lined by simple columnar epithelium (Figure 5). For the anterior midgut caeca in the mud crab zoeae was similar to that of the lobster *Homarus americanus* (Factor, 1995) which is bifurcate whereas the tiger shrimp *Penaeus monodon* is a single diverticulum (Arkarajamorn, 1991). The function of anterior midgut caeca is ambiguous, however, it may be the site for enzymatic digestion of food which is delivered from stomach before entering the intestine (Arkarajamorn, 1991) or may be involved in the contribution of components which can activate the enzymes (Dall and Moriaty, 1983). The posterior midgut caeca was also lined by simple columnar epithelium (Figure 5), it protruded anteriorly above the dorsal wall of intestine (Figure 4). This result did not agree with the study in lobster (*Homarus americanus*) reported by Factor (1995) who found posterior midgut caeca protrudes posteriorly above the hindgut. However, this study agreed with the study in tiger shrimp *Penaeus monodon* by Arkarajamorn (1991) who described posterior midgut caeca projects anteriorly. The specific function of posterior midgut caeca is not conclusive, it may be involved in an ion transport especially calcium and water transport (Arkarajamorn, 1991; Factor, 1995). Both anterior midgut caeca and posterior midgut caeca were not found in the first and the early second zoeal stages of *Scylla olivacea* but from the third zoea to the fifth zoea , these structures were progressively increased. In the midgut area, the structure called the midgut gland or hepatopancreas was located on both sides of the digestive tract (Figure 6). The epithelial lining of

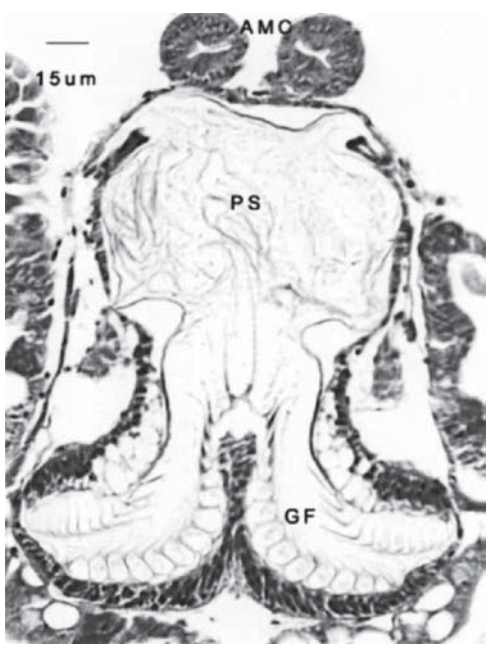

Figure 2 Photomicrograph of a cross section of third zoeal stage. AMC: anterior midgut caeca, GF: gland filter, PS : pyloric stomach.

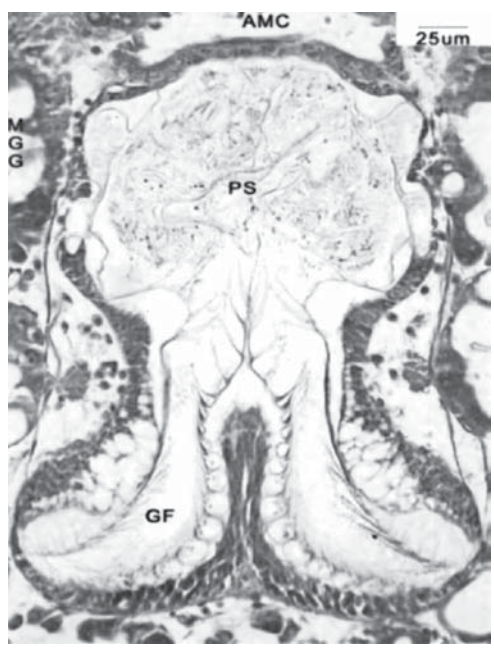

Figure 3 Photomicrograph of a cross section of fourth zoeal stage. AMC: anterior midgut caeca, GF : gland filter, MGG: midgut gland, PS: pyloric stomach.

the midgut gland was a simple columnar epithelium with many vacuolated cells. At these sites; the enzymatic digestion, food absorption and food transportation were taking place. The midgut glands could be found throughout the zoeal development (zoea 1-zoea 5). In the later zoeal stage, the more branched and larger size of this gland were dominated in the cephalothorac region (Figures 6, 7, 8).

The hindgut of the zoeae was a simple tube which began in abdomen and continued to anus and was lined by simple columnar epithelium .The thick, muscular wall of hindgut formed itself into six longitudinal ridges which protruded into the lumen (Figure 9).

From histological study of the digestive system throughout the zoeal stage, the third zoeae were completely developed. Therefore, it required high energy for development. Consequently, the energy left for molting may not be sufficient and

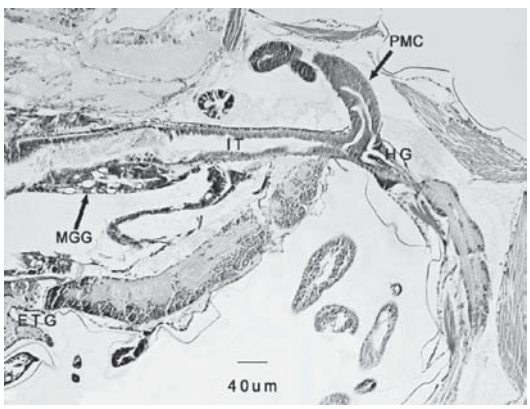

Figure 4 Photomicrograph of a long section of fifth zoeal stage (posterior region). ETG : esophageal tegumental gland, HG : hindgut, MGG: midgut gland, PMC: posterior midgut caeca.

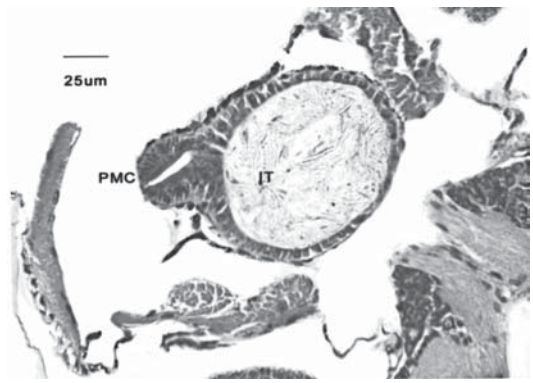

Figure 5 Photomicrograph of a cross section of fourth zoeal stage. IT: intestine, PMC : posterior midgut caeca.

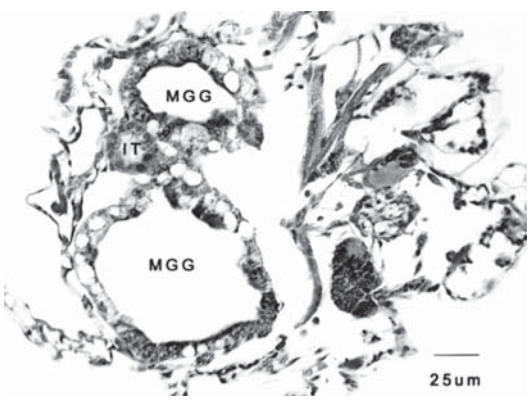

Figure 6 Photomicrograph of a cross section of first zoeal stage through the anterior midgut. IT : intestine, MGG : midgut gland.

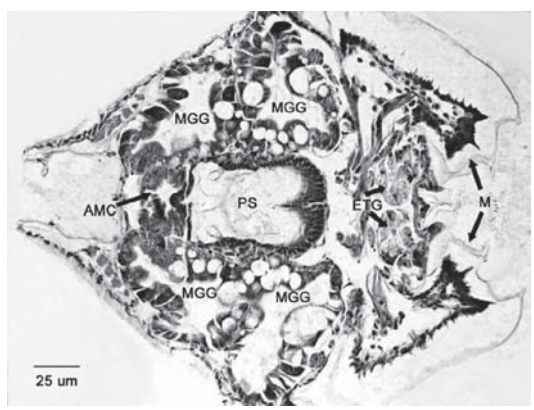

Figure 7 Photomicrograph of a cross section of second zoeal stage. AMC: anterior midgut caeca, ETG : esophageal tegumental gland, MGG : midgut gland, PS : pyloric stomach.

thus cause high mortality during their development from the second to the third zoeal stage. Therefore, live feed is necessary during these zoeal stages because it contains complete nutrients including free amino acid. In addition live feeds can be self autolyze within the incompleted digestive tract. (Warner, 1977; Lebour, 1928). However, in the later zoeal stages (zoea 3- zoea 5), the non living feed could be more appropriate for supplemented or substituted live feed due to the fully development of *Scylla olivacea* digestive system.

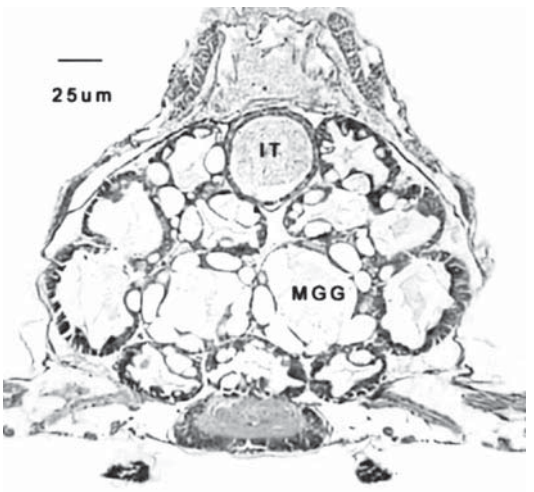

Figure 8 Photomicrograph of a cross section of fourth zoeal stage. IT : intestine, MGG : midgut gland.

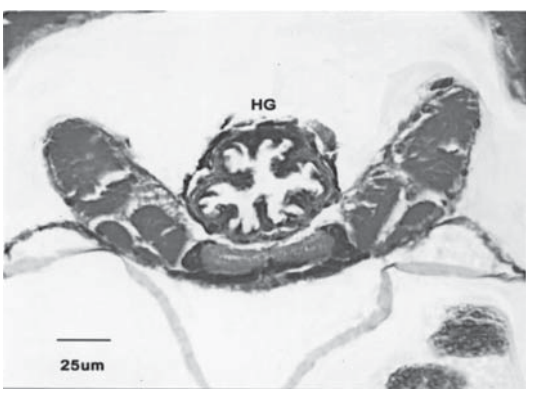

Figure 9 Photomicrograph of a cross section of fourth zoeal stage. HG : hindgut.

CONCLUSION

The digestive system of mud crab (*Scylla olivacea*) zoeae could be divided into three parts; foregut, midgut and hindgut. The foregut was composed of mouth , esophagus, cardiac stomach and pyloric stomach . The gastric filter was not found in the pyloric stomach of the first and the early second zoeal stages. The gastric mill was not developed in the cardiac stomach of *S. olivacea*. The midgut consisted of intestine, anterior midgut caeca , posterior midgut caeca and midgut glands or hepatopancreas. Anterior midgut caeca and posterior midgut caeca were not developed in the first and the early second zoeae. However; these structures in the third zoeae had developed and progressively increased in size at the later stage. The midgut glands or hepatopancreas had developed throughout the zoeal stages (zoea 1- zoea 5). At the later zoeal stage, the more branched and larger size of these structures were presented. The hindgut of the zoeae was lined by a simple columnar epithelium and it protruded into the lumen.

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