Reproductive morphology, morphometric aspects and molecular identification of *Scylla* (Mud Crab) species in Sri Lanka

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Abstract

This study was conducted to identify and determine the reproductive morphology and morphometric relationships of green mud crab, *Scylla* species in Sri Lanka. A total of 231 individuals representing different stocks around the country were studied. The size range of females was between 7.5 - 20.3 cm, while that of the males was from 6.7 - 20.6 cm. The length at 50% maturity (L_{50}) of female green mud crab was 14.1 cm, while the same for males was 13.0 cm. Length-weight relationship of *Scylla* showed a negative allometric growth for female crabs, whereas a positive allometric growth was observed for the male. The corresponding weight for the L_{50} of females was 426.7 g, while the corresponding value for males was 367.3 g. These maturity estimations can be used as a basis for recommending the precise legal size for harvest. Molecular analysis was also carried out under this study to identify the morphologically different mud crab species in the catch. The occurrence of both *Scylla serrata* and *Scylla olivacea* was confirmed in the country.

Keywords: length at maturity, size regulation, gonads, molecular identification, *Scylla*

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Introduction

Among the different crab species recorded in the world, the edible crabs (Crustacea: Decapoda: Portunidae) have a high level of importance as they comprise a distinct taste with a unique nutritive value (De Silva and Munasinghe, 2016). Mud crabs are considered one of the most delicious seafood and have a high economic value around the world, especially in tropical and subtropical countries. The mud crab or mangrove crab; *Scylla serrata* (Order Decapoda, Family Portunidae) is an ecologically important edible crab species found in low saline habitats such as estuaries and mangroves in many countries in Asia, Africa, Australia and many Indo-Pacific nations (Bir *et al*., 2020). Though there is a huge worldwide consumer demand for this species, aquaculture hatchery rearing is still a challenge (Paran *et al*., 2022). Therefore, to reach consumer demand, wild capture of mud crabs is being heavily practiced, in addition to capture based aquaculture attempts in some countries (Shelley, 2008; Waiho *et al*., 2018). Le Vay in 2001 also emphasized that it is crucial to understand the biology and ecology of mud crabs for sustainable management of the mud crab fisheries.

Like the other crabs, mud crabs also show sexual dimorphism (Hidir *et al*., 2021) and habitat specificity. Adult mud crabs are generally found in muddy, mangrove-lined brackish waters, and the ovigerous females move offshore to spawn (Hill, 1994). The females can grow up to 3.5 kg in size and have a shell width of up to 24 cm. The length at maturity of *S. serrata* differs greatly in different geographic regions. It is suggested that the different maturation sizes depend on the latitudinal distribution of the crabs, resulting in a faster maturation in tropical regions (Quinn & Kojis, 1987; Robertson & Kruger, 1994). In both males and females, a distinction between physiological, morphological, and functional maturities can be made.

Among South Asian countries, Sri Lanka contributes a considerable amount of mud crabs to the global market. Due to high export demand, there is a growing trend to harvest mud crabs extensively. With high fishing pressure on the resources (MOF, 2021), there is a risk of the crab stocks depleting. Furthermore, the situation will be much more critical if immature crabs are caught. As a common form of intervention in fisheries management, a minimum legal size of capture can be set, based on the size at first maturity which allows the adult stock to mate and spawn at least once before capture (Stevens *et al*., 1993). However, the use of different basis in determining the maturity of crabs, as morphological, physiological and functional, has also resulted in a range of values for maturity, hence a thorough understanding is required when imposing the restrictions.

In Sri Lanka, a limited number of studies have been focused on the biological aspects of mud crabs, especially on length at maturity of male mud crabs based on physiological

maturity. Therefore, precise information is vital to compose size regulations in the fishery and thereby to ensure a sustainable crab industry in the country.

The species belonging to the genus *Scylla* has been controversial for some time as there are overlapping morphological and morphometric characteristics. The two species *S. serrata* and *S. olivacea* have been reported from Sri Lankan waters (De Silva and Munasinghe, 2013). Three species and one subspecies have been reported from the Philipines (*S. serrata*, *S. oceanica*, *S. tranquebarica* and *S. serrata* var. *paramamosain*) (Estampador, 1949) and the two species *S. serrata* and *S. olivacea* have been recorded in Bangladesh (Rouf *et al*., 2016). Keenan *et al*., (1998) have reported that the genus included 4 species *S. serrata*, *S. olivacea*, *S. tranquebarica* and *S. paramamosain* by using morphometrics, morphology and allozyme variability. A number of studies carried out in the coastal regions of India had initially reported the occurrence of the three species, *S. serrata*, *S. olivacea* and *S. tranquebarica*, but it has been later confirmed that the Indian coastal region has only two species; *S. olivacea* and *S. serrata* (Balasubramanian *et al*., 2016). *S. serrata* is the dominant species in India, while the dominant species in Bangladesh is *S. olivacea* (Asaduzzama *et al*., 2020).

Realizing the above information gaps, the present study was carried out to ascertain the precise legal size and weight of crabs to be harvested based on their biological and morphometric characteristics. Further, under this study molecular identification was carried out to confirm the identity of the *Scylla* species that are found in Sri Lankan waters.

Materials and Methods

Mud crab Sample collection

The present study was carried out based on the mud crab samples obtained from the collecting centres around Negombo, Sri Lanka in September and October 2021. Negombo has well known crab collection centres, receiving crabs from the various areas of the country such as Jaffna, Kalpitiya, Negombo, Chilaw, Palakuda, Talaimannar and Batticaloa in Sri Lanka (Figure 1).

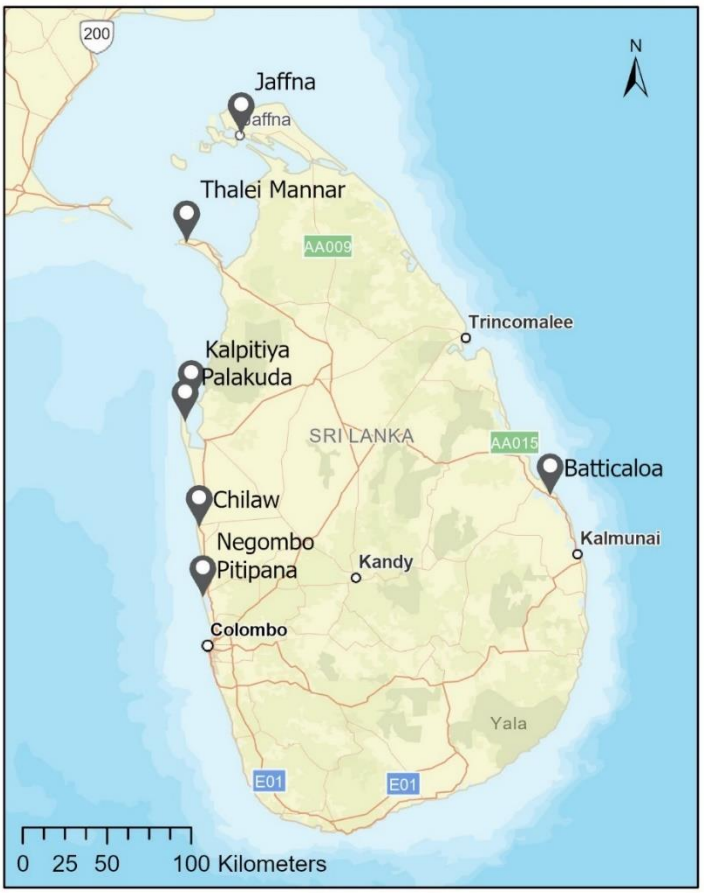


Figure 1: Major Mud crab collecting sites

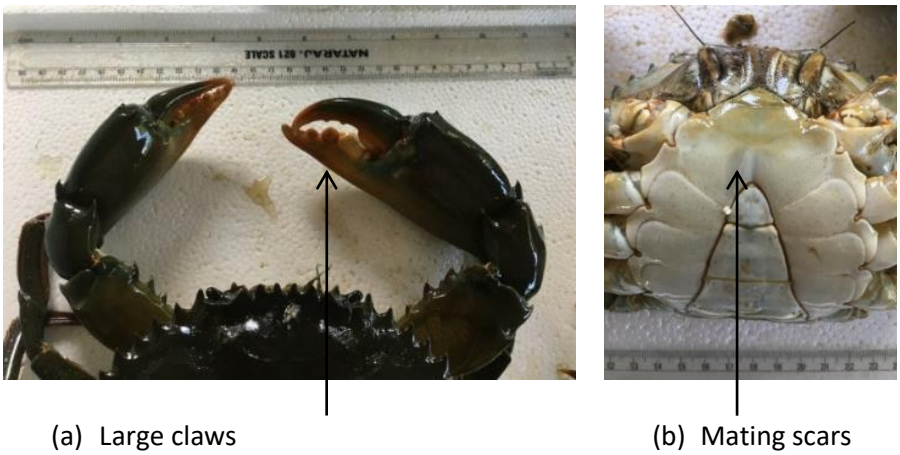
A total of 231 individuals of *S. serrata* (green mud crabs) were collected and analyzed for their biological characteristics; length-weight relationship and length at maturity. Freshly collected samples were transported to the laboratory at the Negombo Regional Research Centre of the National Aquatic Resources Research and Development Agency (NARA) by preserving on ice.

At the laboratory, individual crabs were examined for the following: Carapace Width (CW), total weight (g), and maturity stage. The length measurements were taken using a Vernier Caliper with an accuracy of up to 0.1 cm and the weight up to 0.1 g. The maturity of females was determined based on the pigmented abdominal flap (Srinivasagam *et al*., 2000). In addition, female crabs were dissected and categorized using the appearance of their ovaries (Figure 2).

(a) (b) (c)

Figure 2. Assessment of female mud crabs for sex and maturity (a) immature female with a narrow and less pigmented abdominal flap (b) mature female with a broad and heavily pigmented abdominal flap (c) body cavity of a mature female with orange color ovaries occupying full body cavity

Based on the ovarian development, three categories of females were determined as (i) Immature - Yellowish/transparent colour and absence of prominent seminal receptacle (ii) Maturing - Pink in colour occupy one third of the body cavity and (iii) Mature- Orange-red colour with prominent seminal receptacle occupying full body cavity (Srinivasagam *et al*., 2000). Unlike the females, it has been noted that size at maturity of males could not be determined using only the external characteristics such as mating scars and enlarged claws. Therefore, maturity of males was determined based on the physiological maturity where internal appearance of gonads was taken into consideration. Dissections of males were done to examine the internal appearance of reproductive organs and sexual maturity was determined as (i) Immature - Creamy/transparent colour and absence of prominent vas deferens (ii) Mature - Milky white vas deferens occupying half to full body cavity (Alberts-Hubatsch *et al*., 2016). In addition, the presence of enlarged claws and mating scars were also recorded for further confirmation (Figure 3).



(a) Large claws (b) Mating scars

(c) Milky white vas deferens

(d) Close up image of vas deference

Figure 3. Identification of mature male crabs (a) Large claws (b) Presence of mating scars (c) Milky white vas deference (c) Close up image of vas deference occupying half of body cavity

In addition to these samples, a morphologically different mud crab species that morphologically resembled the species *Scylla olivacea* (Orange mud crab), which is also a commercially important species (Keenan *et al.*, 1998) was recorded. Therefore, molecular analysis was carried out for one sample of *S. serrata* (Sample A) and a sample of the species believed to belong to *S. olivacea* (Sample B) was carried out for further confirmation of the species. DNA of the samples was extracted using the Promega Wizard genomic DNA extraction kit. PCR was carried out for this extracted DNA with universal invertebrate mitochondrial COI primers LCO1490 and HC02198. The PCR products were sequenced at Macrogen Inc., Korea and the consensus sequences were used to identify the species by using the NCBI blast service.

Data analysis

The following log regression model was used to estimate the proportion of mature *S. serrata* separately for males and females by *CW* (King, 1995):

$$P = 1/(1 + \exp(-a(CW - b)))$$

Where *P* is the predicted proportion of mature crabs at a particular *CW* and *a* and *b* are estimated parameters (McCullagh &Nelder 1989), *a* describing the shape of the curve and *b* being the inflection point where 50% of crab for that *CW* are mature, L_{50}. The negative log likelihood ($-\log_e L$) was calculated as below where *M* is the number of mature and *I* is the number of immature *S. serrata*.

$$-log_e L = [-M\, log_e P - I log_e(1 - P)]$$

Length-weight relationship of mud crabs was estimated using the following equation (Ricker, 1975).

$$W = aL^b \text{.....(1)}$$

linearized form;

$$\log W = loga + b * logL \text{.....(2)}$$

Data and statistical analyses were performed using R packages size Mat (Torrejon-Magallanes, 2016) and FSA (Ogle, 2021) in the R environment v. 4.1.2 (R Core Team, 2021).

Results

Length at maturity

The size range of females was between 7.5 - 20.3 cm while the respective values of males ranged from 6.7 - 20.6 cm. The maturity curve for females was derived based on morphological maturity (pigmented and wider appearance of the abdominal flap), while for males it was based on physiological maturity (appearance of vas deference). The estimated sizes at maturity of females and males were respectively 14.1 and 13.0 cm (Figure 4 a, b).

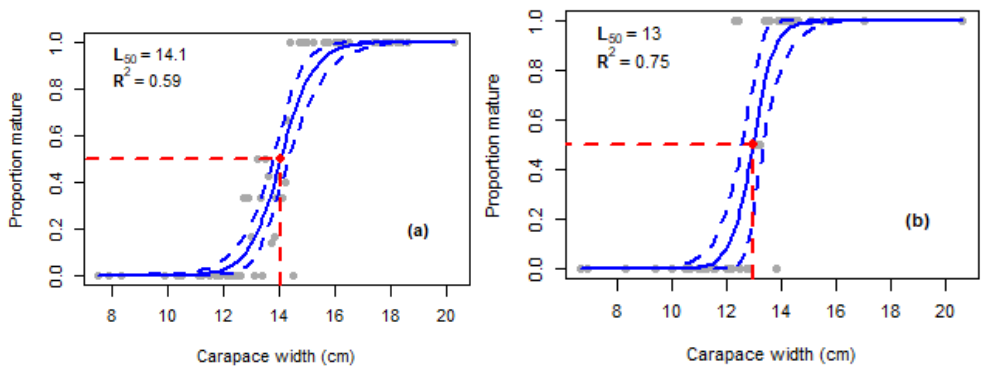


Figure 4. Estimated sizes at maturity of (a) female and (b) male mud crab

Length-weight relationship

The scatter plots between carapace width and weight of female and male mud crabs are shown in Figure 5 (a, b) and Table 1. Accordingly, a positive allometric growth (b > 3) was observed for male mud crabs, whereas a negative allometric growth (b < 3) was observed for female mud crabs. The corresponding weight for the L_{50} of the female was 426.7 g, while the respective value for the male was 367.3 g.

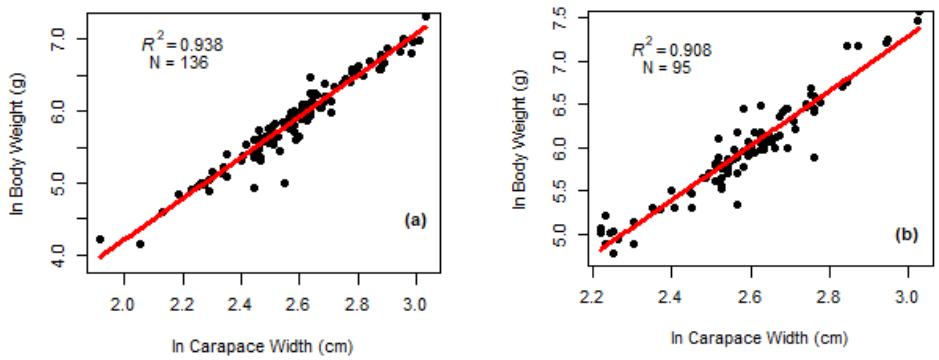


Figure 5. Length-weight relationship of (a) female and (b) male mud crab (N = number of individuals)

Table 1. Length–weight relationship and growth parameters of the mud crabs.

Sex	No. individuals	Ln *a*	Std. error *a*	*b*	Std. error *b*	R^2	T	p
Female	136	-1.5168	0.0634	2.8619	0.1642	0.9383	45.14	< 0.001
Male	95	-2.1733	0.2678	3.1506	0.1032	0.9093	30.526	< 0.001

Molecular identification

When the consensus sequences were matched with the NCBI database, sample A matched with *S. serrata* with 99.58 % percentage identity and sample B matched with *S. olivacea* with 97.33 % percentage identity. The identification confirmed that both species are occurring in Sri Lankan waters. The consensus sequences were deposited in the GenBank database and the relevant accession numbers are ON527742 and ON631787. The Sample B sequence shows 98.86 % similarity with a specimen identified from Sri Lanka in 2015, it shows 98.48 % identity with the *S. olivacea* recorded from India. This shows that the *S. olivacea* species in India and Sri Lanka are very closely related. The similarity with the Indian species shows that the 2 populations may be originating from a common ancestor.

Discussion

Lack of fundamental information on the biology and fishery of mud crabs could hinder the ability to provide precise scientific recommendations to natural stock management. In Sri Lanka, the mud crab fishery is practiced in most of the coastal areas without any size regulations for capture and therefore, it is very important to impose regulations on size at first capture.

Moreover, the maturity of *S. serrata* starts at different sizes in different geographic regions indicating the dependency on the latitudinal distribution of crabs (Hubatsch 2015). Hence, it is important to adopt the most suitable maturity value for geographical region for an effective implementation of these regulations at the national level.

Unlike females, clearly visible external morphological changes during maturity cannot be recognized in male mud crabs (Amarasekara *et al*., 2016). Further, Robertson and Kruger, (1994); Knuckey, (1996) explained that physiological maturity in males is characterized when spermatophores develop in the vas deferens after the pubertal molt, but almost similar external appearance of it is shown as adolescent/juvenile crabs. Male crabs attain physiological maturity well before they show distinct secondary sexual morphological characteristics such as allometric growth of male chelae (Hartnoll, 1974; Hall *et al.*, 2006). Once the attainment of morphological maturity, which is shown by the growth of large claws in males (Knuckey, 1996), they get the ability to successfully mate (Hall *et al*., 2006). Hall *et al*., (2006) further mentioned that, though some adolescent males have mated, functional maturity was far more prevalent in morphologically mature males.

In females, physiological maturity is reached during the last pubertal molt when the ovaries are fully developed. In contrast to males, functional maturity is often reached at the same time as physiological maturity, since successful mating can occur during the last pubertal molt when the female is soft-shelled. The abdominal flap of females is much broader than that of males and becomes heavily pigmented when the female reaches maturity. Morphological maturity is characterized by the development of a mature abdominal flap (Robertson & Kruger, 1994) and sometimes by the ability to extrude eggs (Hill, 1975). Therefore, comparing female sizes at maturity from different geographic regions can be confusing, since some authors use the shape of the abdominal flap as an indicator for maturity, whereas others define maturity by the ability to mate or extrude eggs.

The present study revealed that 50% of the female green mud crabs reach maturity at the size of 14.1 cm in Sri Lankan waters. The conversion of L_{50} of females into weight was 426.7 g. This is comparable to the 12.0 cm which was size of female mud crab at first maturity depending on ovarian development in Negombo Lagoon (Jayamanna and Jinadasa, 1993) and 12.17 cm in Koggala Lagoon based on the appearance of the abdominal flap (Amarasekera *et al*., 2016). The difference can be mainly dependent on the biological characteristic used to measure the L_{50} or the studies which were confined to one localized habitat. However, a considerable deviation ranging from 8.5 to 18.0 cm could be observed for the same parameter in different studies carried out in the world (Alberts-Hubatsch *et al*., 2016), perhaps influenced by the different latitudinal distributions (Le Vay, 2001).

No published information was available for the maturity of male mud crabs in Sri Lankan waters as the determination of maturity is difficult based on external features. Present study revealed that the 50% of male *S. serrata* reach physiological maturity at the size of 13.0 cm and a corresponding weight of 367.3 g in Sri Lankan waters. Furthermore,

the b values obtained for length weight relationship of male and female mud crabs indicated the tendency of males to be heavier than females at same age in their habitat.

The present findings will be fundamental to impose the regulations on mud crab fishery to regulate the immature catches and thereby to ensure the sustainability of the resource. Since male and female mud crabs could easily be externally distinguished using their distinct characteristic features, it is further suggested to consider females and males separately in the implementation of legal size for harvest to minimize immediate economic impacts resulting from the law enforcement.

The identification of the two species *S. serrata* and *S. olivacea* indicates that both species are present in the Sri Lankan coastal waters and further studies could be done to determine the existence of other *Scylla* species and to determine the dominant species in mud crab catches of Sri Lanka.

Conclusion

The L_{50} estimations of carapace width of *S. serrata* were 14.1 and 13.0 cm respectively for females and males while the corresponding weight for the L_{50} values of females and males were respectively 426.7 and 367.3 g. These estimations can be used as the basis for minimum legal size to be harvested. In the Sri Lankan mud crab catches, two species of *Scylla*; *S. serrata* and *S. olivacea* were confirmed by molecular analysis.

Acknowledgements

The financial and other support provided by the National Aquatic Resources Research and Development Agency (NARA); Sri Lanka is highly appreciated. Special thanks are due to P&N Fernando & Company Pvt. Ltd., Ja-Ela and to the other collecting companies for their fullest support given for data and sample collection.

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