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Age and size at sexual maturity of same age group of male green mud crab (*Scylla paramamosain*)

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Abstract
The present study was conducted to elucidate the age at sexual maturity and transformation of secondary organs of male mud crab. Five crabs were dissected at 15 days interval; testes were collected and preserved in Davidson's fixatives for 24 hours, then transferred to 50% ethanol. Samples were chronologically dehydrated in 70% to 100% ethanol at 10% intervals; embedded, sectioned at 6 µm, stained with Haematoxylene-Eosin and observed under stereo microscope. Present observation revealed three distinct sexual maturation stages of male mud crab (*Scylla paramamosain*) with first sexual maturation in four months of age. Internal carapace width (ICW) showed discrete relation with external morphological features at ICW of 10.20 cm at pubertal molting. Asynchronies maturation was noticed with first fully sexually matured crab (20%) in 5th month of age and the median sexual maturity (MA_{50}) occurs in 6.5 month of age. Findings of the present study suggested to being ready for captive mating of mud crab within 4 months of age after crab instars.

Keywords: Age, sexual maturity, same age group, male, mud crab

1. Introduction
Mud crab is considered as nonconventional commercial species in south-east Asian countries [1], mostly exploited from wild sources [2], for livelihoods in the Indo-Pacific countries [1, 3, 4]. High international market price and thriving demand forced the fisher folk towards over exploitation of mud crabs [1, 5-7]. Massive harvest of all size groups hindering regular recruitment in the natural stock [8]. A clear concept on reproductive biology is worthy for efficient management and planning strategy for protecting the potential breeders in order to ensure regular recruitment in natural stocks of commercially exploited species [9, 10].

Likelihood of stock management, successful copulation/fertilization and efficient hatchery management protocols utterly dependent on the understanding of degree of sexual maturity of male and female crabs [11]. The onset of maturity in crustaceans is signaled by a series of morphological, physiological and behavioral transformations through which immature individuals become able to produce reproductive cells and copulate [12]. When organisms grow some of their morphological dimensions increase at a different rate from others that results in a change in body proportions known as relative growth and relative growth data have been widely used to predict the onset of morphometric sexual maturity in a number of organisms [12].On the other hand, histological observation on gonad maturation stages provide invaluable information to establish a relationship between sexual maturity and morphological features [13-15].

However, it is strongly believed that, a clear concept of age, size and morphological features at first sexual maturity and gonad maturation stages of both male and female is pre-requisite for successful copulation (mating), breeding and establishing of hatchery management protocol [16]. Reproductive biological features like age and size at sexual maturity of many species have been derived from the study on gonad maturation stages through histological observation [17]. Similarly, studies on gonad development of *Scylla serrata* [18, 19], *S. paramamosain* [13, 20], *S. olivacea* [14, 21] have been conducted and these studies have classified the gonad development stages. Unfortunately, all the above reproductive biology and maturity studies have been conducted on wild sourced samples with unknown age, contain various age groups, originate from different parents or had more than a single species. Mud crab produced from hatchery with known uniform age group might able to provide accurate information regarding growth, sexual maturity and reproductive capability. In addition, a captive broodstock might open other areas of future research on nutritional requirements and genetic selection for resistance to

disease [22]. But, information on biological study and sexual maturity of the same age group of mud crab is very limited. However, the ultimate aims of this study are to identify the age at first maturity, major features of gonad at different development stages and to establish the maturity levels with external morphological features of the male green mud crab for setting proper matting protocols under captive condition.

2. Materials and Methods

2.1 Study site

The experiment was conducted at the Centre for Marine and Coastal Studies (CEMACS) under Universiti Sains Malaysia (USM), Penang, Malaysia during 2012 to 2015. The study site located at North-East part of Penang Island under 5° 28' 2.3664"N and 100° 12' 2.8728"E in Global Positioning System (GPS).

2.2 Source of experimental crabs

Animal samples were managed by breeding the natural base female (P_0) and consequent rearing the larvae in hatchery condition followed by communal growing the juvenile male crabs under captive condition in fiber glass tanks with a density of 2 crabs/m^2.

2.3 Collection of crabs and measurement

As the crabs reaches 4 months of age, 5 male crabs were randomly collected at 15 days intervals, euthanized by thermal-shock at −20 °C for 30 min, measured (TW: total weight and CW: carapace width) and recorded for each crabs. The allometric growth organs (propodus length, propodus width and chela height) were measured for each crab with the help of slide caliper following standard methods.

2.4 Dissection of crabs, gonad sample collection and preservation

The anesthetized crabs were taken out from the freezer, the anterolateral and frontal spines were dissected. The upper shell (carapace) was gently removed to open the ovary/gonad. Necessary pictures on gonad morphological features were taken with a digital camera (Panasonic, DMC-FH25). The testis was gently removed, weighed followed by drying with blotting papers. The whole testes for each crab was separately preserved in Eppendorf tube filled with Davidson's fixatives for 24 hours, then transferred to 50% ethanol until histological process performed.

2.5 Histological slide preparation and observation

Histological slide of gonad tissues were prepared through routine histological procedures of dehydration in ascending order of ethanol starting from 70% and ended at 100% at 10% intervals, xylene treatments, embedded in paraffin wax blocks, sectioning at 6 µm, placed onto a glass slide and stained with Haematoxylene-Eosin [23]. The stained tissues were covered with a cover slip and observed under a light stereo microscope. Images of the cells of gonad development stages were taken by using a digital computer with an image capture analysis system (Image Cell B, Stereomicroscope, Camera-X cam-α, Olympus SZX91CAS, Japan). Histological classification of gonad development stages was performed according to previous author [14]. Data was analyzed using Microsoft Excels 2010 and SPSS 20.

3. Results

3.1 Histological classification

Histological classification of gonad development stages: From the histological observations, three distinct stages were identified in gonad development process of the male mud crabs (Plate 1 and described in Table 1).

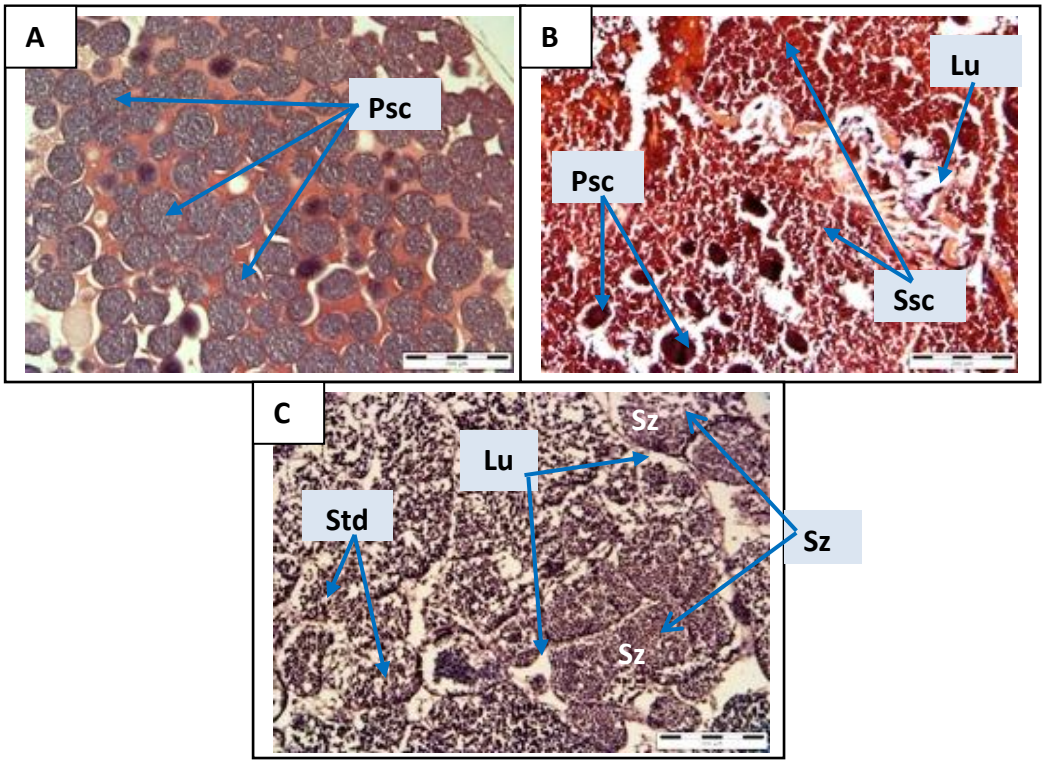

Plate 1: Histological features of three different gonad development stages of male mud crab in captivity; A= Immature (spermatogonia), B= Maturing (spermatocytes), C= Mature (spermatids and spermatozoa); Note: [Psc= Primary spermatocytes, Ssc= Secondary spermatocytes, Std= Spermatids, Sz= Spermatozoa, Lu= Lumen]

The stages were distinguished on the basis of formation of most advanced oocytes. The immature stage (Stage-1) was characterized with the presence of spermatogonia and primary spermatocytes in gonad with the dominancy (>80%) of spermatocytes over spermatogonia (Plate 1 A and Table 1). The intermediate or maturing stage (stage-2) was marked with the occurrence of both primary and secondary spermatocytes with the prevailing (>80%) of secondary spermatocytes (Plate 1 B and Table 1). The mature stage (stage-3) typically contained bulk numbers of spermatozoa (>80%) and a few numbers of spermatids (Plate 1 C and Table 1). Among the samples, 33.34%, 27.28% and 39.38% of the crabs were categorized under immature, maturing and mature stages, respectively (Table 1).

Table 1: Gonad morphological characteristic, histological features and body size (weight and CW) at different sexual maturity stages in male mud crab

Stages	Histological features	Composition of stages based on histology (%)	Comments
1	Spermatogonia and dominant with primary spermatocytes (>80%)	33.34	Immature
2	Primary spermatocytes and dominancy of secondary spermatocytes (>80%)	27.28	Maturing
3	Dominancy of spermatozoa (>80%), presence of spermatids	39.38	Mature

3.2 Gonad morphological features at different development stages

Based on the histological classification, the features in gonad morphology at different development stages of male mud crab are shown in Plate 2 and Table 2. In stage-1 (immature), the testes were difficult to distinguish from other internal organs, especially from hepatopancreas. Vas deferens looked like filaments and overall appearance seemed lucid-watery (Plate 2 A). The immature male had the highest carapace width of 10.0 cm and body weight of 188.5 g (Table 2). In stage-2 (maturing), the testes seemed like coiled shaped small kidney bean and watery-whitish in color. Vas deferens found small, thin, and coiled. Testes situated between the cardiac stomach and heart (Plate 2 B).

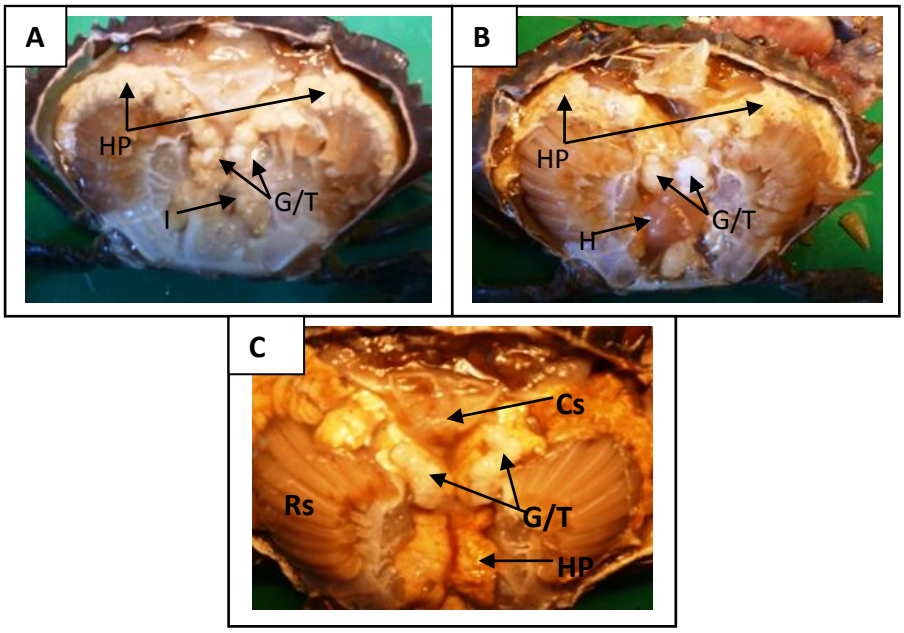

Plate 2: Anatomical view of different gonad developmental stages of male mud crab in captivity; A= Immature (spermatogonia), B= Maturing (spermatocytes), C= Mature (spermatids and spermatozoa); Note: [HP= Hepatopancreas, G/T= Gonad/Testes, I= Intestine, H= Heart, Cs= Cardiac stomach, Rs= Respiratory organ]

In the maturing stage, male crab had the lowest carapace width of 8.0 cm and lowest weight of 138.4 g., whereas, the highest carapace width and body weight was 10.2 cm and 266.1g, respectively (Table 2). In the mature stage (stage-3), swollen and prominent testes were noticed with opaque and whitish in color. The vas deferens was also swollen and in coiled forms, off white to pinkish in color. Testes elongated from the base of the eye organ to beneath the heart and vas deferens elongated to the base of 4th walking leg (Plate 2 C). The mature male had the lowest carapace width of 8.2 cm and body weight of 154.4g (Table 2).

Table 2: Gonad morphological characteristic and body size (weight and CW) at different sexual maturity stages in male mud crab

Stages	Gonad morphology	Crab size	
		Body weight (g)	Carapace width (cm)
1. Immature	Testes and vas deferens invisible in naked eye; vas deferens looks like filamentous and lucid	86.1-188.5	6.2-10.0
2. Maturing	Small coiled shape testes, whitish; vas deferens lean, small, coiled shape; located at middle of cardiac stomach and heart	138.4-266.1	8.0-10.2
3. Mature	Testes swollen, opaque, whitish; vas deferens swollen, coiled & off white-pinkish	154.4-435.0	8.2-12.1

3.3 Age at first sexual maturity and composition of gonad development stages

First maturation and progress in gonad development stages is presented in Fig. 1. First sexual maturation (stage-1) of male *S. paramamosain* was traced in four month of age among 20% of the samples only. Gonads/testes were not traced in the rest of the samples for that day, indicated as sub-adult (Fig. 1).

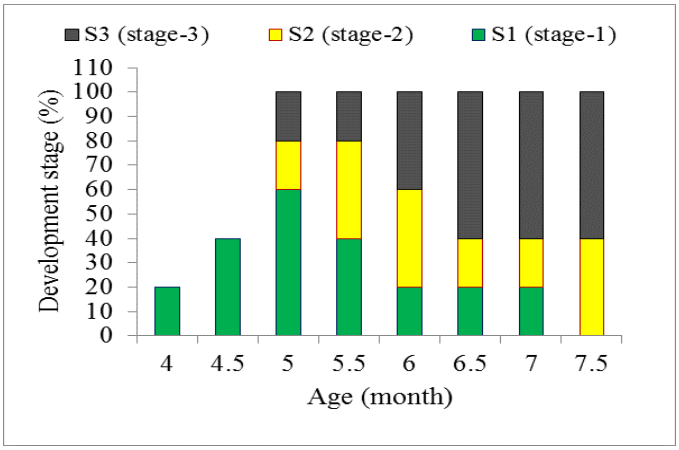

Fig 1: Composition (%) of different gonad maturity stages in relation to the age (stage-1: immature, stage-2: maturing and stage-3: mature) of the male mud crab

The proportion of first stage increased to 40% and 60% in 4.5th and 5th month of age, respectively. First fully matured male crab (20%) was observed at 5th month of age, indicated onset of full maturity. Despite of asynchronies maturation trend, >50% of the sample were found to be matured (stage-3) in an age of 6.5 months (Fig. 1) indicated the age of median sexual maturity (MA_{50}) in captive condition.

3.4 Relationship between body size and allometric growth organs

The relationship between internal carapace width (ICW) and external allometric growth organs *viz*, left propodus length (LPL), left propodus width (LPW) and left chelae height (LCH) has been scattered plotted (Fig 2). Allometric growth organs in relation to the ICW showed discontinuity with sudden changes in allometric growth organs after ICW of 10.20 cm and began again at ICW of 11.00 cm.

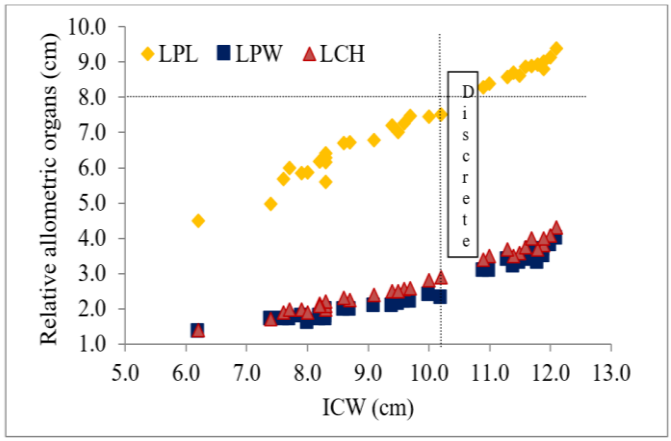

Fig 2: Scatter plot relationship between internal carapace width (ICW) and allometric secondary growth organs of the male mud crab (LPL= left propodus length; LPW= left propodus width and LCH= left chelae height) showing discrete growth at maturity

4. Discussion

Virtually, all the essential organs of an animal were developed during gamete formation and embryogenesis, but some of the organs remained in rudimentary forms and developed slowly with progress in age and also with the increase in size. First sexual maturity denotes the ability of an organism to take part in sexual activities for reproduction purposes for the first time of the life span. As the male green mud crab (*S. paramamosain*) was sequentially dissected at various ages, internal sexual organs were not traced before 4 months of age (Fig. 1). The testes and vas deferens were visible and developed stepwise until maturity.

The practical maturity of male mud crab could be recognized from the mating scars on the chelapeds of the male [4], on the thoracic region and on the first walking legs [24]. Whereas, mating scares among less than one third of the adult crabs was absent reveals that the absence of mating scars did not indicate that the male crab has not mated [25]. Mating scares might be lost as the crab moult and disappear after a certain time of mating if it was not well developed [19, 25]. In this study, male mud crabs were kept separate from that of the females, thus mating was not possible and mating scares was absent, thus estimation of functional maturity from the mating scares mentioned by various authors was not possible [1, 4, 24, 25].

In many species, morphological development seemed prominent and has been regarded as functional maturity [19]. In Australia, scared male was recorded at minimum ICW (internal carapace width) of 12.5 cm [25], whereas, functional maturity was recorded at 13.8 cm ICW in Ponape [26]. Matting scares within smaller male crabs was recorded in Asia [27]. Similarly, MD_{50} at ICW of 10.3 cm for wild *S. paramamosain* was observed in Thailand [14]. In this study, size distribution of male mud crab ranged between 6.2 cm to 12.1 cm of ICW, of which the size distribution in matured crabs ranged between 8.2 cm to 12.1 cm of the ICW (Table 2), thus, supported by the above mentioned authors.

This study showed discontinuity and sudden changes in allometric growth organs with ICW (Fig. 2). Such type of discrete change and relationship between ICW and LCH has been reported as a general scale for identifying sexual maturity in male brachyurans [25], in freshwater crab *Dilocarcinus pagei* [28] and also found in wild sourced mud crabs [14]. Meanwhile, allometric change in the chelae height to external carapace width at 106.4 mm was observed [29], thus coincided with this study.

On the other hand, the presence of spermatophores in vas deferens has been regarded as functional maturity [14, 19, 30]. In this study, three distinct gonad development stages were identified from external observation (Plate 2) and simultaneously from the histological scrutiny of the gonad samples (Plate 1). In stage-1 (immature), the vas deferens was undistinguished and watery (Plate 2 A), but in stage-2 (maturing), the vas deferens was distinct while absence of spermatophores (Plate 2 B). In the case of the last stage-3 (mature), the prominent testes and vas deferens was noticed (Plate 2 C) that filled with spermatophores (Plate 1 C). The observation of this study seemed to be corresponding to the stages described in portunid crab [31], in anomuran crab [32] and in mud crab [14].

As the histological gonad development samples were fitted against age (month) of the crabs, it was found that first appearance (immature) of gonad was at four months of age and fully mature (first sexually mature) male was perceived at

five months of age (Fig. 1). The immature male represented from the fourth month until the age of the seventh month and indicated asynchronies maturation might due to nutrition and obesity [33]. Despite the asynchronies in maturation, 50% of maturity was observed at the age of 6.5 months (Fig. 1). It has been reported that all mud crabs attained maturity within their first year of life span [1]. *S. Serrata* has shown maturity signs at day 147 of age [34], while wild *S. paramamosain* attained maturity at approximately 160 days from first settlement [8]. Thus the observations of this study showed similarity with the observation of the above mentioned authors.

The observation of this study found the highest frequency for the mature stage (39.38%) and lowest frequency (33.34%) for that of the immature stage (Table 1) crabs. In a study, highest frequency for the immature stage (56%) and the lowest (18%) for the mature stage was recorded with wild source samples [14] collected from mangrove swamps. Juveniles and sub-adult crabs are noticeably plentiful in the mangrove swamps [4, 5, 35, 36] and led to the highest frequency for the immature stage. Most of the matured crabs might move towards the estuary and inshore region for mating and reproduction purposes [1, 37-39], thus, the matured population may have escapes from sampling. While in the present study, crabs were reared under captive condition and sampling has been done from the onset of gonad appearance up to the mature stage with a specific interval (15 days) to ensure the occurrence of all stages in the sample.

5. Conclusion

Mud crab as a marine breeder crustacean required suitable environmental conditions for growth, maturity and reproductive development. Male mud crabs showed stepwise maturation with progress of age and size. Despite the same age group of population, a portion of male showed faster sexual maturity, whereas, the majority began maturing uniformly and the least portion showed slow maturation. First sexual maturity was noticed in 4 month of age and attained fully mature in 5 month of age. Depending on the age of male sexual maturity, this study suggested to being ready within 5 month of age for captive mating. However, this noteworthy observation on age at sexual maturity of male mud crab could be the footprint for setting up of further research on captive mating for genetic selection, line breeding and disease resistance.

6. Acknowledgement

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