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Reproductive biology of the three-spot swimming crab (*Portunus sanguinolentus*) from the west coast of Sri Lanka with a novel approach to determine the maturity stage of male gonads

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ABSTRACT

Aspects of the reproductive biology of *Portunus sanguinolentus* from the west coast of Sri Lanka were studied from February 2014 to January 2015. Berried females were observed throughout the year, confirming that they were continuous spawners; however, peak spawning was in October. The gonadosomatic index of females was significantly higher than the males (GLM; $p < 0.05$). Sex ratio fluctuated seasonally and a significantly higher male to female ratio was observed in October (χ^2 test). Size at first sexual maturity was estimated at 9.75 and 9.40 cm carapace width for males and females, respectively. Three types of external egg masses, stages I, II and III, were identified based on the colour of berried eggs. The mean (±SD) diameter of eggs in each stage was 253.8 ± 3.19, 281.8 ± 6.79 and 316.5 ± 9.78 µm, respectively. The estimated fecundity of *P. sanguinolentus* varied from 1.12×10^5 to 1.38×10^6. We propose a new method to identify the maturity stage of the male gonad using external characteristics. Males with mature gonads have blue colour patches on the ventral side of the chelar propodus and merus but this was not prominent in males with immature gonads and absent in females.

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KEYWORDS
Gonadosomatic Index (GSI); fecundity; chelar propodus; merus; size at first sexual maturity

Introduction

Portunus sanguinolentus, commonly known as the three-spot swimming crab, belongs to the family Portunidae. These marine crabs mainly inhabit near shore and offshore waters from South Africa to Hawaii but are common in sandy oceanic habitats to a depth of 30 m (Wenner & Williams 1972; Campbell & Fielder 1986; Apel & Spiridonov 1998; Rasheed & Mustaquim 2010).

The crab fishery in Sri Lanka has a long history and mud crabs (*Scylla serrata*) and blue swimming crabs (*Portunus pelagicus*) have been widely exploited since the beginning of this fishery. Crabs are utilized locally, and a considerable portion is exported as chilled, frozen, fresh and pasteurized crab meat (canned) to different countries including the US, EU and Japan (Sivanthan & De Croos 2012). About 260 metric tons of crabs worth about US$ 16.1 million were exported from Sri Lanka in 2014 (www.fisheriesdept.gov.lk). As in many other countries, both *S. serrata* and *P. pelagicus* in the coastal waters of Sri Lanka are showing some signs of stock depletion, including reduced volume of catches and frequent harvesting of immature or undersize individuals. To cater for the ever-increasing market demand, both locally and internationally, there is increasing exploitation of previously discarded crab species like *P. sanguinolentus* from the coastal waters of Sri Lanka. Though there is an emerging fishery for *P. sanguinolentus* in the coastal waters of Asian and Pacific regions, including Sri Lanka, no comprehensive studies have been conducted to understand the biology of these crabs, their distribution, status of the fishery and population dynamics which are crucial components for formulating fishery management decisions (Rasheed & Mustaquim 2010; Soundarapandian et al. 2013).

Information on reproductive biology plays an important role when formulating management decisions and policy advice in any fishery as spawning is the basis for recruitment (Annala & Eayrs 2010; De Croos et al. 2011). Many studies have been conducted on various aspects of the reproductive biology of crustaceans including brachyuran crabs. However, limited attempts have been made to study the reproductive biology of *P. sanguinolentus* and no such information is available in Sri Lankan waters.

This study was designed to achieve two major objectives. First, we planned to examine various aspects of the reproductive biology of *P. sanguinolentus* inhabiting the coastal waters off Negombo, Sri Lanka, to provide information to manage this fishery. To achieve this objective,

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we aimed to (i) study the development stages of male and female gonads, (ii) define the spawning seasonality, (iii) study the fecundity characteristics and (iv) estimate the size at first sexual maturity (L_{50}) for females and males.

P. sanguinolentus are sexually dimorphic (Araújo et al. 2012; Soundarapandian et al. 2013). Different methods are used to determine the gonad maturity stages of portunid crabs and size at which they attain sexual maturity. The most commonly used methods include macroscopic and microscopic observation of gonads, development of vas deferentia of male crabs, changes in carapace width and growth of chelae and pleopods in males and abdomen in females (De Lestang et al. 2003; Rasheed & Mustaquim 2010). We propose a new method to identify the maturity stage of male gonads (i.e. mature or immature) using external morphological characteristics.

Materials and methods

Study site and sample collection

Portunus sangulentus samples were collected at Negombo fish landing site (7°12′ 37.22 N, 79° 49′ 51.39 E) where more than 90% of catches of this species are landed by sail-driven shrimp trawls. On average, 90 ± 6 sail-driven shrimp trawls with a crab catch of 2 ± 1.5 kg are landed daily at this site. More than 30 unloaded trawls were sampled randomly on each sampling day, making fortnightly field visits from February 2014 to January 2015. Crab samples each with 1 kg were collected randomly from unloaded boats and these samples were packed in ice and transported to the laboratory of the University of Sri Jayewardenepura, Nugegoda, Sri Lanka, for further analysis.

Laboratory analysis

At the laboratory, the sex of *P. sanguinolentus* was determined by examining their abdominal morphology. Size and the colour of the external egg mass of berried females were recorded categorizing them into three different stages following the classification proposed by Wehrtmann (1990). Carapace width (CW) and carapace length (CL) of each specimen were measured to the nearest 0.1 cm using a measuring board and the total body weight (TW) was taken to the nearest 0.1 g. Distance between the tips of the ninth anterolateral teeth was considered as the (CW) and the distance between the frontal notch and posterior margin was considered as (CL) as defined by Rasheed and Mustaquim (2010). A total of 1087 *P. sanguinolentus* were in the collected samples of which 574 were female and 513 male.

The mean carapace width (CW), carapace length (CL) and total body weight (TW) of male and female *P. sanguinolentus* were computed and compared using the Mann–Whitney test.

Sex ratio

Male to female sex ratio was determined every month as the proportion of females to males. To assess if the calculated monthly sex ratio was significantly different from the expected ratio (1:1), a chi-square goodness of fit test (χ^2) was used.

Gonad development stages

The gonad development stages of males and females were identified based on visual examination and confirmed by histology. Five gonad development stages were identified for females and two for males following the macroscopic and histological gonad staging criteria proposed by Soundarapandian et al. (2013). Although three gonad development stages (immature, maturing and mature) have been proposed for male *P. sanguinolentus* by Soundarapandian et al. (2013), only two gonad development stages, immature and mature, were considered in this study.

For histological examination, gonads were fixed in aqueous Bouin's solution for 24 h, stored in 70% alcohol and subsequently dehydrated by increasing the alcohol concentration (70–100%) diaphonized in xylol, infiltrated and embedded in paraffin wax. Sections of 5 µm were cut using a microtome and slides with the histological sections were stained with hematoxylin and eosin (H&E).

External morphology of male and female crabs was carefully observed with respect to each gonad development stage.

Gonadosomatic Index

Crabs were dissected individually and the entire gonad was removed and measured to the nearest 0.01 g (GW). Somatic weight of each dissected crab was also measured. GSI was calculated using the following formula proposed by Sukumaran and Neelakantan (1997).

$$\text{GSI} = (\text{Gonad weight}/\text{Somatic weight}) \times 100$$

Gonadosomatic index was calculated for each individual separately and values were compared using a generalized linear model (GLM) considering sex and months as factors.

Percentage occurrence of each maturity stage of *P. sanguinolentus* female was plotted to determine gametogenic cycle. Monthly variation of percentage berried (with external egg masses) crabs with respect to female crabs with mature gonads was computed and compared.

Size at first sexual maturity (L_{50})

To obtain high precision in the size at first sexual maturity estimates, a large number of males (n = 348) and females (n = 353) were obtained from commercial catches during the peak spawning period (September and October). Males with 'mature' gonads and females with 'ripe' gonads were considered in calculating the mean length at first sexual maturity. The length at first sexual maturity was estimated for male and female crabs separately using the GLM described by King (2007).

Fecundity

The berried females with bright yellow or bright orange egg masses were selected to estimate fecundity. The carapace width of these crabs ranged from 8.7 to 16.2 cm. Pleopods bearing egg masses were removed carefully and the weight of the whole egg mass (berry) was taken to the nearest 0.1 mg. Following the gravimetric method, three subsamples (~2 mg) were taken from different locations of the egg mass, weighed to the nearest 0.1 mg and immersed in buffered Gilson's fluid (De Croos et al. 2011). Egg counts were made under a dissecting microscope and fecundity (F) was calculated according to the following formula.

$$F = \frac{\sum_{i=1}^{n} \frac{o_i}{w_i}}{n} \times W$$

where o_i – number of oocytes in a subsample, w_i – weight of the subsample, n – number of subsamples and W – total weight of the ovary.

The relationship between the carapace width and fecundity was estimated through a linear regression analysis.

Egg diameter

External appearance and the diameter of three different stages of external eggs were determined separately. A sample of 250 eggs was taken from each stage and the egg diameter was measured using an ocular micrometer scale. When eggs are round in shape, three different diameter readings were taken for an egg and mean value was calculated, but for oval-shaped eggs, long axis diameter was taken as the egg diameter. Average egg diameter (±SD) of each stage of the egg was calculated.

Statistical analysis

All the statistical analysis tests were performed using R version 2.8.1 (R Development Core Team, 2009, http://www.rproject.org). Prior to analysis, data were examined for homogeneity of variances (F test). Differences were considered significant at a probability level of 0.05.

Results

The mean (±SD) carapace width and length were greater in males (10.34 ± 1.90 cm; 4.51 ± 0.86 cm) than the females (10.30 ± 1.87 cm; 4.42 ± 0.81 cm) while the average body weight of females (60.95 ± 37.09 g) was higher than the males (59.84 ± 35.05 g). However, carapace width, carapace length, and body weight of male and female crabs were not significantly different (Mann–Whitney test, $p > 0.05$).

The regression relationship between the carapace width and body weight of *P. sanguinolentus* showed that both male (b = 2.97) and female (b = 2.98) have isometric growth.

Sex ratio

During the study period, the expected sex ratio (1:1) was recorded only in August and November. Male crabs were predominant in the catches during February, March, June, September and December while the females were dominant in the other months (Table 1). However, the calculated monthly male to female ratio was significantly different only in October.

Macroscopic and histological gonad staging

Two developmental stages of male gonads namely 'immature' and 'mature' were identified. Immature gonads are small and creamy white in colour (Figure 1(a)). They are located on either side of the stomach and testes and vas differentia are not clearly differentiated. Immature

Table 1. Monthly variation of sex ratio (male: female) and p-value (obtained from chi-square test) of *P. sanguinolentus* landed by sail-driven shrimp trawls at Negombo fish landing site, Sri Lanka, from February 2014 to January 2015.

Month	Sex ratio (male: female)	p value
February	1.1:1.0 (n = 80)	0.487
March	1.1:1.0 (n = 117)	0.486
April	0.8:1.0 (n = 92)	0.297
May	0.7:1.0 (n = 118)	0.143
June	1.1:1.0 (n = 75)	0.564
July	0.7:1.0 (n = 106)	0.153
August	1.0:1.0 (n = 85)	0.914
September	1.1:1.0 (n = 89)	0.635
October	0.5:1.0 (n = 82)	0.015*
November	1.0:1.0 (n = 86)	0.524
December	1.1:1.0 (n = 76)	0.617
January	1.0:1.1 (n = 81)	0.522

Notes: Number of crabs (n) used to estimate sex ratio in each month is given within brackets.
*Significant difference at a level of 95%; $p < 0.05$.

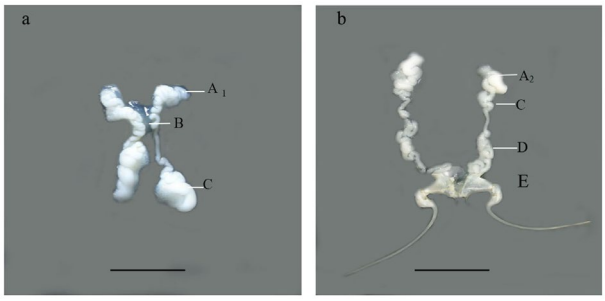

Figure 1. Macroscopic appearance of maturity stages of *P. sanguinolentus* male gonads a: Immature (macroscopic); b: Mature (microscopic). A1, immature testis; A2, mature testis; B, commissure; C, anterior vas deferens; D, posterior vas deferens; and E, ejaculatory duct.
Note: Scale bars: 1 cm.

gonads mainly contain spermatogonia and primary spermatocytes.

In mature gonads, testes are large; vas-differentia are coiled and swollen. They occupy a larger area in the body cavity than the immature gonads (Figure 1(b)). Spermatozoa are dominant in mature gonads and spermatids are also present.

Five developmental stages of female gonads, i.e. immature, early maturing, late maturing, ripe and spent, were identified during this study. Immature ovaries are small, flattened and have a ribbon-like appearance. They are white or whitish yellow in colour and occupy a small area of the body cavity (Figure 2(a)). In this stage, ovaries are difficult to recognize from the rest of the tissues. Early maturing ovaries are larger than immature ovaries and their colour ranges from yellow to orange. These ovaries do not extend to the hepatopancreatic region (Figure 2(b)). There are fewer perinucleolar oocytes in these ovaries and yolkless oocytes appeared to be more abundant. Late maturing ovaries are large and yellow to orange in colour. These ovaries extend to the hepatopancreatic region (Figure 2(c)). A small number of perinucleolar oocytes are present, but yolky oocytes appeared to be more abundant and yolk granules are predominant in the cytoplasmic region. Ripe ovaries are very large and they are bright yellow in colour. They occupy a larger area of the body cavity and they are highly nodulated (Figure 2(d)). Perinucleolar oocytes are present in small numbers. Each oocyte is surrounded by cortical bodies. Spent ovaries are pinkish white in colour; they are not clearly visible (Figure 2(e)) and contain a few irregularly shaped oocytes.

Fluctuations of percentage berried females and characteristics of external egg masses

At the time of spawning, the eggs are extruded through their gonopores and attached to the cluster of long and very smooth setae on endopodites of the pleopods. Based on the colour, external egg masses were categorized into stage I, stage II and stage III. Stage I egg masses are pale yellow to deep yellow in colour while stage II egg masses are yellow to gray. The colour of stage III egg masses ranges from grey to black.

There are slight differences in the appearance of eggs in each stage. Stage I eggs are spherical in shape and eye spots are not visible in the eggs. The eggs in stage II are also spherical in shape and eye spots are present. Stage III eggs are oval in shape and eyespots and chromatophores are clearly visible inside the egg. Average diameters (±SD) of stage I, stage II and stage III eggs were 253.8 ± 3.19, 281.8 ± 6.79, and 316.5 ± 9.78 µm, respectively.

Egg-bearing females were observed throughout the study period. The highest percentage of ovigerous females with respect to females with mature gonads was observed in October and the lowest in November (Figure 3).

Gonadosomatic Index and reproductive cycle

There were significant variations in GSI with respect to sex and time, and the GSI of females was significantly higher than the males (GLM, $p < 0.05$, df = 1). The estimated GSI of males ranged from 0.02 to 3.69 with a mean (±SD) value of 0.75 ± 0.58 ($n = 574$). In females, this value ranged from 0.12 to 9.44 (3.29 ± 2.13, $n = 513$).

In both sexes, GSI showed a seasonal pattern. In males, GSI increased from February to August with small peaks in March and May. The highest GSI for males was in August and thereafter, GSI declined. In females, GSI values increased from February to July and declined in August. GSI increased again with the highest values in October, declining thereafter (Figure 4). Furthermore, GSI increased with increasing carapace width. The highest GSI value for females was recorded in the carapace width range of 11.0–11.9 cm while for males, it was in the 12.0–12.9 cm range (Table 2).

Percentage maturity stage of *P. sanguinolentus* females collected in each month was plotted and results showed that they were sexually active throughout the year. Individuals with 'Ripe gonads' were evident throughout the study period with a peak in October. The highest percentage of females with 'immature' gonads was observed in May and individuals with 'late maturing gonads' reached a peak in April followed by March. Spent females were dominant in the catches from August to November, though they were present in the other months too (Figure 5).

Size at first sexual maturity (L_{50})

The estimated size at first sexual maturity of *P. sanguinolentus* males was 9.75 cm CW while females attained sexually mature at 9.40 cm (Figure 6).

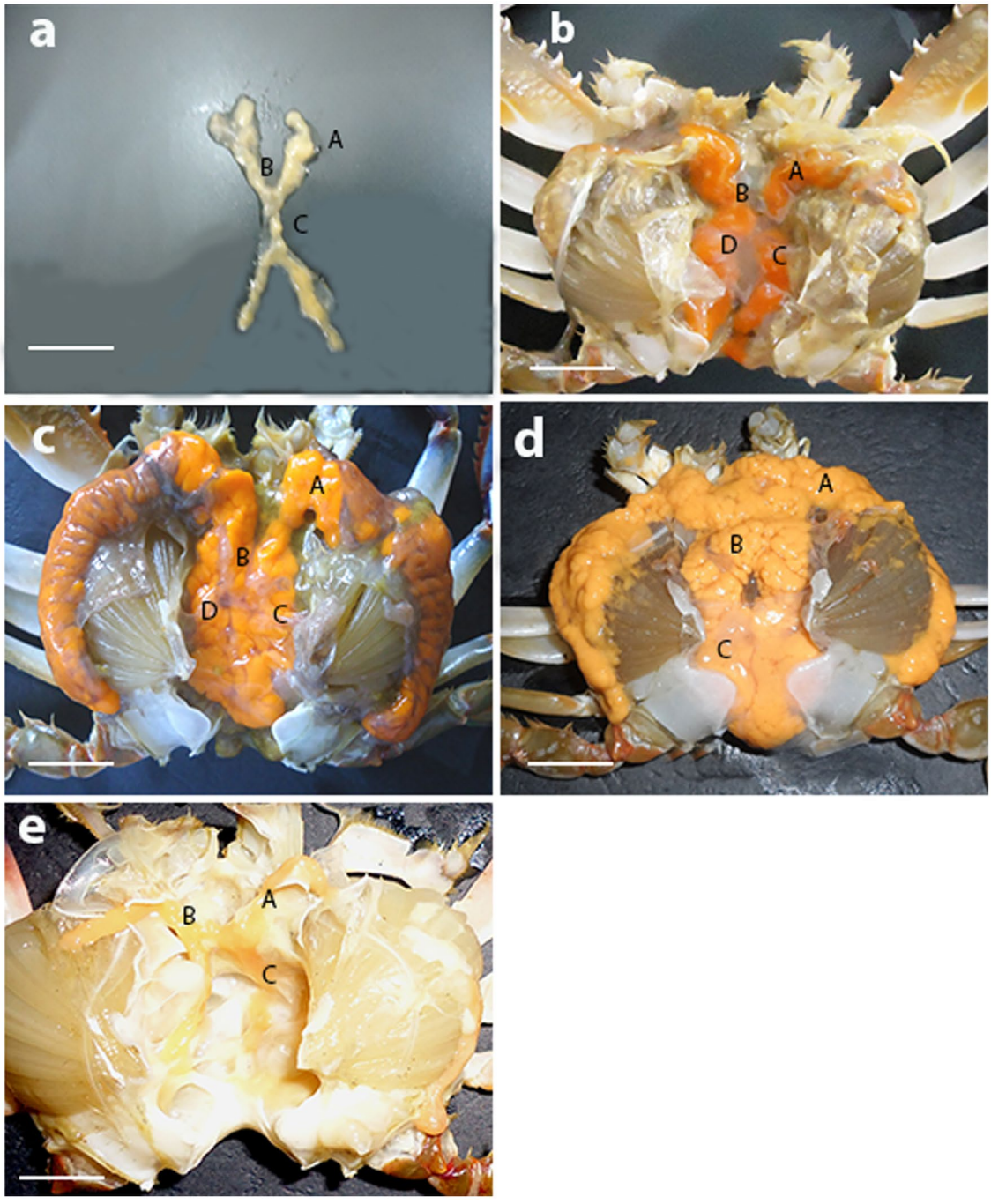

Figure 2. Macroscopic appearance of maturity stages of *P. sanguinolentus* female gonads (a) immature; (b) early maturing; (c) late maturing; (d) ripe; and (e) spent. A, anterior horn; B, commissure; C, posterior horn; and D, spermatheca.
Note: Scale bars = 1 cm.

Fecundity characteristics

During the study period, 46 egg masses were analysed to estimate fecundity. The estimated fecundity ranged from 1.12×10^5 to 1.38×10^6 with a mean (±SD) of $6.48 \times 10^5 \pm 1.96 \times 10^5$. Fecundity of *P. sanguinolentus* increased with increasing carapace width (Figure 7).

New method to determine maturity stages of P. sanguinolentus *male*

It was observed that there were some colour changes in the chela propodus and merus of *P. sanguinolentus* males with respect to their gonad maturity stage (n = 513). A blue colour patch was observed on the ventral side of the

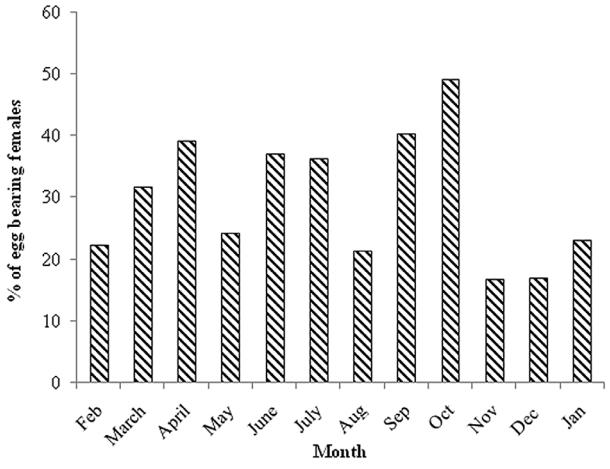

Figure 3. Monthly variation of percentage egg-bearing *P. sanguinolentus* with respect to female crabs with mature gonads in the coastal waters off Negombo, Sri Lanka, from February 2014 to January 2015.

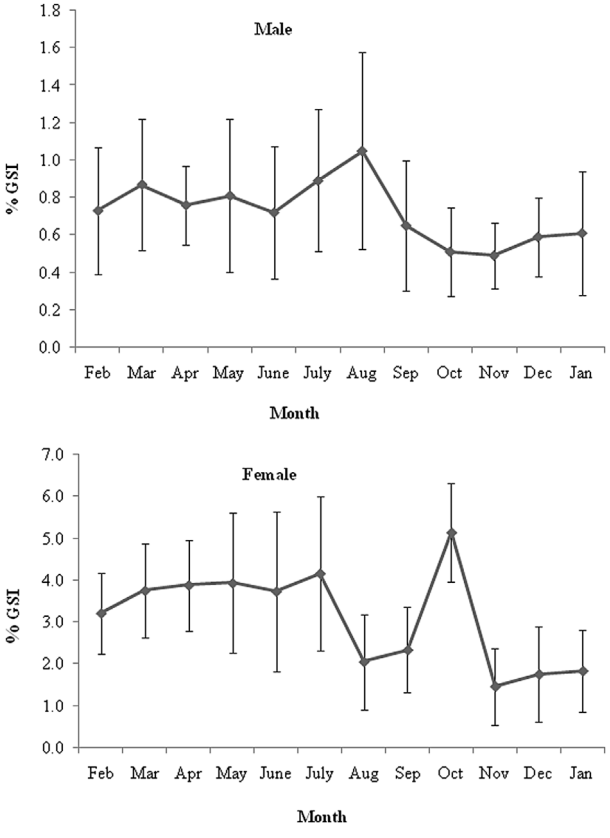

Figure 4. Monthly variation of % GSI of male and female *P. sanguinolentus* in the coastal waters off Negombo, Sri Lanka, from February 2014 to January 2015.

chela propodus and merus of males with mature gonads, but this colour pattern was not prominent and appeared as blue dots in males with immature gonads. Furthermore, this colour pattern was absent in female crabs (Figure 8).

The carapace width of crabs with a prominent blue colour patch (with mature gonads) ranged from 8.6 to 16.5 cm. While for the crabs with blue colour dots (immature gonad), carapace width was from 3.1 to 10.3 cm. We did not observe any male crabs with blue colour dots above a carapace width of 10.3 cm. It was further noted that when a blue colour patch appears, it does not disappear but the colour intensity seems to increase with increasing carapace width of males.

Apart from identifying the gonad maturity stages based on the colour pattern of the chela propodus and merus of *P. sanguinolentus* males, there is the possibility of using this colour change to determine whether male crabs are sexually mature or not. Therefore, male crabs with blue dots and a dominant blue patch on the ventral side of the chela propodus and merus can be considered sexually immature and mature individuals, respectively.

Discussion

P. sanguinolentus are widely exploited using trawl gear, bottom set gill nets, traps and pots in many parts of the world (Dineshbabu et al. 2007; Pillai & Thirumilu 2012). In Sri Lanka, they are mainly taken as a by-catch of the sail-driven shrimp trawlers and occasionally from the bottom set gill nets operated for rock fish and rays.

Though previous studies have reported that *P. sanguinolentus* males are heavier than females (Sukumaran & Neelakantan 1997; Dineshbabu et al. 2007), this study confirmed that the average body weight of males and females was not significantly different. Furthermore, the findings of this study revealed that *P. sanguinolentus* has isometric growth which is similar to observations of previous studies (Dineshbabu et al. 2007; Rasheed & Mustaquim 2010; Pillai & Thirumilu 2012).

The observed differences in the expected sex ratio could be owing to behavioural changes and local migration patterns. According to Carpenter et al. (1997), there are differences in habitat preference of mature males and females. Females seem to be more abundant in deeper water than males (Lee & Hsu 2003). However, the exact reason for the presence of significantly high numbers of females in commercial catches during the peak spawning period is not clear.

Berried females were found throughout the study period and similar observations have been reported in the coastal waters of India (Pillai & Nair 1971; Varadharajan et al. 2009; Rasheed & Mustaquim 2010; Pillai & Thirumilu 2012). In our study, the highest percentage of berried females as well as the highest GSI for females was observed in October. These findings confirm that *P. sanguinolentus* reproduce throughout the year in the coastal waters of Sri Lanka having a peak spawning in October. Rasheed

Table 2. Mean body weight ((±SD), mean gonad weight (±SD) and percentage Gonado somatic Index (%GSI) (±SD) of *P. sanguinolentus* females and males collected from the shrimp fishing ground off Negombo, Sri Lanka, from February 2014 to January 2015.

	Females			Males		
Carapace width (cm)	Mean body weight (g)	Mean gonad weight (g)	% GSI	Mean body weight (g)	Mean gonad weight (g)	% GSI
5.0–5.9 (*n* = 11.0)	22.32 ± 3.45	0.08 ± 0.23	0.36 ± 1.01	NA	NA	NA
6.0–6.9 (*n* = 78.61)	26.55 ± 2.43	0.39 ± 0.13	1.48 ± 1.21	16.12 ± 2.33	0.10 ± 0.01	0.61 ± 0.14
7.0–7.9 (*n* = 39.47)	28.24 ± 4.78	0.46 ± 0.18	1.67 ± 0.98	21.18 ± 2.69	0.14 ± 0.04	0.63 ± 0.28
8.0–8.9 (*n* = 57.63)	31.40 ± 15.40	0.58 ± 0.32	2.08 ± 1.36	30.18 ± 5.49	0.20 ± 0.34	0.65 ± 0.94
9.0–9.9 (*n* = 65.67)	41.94 ± 13.77	1.10 ± 0.76	2.84 ± 2.02	38.57 ± 8.53	0.28 ± 0.28	0.69 ± 0.75
10.0–10.9 (*n* = 99.72)	54.26 ± 9.88	1.83 ± 1.19	3.61 ± 2.3	55.83 ± 8.23	0.34 ± 0.21	0.67 ± 0.36
11.0–11.9 (*n* = 91.70)	74.83 ± 13.51	3.05 ± 1.31	4.49 ± 2.54	71.57 ± 11.61	0.46 ± 0.17	0.66 ± 0.26
12.0–12.9 (*n* = 77.72)	89.91 ± 29.49	3.37 ± 3.37	4.34 ± 3.95	86.43 ± 12.88	0.92 ± 0.70	1.07 ± 0.71
13.0–13.9 (*n* = 47.55)	113.38 ± 22.54	4.34 ± 4.24	4.21 ± 3.71	127.06 ± 16.2	0.93 ± 0.26	0.75 ± 0.23
14.0–14.9 (*n* = 1.1)	153.3	2.28	1.49	145.7	1.04	0.71
15.0–15.9 (*n* = 0.4)	NA	NA	NA	159.94 ± 35.34	0.93 ± 0.47	0.60 ± 0.16
16.0–16.9 (*n* = 5.1)	210.5 ± 12.73	3.07 ± 1.94	1.47 ± 1.68	167.04	1.18	0.71

Notes: Symbol NA indicates that data are not available and *n* within brackets indicates the number of females and males measured under each carapace width category, respectively.

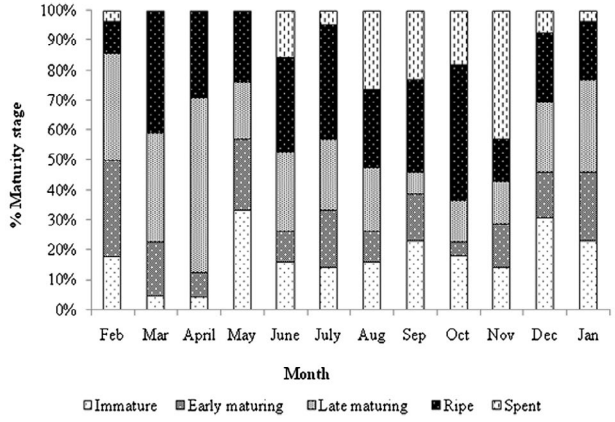

Figure 5. Gametogenic cycle of *P. sanguinolentus* females inhabiting the coastal waters off Negombo, Sri Lanka, from February 2014 to January 2015.

and Mustaquim (2010) reported a similar reproductive pattern for *P. sanguinolentus* in the coastal waters of Karachi, Pakistan. Some other studies reported that egg-bearing females occur throughout the year in the coastal waters of India with peaks during August, January and March along the Parangipettai coast (Soundarapandian et al. 2013), November to March along the south-west coast (Hines 1989), and December to May and July to August from the Calicut coast (Fisher 1999). Though the exact reason for differences in peak spawing period of *P. sanguinolentus* inhabiting slightly different geographical regions is not clear, it may be because of changes in water conditions associated with the monsoon pattern. The role of environmental factors in controlling gametogenesis and spawning of brachyuran crabs has been well documented. Temperature, salinity, food availability, rainfall, lunar pattern and photoperiod have been considered as the major environmental factors that influence the spawning intensity and spawning periodicity of crabs (Pillai & Nair 1971). However, previous studies proposed that brachyuran crabs inhabiting tropical waters usually breed throughout the year (Emmerson 1994; Saradha 1998) and the results of the present study support these findings.

In our study, the highest GSI for males was recorded in August and for females, it was in October. This suggests that male and female crabs do not release their gametes synchronously. Dinakaran and Soundarapandian (2009) have reported that male crabs store their sperms inside females body for a few months prior to spawning. We suggest, therefore, that peak mating of *P. sanguinolentus* occurs in August in the coastal waters of Negombo, resulting in peak spawning in October.

We found that the colour differences in external egg masses were associated with the different developmental levels of eggs as stated by Soundarapandian et al. (2013). Furthermore, the reported mean egg diameters for eggs in stages I, II and III were similar to the values reported by Soundarapandian et al. (2013).

According to Reeby et al. (1990), male *P. sanguinolentus* attain sexual maturity at 81–85 mm (LCW). However, Sumpton et al. (1989) reported that male and female *P. sanguinolentus* inhabiting in the coastal waters of Queensland, Australia, attain sexual maturity at 83 and 74 mm (LCW), respectively. We found that males attained sexual maturity at 97.5 mm (CW) while the size at first sexual maturity of female was 94 mm (CW). The exact reason/s for these differences is not clear. However, previous studies have shown that the size at first sexual maturity of crabs may vary with moult increment, the number of moults (Hines 1989; Rasheed & Mustaquim 2010) and environmental factors such as temperature, salinity and rainfall (Fisher 1999). Sumpton et al. (1989) showed that *P. sanguinolentus* females mature earlier than the males in Queensland waters and our study confirms that like in many other portunids, maturity occurs at the same age for both sexes.

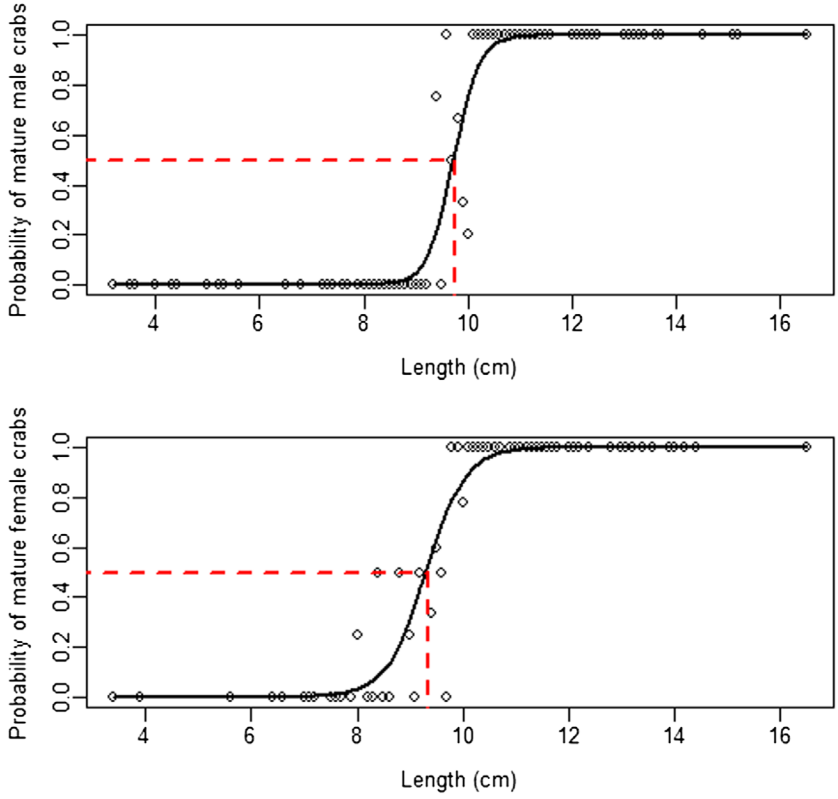

Figure 6. Size at first sexual maturity (L_{50}) of a *P. sanguinolentus* male and female in the coastal waters off Negombo, Sri Lanka.

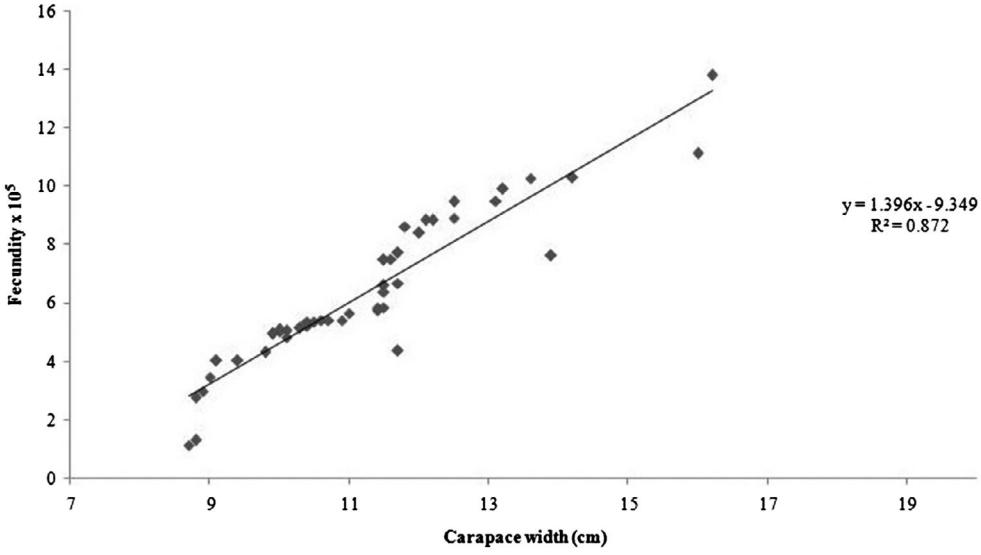

Figure 7. Relationship between carapace width (CW) and fecundity of *P. sanguinolentus* ($n = 46$).

Information on fecundity is very important to manage crab fisheries as it is important to evaluate the reproductive potential of spawning stock biomass as well as commercial potential of crab stocks (Lee & Hsu 2003). According to Dhas et al. (1980), fecundity of *P. sanguinolentus* ranged between 961,000 and 2250,000 eggs in Mangalore while in Karachi, Karwar, Northern Taiwan and Tamil Nadu, India, these values ranged between 225,649 to 524,456 eggs; 158,608 to 712,526 eggs, 410,000 to 2440,000 and 283,963 to 967,293 eggs (Reeby et al. 1990; Kailola et al. 1993; Kumar et al. 2003; Soundarapandian et al. 2013). In the present study, the estimated fecundity ranged between 112,017 and 1380,223. This discrepancy in fecundity of *P. sanguinolentus* may be owing to several

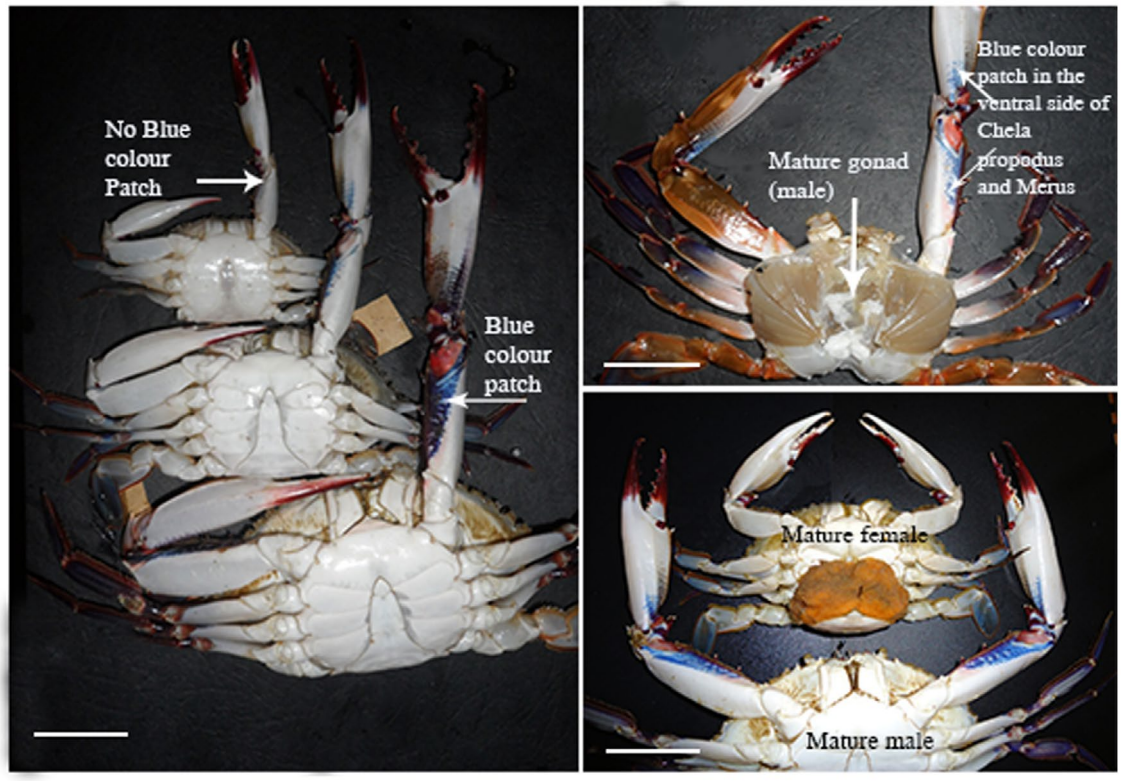

Figure 8. Identification of maturity stage of *P. sanguinolentus* male gonads using external morphological characteristics: (a) variation of colour pattern in the ventral side of chela propodus and merus of a mature and immature *P. sanguinolentus* male; (b) mature *P. sanguinolentus* male with mature gonad and a blue colour patch on the ventral side of chela propodus and merus; and (c) mature male with a blue colour patch on the ventral side of chela propodus and merus and egg-bearing female without this colour patch).
Note: Scale bars = 1 cm.

reasons, including variations in geographical range, habitat structure, crab body size and food availability. Loss of eggs during the incubation period could be another possible reason for this observation as most of the time crabs were obtained from commercial catches to investigate the fecundity (Sastry 1983; Shields 1991). Variations of fecundity with respect to body size are very common in crabs (Sukumaran et al. 1986; Rasheed & Mustaquim 2010; Safaie et al. 2013; Sundarapandian et al. 2013) and this study also showed an increase in fecundity with increasing carapace width. Morphometric parameters such as carapace width, abdominal width and body depth are considered good indicators to give an idea about the reproductive potential of crabs. However, there may be substantial variations in fecundity throughout the year (Sondarapandian et al. 2013).

Morphometric and histological methods are widely used to determine the gonad maturity stages of male crabs (Islam & Hisashi 2012). These methods are time-consuming and need expert assistance. This study revealed a new method to identify the maturity stage of the male gonad using external morphological characteristics and the proposed method is very simple and can be used by a layman. However, as proposed by De Lestang et al. (2003), indirect approaches may not be very precise as any conclusion is based on limited data collected from a specific geographical location. Therefore, further research on this aspect would be worthwhile in the future, covering a wider size range of crabs and different geographical locations with dissimilar habitats. However, the proposed new method would be a milestone in the reproductive biology of *P. sanguinolentus* and it will be easily applied in the field to identify mature and immature *P. sanguinolentus* males.

By considering the estimated size at first sexual maturity (L_{50}) of *P. sanguinolentus*, it is recommend to implement a minimum landing size limit of 9.8 cm for this species to utilize this resource sustainably in the coastal waters of Sri Lanka. As there is an emerging fishery for *P. sanguinolentus* in many parts of the world, information generated through this study will be useful to manage and utilize the resources in a sustainable manner.

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References

Annala J, Eayrs S. 2010. Research priorities for marine fisheries conservation and management. In: Grafton RQ, Hilborn R, Squires D, Tait M, Williams M, editors. Handbook of marine fisheries conservation and management. Oxford: Oxford University Press; p. 714.

Apel M, Spiridonov VS. 1998. Taxonomy and zoogeography of the portunid crabs (Crustacea: Decapoda: Brachyura: Portunidae) of the Arabian Gulf and adjacent waters. Fauna of Arabia. 17: 159–331.

Araújo MSLC, Negromonte AO, Barreto AV. 2012. Sexual maturity of the swimming crab *Callinectes danae* (Crustacea: Portunidae) at the Santa Cruz Channel, a tropical coastal environment. Journal of the Marine Biological Association of the United Kingdom. 92: 287–293.

Campbell GR, Fielder DR. 1986. Size at sexual maturity and occurrence of ovigerous females in three species of commercially exploited portunid crabs in S.E. Queensland. Proceedings of Royal Society of Queensland. 97: 79–87.

Carpenter KE, Jones KF, Zajonz U. 1997. FAO species identification field guide for fishery purpose. The living marine resources of Kuwait, Eastern Saudi Arabia, Bahrain, Qatar, and the United Arab Emirates. FAO Report. 7: 1–293.

De Croos MDST, Pálsson S, Thilakarathna RMGN. 2011. Sex ratios, sexual maturity, fecundity, and spawning seasonality of *Metapenaeus dobsoni* off the western coastal waters of Sri Lanka. Invertebrate Reproduction & Development. 55: 110–123.

De Lestang S, Hall NG, Potter IC. 2003. Reproductive biology of the blue swimmer crab, *Portunus pelagicus* (Decapoda: Portunidae) in five water bodies on the west coast of Australia. Fishery Bulletin. 101: 745–757.

Dhas MA, Subramoniam T, Varadarajan S, Govindharajulu P. 1980. Germinal zone activity and oocyte differentiation in the marine crab *Portunus pelagicus*. Indian Journal of Marine Science. 46: 287–292.

Dinakaran GK, Soundarapandian P. 2009. Mating behaviour and broodstock development of commercially important blue swimming crab, *Portunus sanguinolentus* (Herbst). Indian Journal of Science and Technology. 2: 71–75.

Dineshbabu AP, Sreedhara B, Muniyappa Y. 2007. Fishery and stock assessment of *Portunus sanguinolentus* (Herbst) from south Karnataka coast, India. Journal of the Marine Biological Association of India. 49: 134–140.

Emmerson WD. 1994. Seasonal breeding cycles and sex ratios of eight species of crabs from Mgazana, a mangrove estuary in Transkei. South African Journal of Zoology. 14: 158–168.

Fisher MR. 1999. Effect of temperature and salinity on size at maturity of female blue crabs. Transactions of the American Fisheries Society. 128: 499–506.

Hines AH. 1989. Allometric constraints and variables of reproductive effort in brachyuran crabs. Marine Biology. 69: 309–320.

Islam S, Hisashi K. 2012. Male reproductive biology of mud crab *Scylla olivacea* in a tropical mangrove swamps. Journal of Fisheries & Aquatic Science. 7: 194–199.

Kailola PJ, Williams MJ, Stewart PC, Riechelt RE, McNee A. 1993. Australian fisheries resources. Canberra: Bureau of Resource Sciences; p. 422.

King M. 2007. Fisheries biology, assessment and management. Fishing new books. Oxford: Wiley; p. 70.

Kumar A, Sundaramoorthy B, Jakhar JK. 2003. Standardization of crab bottom set gillnet for reduction of bycatch at Thoothukudi coast, Tamilnadu. India Archives of Applied Science Research. 5: 74–81.

Lee HH, Hsu CC. 2003. Population biology of the swimming crab *Portunus Sanguinolentus* in the waters off Northern Taiwan. Journal of Crustacean Biology. 23: 691–699.

Pillai KK, Nair NB. 1971. The annual reproductive cycles of *Uca annulipes*, *Portunus pelagicus* and *Metapenaeus affinis* (Decapoda, Crustacea) from the southwest coast of India. Marine Biology. 11: 152–166.

Pillai SL, Thirumilu P. 2012. Fishery, biology and yield estimates of *Portunus sanguinolentus* off Chennai. Journal of the Marine Biological Association of India. 54: 73–76.

Rasheed S, Mustaquim J. 2010. Size at sexual maturity, breeding season and fecundity of three-spot swimming crab *Portunus sanguinolentus* (Herbst, 1783) (Decapoda, Brachyura, Portunidae) occurring in the coastal waters of Karachi, Pakistan. Fisheries Research. 103: 56–62.

Reeby J, Prasad PN, Kusuma N. 1990. Fecundity of *Portunus* species from Karwar Waters. Journal of Fisheries Science and Technology. 27: 153–154.

Safaie M, Pazooki J, Kiabi B, Shokri MR. 2013. Reproductive biology of blue swimming crab, *Portunus segnis* (Forskal, 1775) in coastal waters of Persian Gulf and Oman Sea, Iran. Iranian Journal of Fisheries Sciences. 12: 430–444.

Saradha PT. 1998. Crab fishery of the Calicut coast with some aspects of the population characteristics of *Portunus sanguinolentus*, *P. pelagicus* and *Charybdis cruciata*. Indian Journal of Fisheries. 45: 375–386.

Sastry AN. 1983. Ecological aspects of reproduction. In: Vernberg FJ, Vernberg W, editors. *The Biology of Crustacea*. 7: 179–254

Shields JD. 1991. The reproductive ecology and fecundity of Cancer crabs. In: Wenner AM, Kuris A, editors. Crustacean egg production. Rotterdam: Balkema press; p. 193–213.

Sivanthan S, De Croos MDST. 2012. Market chain assessment of the Blue swimming crab industry in Jaffna, Sri Lanka. 1st Ruhuna International Science & Technology Conference; Jaffna, Sri Lanka. p.15.

Soundarapandian P, Varadharajan D, Boopathi A. 2013. Reproductive biology of the commercially important portunid crab, *Portunus sanguinolentus* (Herbst). Journal of Marine Science: Research & Development. 3: 1–9.

Sukumaran KK, Neelakantan B. 1997. Age and growth in two marine portunid crabs, *Portunus (Portunus) sanguinolentus* (Herbst) and *Portunus (Portunus) pelagicus* (Linnaeus) along the south-west coast of India. Indian Journal of Fisheries. 44: 111–131.

Sukumaran KK, Telang KY, Thippeswamy O. 1986. Fishery and biology of the crab *Portunus sanguinolentus* (Herbst) along the South Kanara coast. Indian Journal of Fisheries. 33: 188–200.

Sumpton WD, Smith GS, Potter MA. 1989. Notes on the biology of the portunid crabs, *Portunus sanguinolentus* (Herbst) in subtropical Queensland waters. Australian Journal of Marine and Freshwater Research. 40: 711–717.

Varadharajan D, Soundarapandian P, Dinakaran GK, Vijakumar G. 2009. Crab fishery resources from Arukkattuthurai to Aiyammpattinam, South east coast of India. Current Research Journal of Biological Sciences. 1: 118–122.

Wehrtmann IS. 1990. Distribution and reproduction of *Ambidexter panamense* and *Palaemonetes schmitti* in Pacific Costa Rica (Crustacea, Decapoda). Revista de Biologıa Tropical. 3: 327–329.

Wenner E, Williams A. 1972. Shrimps, lobsters, and crabs of the Atlantic Coast of the Eastern United States, Maine to Florida. Estuaries. 8: 68–77.