



## Morphometric allometry of horseshoe crab, *Tachypleus gigas* at west part of Sarawak waters, Borneo, East Malaysia

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**Abstract.** A study was conducted at west part of Sarawak waters in Borneo, Malaysia to compare the morphometric allometry of coastal horseshoe crab, *Tachypleus gigas* obtained from two different locations, Pasir Putih Village and Gerigat Beach. The specimens were collected by using hand manually and monofilament gill netting during March 2014 to April 2014. All measurements of body parameters of male and female *T. gigas* from Gerigat Beach were recorded higher compared to specimens from Pasir Putih Village. All body parameters between male and female *T. gigas* from both locations showed significant different when  $p < 0.05$ . Allometric analysis was used to compare BW-TL/CW of *T. gigas* in log transform by using Pearson correlation and regression analysis. Male and female *T. gigas* from both locations revealed negative allometric growth for all body parameter relationships since 'b' value was less than 3 excluding BW-CW and TEL-TL of female from Gerigat Beach. Two way ANCOVA analysis indicated significant differences in BW-CW and BL-TL relationships of both sexes from two locations. The dissimilar size of *T. gigas* from different locations in west Sarawak waters is probably due to two discrete populations.

**Key Words:** *Tachypleus gigas*, width-weight relationship, length-weight relationship, horseshoe crab, Borneo).

**Introduction.** Horseshoe crab is known as the "living fossil" after the declaration that their external morphology only slightly changed since the Cambrian period (Rudkin et al 2008). In the current era, only three out of the four species of the horseshoe crab can be obtained in the Asian waters, namely *Tachypleus tridentatus*, *Tachypleus gigas* and *Carcinoscorpius rotundicauda* (Sekiguchi & Nakamura 1979; Chiu & Morton 2003; Faridah et al 2015). It was also found that horseshoe crab is actually highly similar to spider and scorpion, although the name falls under the family of crab (Hickman et al 2007).

The major physical characteristics of the four species types of horseshoe crab demonstrated certain degree of similarities among each other. Therefore, the effective method to describe the differences among closely related species is by conducting a morphometric study (Hussain et al 2009; Sriyaya et al 2010). According to Huxley & Tessier (1936), allometry analysis can be carried out for morphometric analysis to describe the changes of size, shape, and relationship between different body parameters belonged to the same organism. In more detail, an allometric correlation enhances the knowledge of morphometric variations within in the population origin (Chatterji et al 1988). Previous studies showed that the variation of horseshoe crab size was influenced by the changes in habitat, *in-situ* physico-chemicals parameters, diets, stage of maturity, and genetic (Krumholz & Cavanah 1968; Gaspar et al 2002; Graham et al 2009; Shuster & Sekiguchi 2009).

The aim of this study is to determine the morphometric variations of *T. gigas* in term of different habitats, Pasir Putih Village and Gerigat Beach and to highlight the effects of different ecological habitat to the growth of *T. gigas*.

## Material and Method

**Sampling sites and sample collection.** In this study, a total of 125 *T. gigas* specimens were collected in Pasir Putih Village (N 01°39.490' E 110°28.300') and Gerigat Beach (N 02°02.277' E 111°11.305') with the aid from local fisherman. Pasir Putih Village is a sandy beach with replanting mangrove trees area and Gerigat Beach is a sandy beach as shown in Figure 1. The specimens were collected manually by using hand and using a 20.0 × 1.5 m net size; 5.08 cm mesh size monofilament gill net during March 2014 to April 2014.

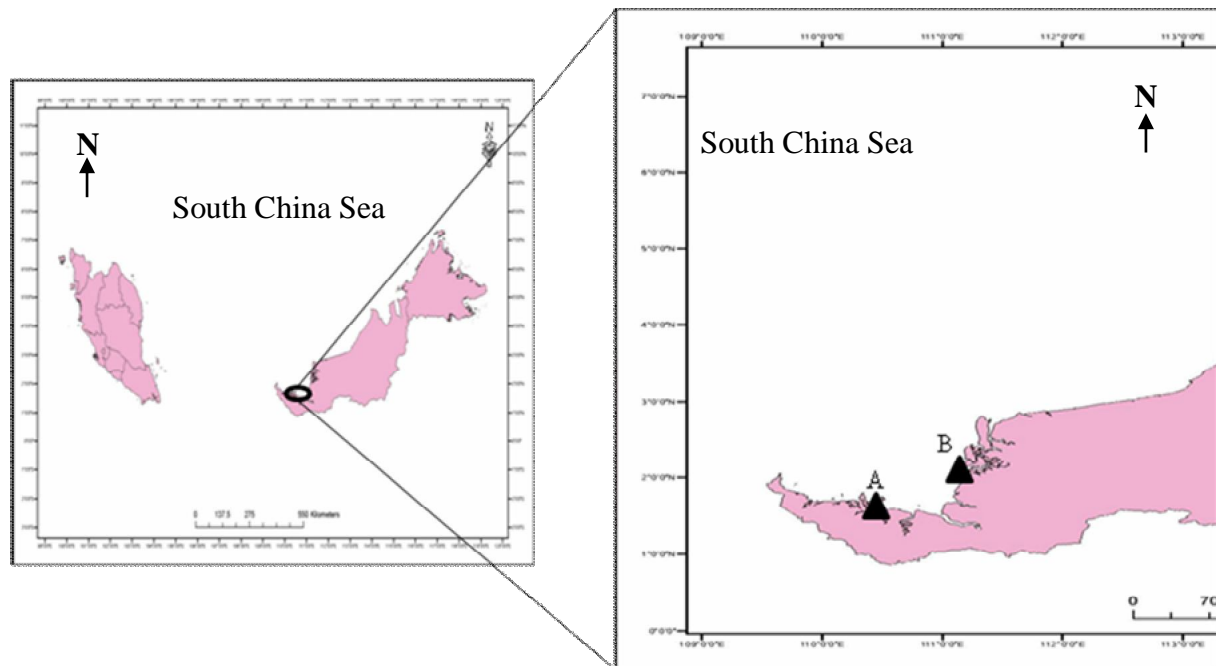


Figure 1. A map of Malaysia showing the locations of Pasir Putih Village and Gerigat Beach at west part of Sarawak waters. ▲ indicated the locations of the sampling site, (A) Pasir Putih Village and (B) Gerigat Beach.

**Morphometric measurement.** Body measurement parameters such as total length (TL), carapace width (CW), telson length (TEL) and body weight (BW) were measured for each individual of horseshoe crab (Figure 2). Total length was measured by using measuring board (Wildco), while carapace width and telson length were measured to the nearest mm by using a digital vernier caliper (Mitutoyo) to the nearest cm. The weight of each horseshoe crab was weighted to the nearest 0.1 g by using digital balance (Adventurer ARA 520).

**Allometric analysis.** The allometric analysis was conducted according to the methodology proposed by Le Cren (1951) and Chatterji (1976). The body weight to carapace width (BW-CW) and body weight to total length (BW-TL) measurements in log transformed of different sexes and between different locations were determine and expressed by using an exponential equation;  $y = bx^a$  in logarithmic form.

**Statistical analysis.** The regression analysis was performed by using Microsoft Excel version 2007 in order to discover the dissimilarity of the association degrees within different body parts, body weight-total length (BW-TL), carapace width-total length (CW-TL), telson length-total length (TEL-TL) and body weight-carapace width (BW-CW) of the horseshoe crabs (Chatterji et al 1988; Srijaya et al 2010). Apart from that, ANCOVA analysis from SPSS software version 21.0 was used to test significant differences between BW-CW and BW-TL relationships of male and female *T. gigas*. Independent samples *T*-test also applied to test differences between male and female from both locations.

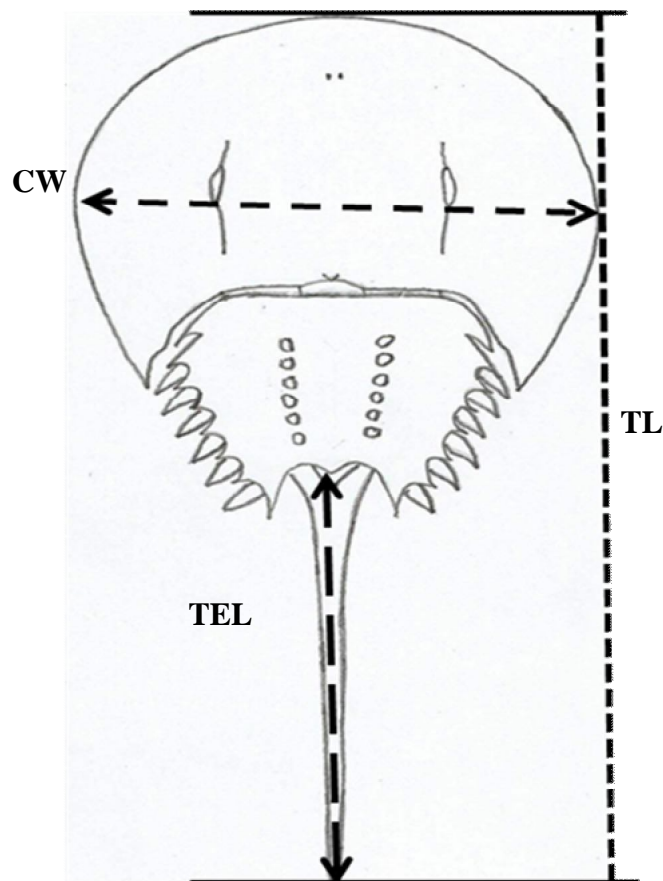


Figure 2. Body measurement used for morphological analysis of a horseshoe crab; TL (total length in cm), CW (carapace width in cm) and TEL (telson length in cm).

## Results and Discussion

**Morphometric analysis of male and female *T. gigas* from Pasir Putih Village and Gerigat Beach.** A total of 83 individuals of *T. gigas* ( $\text{♂} = 48$ ,  $\text{♀} = 35$ ) were collected from Pasir Putih Village and 42 individuals of *T. gigas* ( $\text{♂} = 22$ ,  $\text{♀} = 20$ ) were collected from Gerigat Beach. The mean values of all body parameters of male and female *T. gigas* that obtained from Pasir Putih Village and Gerigat Beach were tabulated in Table 1. For female specimens, the range CW and BW was 16.3-26.4 cm and 260.0-1820.0 g respectively, meanwhile the CW and BW of male specimens ranged between 15.4-22.6 cm and 182.5-370.3 g respectively. Female of *T. gigas* showed significant different in TL, CW, BW and TEL with male of *T. gigas* from both locations. Female specimens from Gerigat Beach showed higher values; 0.24%, 1.81% and 15.95% respectively in difference of body parameters excluding lower value in TEL (1.48%) compared to female from Pasir Putih Village (Table 2). Male from Gerigat Beach also showed higher values in all body parameters compared to male from Pasir Putih Village, 2.33%, 2.82%, 1.34% and 1.13% respectively. Female from both locations showed higher different percentage in body parameters compared to male, same as reported by Yamasaki et al (1988), Chatterji (1994), Chiu & Morton (2003) and Itow et al (2004). Therefore, it is clear that sexual dimorphism features of horseshoe crabs can be clearly observed through the differences of size (Shuster & Sekiguchi 2009; Tan et al 2012; Mohamad et al 2016), which male *T. gigas* need to molt 16 molt times and female *T. gigas* required to molt 17 times to achieve maturity. In addition, female horseshoe crabs contain large volume of eggs in its carapace during spawning season and similarity of fat content (Le Cren 1951; Sekiguchi et al 1988). Besides that, the difference in size of adults male and female of *T. gigas* from Pasir Putih Village and Gerigat Beach probably due to two discrete populations, same as reported for other species of horseshoe crabs, *C. rotundicauda*, *T. tridentatus*

and *Limulus polyphemus* from two different locations (Riska 1981; Chiu & Morton 2003; Pierce et al 2000; Srijaya et al 2010; Mohamad et al 2016). *T. gigas* from Pasir Putih Village also obtained in bulk populations but their mean size was smaller compared to specimens from Gerigat Beach which considered probably there was less food availability since there were a lot of competitions in obtaining food.

Table 1

Morphometric of four body parameters of horseshoe crab male and female of *T. gigas* species collected from Pasir Putih Village and Gerigat Beach

| Parameters | Pasir Putih Village                      |   | Gerigat Beach                            |  |
|------------|--|---|--|--|
|            | ♂ (N = 48)                               | ♀ (N = 35)                                | ♂ (N = 22)                               | ♀ (N = 20)                                 |
| TL (cm)    | 33.6±4.2 <sup>a</sup><br>(13.4-38.6)     | 41.8±5.2 <sup>b</sup><br>(29.8-48.9)      | 34.4±2.9 <sup>a</sup><br>(27.0-38.2)     | 41.9±6.0 <sup>b</sup><br>(22.7-50.1)       |
| CW (cm)    | 17.2±1.0 <sup>a</sup><br>(15.4-22.6)     | 21.7±2.0 <sup>b</sup><br>(16.3-26.4)      | 17.7±0.7 <sup>a</sup><br>(16.6-18.8)     | 22.1±1.0 <sup>b</sup><br>(19.8-23.8)       |
| BW (g)     | 295.4±29.2 <sup>a</sup><br>(225.1-370.3) | 726.3±172.6 <sup>b</sup><br>(260.0-990.1) | 299.4±46.0 <sup>a</sup><br>(182.5-359.9) | 864.1±247.5 <sup>b</sup><br>(682.7-1820.0) |
| TEL (cm)   | 17.5±2.8 <sup>a</sup><br>(8.6-23.2)      | 20.3±4.5 <sup>a</sup><br>(9.2-26.7)       | 17.7±2.7 <sup>a</sup><br>(10.4-21.3)     | 20.0±6.0 <sup>b</sup><br>(12.5-26.3)       |

\*TL = total length, CW = carapace width, BW = body weight, TEL = telson, ♂ = male, ♀ = female. \*Same-superscripts indicated significant difference of TL, CW, BW and TEL between sexes of *T. gigas* from different locations when  $p < 0.05$ .

Table 2

Difference of four body parameters of horseshoe crabs based on sex and different locations (in percentage)

| Parameters | % difference of parameters |       |                     |               |
|------------|----------------------------|-------|---------------------|---------------|
|            | ♂                          | ♀     | Pasir Putih Village | Gerigat Beach |
| TL (cm)    | 2.33                       | 0.24  | 19.62               | 17.90         |
| CW (cm)    | 2.82                       | 1.81  | 20.74               | 19.91         |
| BW (g)     | 1.34                       | 15.95 | 59.33               | 65.35         |
| TEL (cm)   | 1.13                       | 1.48  | 13.79               | 11.5          |

\*% = percentage, TL = total length, CW = carapace width, BW = body weight, TEL = telson, ♂ = male, ♀ = female.

**Allometric analysis of male and female *T. gigas* from Pasir Putih Village and Gerigat Beach.** According to Chatterji et al (1988), the study on the relationship between different body parameters of a living organism will contribute further understanding of their morphometric characteristics and growth. Therefore, regression analysis was applied for selected body measurements of male and female from Pasir Putih Village and Gerigat Beach, where the data is listed in Table 3.

All relationship between body parameters of both sexes *T. gigas* from both location showed significant differences ( $p < 0.05$ ) except CW-TL and BW-CW of male from Pasir Putih Beach, CW-TL of male from Gerigat Beach, and BW-TL and CW-TL of female from Gerigat Beach. Present study showed 'b' value of BW-CW and BW-TL for male and female from Pasir Putih Village and Gerigat Beach was ranging from 0.19 to 3.94 (Table 3). The regression coefficients, 'b' values of both sexes from both locations showed negative allometric growth since 'b' value less than 3 except BW-CW and TEL-TL of female from Gerigat Beach which 'b' values; 3.16 and 3.94. Negative allometric growth ( $b < 3$ ) indicated that the growth of CW/TL was faster than the BW increment after molting (Mohamad et al 2016). Only female *T. gigas* from Gerigat Beach showed positive allometric growth.

The relationship between TEL-TL of male ( $r = 0.918$ ) and female from Gerigat Beach ( $r = 0.901$ ) exhibited stronger correlation compared to others relationship when  $p < 0.05$ . Normally after the molting process, the body weight is increased proportionately

with the continuing rise as their carapace widths grow wider and their total length elongated (Vijayakumar et al 2000).

Table 3

Statistical analysis of body parameters measurement of male and female of *T. gigas* based on two different locations

| Pasir Putih Village | M      |                      |    | F      |                      |    |
|---------------------|--------|----------------------|----|--------|----------------------|----|
|                     | RC (b) | CC (R <sup>2</sup> ) | S  | RC (b) | CC (R <sup>2</sup> ) | S  |
| BW-TL               | 0.19   | 0.097                | S  | 1.00   | 0.171                | S  |
| CW-TL               | 0.06   | 0.038                | NS | 0.43   | 0.351                | S  |
| TEL-TL              | 0.58   | 0.243                | S  | 1.85   | 0.849                | S  |
| BW-CW               | 0.43   | 0.050                | NS | 2.35   | 0.488                | S  |
| Gerigat Beach       |        |                      |    |        |                      |    |
| BW-TL               | 0.83   | 0.194                | S  | 0.52   | 0.159                | NS |
| CW-TL               | 0.10   | 0.053                | NS | 0.07   | 0.072                | NS |
| TEL-TL              | 1.88   | 0.918                | S  | 3.94   | 0.901                | S  |
| BW-CW               | 2.67   | 0.396                | S  | 3.16   | 0.451                | S  |

\*TL = total length, CW = carapace width, BW = body weight, TEL = telson, RC = regression coefficient, CC = correlation coefficient, S = significant, NS = not significant.

**Relationships of CW/TL-BW.** The 'b' value of length-weight ranged from 0.27 to 2.54 for male and female from both locations which does follow the cube law and less than 3.0 which indicated allometric growth. The range of 'b' value for different sexes of *C. rotundicauda* also recorded less than 3.00 (Srijaya et al 2010). Compared to *T. tridentatus* species from Tanjung Limau and Inderasabah, Sabah, 'b' value for BW-CW was reported more than 3 while less than 3 for BW-TL relationship (Mohamad et al 2016). Same as reported for BW-CW ( $b = 5.24$ ) was higher compared to BW-TL ( $b = 2.46$ ) of *T. gigas* specimens from India (Vijayakumar et al 2000). However, present study showed 'b' value of female was higher compared to male and this was supported by Srijaya et al (2010), Ismail et al (2012), Tan et al (2012) and Mohamad et al (2016). This indicated that the length (CW/TL) of female horseshoe crab was higher compared to male.

Negative allometry growth was shown in this study since current 'b' value was less than 3.00. Previous research related with horseshoe crab growth also indicated negative allometric growth (Srijaya et al 2010; Mohamad et al 2016). The relationship between BW-TL ( $r = 0.372$ ), and BW-CW ( $r = 0.687$ ) of female (pooled locations) exhibited higher Pearson's correlation coefficient compared to male ( $r = 0.309$ ,  $r = 0.309$ ). CW indicates higher increase compared to TL in female compared to male.  $CW_{\text{female}}$  raise about three times ( $b = 2.54$ ) compared to  $TL_{\text{female}}$  ( $b = 0.77$ ) and  $CW_{\text{male}}$  showed one time increment rates ( $b = 0.85$ ) compared with  $TL_{\text{male}}$  ( $b = 0.27$ ). Present study showed a better CW increment of male and female *T. gigas* from two locations in Sarawak, same as reported by Vijayakumar et al (2000).

The coefficient of determination,  $R^2$  of BW-CW and BW-TL of male from different locations was recorded lower compared to female except for BW-TL male from Gerigat Beach (Table 3). Through two-way ANCOVA analysis, there is significant different between BW-CW, [ $F(1,119) = 29.99$ ,  $p = 0.00$ ] and BW-TL [ $F(1,119) = 4.63$ ,  $p = 0.03$ ] relationships for both sexes from Pasir Putih Village and Gerigat Beach when  $p < 0.05$ . According to Mohamad et al (2016), the relationship of different body parameters among male and female from different habitat will show similar rates of increment when  $p > 0.05$ . The differences of body parameters between same species from different locations probably due to the different environmental parameters, food availability and population density (Hile & Jobes 1940; Le Cren 1951; Vijayakumar et al 2000; Srijaya et al 2010).

Consequently, the aim of this morphological variations study is to provide crucial data that related with horseshoe crabs in west coast of Sarawak waters since no information on morphological and allometric study of Sarawak horseshoe crabs provided until now.

**Conclusions.** Body parameters of female *T. gigas* originated from Sarawak waters were higher and heavier compared to male. The relationship between CW/TL-BW for *T. gigas* exhibited negative allometric growth ( $b < 3$ ) which indicated the increment of BW was slower than CW/TL. The current estimated size for adult male and female of *T. gigas* during spawning activity can be used for better conservation management to prevent the seriously declining population in future.

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