

## Gonad development and size at maturity of the male mud crab *Scylla paramamosain* (Forsskål, 1755) in a tropical mangrove swamp

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### Abstract

The reproductive traits and size at sexual maturity of the male mud crab *Scylla paramamosain* were investigated in Pak Phanang mangrove swamps, Thailand. Samples were taken seven times from the local middlemen mud crab traders during June 2006 to January 2008. Gonad development was determined based on histological appearance that was classified into three stages: 1) Immature (Spermatogonia), 2) Maturing (Spermatocytes) and 3) Mature (Spermatids and Spermatozoa). Among the sample population, the highest 72% was under gonad development stage I, whereas mature stage III was only 12%. The size at first maturity was estimated by the external allometric growth and histological observation of gonad. The size at which 50% of individuals attain sexual maturity was estimated by the two mathematical models such as probit analysis and logistic curve. The mean size at first sexual maturity and 50% maturation of male *S. paramamosain* were 96 mm and 109 mm internal carapace width (ICW) which revealed that 88% individuals were immature. The present result suggested that the minimum legal size of male *S. paramamosain* capture should be >110 mm ICW.

**Keywords:** Reproductive traits, *Scylla paramamosain*, Legal capture size, Mangroves

### INTRODUCTION

The reproductive information on a commercially exploited species is crucial for understanding its population dynamics, which is fundamental for developing an effective management models. The minimum legal size for catch is one of the popular management regimes in mud crab fisheries with the purpose of protecting the reproductive potential of resource stock (Goshima *et al.* 2000, Conan *et al.* 2001). The restriction of the harvest to males has been considered to have relatively little impact on the

reproductive output of the stock. However, concern for the effects of reduction of males has prompted which triggered out the importance of research on male maturity (Van Engel 1990, Knuckey 1996, Castilho *et al.* 2008).

In male crustaceans, there are three common methods to determine maturity. First is the morphometric method; using change of allometric relationship between sizes of body parts (e.g., Knuckey 1996, Viau *et al.* 2006). Second is the histological (gonad) method; histological examination of the gonad to see if spermatozoa are present in the testes and/or vas deferentia (e.g.,

Robertson and Kruger 1994, Leal *et al.* 2008). The third is functional maturity; usually referred to as ability of mate successfully (Conan and Comeau 1986). In fact, the main criteria for determining functional maturity in crustaceans are the presence of scars on the sternum or forward walking legs, which are produced by abrasion with the female during the precopulatory embrace (Robertson and Kruger 1994, Knuckey 1996). Another commonly considered criteria for the maturity estimation is the presence of spermatozooids or spermatophores in the testes or vas deferentia as well as copulation marks (Viau *et al.* 2006, Islam and Kurokura 2012).

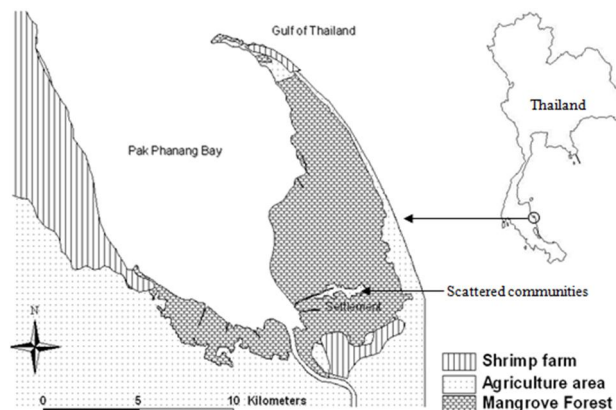
However, the mating scar in male could not find less than 125 mm internal carapace width (ICW) (Knuckey 1996) and completely absence in smaller individuals than 115 mm (Robertson and Kruger 1994). In the present study, it is noticed that no sample crab exceeds the size of 130 mm ICW and very few were > 115 mm ICW. On the other hand, no prominent scars were found in the samples. Moreover, maturity in male crabs is not easily determined from external characteristics (Robertson and Kruger 1994). Thus present study was concentrate on the histological observation of vas deferens/testes to establish the maturity status of wild male mud crab population.

The male reproductive biology and maturity size of mud crab (*Scylla* spp.) has been established in Australia (Knuckey 1996) and in South Africa (Robertson and Kruger 1994). There were very few study have been taken in Asian countries like, Ong (1966) from Malaysia, Lavina (1980) from Philippines and Islam and Kurokura (2012) from Thailand. However, there were no subsequent study. Moreover, all the previous studies focused on *S. serrata* and study on other *Scylla* species is rear. Thus paucity of information existed on the male reproductive biology of *S. paramamosain* in Asian countries was the trigger to conduct the present study to provide detailed reproductive information of male mud crab.

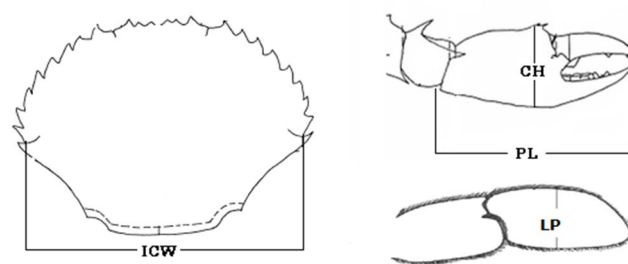
## METHODOLOGY

**Sampling:** Male *S. paramamosain* were collected during June 2006 to January 2008 from the local mud crab middle trades of Pak Phanang estuary, Nakhon Si Thammarat, Southern Thailand (Figure 1). Crab fishing is conducted throughout the year within mangrove channels as well as associated channels connected to the bay. In the present study, survey was focused on the communities within the mangrove. In the laboratory, 81 male crabs were examined which did not include any morphological anomalies. The following measurements of body size were taken with a digital caliper to the nearest

0.1 mm: internal carapace width (ICW), lower paddle width (LPW), propodus length (PL) and chela height (CH) of left cheliped (Figure 2).



**Figure 1:** Study area, Pak Phanang mangrove ecosystem and the sampling place (scattered communities) inside of the mangrove



**Figure 2:** Measurements of morphometric characteristics such as internal carapace width (ICW), propodus length (PL), chela height (CH) and lower paddle width (LPW) of male *Scylla paramamosain* collected from Pak Phanang mangrove swamps, Thailand.

**Histological study:** Tissue from the middle vas deferens/testes from each male was dissected and preserved in Davidson's fixation for further histological examination. The tissues were dehydrated in ascending ethanol concentrations from 70 to 100%, transferred to Lemosol (Wako Pure Chemical Industries, Osaka, Japan) and embedded in paraffin. The tissues were sectioned to 5  $\mu$ m and stained with Mayer's hematoxylin-eosin (HE). The histological stage of development was determined by making reference to those of other crustaceans (for example, Lestang *et al.* 2003, Mura *et al.* 2005; Viau *et al.* 2006 and Islam and Kurokura 2012). The development stages of the gonad were determined by the macroscopic appearance based on the formation of spermatoocytes and presence of spermatozoa.

**Estimation of size at maturity:** The middle vas deferentia were used for the histological study and maturity estimation. Sexual maturity was classified with the visibility of vas deferentia and the presence of spermatophores within their lumina. The relative

frequencies of each stage of sexual maturity in the samples were analyzed to describe the reproductive cycle of males.

The size at first maturity was estimated by three methods that are described following:

1. Allometric increments proportional to ICW; the allometric growth increments of CH, PL and LPW were plotted against the increments of ICW and existence of the flexion point was investigated and treated at the size of first maturation in male crab.
2. The minimum size at maturity recorded through histological studies of the male mud crabs (> 70 mm ICW).
3. Chela height (CH)/internal carapace width (ICW) index is another method which calculated as the divides of CH by ICW. The samples were categorized to two groups by the critical point of CH/ICW and the histological developments of the gonad were compared between two groups. Regression analysis was performed to determine the relation of Chela height (CH) with internal carapace width (ICW). A significant level of  $P > 0.05$  was considered.

Two mathematical models (1 and 2) were used to estimate the size at which 50% of the individuals had reached sexual maturity that are described below.

Model 1: Probit analysis (Robertson and Kruger 1994) was performed using abdomen-width data using probit analysis to determine the size at which 50% of females reach sexual maturity ( $ICW_{50}$ ). The data from sample crabs were allocated to 10 mm ICW size classes. The proportion ( $p$ ) of mature male in each size class was calculated according to Mikhaylyuk (1985) that was converted to logit [ $\text{logit}(p) = \ln(p/1-p)$ ]. The logistic data were then converted to probit ( $P$ ) =  $p+5$ . Finally, the probit data were plotted against ICW, and a regression line was fitted to the data points. The ICW value equivalent to probit 5 was extrapolated as the median size at sexual maturity.

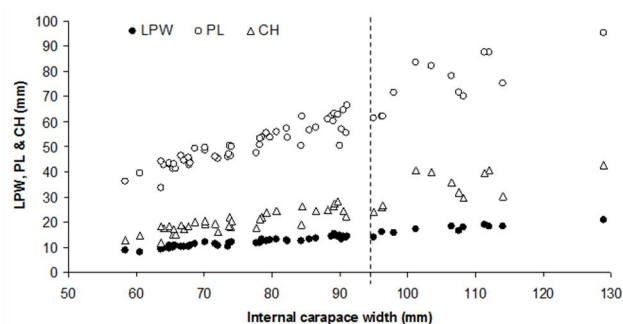
Model 2: Ratio of mature individuals determined by the histological observation in each size class was fitted to the sigmoid curve (see the formula below).

$$P_{ICW} = \frac{1}{1 + e^{(M_1 - M_2/ICW)}} \quad (\text{Koolkalya et al. 2006})$$

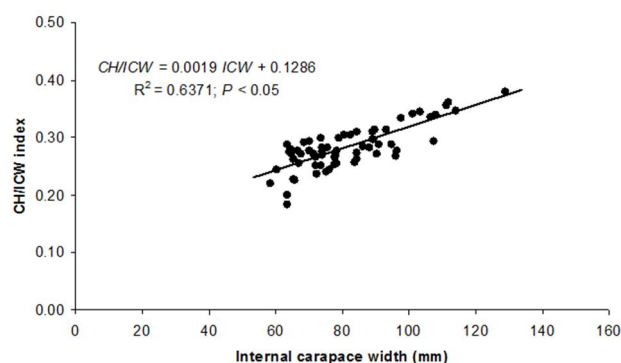
Where  $P_{ICW}$  is the proportion of mature to immature crabs in each ICW class (10 mm interval), and  $M_1$  and  $M_2$  are the equation coefficients. The best fit curve was estimated by the using of Kaleida graph software (Kaleida graph, version 3.6).

## RESULTS

**Allometric growth:** The male chela height (CH), propodus length (PL) and lower paddle width (LPW) were scattered plotted against the internal carapace width (ICW) and found that the relative growth of these secondary sexual allometric parts were increased sharply at the size of 95 mm ICW (Figure 3). Figure 4 shows the relation between CH/ICW and ICW that represent a clear allometrical growth of CH/ICW with the increments of body size.



**Figure 3:** Scatter plotted of chela height (mm), propodus length (PL) and lower paddle width (LPW) against ICW (mm) of male *Scylla paramamosain* collected from Pak Phanang mangrove swamps, Thailand. Vertical dash line represents the probable discontinuity increments (growth at maturity) with observed body parts at the ICW of 94.94 mm



**Figure 4:** The relationship of CH/ICW values regarding body size of male *Scylla paramamosain* collected at Pak Phanang mangrove swamps during June 2006 to January 2008. The critical CH/ICW values estimated to be 0.36 at the body size of about 110 mm ICW

**Gonad development:** A total of 72 male crabs ( $\geq 70$  mm ICW) were assessed to observe the gonodal condition. The progress of gonad maturation was classified into five stages based on external appearance of testes and the development stage of the most advanced spermatocytes with the histological observation (Table 1; Figure 5). The histological examination showed that among the sample population, the highest 72% was under gonad development stage I whereas only 12% was belong to the mature stage III (Table 1). The smallest mature male and

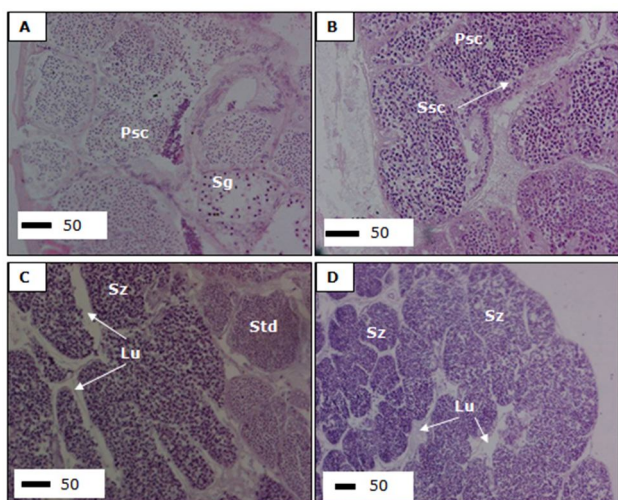
largest immature male were recorded 84 mm ICW and 102 mm ICW respectively.

**Table 1:** Stages of physiological sexual maturity of male *Scylla paramamosain* and their composition in respective stage

Stage	Characteristics	Remarks	% of individuals
I	Testes not visible to the naked eye; vas deferens resemble translucent filaments; presence of spermatogonia	Immature	72%
II	Small testes and thin vas deferentia; formation of primary and secondary spermatocytes	Maturing	16%
III	Testes swollen, opaque and white; vas deferens swollen and pink; containing spermatophores	Mature	12%

The immature stage (stage I) was represented by gonad with spermatogonia and primary spermatocytes (Figure 5A). The maturing stage (stage II) was characterized with the containing of secondary spermatocytes as predominately, but with the presence of primary spermatocytes in few (Figure 5B). The mature stage was defined as the dominancy of spermatozoa with the presence of spermatids (Figure 5C and 5D).

The size distribution of stage I, II and III ranged from 60-98 mm, 78-107 mm and 95-128 mm ICW respectively. No spent males were registered during the study period. In the present study, sexually mature male were defined with the gonad stage of III as the presence of spermatozoa.



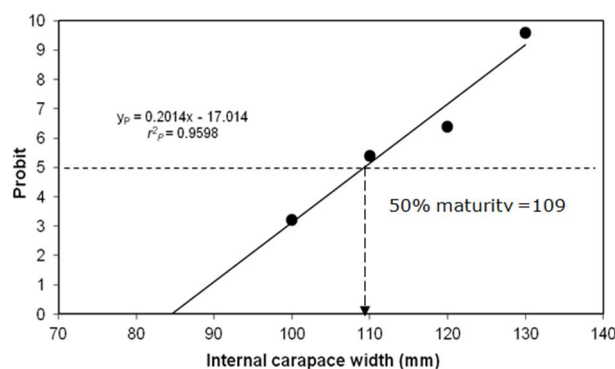
**Figure 5 (A-D):** Histological sections of Vas deference in *Scylla paramamosain* A: Immature (Spermatogonia), B: Maturing (Spermatocytes) and C (Spermatids) & D (Spermatozoa): Mature. Sg, spermatogonia; Psc, primary spermatocyte; Ssc, secondary spermatocyte; Sz, spermatozoa; Std, spermatid; Lu, lumen.

**Relation between allometric growth and maturation:** A significant relationship was observed between CH/ICW and carapace width ( $r^2 = 0.63, P < 0.05$ ) (Figure 4). Table 2 shows the assemblage of the CH/ICW index relating to stages of the gonad development. The CH/ICW values ranging from 0.15 to 0.25 were completely belongs to stage I-II, indicating the immature male. On the contrary, majority of the individuals were stage III in 0.36-0.40 class in and no individual was in stage I within mentioned ranged. Thus, the critical values of CH/ICW for maturation were estimated to be 0.36 in *S. paramamosain*.

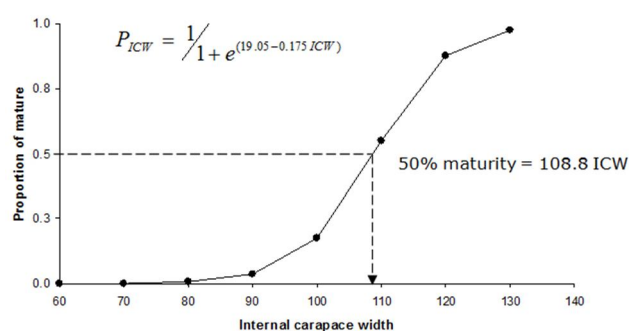
**Table 2:** Proportion of male chela height (CH) and internal carapace width (ICW) of *Scylla paramamosain* with reflecting the different stages of gonadal development

Range of CH/ICW	Number of samples	Percentage of gonad development		
		Immature		
		Stage I	Stage II	Stage III
0.15-0.20	2	100	0	0
0.21-0.25	22	90.9	9.1	0
0.26-0.30	43	76.7	16.3	7
0.31-0.35	8	37.5	37.5	25
0.36-0.40	6	0	20	80

**Estimation of size at 50% maturity:** Probit analysis of the abdomen-width data resulted in an  $M_{50}$  value of ICW 109 mm (Figure 6). This value represents the body size at which 50% of the males are assumed to reach sexual maturity. In another model, the logistic curve fitting with the equation of Koolkalya *et al.* (2006), it is also noticed that 50% of individuals attained sexual maturity at the size of about 109 mm (Figure 7). So, the mean size of the above two mathematical analysis indicate that 50% individuals reached sexual maturity in 109 mm ICW in the Pak Phanang mangrove swamps.



**Figure 6:** Percentage maturity, after histological observation, plotted on a probit scale against internal carapace width to obtain the size at  $M_{50}$  (the point at which 50% of the male crabs are mature) of *Scylla paramamosain* from Pak Phanang mangrove swamps, Thailand.



**Figure 7:** Logistic fitted curve to the proportion of mature *Scylla paramamosain* males in Pak Phanang mangrove swamps at each internal carapace width class (size class = 10 mm).

## DISCUSSIONS

**Possibility of external morphological observation for maturity:** In the present study there were no clear mating scars noticed, and hence the estimate of functional maturity is unclear. Knuckey (1996) found less than a third of all adult crabs are scarred and pointed out that there would be no evidence that every mated crabs developed scars and mating scars lost during molt. Thus the absence of scars does not necessarily mean that a crab has never mated. In the present study, the size-distribution ranged 60-130 mm ICW and 98% of individuals were less than 110 mm ICW. Whereas, 125 mm ICW was the minimum size recorded of scarred crabs in Australia (Knuckey 1996). Though Perrine (1978) noted that the majority of males in Ponape only mate after they have attained a size of 138 mm ICW, smaller scarred crabs noticed in Asia (Ong 1966). Thus, it is questionable to adopt the absence of the mating scars as the evidence of the virginity of males.

On the other hand, crabs can produce spermatozoa without mating (Conan and Comeau 1986, Robertson and Kruger 1994). Several authors have regarded the presence of spermatophores as indication of functional maturity (Paul 1992), although this relationship is debated in other species where morphological development seems to be an important determinant of functional maturity (Sainte-Marie *et al.* 1995, Knuckey 1996). Thus, in the present study, maturity estimation of male mud crab was tried with the pattern of allometric relationship as well as presence of spermatozoa.

Although all allometric variables showed discontinuity in relative growth associated with sexual maturity, propodus length and chela height showed a clear sharply increment in its relative growth. Although, the ratio of chela height to ICW is commonly used to identify maturity stages in male brachyurans (e.g., Knuckey 1996), other parameters like PL, CH can also be used to indicate the

sexual maturity of *S. paramamosain*. This sexual character in present study also showed different growth rates before and after sexual maturity, therefore indicating the end of the immature phase and the beginning of the adult phase such as in major crustaceans (Hartnoll 1978).

**Gonad development:** Histological examination on male gonad development is scarce in case of crustacean and particularly on *Scylla* spp. Robertson and Kruger (1994) described the presence of spermatophores in anterior vas deferens (AVD) in *S. serrata* but did not describe details on the development stages. However, Islam and Kurokura (2012) identified three development stages for the species *S. olivacea*. Sainte-Marie and Sainte-Marie (1999) described the formation and development of spermatophores in snow crab (*Chionoecetes opilio*). Spermatophore formation has also been studied for a number of crustaceans using light microscopy, including the crab species; *Scylla serrata* (Uma and Subramoniam 1979), *Callinectes sapidus* (Johnson 1980), *C. opilio* (Beninger *et al.* 1988), *Portunus pelagicus* (El-Sherief 1991), and *Lithodes maja* (Tudge *et al.* 1998).

In the present study, three gonad development stages were observed by histological microscopic observations and appear to be equivalent to those (Islam and Kurokura 2012) in *S. olivacea*; (Lestang *et al.* 2003) in portunid crab; (Viau *et al.* 2006) in anomuran crab, and the first three of the five stages defined by (Leal *et al.* 2008) in stone crab. The stage I and II defined as the males with undifferentiated vas deferens and males with differentiated vas deferens but no spermatophores. The final stage characterized as males with prominent and convoluted vas deferens containing spermatophores. There was no spent or postovulatory stage in both studied species. Therefore it was not clear whether male crabs are multiple breeders or not which found in other crustacean (Leal *et al.* 2008).

However, as female mud crab showed continuous multiple breeds and they stored sperm at the first time of mating and used that for subsequent breeding (Onyango 2002, Moser *et al.* 2005), male probably also have multiple breed patterns. In the gonad development stages, the rarity of stage III could be due to a short duration of existence and probably turned back to stage I.

**Legal size for management:** In fishery management, minimum legal size limit is usually determined based on size at maturity, allowing individuals to mate at least once after reaching maturity before they are large enough to harvest in order to protect reproductive potential of the stocks (e.g., Stevens *et al.* 1993).

The 50% maturity size is the common minimum legal size used in many open water mud crab fisheries but exclusively for female crab (Robertson and Kruger 1994, Overton and Macintosh 2002). In addition, Overton and Macintosh (2002) emphasized on the account of male in maturity estimation. In the present study, though maturity started at the size of about 95 mm ICW, the two mathematical models showed 50% maturity was 109 mm ICW (Figure 6 and 7). Although each method has its limitations and the small sample size in the present study, their close agreements in both aspects of maturity make the results credible. Thus, to conserve mature stock in Pak Phanang mangrove swamps, an effective minimum legal size of capture for male *S. paramamosain* would be > 110 mm ICW. This size limit is higher than the male *S. olivacea* in the same area (Islam and Kurokura 2012) where they suggested > 100 mm ICW. However, female *S. paramamosain* attained 50% maturity size at about 110 mm ICW (Islam 2008) whereas female *S. olivacea* at 100 mm ICW (Islam *et al.* 2010). This finding indicates though maturity size does not vary widely with the sexes but may vary with the species in the particular area. Thus, the legal size of capture for mud crab should be species-specific which also suggested by Overton and Macintosh (2002).

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