Comparison of horseshoe crabs (*Tachypleus gigas*) morphometry between different populations using allometric analysis

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Abstract. Studies on horseshoe crabs morphometrics found that they have maintained their descendent features from the Late Ordovician Period to present day. In the present study, we applied the allometric study to evaluate the correlation of body growth in three populations of the Asian horseshoe crab (*Tachypleus gigas*) collected from Balok (Pahang), Cherok Paloh (Pahang) and Merlimau (Melaka), Malaysia, coastal areas. The aims of this study are to examine the logarithmic growth of horseshoe crabs between three populations by analyzing the variation of their body weight (BW), carapace length (CL), carapace width (CW) and telson length (TEL) to determine their growth and maturity. Their body parameters were analyzed by the allometric method. There are no significant differences between males weight in all populations ($p>0.05$). However, females from Merlimau were smallest (BW: 519.7±66.3 g; CL: 21.1±1.1 cm; CW: 19.6±0.9 cm) among the three populations; Balok (BW: 928.5±123.2 g; CL: 23.8±1.0 cm; CW: 23.3±1.0 cm) and Cherok Paloh (BW: 939.8±125.7 g; CL: 25.4±1.5 cm; CW: 25.1±1.6 cm). Males and females of *T. gigas* in Merlimau could be classified as less matured among Balok and Cherok Paloh, since the increment of CL/CW were higher than their BW. Further study on *T. gigas* allometry along Malaysian coastal area is needed to understand the variation growth between populations. The study could be an alarming condition to a particular *T. gigas* population.

Key Words: body weight, carapace length, telson length, maturity, logarithmic growth.

Introduction. Horseshoe crabs are existed since 300 million years ago and known as a living fossil according to its ancient morphological appearance (Mohd Razali & Zaleha 2017). Studies on their morphometric found that they have maintained their descendent features from the Late Ordovician Period to present day (Rudkin et al 2008; Rudkin & Young 2009). The concept of allometry was first explained by Huxley & Tessier (1936). Allometry method is an efficient way to study the variation and changes in organisms form, size and shape (Webster 2007; Hussain et al 2009; Srijaya et al 2010) and describe the relationship between differences in one body parameter to the other within same species. The allometric relationship study is used to assess the correlation growth of various body parameters (Chatterji et al 1988; Christopher 1996; Vijayakumar et al 2000).

The variation of horseshoe crabs morphometric characteristics between population and genus has been reported previously by many researchers (Chatterji 1994; Vijayakumar et al 2000; Hussain et al 2009; Srijaya et al 2010; Mohamad et al 2016; Noor Jawahir et al 2017). Chatterji et al (1988) stated that, understanding the correlation of the allometric relationship in horseshoe crab is important to understand the major physical characteristics between different populations. Previous studies found those differences due to environmental conditions (Daniels et al 1998); habitat and in-situ physio-chemicals parameters and horseshoe crabs' condition; diets, stage of maturity, genetic and population density would influence the variation of horseshoe crab size (Krumholz & Cavanah 1968; Hickman 1979; Schaefer et al 1985; Chatterji et al 1988; Gaspar et al 2002; Graham et al 2009; Shuster & Sekiguchi 2009). Therefore, analysis of allometric parameters would help in evaluating the relationship between ecosystems and horseshoe crabs morphological parameters.

In the present study, we applied the allometric study to evaluate the correlation of body growth in three populations of the Asian horseshoe crab (*Tachypleus gigas* [Müller, 1785]) collected from Balok (Pahang), Cherok Paloh (Pahang) and Merlimau (Melaka), Malaysia coastal areas. Sexual dimorphism features of *T. gigas* could be observed through the differences of size (Shuster & Sekiguchi 2009; Tan et al 2012; Mohamad et al 2016); male *T. gigas* need to molt 16 times and female *T. gigas* required to molt 17 times to achieve maturity (Kumar et al 2015; Noor Jawahir et al 2017) within 9 to 10 years. Besides that, it is a well-known fact that the logarithmic growth of horseshoe crabs would be slowed down at each growth stage (Shuster 1982) and would be totally stopped once they archived their maturity (Figure 1). The weight of the matured female horseshoe crabs would be mainly influenced by the present of the eggs and fat content that varies among season (Sekiguchi et al 1988; Atar & Seçer 2003; Mohanty et al 2014) rather than the by the size of the carapace.

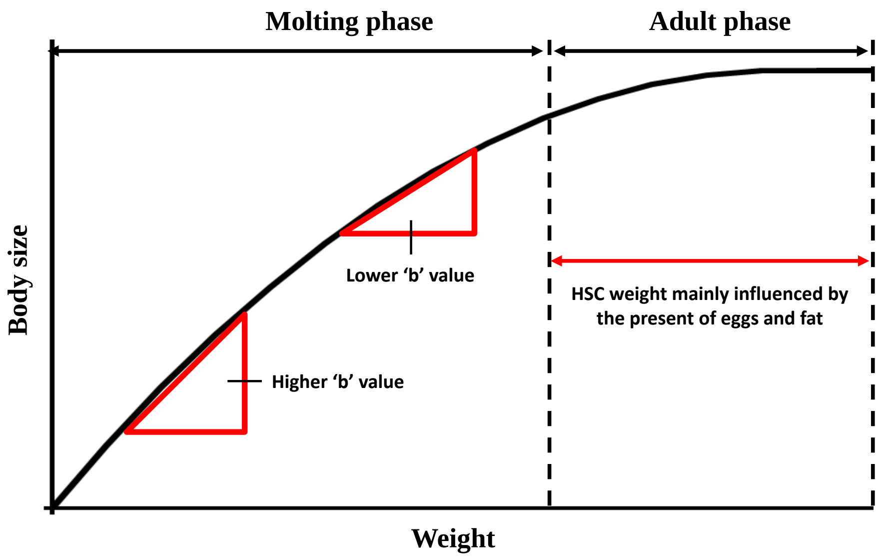

Figure 1. Horseshoe crabs' growing pattern.

Studies on the relationships of size and weight are important in estimating the condition of a particular *T. gigas* population (Lawson et al 2013) such as, variation in growth and maturity of species (Karnik & Chakraborty 2001; Chatterji & Pati 2014; Mohanty et al 2014; Susanto & Irnawati 2014; Mohamad et al 2016). However, only a few studies were carried out on the relationships of these parameters on *T. gigas* in east coast of Malaysian peninsula by Ismail et al (2012) and Sarawak by Noor Jawahir et al (2017) and on *Tachypleus tridentatus* in Papar, Sabah by Chatterji & Pati (2014) and Mohamad et al (2016). No previous study has been conducted to compare the differences of *T. gigas* morphometrics between the west and east coast of Malaysian peninsula. The aim of this study is to examine the logarithmic growth of *T. gigas* between three populations by analyzing the relationship of carapace length (CL)/carapace width (CW) – body weight (BW) to determine the increment in *T. gigas* growth.

Material and Method

Samples collection. Balok and Cherok Paloh in Pahang and Merlimau in Malacca, Malaysia are among the active fishing ports engaged in *T. gigas* harvesting activity. For the purpose of this study, a total of 205 *T. gigas* samples were collected in Balok, Pahang (N 03°56'14.9" E 103°22'30.2"), Cherok Paloh, Pahang (N 03°36'17.1" E 103°22'51.0") and Merlimau, Melaka (N 02°07'44.6" E 102°24'54.3") (Figure 2). We used the same harvesting method (fishing net of 400 m long, 6 m wide with mesh size of 10 mm) in three sampling locations to decrease the biasness toward the selection of the matured crabs in spawning site. Fishing net was deployed into the sea during the incoming high tide until 24 hours. The sampling activities were conducted during April – May 2017.

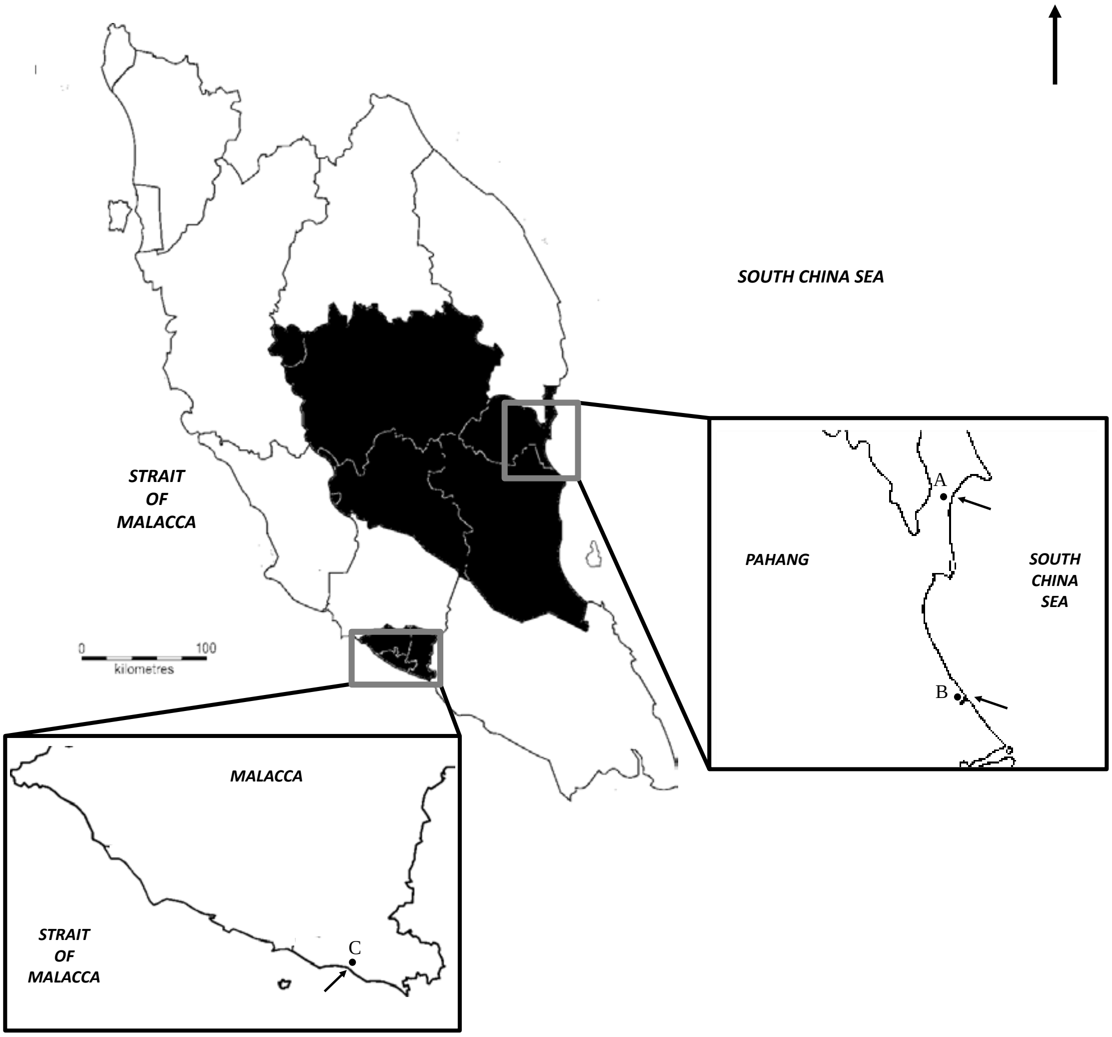

Figure 2. Locations of the sampling area. A - Balok, Pahang (N 03°56'14.9" E 103°22'30.2"); B - Cherok Paloh, Pahang (N 03°36'17.1" E 103°22'51.0"); C - Merlimau, Melaka (N 02°07'44.6" E 102°24'54.3").

Morphometric measurement*.* Samples were sexed and their body measurement parameters namely, body weight (BW), carapace length (CL), carapace width (CW) and telson length (TEL) were measured (Figure 3). Length parameters were measured to the nearest 0.1 cm by using a measuring tape. The weight of each *T. gigas* sample was weighted with 0.1 g accuracy, using digital balance.

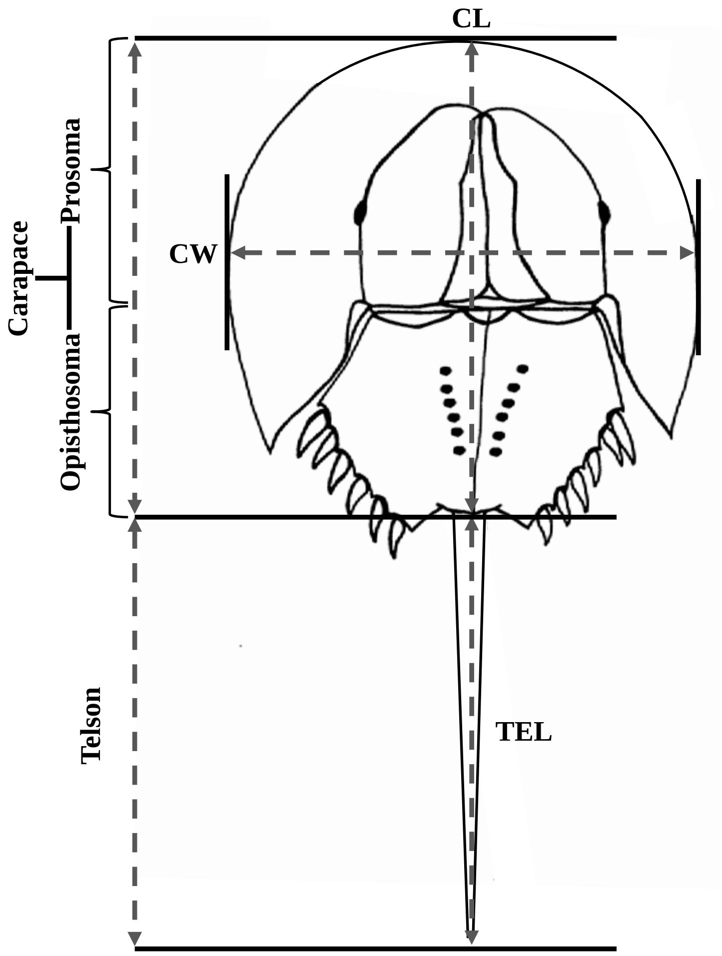

Figure 3. Body measurement used for morphometric analysis *T. gigas*; CL - carapace length, CW - carapace width, TEL - telson length.

Allometric analysis. Data collected for all measurements were arranged and compared according to the sexes and sampling location. All values were converted into the logarithmic form:

$$Log\,Y = a + b\,Log\,X$$

$'X'$ is considered as basis of body measurement (BW) and $'Y'$ is the determined body measurement (CL and CW) and $'b'$ is the value of regression coefficient. When $'Y'$ proportions growth higher than $'X'$, $b > 1$, which is called positive allometry. However, if $'Y'$ proportions growth lower than $'X'$, $b < 1$, which is called negative allometry. If $b = 1$, the $'Y'$ proportions growth increase at the same rate of $'X'$ and this condition called isometry.

Data analysis. The correlation coefficient (r^2) and significance analysis of CL – BW, CW – BW and TEL – CL of male and female *T. gigas* were performed by the Pearson Correlation (Sig.2-tailed) analysis. Independent samples T-test was applied to test the differences between male and female from the same locations. Analysis of variance (ANOVA) was performed and means comparisons were done by Tukey post-hoc tests. The analysis was applied to test the significant differences between male and female body measurements between the three sampling locations. Analyses were performed using a SPSS package (SPSS 20.0 for windows, SPSS Inc., Chicago, IL, USA). P values of less than 0.05 were considered statistically significant.

Results

Morphometric analyses. Morphometric data of males and females of *T. gigas* from the three sampling locations is presented in Table 1 and the percentage of parameter differences are presented in Table 2.

Table 1

Mean of the different body measurements with their standard deviation

Parameters	Sampling location		
	Balok, Pahang	*Cherok Paloh, Pahang*	*Merlimau, Melaka*
		Males	
BW (g)	351.3±54.1[a]	348.6±42.7[a]	378.5±22.1[b]
CL (cm)	17.5±1.0[a]	18.4±0.8[b]	19.0±1.1[c]
CW (cm)	17.3±0.8[a]	18.1±1.0[b]	18.3±0.8[b]
TEL (cm)	17.5±1.4[a]	17.0±1.4[a]	16.0±2.2[b]
		Females	
BW (g)	928.5±123.2[a]	939.8±125.7[a]	519.7±66.3[b]
CL (cm)	23.8±1.0[a]	25.4±1.5[b]	21.1±1.1[c]
CW (cm)	23.3±1.0[a]	25.1±1.6[b]	19.6±0.9[c]
TEL (cm)	21.0±2.1[a]	19.3±3.0[b]	15.5±2.7[c]

BW - body weight, CL - carapace length, CW = carapace weight, TEL - telson length. Different superscripts indicated significant difference of BW, CL, CW and TEL between different sampling locations ($p<0.05$).

Table 2

Percent difference of various measurements of Balok, Cherok Paloh and Merlimau population

Parameters	B/CP (%)	B/M (%)	CP/B (%)	CP/M (%)	M/B (%)	M/CP (%)
			Male			
BW (g)	0.77	-	-	-	7.74	8.58
CL (cm)	-	-	5.14	-	8.57	3.26
CW (cm)	-	-	4.62	-	5.78	1.10
TEL (cm)	2.94	9.38	-	6.25	-	-
			Female			
BW (g)	-	78.66	1.22	80.84	-	-
CL (cm)	-	12.80	6.72	20.38	-	-
CW (cm)	-	18.88	7.73	28.06	-	-
TEL (cm)	8.81	35.48	-	24.52	-	-

–: negative percentage value. B - Balok, Pahang; CP - Cherok Paloh; M - Merlimau, Melaka.

Result of males body weight showed no significant different between populations ($p>0.05$) (Figure 4). Two-way ANOVA showed the sizes (CL and CW) of males from all populations were not significantly different ($F = 0.962$, $p>0.05$). Contrast results are showed by females, where the body weight of specimens collected from Merlimau were significantly lowest ($p<0.05$) compared to Balok and Cherok Paloh samples (Figure 5). Body weight of females in Balok and Cherok Paloh were 78.66% and 80.84% heavier respectively then females in Merlimau. Besides that, the sizes (CL and CW) of females from Merlimau were significantly smaller compared to Balok and Cherok Paloh ($F = 4.672$, $p<0.05$). The weight of *T. gigas* males were significantly lower compared to females in all population ($F = 1634.44$, $p<0.05$).

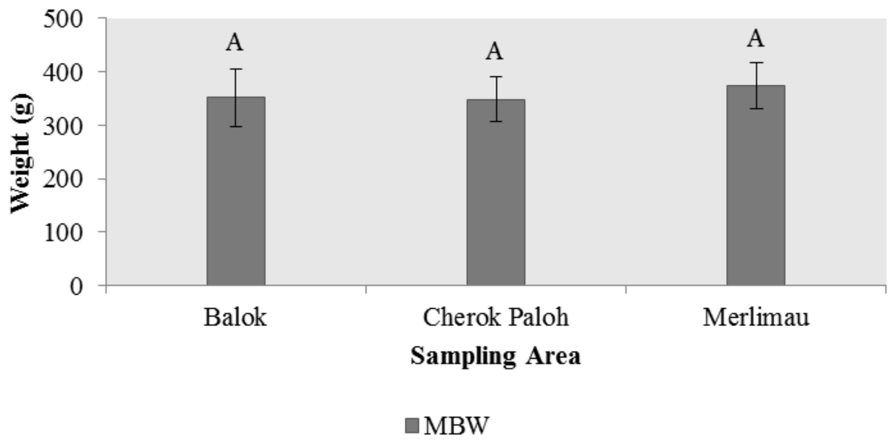

Figure 4. Comparisons of MBW (male's body weight) of *Tachypleus gigas* between populations.

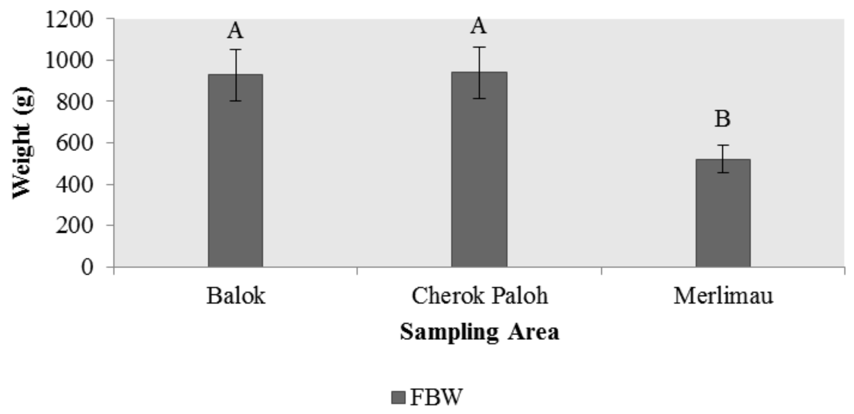

Figure 5. Camparisons of FBW (females' body weight) of *Tachypleus gigas* between populations.

Allometric analyses. Values of the allometric analysis (RC: regression coefficient, b; CC: correlation coefficient, r^2; S: significance) of CL – BW and CW – BW of males and females from the three sampling locations are presented in Table 3 and Figure 6-11. Generally, the CL/CW – BW relationship of *T. gigas* from all populations followed the cube law with the range (Isometry: m1/m3, b = 1/3 = 0.33) of 'b' values varied from 0.21 to 0.48 indicates allometry growth. The relationships of CL/CW – BW were not significant for all populations. However, the increment of *T. gigas* CL/CW – BW in Balok showed negative allometry growth (b<0.33). Result showed that, the increment of CL/CW – BW of males and females of *T. gigas* in Merlimau was higher compared to Balok and Cherok paloh. Males and females in Balok have lowest increments of CL/CW – BW among the three populations. There was no fix relationships pattern on TEL – CL correlation among the three sampling locations. Male (b: -0.17) and females (b: -1.72) from Merlimau and female (b: 0.20) from Balok showed negative allometry growth of TEL – CL (b<0.33). While males from Balok (b = 0.65) and Cherok Paloh (b = 0.35) and females from Balok (b = 1.08) showed positive allometry growth of TEL – CL (b>0.33).

Table 3

Statistical analyses result of carapace length and body weight, carapace width and body weight and carapace length and carapace width relationships

Parameter	Balok, Pahang			Cherok Paloh, Pahang			Merlimau, Melaka		
	RC (b)	*CC (r^2)*	*S*	*RC (b)*	*CC (r^2)*	*S*	*RC (b)*	*CC (r^2)*	*S*
				Males					
CL – BW	0.28	0.65	S	0.30	0.68	S	0.75	0.60	S
CW – BW	0.21	0.59	S	0.42	0.79	S	0.55	0.54	S
TEL – CL	0.65	0.19	S	0.35	0.03	NS	-0.37	0.02	NS
				Females					
CL – BW	0.27	0.73	S	0.30	0.46	S	0.33	0.64	S
CW – BW	0.29	0.80	S	0.29	0.43	S	0.30	0.74	S
TEL – CL	0.20	0.01	NS	1.08	0.11	NS	-1.72	0.24	S

RC - regression coefficient, CC - correlation coefficient, S - significant, NS - not significant, BW - body weight, CL - carapace length, CW - carapace width TEL - telson length.

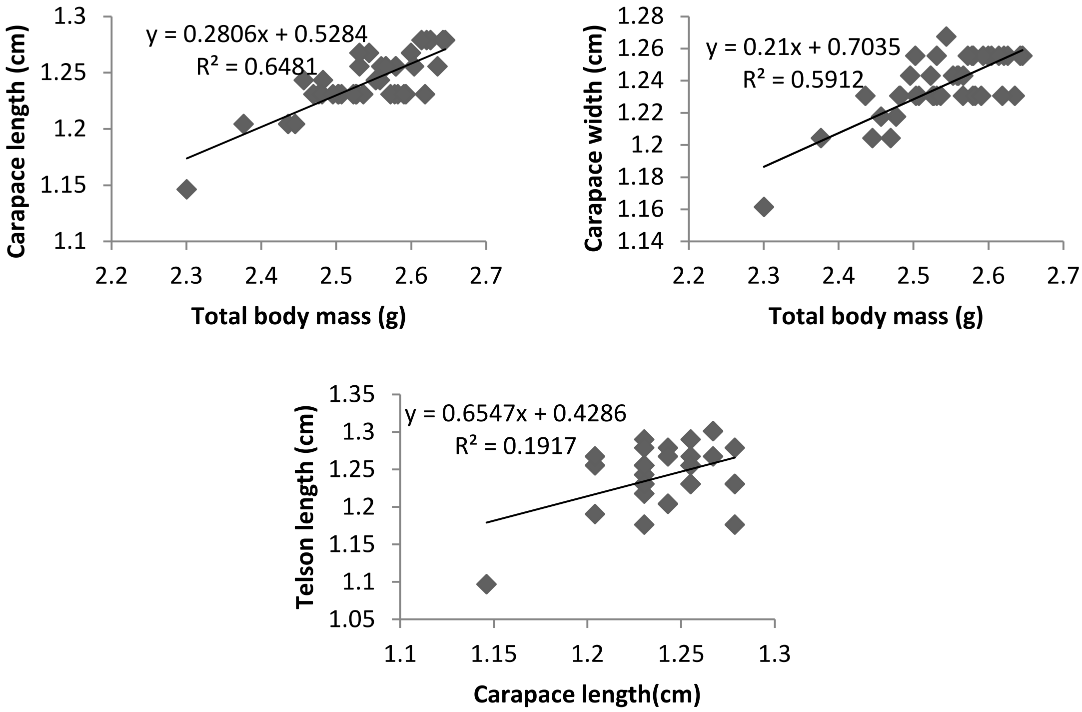

Figure 6. The CL – BW relationships of male *Tachypleus gigas* from Balok. A - Regression linear of log CL – BW. B - Regression linear of log CW – BW. C- Regression linear of log TEL – CL.

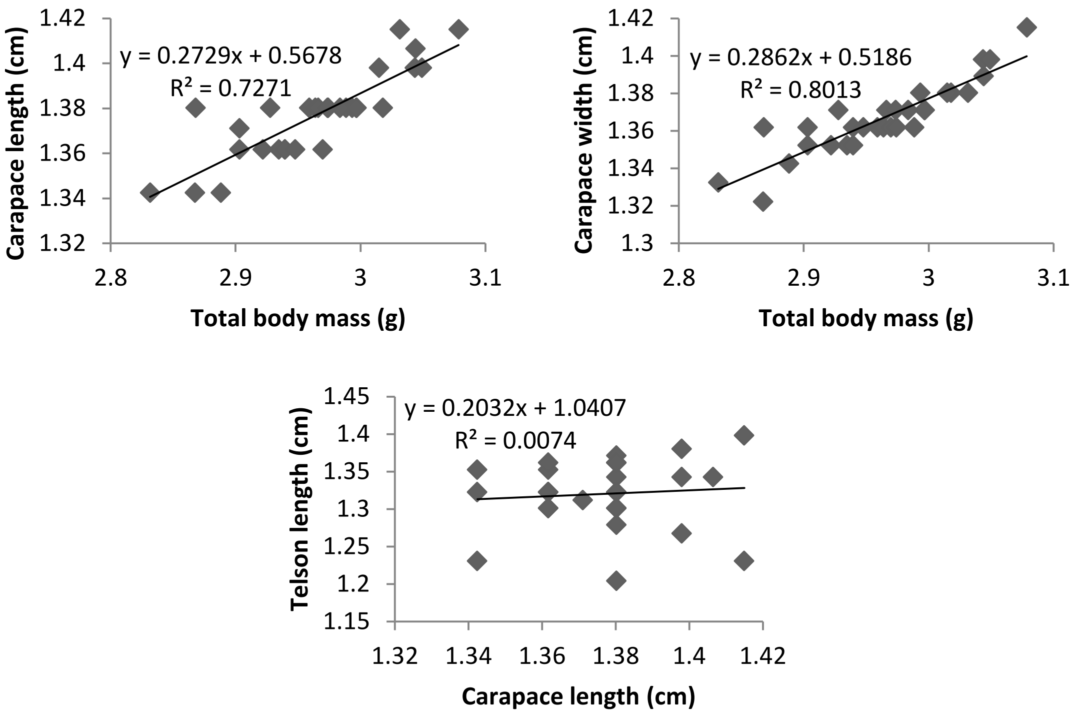

Figure 7. The CL – BW relationships of female *Tachypleus gigas* from Balok. A - Regression linear of log CL – BW. B - Regression linear of log CW – BW. C - Regression linear of log TEL – CL.

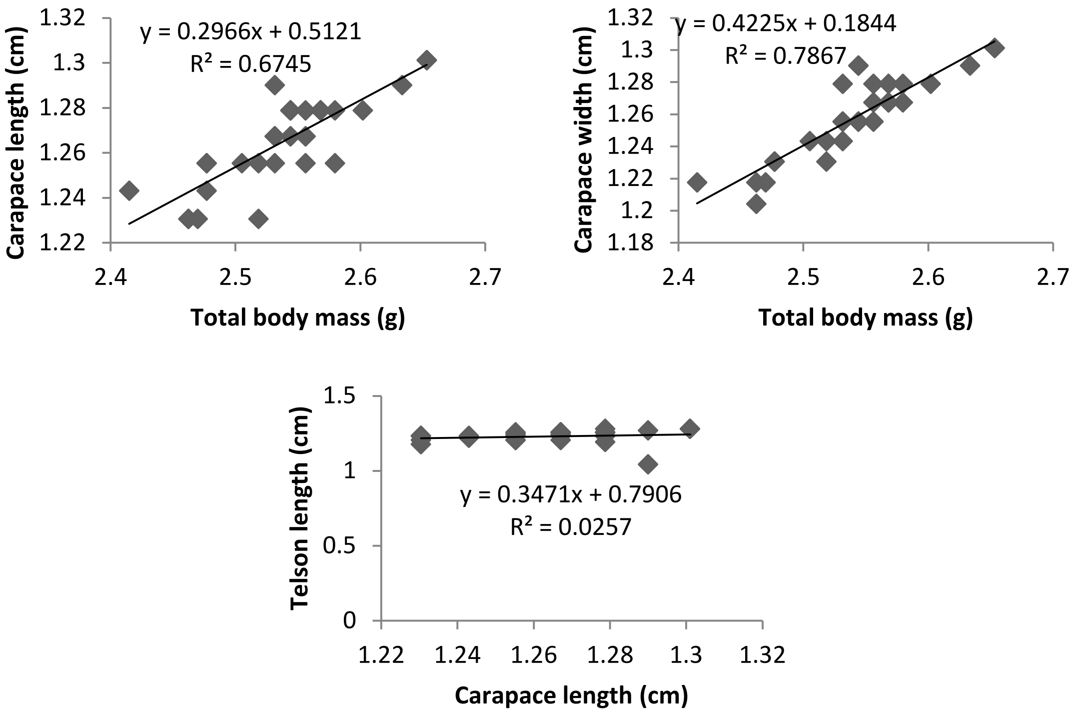

Figure 8. The CL – BW relationships of male *Tachypleus gigas* from Cherok Paloh. A - Regression linear of log CL – BW. B - Regression linear of log CW – BW. C - Regression linear of log TEL – CL.

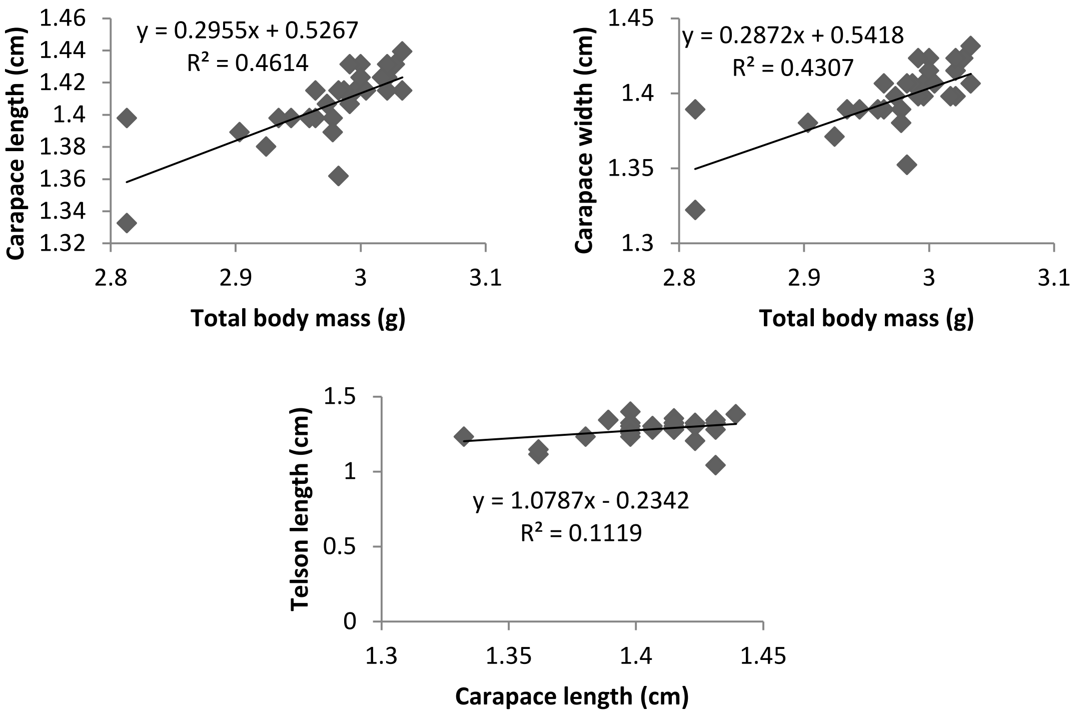

Figure 9. The CL – BW relationships of female *Tachypleus gigas* from Cherok Paloh. A - Regression linear of log CL – BW. B - Regression linear of log CW – BW. C - Regression linear of log TEL – CL.

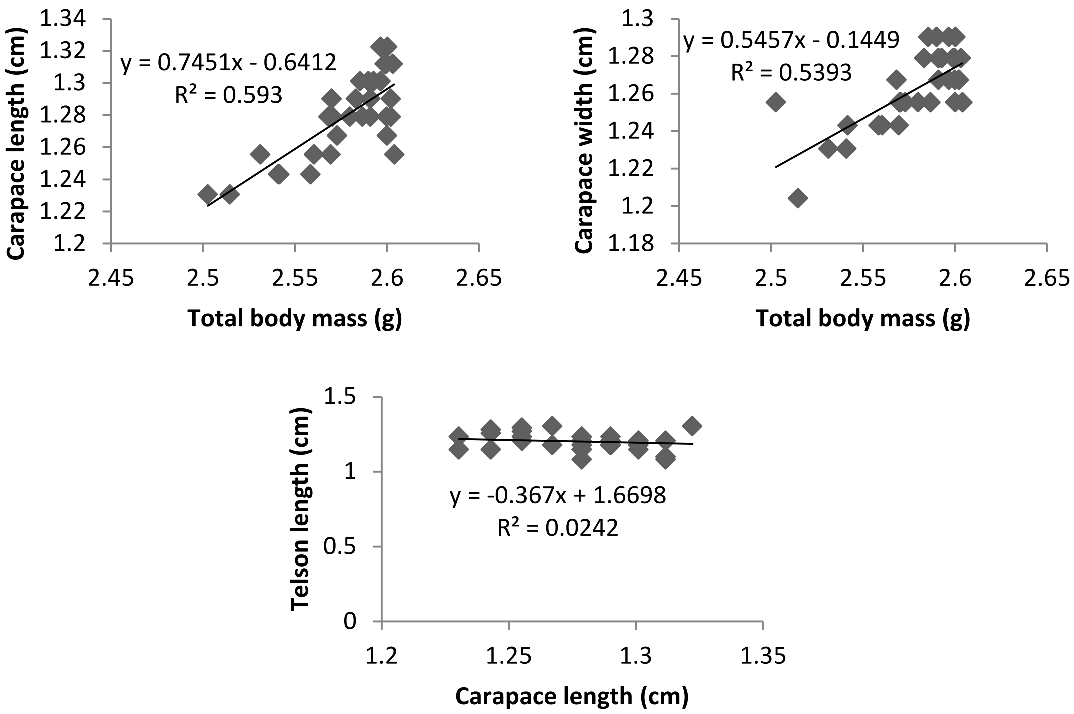

Figure 10. The CL – BW relationships of male *Tachypleus gigas* from Merlimau. A - Regression linear of log CL – BW. B - Regression linear of log CW – BW. C - Regression linear of log TEL – CL.

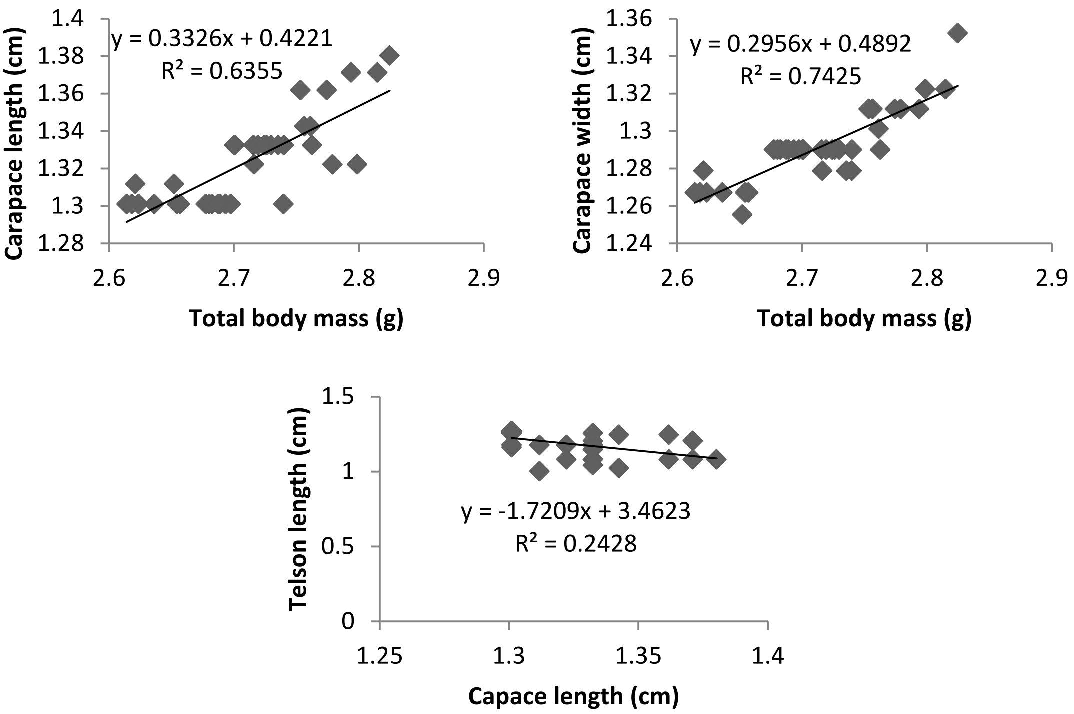

Figure 11. The CL – BW relationships of female *Tachypleus gigas* from Merlimau. A - Regression linear of log CL – BW. B - Regression linear of log CW – BW. C - Regression linear of log TEL – CL.

Discussion. Previously, many researchers conducted analysis of *T. gigas* body allometrics by studying the relationship of total length (CL + TEL = TL) and body weight (BW) (Vijayakumar et al 2000; Ismail et al 2012; Chatterji & Pati 2014; Mohamad et al 2016). Nevertheless in this present study, we were not studied the relationships of TL – BW since some of the samples telson were deformed such as broken or short (early regeneration). Moreover, there were no fix relationships pattern of TEL – CL among all populations hence, deformed telson would distort the result of TL – BW increment and cause biasness to shorter telson *T. gigas*. Excluding the telson in the analysis would decrease the biasness in the analysis of body size increment, since the contribution of telson in horseshoe crabs' weight proportion is less than the contribution on horseshoe crabs size.

Females from the three sampling locations were significantly heavier and bigger than males. Different in weight and size between sexes are one of the characteristics for the horseshoe crab species (Chatterji et al 1994; Botton et al 1996; Chiu & Morton 2001; Suggs et al 2002; Sekiguchi & Shuster 2009). These variations were due to the differences of maturity stage between the males and females; females of *T. gigas* reaching maturity with one additional instar stage compared to the males (Sekiguchi et al 1988; Kumar et al 2015; Noor Jawahir et al 2017). Female horseshoe crabs may grow larger than males due to the continuous juvenile hormone productions (Levin 2003). Besides that, the weight of females would be directly influenced by the present of eggs inside the prosoma (Sekiguchi et al 1988; Atar & Seçer 2003; Mohanty et al 2014). Differences in size between males and females were caused by the adaptation and evolution on their mating behavior. Suggs et al (2002) stated that smaller males were generally more successful to amplex on females' opisthosoma than larger males. This would lead the larger crabs to engage the mating as satellite male. Brockmann et al (1994) encountered that 51% of the females' egg would be fertilized by the attached male while, 40% would be fertilized by the satellite males and 9% unidentified. This natural selection would maintain the smaller size of male *T. gigas* in the nature.

Present study found that female *T. gigas* size and weight in Merlimau were smallest and lowest respectively among three sampling locations. These showed a significant variation in *T. gigas* morphometrics between the west and east coast of Malaysian peninsula. There are many factors that could influence the phenotypic variation of *T. gigas* including environmental conditions (Daniels et al 1998) namely, habitat and in-situ physio-chemicals parameters and horseshoe crabs' condition namely, diets, stage of maturity, genetic and population density would influence the variation of its size (Krumholz & Cavanah 1968; Hickman 1979; Schaefer et al 1985; Chatterji et al 1988; Gaspar et al 2002; Graham et al 2009; Shuster & Sekiguchi 2009). As a philopatric organism, horseshoe crabs have high genotypic variations with low genetic connectivity between populations (Obst et al 2012; Rozihan et al 2013) that would lead to the higher variation in phenotype characteristic (Liew et al 2015) .

Horseshoe crabs growth pattern could be classified as logarithmic growth. Their growth would be slowed down at each growth stage and would be totally stopped once they have achieved the mature phase after several molting cycles (Shuster 1982; John 2012). Chatterji et al (1988) stated that the allometric relationship between carapace length and soft body parts provides a better understanding of the growth of a species. Present allometric study showed that male and female *T. gigas* in Merlimau, Malacca were still on their earlier growing phase (less mature) compared to Balok and Cherok Paloh since, the increments of their CL/CW – BW were highest compared to Balok and Cherok Paloh. Male and females in Balok were the most matured among the three populations, as the increments of their CL/CW – BW were lowest compared to Cherok Paloh and Merlimau. The weight of the matured female *T. gigas* would be mainly influenced by the present of the eggs and fat content which varies among seasons (Sekiguchi et al 1988; Atar & Seçer 2003; Mohanty et al 2014) rather than the size of the carapace. Nonetheless, the environmental condition such as food availability and pollution also could influence the vulnerability of crabs' weight in a particular population (Daniels et al 1998; Mohamad et al 2016).

Differences in maturity level of the *T. gigas* among populations could be related with the exploitation status in a particular habitat. Over the past century, horseshoe crabs are subjected to commercial exploitation for human consumption, fertilizer, and bait purposes (Rudloe 1982; Berkson & Shuster 1999; Widener & Barlow 1999; Carmichael et al 2003). Recently, horseshoe crabs have been utilized in the medical field in many parts of the world (Widener & Barlow 1999; Naqvi et al 2004; Gerhart 2007; Shin et al 2009). In Malaysia, the matured female *T. gigas* are known to be mainly as food for local consumption or marketed to the neighbor countries (Kassim et al 2008; John et al 2010; Manca et al 2016). Besides that, *T. gigas* exploitation in Malacca is higher compared to Pahang. Locals in Malacca prefer to apply the higher fishing effort (fishing net) to harvest the matured *T. gigas* which migrate inshore to spawn compared to Pahang (Mohd Razali & Zaleha 2017). Heavy exploitation might decrease the proportions of the matured crabs in the wild. Increase in concentrate urbanization along the Malacca shoreline would probably expose the aquatic environment to pollution. While, most of the shoreline areas in Pahang are remains in coastal mangrove forest. Further study on *T. gigas* allometry along Malaysian coastal area is needed to understand the variation in growth between populations.

Conclusions. The sizes of males *T. gigas* in Merlimau were biggest than of the two populations in Pahang; Balok and Cherok Paloh. However, females from Merlimau were smallest compared to Balok and Cherok Paloh populations. Females from Cherok Paloh were biggest among the three analyzed populations. The relationships between CL/CW – BW for *T. gigas* between the three populations showed negative allometric growth ($b < 1$). Which means that the growth of BW were faster than CL and CW. Population of male and female *T. gigas* in Merlimau is in the earliest growth phase, since the increments of male and female CL/CW – BW were highest compared to Balok and Cherok Paloh.

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