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Morphometric variation in the horseshoe crab *Tachypleus gigas* (Xiphosura: Limulidae) from Banyuasin estuarine of South Sumatra, Indonesia

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Abstract

Two Asian horseshoe crabs (*Tachypleus gigas* and *Carcinoscorpius rotundicauda*) from Banyuasin estuarine had been reported in a previous study, however, poor samples of *T. gigas* were found thus it's not possible for morphometric analysis. Therefore, a further study intensively was needed. The present study aimed to apply the allometric relationships to describe the morphometric variability for *T. gigas* obtained from Banyuasin estuarine waters. The prosoma width (PW), carapace length (CL), telson length (TEL), total length (TL), and body weight (BW) were recorded and pooled by sexes. The Independent Sample T-test was used to test the difference in body parameters for both sexes. Both power equations ($W = aL^b$) and linear equations ($Y = a + bX$) were used to analyze the length-weight/width relationship. A Student's t-test (Bailey's t-test) was applied at a significant level at 5% for determining their growth pattern. No significant difference between males and females for all major body parameters. The male's growth pattern indicated that the BW grew slower than the PW (negative allometric) but grew at the same rate concerning the CL, TEL, and TL (isometric). The female's BW also grew at the same rate as the PW, CL, TEL, and TL. Based on length-length relationships, the male's growth pattern indicated that the PW grew rapidly than the CL (positive allometric), grew slower than the TL, and grew at the same rate as the TEL. While for female's growth pattern indicated that the PW grew slower than the TL, but grew at the same rate with the CL and TEL. The negative allometric was shown by the CL-TL relationships and the TEL-TL relationship for females but the CL-TEL relationship indicated an isometric growth. These results were expected to be used as a basis for managing the horseshoe crabs population-based conservation.

Keywords: Allometric, Banyuasin estuarine, Horseshoe crabs, *Tachypleus gigas*

Introduction

Many researchers have explained an essential of the morphometric study as well as the allometric studies for assessing the growth characteristics, body shape changes, population diversity of organisms, and its related with their ecosystems condition (Chiu and Morton 2001; Webster 2007; Sriwijaya et al. 2010; Syuhaida et al. 2019; Fauziyah et al. 2019c). These morphological structure variations were useful contributed to studying the classification and identification of various species, whereas in the term of morphometric study, the allometric studies could usefully evaluate the variation in the body part parameters of species living in various ecosystems (Sriwijaya et al. 2010). The allometric study of horseshoe crabs played a key role in understanding the comparative of morphometric and growth in various body parameters (Chatterji et al. 1988). Additionally, the changes in the maturity stage, genetic, diets, in-situ physico-chemicals parameters, and habitat were factors that influenced the size variation of horseshoe crab (Gaspar et al. 2002; Graham et al. 2009; Jawahir et al. 2017).

Banyuasin estuarine waters indicated good fish diversity and stable condition for their fish community structure as well as the water quality parameters appropriate for the fish sustainability (Fauziyah et al. 2018; Fauziyah et al. 2019a). Two horseshoe crabs, *Tachypleus gigas* and

Carcinoscorpius rotundicauda were found in these waters (Fauziyah et al. 2018; Fauziyah et al. 2019b; Fauziyah et al. 2019c). In the first investigation (Fauziyah et al. 2019b), such a small amount of *T. gigas* was found thus it's not possible for further analysis. Hence, further investigations were needed for describing their morphometric variability.

In Indonesian waters, *T. gigas* found in Muara Badak Waters of Kutai Kartanegara, East Kalimantan (Ahmad et al. 2017), distributed along the Java Northern Coastal and Madura Southern Coastal (Meilana et al. 2016; Mashar et al. 2017), found in Kuala Tungkal Waters of Tanjung Jabung Barat, Jambi (Rubiyanto 2012), found in Maluku waters (Dolejš and Vaňousová 2015; John et al. 2018) and then no scientific record on the occurrence of *T. gigas* in other waters of Indonesia. The horseshoe crabs in Indonesia were found as discard or by-catch and were not an essential fishery (John et al. 2018; Fauziyah et al. 2018). However recently, these horseshoe crabs under-protected animals according to the decree of the Ministry of Environment and Forestry No. P.20/2018 on protected plants and animals. Additionally, the IUCN Red List assessment on *T. gigas* listed these species under Data Deficient (World Conservation Monitoring Centre 1996).

The present study aimed to apply the allometric relationships to describe the morphometric variability for *T. gigas* obtained from Banyuasin estuarine waters. These results were expected to be used as a basis for managing the horseshoe crabs population-based conservation.

Material and methods

A total of 27 *T. gigas* samples were obtained from the Banyuasin estuarine waters of South Sumatera, Indonesia (Figure 1) during July-November 2019. The samples were collected using a trammel net fishing and bottom gillnet whereas the sampling location referred to the previous survey as well as the information of local fishermen.

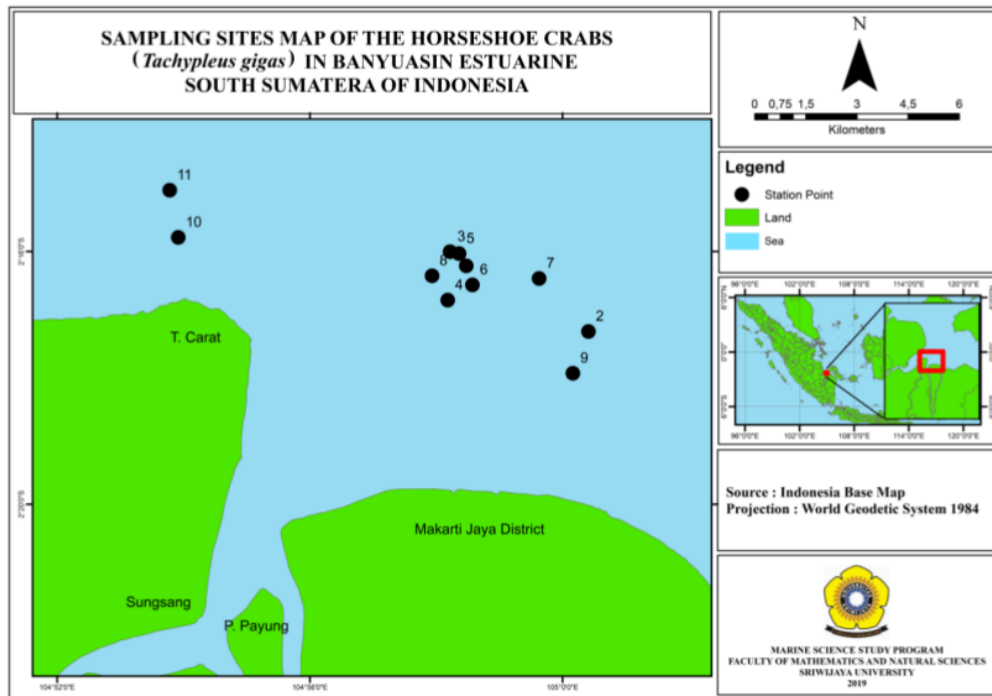


Figure 1. The sampling sites map in Banyuasin Estuarine of South Sumatra, Indonesia.

Identification of *T. gigas* referred to the previous study conducted in Banyuasin estuarine (Fauziyah et al. 2019c; Fauziyah et al. 2019b). For *T. gigas*, their cross-section morphology of the telson is a triangle shape with only has one spine on the rear part of the opisthosoma whereas *T. tridentatus* besides has a triangle shape of the telson (similarly to *T. gigas*), there is three spines on the rear part of the opisthosoma (only one spine for *T. gigas*) and many tiny spines on opisthosoma (back carapace) than others (Cartwright-Taylor et al. 2009; Tanacredi et al. 2009; Cartwright-Taylor et al. 2011; Dolejš and Vaňousová 2015). While *C. rotundicauda* is easy to distinguish due to their cross-section morphology of the telson is a rounded shape (Yang and Ko 2015). The clasper shape on the first and second walking legs is used to distinguish between male and female (Figure 2) due to a hemichelate clasper like hooks is only belonged by the male while a chelate clasper like scissors is belonged by the female (Fauziyah et al. 2019c; Fauziyah et al. 2019b).

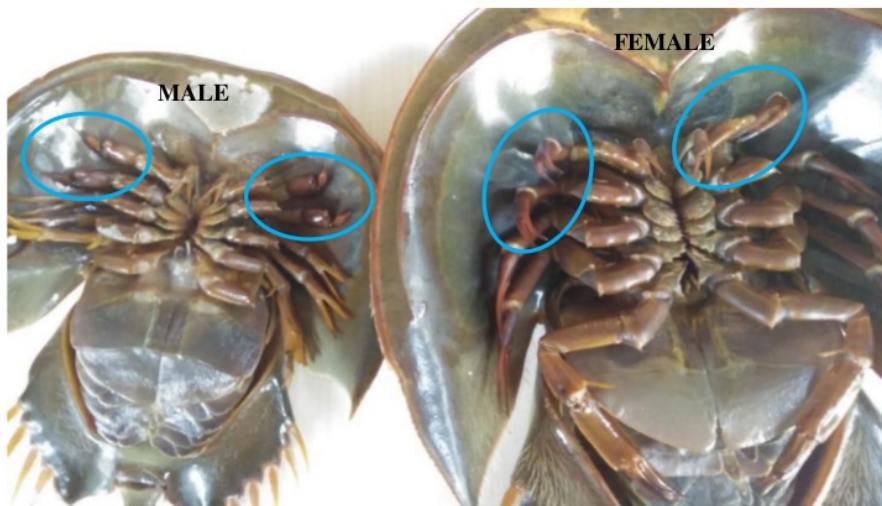


Figure 2. The differences in body parts of male and female for *T. gigas* species. The male has a hemichelate clasper like hooks while the female has a chelate clasper like scissors.

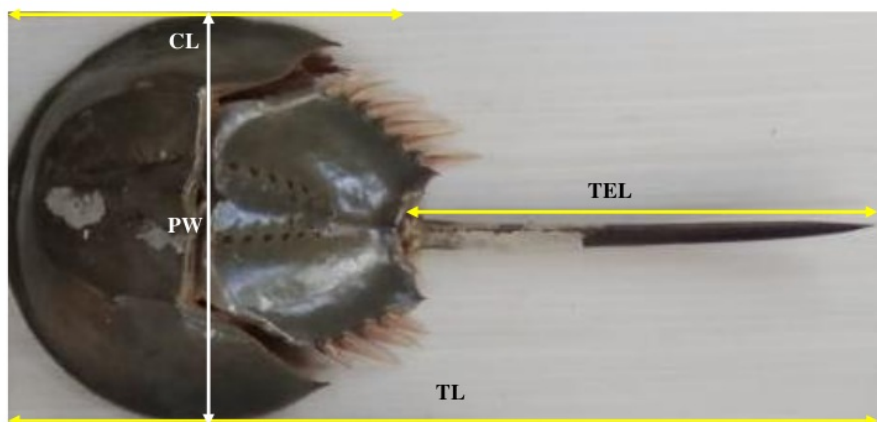


Figure 3. The major morphometric measurement of the horseshoe crab *T. gigas* consists of prosoma width (PW), carapace length (CL), telson length (TEL), and total length (TL). All body parameters were recorded to 1 mm accuracy.

The prosoma width (PW), carapace length (CL), telson length (TEL), and total length (TL) were measured to 1 mm accuracy (Figure 3) whereas body weight (BW) was weighted to 1 gram accuracy. All measurement data were pooled according to females and males. The Independent Sample T-test (Two-Sample Assuming Unequal Variances) was used to test the difference in body parameters for both sexes. The power equations ($W = aL^b$) was applied to analyze the length-weight relationship (Le Cren 1951; Froese 2006; Graham et al. 2009; Jawahir et al. 2017) while the linear equations ($Y = a + bX$) was used to analyze the length-length/width relationship (Chiu and Morton 2001; Aydin and Aydin 2011; Amaral et al. 2014; Ming et al. 2016).

In the term of the TEL-TL, PW-TL, and PW-CL relationships (Amaral et al. 2014; Ming et al. 2016), the b value < 1 indicated that Y-axis (PW, LT) grown relatively slower than X-axis (TL, CL) and it's called a negative-allometric growth, whilst the b value > 1 indicated a positive-allometric growth and the b value $= 1$ indicated an isometric growth. Due to the dimensions of X and Y were not the same at the length-weight relationships (BW-PW, BW-TL, BW-CL) hence called a negative-allometric growth when the b value < 3 whereas the b value > 3 indicated a positive-allometric growth and the b value $= 3$ indicated an isometric growth. Thus the b value is indicating growth pattern (Syuhaida et al. 2019) and for determining significant differences from the isometric value ($b = 3$ or $b = 1$), the Student's t-test (Bailey's t-test) was applied with the significant level at 5% (Thomas 2013; Hegele-drywa et al. 2014; Nair et al. 2015).

Results

The mean of the major body parameters of *T. gigas* by sex and their normality test were presented in Table 1 while testing the difference in body parameters for both sexes was presented in Table 2. All data of the major body parameters have been normally distributed based on the Kolmogorov-Smirnov Test thus the independent sample t-test could be examined. The mean size of the female's body parameters (BW, CL, TL, and TL unless PW) were higher than males (Table 2) however there was no significant difference between males and females for all major body parameters ($p > 0.05$).

Table 1. The normality test results of the body parameters data by sex using the Kolmogorov-Smirnov Test. The values of Asymp. Sig. (2-tailed) > 0.05 indicated a normal distribution.

Statistic parameters	The major body parameters				
	BW	CL	TEL	TL	PW
Males					
Mean	251.8469	155.3846	158.3846	317.6154	169.1538
Standar Deviation	91.19048	16.19433	32.26334	42.0546	26.5388
Kolmogorov-Smirnov	0.547	0.937	0.82	0.801	0.747
Asymp. Sig. (2-tailed)	0.926	0.344	0.512	0.543	0.633
Females					
Mean	352.5923	157.9231	167.0769	321.3846	161.6154
Standar Deviation	284.7488	37.55987	33.37529	71.01589	42.56669
Kolmogorov-Smirnov	1.159	0.604	0.754	0.329	0.672
Asymp. Sig. (2-tailed)	0.136	0.859	0.62	1.0	0.757

1 BW = body weight, CL = carapace length, TEL = telson length, TL = total length, PW = prosoma width, The significance level (α) of 0.05

The parameters of the allometric analysis for *T. gigas* by sex were presented in Table 3 and Figure 4-5. Generally, all relationships between each body parameter indicated variation in the coefficient of determination (R^2 ranged between 0.672 to 0.94). Variation in the allometric value (b) ranged between 1.689 to 3.4 for the weight-length relationships while for length-length relationships ranged between 0.428 to 1.523. These values indicated variation in growth patterns such as positive allometric, negative allometric or isometric depend on the student's t-test results.

Table 2. The mean of the different body parameters of *T. gigas* by sex with their variance using the independent sample t-test. There was no significant difference between males and females for all body parameters

Body Parameters	Males (N = 13)		Females (N = 13)	
	Mean	Variance	Mean	Variance
BW	251.8469 ^a	8315.7038	352.5923 ^a	81081.8923
CL	155.3846 ^a	262.2564	157.9231 ^a	1410.7436
TEL	158.3846 ^a	1040.9231	167.0769 ^a	1113.9103
TL	317.6154 ^a	1768.5897	321.3846 ^a	5043.2564
PW	169.1538 ^a	704.3077	161.6154 ^a	1811.9231

¹ BW = body weight, CL = carapace length, TEL = telson length, TL = total length, PW = prosoma width, and the same superscripts of each parameters indicated not significant difference between males and females at $\alpha = 0.05$.

Table 3. The body parameters relationships and growth pattern for *T. gigas* by sex. The value of $t_s < t_{tab}$ indicated isometric growth while the value of $t_s > t_{tab}$ indicated allometric growth.

Body parameters relationships (Y-X)	Parameters of the relationship					Bailey's t-test			Growth Pattern
	b	Sb	t_b	Sig. F	R ²	β	t_{tab}	t_s	
Males									
BW-PW	1.689	0.252	6.697	0.000	0.849	3	2.306	5.197 ^S	Allometric (-)
BW-CL	3.186	0.509	6.255	0.000	0.781	3	2.201	0.365 ^{NS}	Isometric
BW-TEL	3.400	0.785	4.329	0.002	0.676	3	2.262	0.509 ^{NS}	Isometric
BW-TL	3.371	0.597	5.644	0.000	0.780	3	2.262	0.621 ^{NS}	Isometric
PW-CL	1.523	0.182	8.352	0.000	0.864	1	2.365	2.868 ^S	Allometric (+)
PW-TEL	1.423	0.265	5.365	0.001	0.804	1	2.365	1.596 ^{NS}	Isometric
PW-TL	0.736	0.102	7.242	0.000	0.854	1	2.262	2.592 ^S	Allometric (-)
CL-TEL	0.715	0.140	5.098	0.001	0.788	1	2.365	2.037 ^{NS}	Isometric
CL-TL	0.451	0.062	7.233	0.000	0.853	1	2.262	8.810 ^S	Allometric (-)
TEL-TL	0.694	0.099	7.013	0.000	0.817	1	2.201	3.099 ^S	Allometric (-)
Females									
BW-PW	2.850	0.347	8.219	0.000	0.860	3	2.201	0.432 ^{NS}	Isometric
BW-CL	3.223	0.367	8.788	0.000	0.875	3	2.201	0.607 ^{NS}	Isometric
BW-TEL	2.989	0.630	4.746	0.001	0.672	3	2.201	0.018 ^{NS}	Isometric
BW-TL	3.177	0.379	8.388	0.000	0.865	3	2.201	0.466 ^{NS}	Isometric
PW-CL	1.099	0.084	13.110	0.000	0.940	1	2.201	1.178 ^{NS}	Isometric
PW-TEL	0.976	0.160	6.103	0.000	0.842	1	2.365	0.151 ^{NS}	Isometric
PW-TL	0.565	0.061	9.309	0.000	0.887	1	2.201	7.178 ^S	Allometric (-)
CL-TEL	0.845	0.160	5.293	0.001	0.800	1	2.365	0.973 ^{NS}	Isometric
CL-TL	0.498	0.054	9.224	0.000	0.886	1	2.201	9.310 ^S	Allometric (-)
TEL-TL	0.428	0.059	7.290	0.000	0.829	1	2.201	9.751 ^S	Allometric (-)

¹ BW = body weight, CL = carapace length, TEL = telson length, TL = total length, PW = prosoma width, Sb = standard error of the b coefficients, Sig. F = significance F-test, R² = the coefficient of determination, t_b = the t-test statistic for H₀ of b = 0, β = allometric value, t_{tab} = critical values of the t distribution, t_s = Bailey's t-test for allometric values, S = Significant, and NS = Not significant

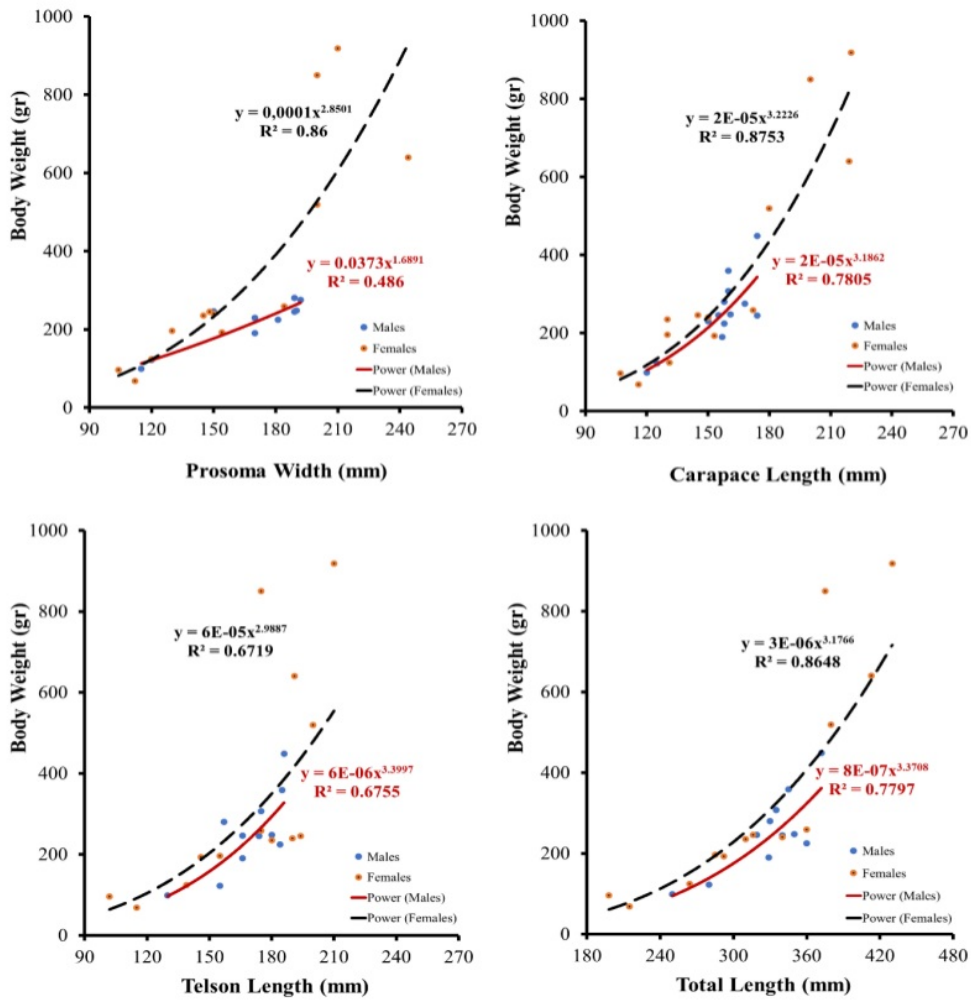


Figure 4. Total weight-length relationship of *T. gigas* by sex. The male growth pattern indicated that the bodyweight grew slowly with respect to the prosoma width (negative allometric, $b < 3$, $t_s > t_{tab}$, $p < 0.05$) but grew at the same rate with respect to the carapace length, telson length, and total length (isometric, $t_s < t_{tab}$, $p < 0.05$). The body weight also grew at the same rate with respect to the prosoma width, carapace length, telson length, and total length for the females.

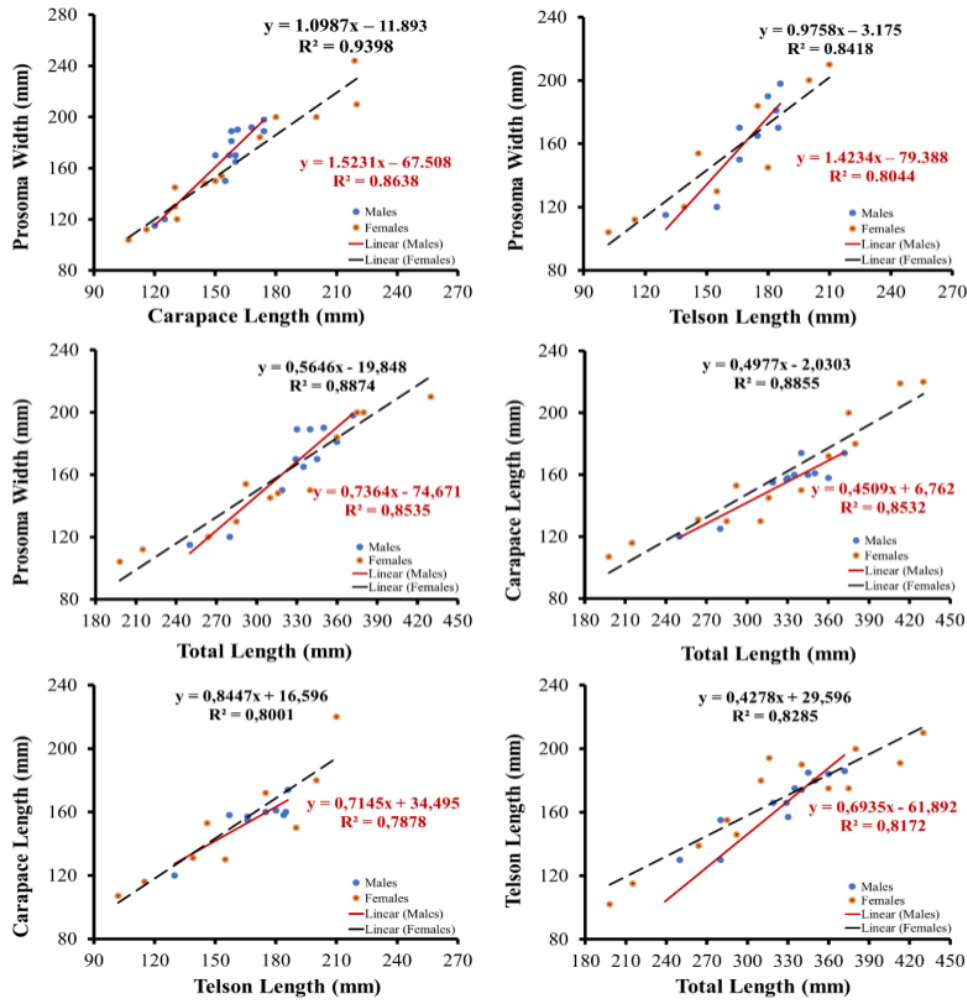


Figure 5. The length-length relationship of *T. gigas* by sex. The male growth pattern indicated that the prosoma width grew rapidly with respect to the carapace length (positive allometric, $b > 1$, $t_s > t_{tab}$, $p < 0.05$), grew slowly with respect to total length and grew in the same rate with respect to telson length. While for females growth indicated that the prosoma width grew slowly with respect to the total length (negative allometric, $b < 1$, $t_s > t_{tab}$, $p < 0.05$), but grew at the same rate with respect to carapace length and telson length (isometric, $t_s < t_{tab}$, $p < 0.05$). The negative allometric was shown by the CL-TL relationships and the TEL-TL relationship for females but the CL-TEL relationship indicated an isometric growth.

The weight-length relationships indicated that males and females of *T. gigas* had an isometric growth pattern ($b < 3$, $t_s < t_{tab}$, $p < 0.05$) unless the BW-PW relationship of males indicated a negative allometric growth ($b < 3$, $t_s > t_{tab}$, $p < 0.05$). These growth patterns indicated that the BW parameter of the males grew relatively slower than the PW parameter however the BW parameter grew at the same rate concerning the CL, TEL, and TL parameters for both sexes.

In the term of the length-length relationships indicated that the PW parameter of the male grew relatively faster than the CL parameter (positive allometric, $b > 1$, $t_s > t_{tab}$, $p < 0.05$) contrarily, the PW parameter grew relatively slower than the CL parameter (negative allometric, $b < 1$, $t_s > t_{tab}$, $p < 0.05$) but grew relatively at the same rate with respect to the TEL parameter (isometric, $t_s < t_{tab}$, $p < 0.05$). Besides that, the CL parameter grew relatively slower than the TL parameter but grew at the same rate

with respect to TEL parameter (isometric, $t_s < t_{tab}$, $p < 0.05$) whereas the TEL parameter grew relatively slower than the TL parameter (negative allometric, $b < 1$, $t_s > t_{tab}$, $p < 0.05$).

The length-length relationships for females indicated that the PW parameters grew at the same rate with the CL and TEL parameters (isometric, $t_s < t_{tab}$, $p < 0.05$) but grew relatively slower than the TL parameter (negative allometric, $b < 1$, $t_s > t_{tab}$, $p < 0.05$). The CL parameter grew at the same rate with the TEL parameter (isometric, $t_s < t_{tab}$, $p < 0.05$), however grew relatively slower than TL (negative allometric, $b < 1$, $t_s > t_{tab}$, $p < 0.05$), whereas the TEL parameter grew relatively slower than the TL parameter (negative allometric, $b < 1$, $t_s > t_{tab}$, $p < 0.05$).

Discussion

The first record of *T. gigas* from Banyuasin Estuarine, South Sumatra of Indonesia, has been published since 2019 (Fauziyah et al. 2019b) however no information in detail to the morphometric variability. This study's results provided the data and information on the morphometric of *T. gigas* for complementing the previous study. Many researchers carried out the analysis of the body allometric for *T. gigas* that obtained from the different populations in Malaysia or India Waters (Chatterji et al. 1994; Vijayakumar et al. 2000; Ismail et al. 2012; Jawahir et al. 2017; Razak and Kassim 2018). Compared with observation in Malaysia (Jawahir et al. 2017; Razak and Kassim 2018) the major body parameters of *T. gigas* in this study tended to attain smaller in sizes for both the sexes.

The body allometric (growth pattern) of this horseshoe crab could be described by the relationship between one body parameter and the other (Panda and Naik 2017), and the morphometric variation in length width and weight also could represent the population structure from immature to mature (Syuhaida et al. 2019). All the PW parameters of this specimen > 8 indicated a mature species for both the sexes (Cartwright-Taylor et al. 2009).

The females were not statistically larger than males and it's not in line with the morphometric characteristic of *T. gigas* from the other's location (Jawahir et al. 2017; Razak and Kassim 2018). However, this results in similar to the morphometric characteristic of *T. gigas* from Pahang, peninsular Malaysia (Ismail et al. 2012). Although these results study indicated no statistically different between males and females for all major body parameters (BW, PW, CL, TEL, and TL) however there was the variation in body allometric for both the sexes.

The male's BW parameter grew relatively slower than the PW parameter (negative allometric) whereas the female's BW and it's PW grew at the same rate (isometric). This result indicated that the male's *T. gigas* were lighter in weight than it's females. The BW parameters also grew at the same rate concerning the CL, TEL, and TL parameters for both the sexes. The weight of these horseshoe crabs increased sharply in the length range 300-400 mm while the increase in the soft body parameters could in consequence of increased food availability and feeding efficiency to the horseshoe crabs (Vijayakumar et al. 2000).

The negative allometric growth for the male's *T. gigas* was shown in this study and it's in line with previous research on *T. gigas* in Malaysia waters (Jawahir et al. 2017; Razak and Kassim 2018) and India waters (Chatterji et al. 1994). The BW-PW relationships indicated that the female's b value (isometric) was higher than the male's b value (negative allometric). The b value was higher in females than males similar to the previous research (Chatterji et al. 1994; Jawahir et al. 2017; Razak and Kassim 2018).

The morphometric variability for the male's *T. gigas* indicated that the BW parameter grew at the same rate concerning the CL, TEL, and TL parameter, the PW parameter grew relatively faster than the CL parameter conversely it grew relatively slower than the TL parameter but grew at the same rate with the TEL parameter, the CL parameter grew relatively slower than the TL parameter but it grew at the same rate with the TEL parameter, while the TL and TL parameters grew at the same rate. The morphometric variability for the female's *T. gigas* indicated that the BW parameter grew at the same rate concerning the PW, CL, TEL, and TL parameters, the PW parameter also grew at the same rate with the CL and TEL parameters but it grew relatively slower than the TL parameter, the CL parameter grew at the same rate with TEL parameter but it grew relatively slower than the TL parameter, while the TEL parameter grew relatively slower than the TL parameter.

Variation in morphometric of the horseshoe crabs could be influenced by many factors (Chatterji et al. 1988; Daniels et al. 1998; Gaspar et al. 2002; Graham et al. 2009) including environmental conditions (habitat and waters quality parameters), and the condition's horseshoe crab (the maturity

stage, diets, population density, and genetic). The fat content, eggs present, and exploitation status influenced the mature female weight of *T. gigas* (Sekiguchi et al. 1988; Mohd Razali and Zaleha 2017; Razak and Kassim 2018). There was the sex ratio balance (Table 2) indicated that their population in a health condition (Cartwright-Taylor et al. 2009). It's one of the essential indicators for the horseshoe crab conservation and management.

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