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Using Applied Statistics for Accurate Size Classification of the Endangered *Tachypleus tridentatus* Horseshoe Crab

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ABSTRACT

The *Tachypleus tridentatus* (Chelicerata: Xiphosura) is an arthropod that usually displays high site fidelity by restricting its distribution to natal vicinities. Briefly, shore perturbation from boating (Kudat), electric, cyanide and bombing (Kunak) and tourism (Semporna) in Sabah can impair the *T. tridentatus* growth and produce sexually mature adults with unusual size. The 8-point morphometry of *Tachypleus tridentatus* from Kudat (Sulu Sea), Kunak and Semporna (Celebes Sea), produces the final output constituting accurate size classification for the species. Meanwhile, *T. tridentatus* are sexually dimorphic ($p = 0.968–0.989$), where male *T. tridentatus* (from Kudat) has prosoma width (27.75 ± 2.68 cm) and weight (1050 ± 610 g) in smaller ranges when compared to the female (33.27 ± 4.68 cm; 3020 ± 1480 g) using the same comparison. In addition, the *T. tridentatus* populations from Kunak (prosoma width: male = 28.91 ± 1.48 cm, female = 29.44 ± 5.47 cm; weight: male = 800 ± 275 g; female = 2550 ± 155 g) and Semporna (prosoma width: male = 21.73 ± 1.34 cm, female = 24.42 ± 1.36 cm; weight: male = 485 ± 306 g, female = 1320 ± 640 g) differed site-wise. The descriptive statistics (average and standard deviation) relate 7.1–23.0% of *T. tridentatus* with negative size class. However, the applied statistics using stepwise analysis and regression curve ($r^2 = 0.566–0.833$), relates 30.4% of *T. tridentatus* with negative size class. Considering weight to produce 100% *T. tridentatus* with positive size class, it is biased because some crabs are gravid with eggs and if attained unpaired (male and female), they may have recently fed. Therefore, the prosoma (male), interorbit (female) and telson lengths are identified as most appropriate for the size classification of *T. tridentatus*. Thus, the findings provide a novel baseline for conservation studies that monitor symmetrical and unusual growth in the *T. Tridentatus* wild stocks.

Keywords: conservation, fisheries, indicator, horseshoe crab, ecology, wild stock

INTRODUCTION

The tri-spine horseshoe crab, *Tachypleus tridentatus* is listed as 'endangered' in IUCN Red List and coexists with the 'data deficient' species like *Tachypleus gigas* and *Carcinoscorpius rotundicauda* in Borneo (Malaysia) (Chen et al., 2019; John et al., 2018). Our knowledge of the *T. gigas* and *C. rotundicauda* biology is recent and up-to-date in Peninsular Malaysia but not for the case of

T. tridentatus. Presently, the published works on *T. tridentatus* include their distribution in Borneo (John et al., 2018), opportunistic mussel predation (Wan et al., 2018) and capture fisheries (Tan, et al., 2012; Zadeh et al., 2009). We learnt that *T. tridentatus* is distributed from Borneo to Honshu islands and is subject to threats like bycatch, biomedical bleeding, sold for consumption, used as decoration or to wade-away evil spirits (John et al., 2021, John et al., 2018; Manca et al., 2017).

Inland forest fragmentation from deforesting lands for agriculture, tourism and urbanization changes the biology of wildlife and introduces fluvial sediments into shallow water bodies. The sediments transported downstream distribute and change the morphology of river banks and shores, redistributes the prey and predator species and also adds stress to benthic assemblages (Chatterji et al., 2012; Nelson et al., 2019). Stress evaluations to benthic communities (c.a. *T. gigas* and *T. tridentatus*) adopted allometric census. While the *T. gigas* allometry is available to indicate its foraging behavior (Tan et al., 2012; Sahu & Dey, 2013), it is yet to emerge for *T. tridentatus*. Meanwhile, the weight-length relationships are used in conservation studies that assess the wild stock health (Chatterji & Pati, 2014; Mohamad et al., 2016), while absence-presence is used to predict distribution change within an area (Pati et al., 2020; 2021).

Unfortunately, the use of allometry is extremely descriptive, the morphology indicators are different with every population and reliability is tested on small sample size (<40 crabs per attempt). Therefore, the use of allometry to indicate unusual horseshoe crab (*T. tridentatus* and *T. gigas*) growth is inaccurate. By increasing the number of indicators to eight and using applied statistics (stepwise analysis and regression) on the samples from Kunak, Kudat and Semporna (Borneo, Malaysia), our aim was to produce an accurate size classification for *T. tridentatus*. This study uses applied statistics like square root ($\sqrt{Xi}$) transformation, Pearson's correlation, Bray-Curtis cluster analysis, stepwise and principal component analysis to produce a novel size baseline for both *T. tridentatus* genders that is flexible for use in future studies.

MATERIALS AND METHODS

Study site description

Sabah (Borneo, Malaysia) is surrounded by seas like South China, Sulu and Celebes while also benefiting from tourism activities in Spratleys, Sida, Labuan, Sipadan and Semporna. Similarly, to the Bajau and Suluk people of Sabah, the coastal communities use marine resources to sustain, which means, *T. gigas* and *T. tridentatus* are consumed in Kudat, Kunak and Semporna. Wild *T. tridentatus* were caught in July-August 2018 for Site 1 (Sungai Karang, Kudat: N 6°53'9.41"; E 116°49'18.69"), a near-pristine area with boating activities; Site 2 (Kampung Madai, Kunak: N 4°43'58.50"; E 118°12'59.68"), an area associated with fish bombing, electric fishing and cyanide poisoning as well as Site 3 (Kampung Simunul, Semporna: N 4°28'2.07"; E 118°37'31.01"), a location frequented by tourists before departure to the islands (Figure 1).

Data collection

Fishermen deployed gill nets (11.4 cm ~ 4.5" mesh size; 400 m × 1 m) during the tide rise and retrieved the nets during the maximum tide rise (soak-time of 2.5–3 hours). The horseshoe crabs that became entangled were removed with caution to avoid limb detachment or carapace damage, a process that could take between 5 to 10 minutes per crab. Simultaneously, the 8-point morphometric indicators (total length, interorbit length, opisthosoma length, prosoma width and length, carapace length, telson length and weight) were measured using a measuring tape (sensitive 0.1 cm) and portable scales (0.01 g sensitivity; see Figure 2). All *T. tridentatus* were released into the water at site of capture while the fishermen were compensated with fuel and monetary payment for boat rental.

Construction of an accurate size classification for *Tachypleus tridentatus*

The morphometric indicators for both, male and female *T. tridentatus* were inserted into Microsoft Excel (2019), before it is exported into Primer v.6.1+Permanova following instructions of the manual. The measurement data (presented as parametric) were transformed using square root ($\sqrt{Xi}$) before the Bray-Curtis cluster analysis. The resemblance matrices were amalgamated into stepwise analysis where the data were

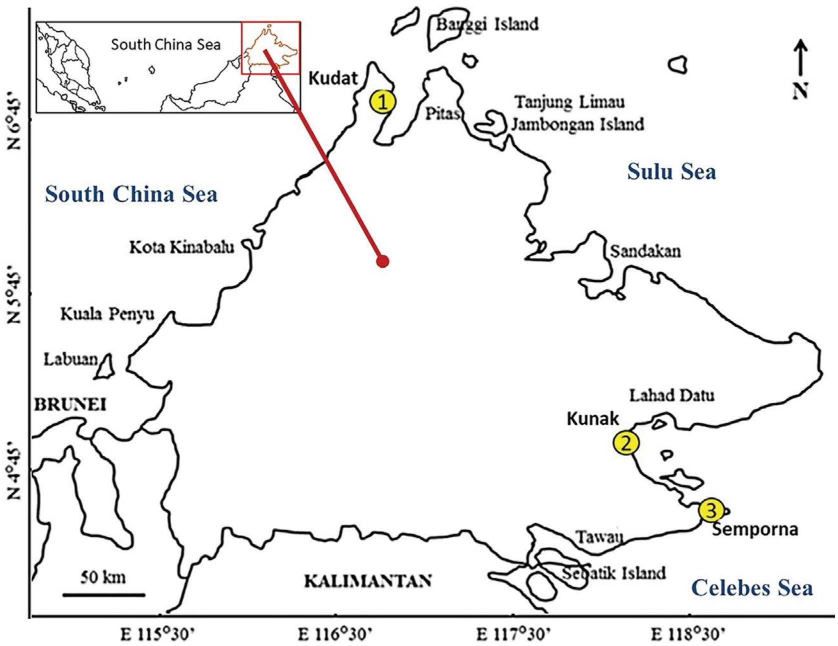

Figure 1. The Tachypleus tridentatus sampling sites in Sabah (Malaysian Borneo). Sampling sites are indicated with 1 = Kudat, 2 = Kunak and 3 = Semporna in which site 1 is facing the Sulu Sea whereas sites 2 and 3 are situated adjacent to Celebes Sea

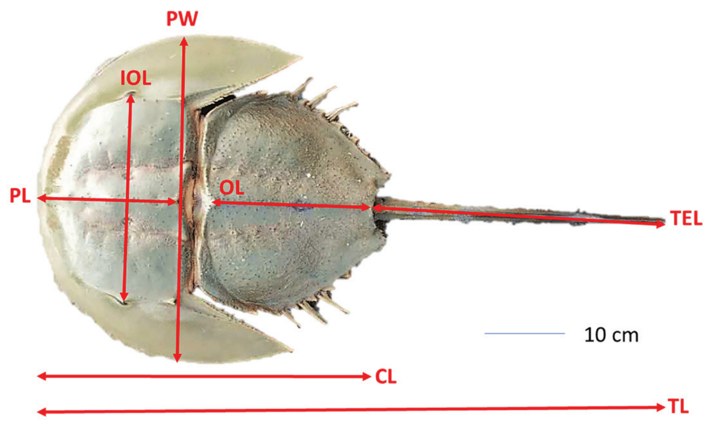

Figure 2. Morphology measurements collected from a Tachypleus tridentatus (female) in the present study. These measurements are collectively known as morphometric indicators, can be collected from both, the male and female horseshoe crab and, are abbreviated as CL = carapace length, IOL = Interorbit length, OL = opisthosoma length, PL = prosoma length, PW = prosoma width, TEL = telson length and TL = total length

sorted using Pearson's correlation. Simultaneously, principal components were constructed so that both analyses identified the best morphometric indicators for *T. tridentatus*. After the selection, a linear plot (y = mx+c) was constructed in Microsoft Excel using the function 'display equation' and 'display r2' function. Then, average and standard deviation values were inserted into the equation manually to establish a median range. Outliers (unusual size) denoted with negative and positive size class were identified and counted. The *T. tridentatus* size classifications as negative, neutral and positive is a novel baseline to benchmark symmetrical and unusual growth in wild stocks.

RESULTS

General size class

A total of 96 *T. tridentatus* (male = 43, female = 53) were sampled from Kudat (n = 34; male = 15, female = 19), Kunak (n = 31; male = 14, female = 17) and Semporna (n = 31; male = 14, female = 17). Living in an environment with minimal anthropic influences, the *T. tridentatus* from Kudat had total length (male = 54.21 ± 5.21 cm; female = 68.42 ± 8.20 cm), carapace length (male = 27.01±2.68 cm; female = 33.72±3.86 cm), prosoma length (male = 27.12 ± 2.37 cm; female = 34.34 ± 3.58 cm), interorbit length (male = 14.51 ± 1.40 cm; female = 18.13 ± 2.49 cm), opisthosoma length (male = 12.73 ± 0.94 cm; female = 16.65 ± 1.59 cm), telson length (male = 25.47 ± 2.64 cm; female = 32.53 ± 3.54 cm) and weight (male = 1050 ± 610 g; female = 3020 ± 1480 g) that appeared larger than the crabs from Kunak and Semporna. The prosoma width of the horseshoe crabs from Kunak were large (male = 28.91 ± 1.60 cm; female = 33.27 ± 4.68 cm) although their prosoma length (male = 16.39 ± 3.57 cm; female = 25.27 ± 2.97 cm) did not exceed the measurements of the crabs from Kudat, whereas the *T. tridentatus* of Semporna had the least values of morphology measurements (Table 1).

The morphometric indicators for male and female *T. tridentatus* were differentiated by Kudat, Kunak and Semporna populations as well as by their gender (Table 2). In this arrangement, we observe maximum (81.3%) *T. tridentatus* possessing symmetrical size class which means their size and weight increases proportionally. We learnt that the maximum number of 22 horseshoe crabs had negative size class, whereas 23 crabs were associated with positive size class. This would mean that 10 male and 12 female crabs had smaller size if compared to the other 10 male and 13 female *T. tridentatus* from the entire pool (n = 45). The 34 *T. tridentatus* from Kudat are associated with less stressful environments if compared to Kunak and Semporna, where 31 crabs were sampled from each site. Therefore, the Kudat populations are anticipated to have the highest morphometric measurements showing viable or healthy *T. tridentatus*. Instead, a maximum number of 10 male and 15 female horseshoe crabs (n = 25) in Kudat, 13 male and 12 female crabs (n = 25) in Kunak and, 12 male and 16 female *T. tridentatus* (n = 28) in Semporna had symmetrical size class. Hence, the morphology indicators like weight, telson length and interorbit length were ideal for conventional size classifications of male and female *T. tridentatus* (Table 2).

Construction of *Tachypleus tridentatus* morphometric census

The 8-point *T. tridentatus* morphometric indicators were transformed into linear values using square root ($\sqrt{Xi}$). Site-wise (Kudat, Kunak and Semporna) comparison of male *T. tridentatus* morphometric indicators showed that prosoma (p = 0.970) and telson length (p = 0.968) and also weight (p = 0.989) achieved the highest correlation values. Comparatively, it was interorbit length (p = 0.984), telson length (p = 0.979) and weight (p = 0.977) for the female *T. tridentatus*. Gender-wise, the male morphometric values are distributed on the positive axis whereas the female crabs have theirs on the negative axis of the principal components (eigenvalue 0.306; cumulative variation 78.2%; Figure 3A) which include a similar outcome for site-wise principal components (Figure 3B). Since descriptive statistics (average ± standard deviation) are applied (male and female separately) to morphometric indicators with highest correlation, the linear equations (y = mx +c) from the graphs generated in Microsoft Excel produce accurate size classifications for *T. tridentatus* if compared to the size classifications (Table 3).

With applied statistics, site-wise, between 2 and 7 male and 5 female *T. tridentatus* possessed symmetrical size class for their respective prosoma and interorbit lengths (Table 3). Meanwhile, gender-wise, the prosoma (male), interorbit (female) and telson lengths of *T. tridentatus* had negative size range (unusual = diminished) whereas for weight, it was positive (unusual = overweight). With 43 male and 53 female crabs associated to 43 *T. tridentatus* pairs (amplexus), the sampling of these crabs during the lunar ebb tides (monthly spawning period) associate them with mature sexual organs (female = gravid with eggs; male = sperm sac swelling) and thus, the attaining of overweight classification.

DISCUSSIONS

The presence of single crabs in Kunak, Kudat and Semporna occur after mechanical separation from wave action (loss of grip by the male) or, the crabs were forcefully separated (after becoming bycatch in fishermen nets) on land or in the shallow waters. Horseshoe crabs that are separated may become stranded on the beach because they could not reach the water during tide fall (Zauki et al., 2019a; Kwan et al., 2017; Robert et al., 2014). This natural selection enables researchers to randomly obtain samples of different age and health conditions (Razak & Kassim, 2018). With the availability of samples, researchers were

Table 1. Morphometric indicators of *Tachypleus tridentatus* from Sabah

No.	TL (cm)		CL (cm)		PW (cm)		PL (cm)		OL (cm)		IOL (cm)		TEL (cm)		BW (kg)	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Kudat																
1	52.8	83.2	26.2	41.5	29.5	41.7	14.2	21.8	12.0	19.7	14.0	22.1	26.6	39.0	0.52	3.27
2	50.2	66.5	29.5	32.0	24.8	34.5	16.3	17.2	13.2	14.8	13.0	17.5	20.7	31.0	0.72	2.69
3	60.0	81.2	29.8	40.0	28.8	41.2	16.1	20.7	13.7	19.3	16.5	21.5	29.5	38.0	0.93	2.59
4	55.1	60.8	28.3	30.8	28.5	30.0	14.7	18.0	13.6	14.8	15.5	17.0	27.0	30.0	1.03	1.55
5	57.6	61.2	27.9	32.4	29.7	28.8	15.3	18.0	12.6	14.4	15.4	17.0	25.5	30.3	1.25	2.28
6	46.3	78.4	23.0	37.8	23.5	35.0	17.9	20.2	11.1	17.6	13.1	22.9	23.1	37.8	0.62	3.10
7	54.0	81.4	25.5	40.5	28.5	40.9	13.5	21.1	12.0	19.4	14.0	21.7	25.2	38.5	0.83	2.59
8	53.2	65.2	25.9	32.5	27.3	30.0	14.0	16.5	11.9	16.0	13.3	17.2	24.0	30.9	0.83	2.90
9	52.2	65.1	24.5	32.5	27.7	30.3	12.4	16.4	12.1	16.1	13.0	17.2	23.5	30.9	0.62	1.86
10	50.0	74.3	25.0	36.6	24.1	37.7	13.2	19.5	11.8	17.1	13.2	20.4	23.1	35.4	0.52	2.28
11	43.9	61.4	24.2	31.2	23.0	30.2	12.1	16.0	12.1	15.2	13.0	17.0	23.0	30.1	0.31	2.28
12	58.0	68.6	32.2	35.1	25.8	33.5	17.6	18.0	14.6	17.1	16.7	17.3	29.1	32.3	1.03	1.86
13	60.2	70.0	29.1	34.2	27.7	35.8	18.5	17.2	16.6	17.0	12.5	17.4	31.1	32.5	1.14	3.03
14	-	71.8	-	35.7	-	36.1	-	18.3	-	17.4	-	17.4	-	32.6	-	3.29
15	-	68.9	-	34.4	-	32.0	-	17.5	-	16.9	-	17.3	-	31.2	-	3.10
16	-	67.5	-	33.9	-	31.0	-	17.5	-	16.4	-	17.2	-	31.1	-	2.59
17	-	56.2	-	30.0	-	30.0	-	17.0	-	16.0	-	17.1	-	26.2	-	2.07
18	-	62.4	-	30.9	-	31.5	-	16.5	-	14.4	-	17.0	-	30.2	-	2.28
19	-	59.5	-	28.6	-	30.9	-	15.0	-	13.6	-	15.6	-	27.2	-	2.07
20	-	58.7	-	29.1	-	29.6	-	15.8	-	13.3	-	15.6	-	28.0	-	1.97
21	-	57.2	-	28.4	-	28.8	-	14.9	-	13.5	-	15.4	-	25.5	-	1.66
Kunak																
1	55.3	74.3	27.0	38.5	28.8	38.8	15.4	28.2	11.6	16.8	14.0	20.4	29.0	35.8	0.51	3.18
2	54.2	70.8	28.9	37.1	28.5	30.8	16.9	26.2	12.0	15.7	14.0	20.1	26.1	35.8	0.82	3.25
3	52.8	67.9	26.2	30.4	27.3	31.9	14.2	27.3	12.0	12.9	13.5	18.0	25.6	33.8	0.72	2.97
4	53.2	68.4	28.5	38.1	29.5	34.8	16.0	27.9	12.5	15.8	14.5	19.1	24.0	31.5	0.82	3.18
5	45.0	59.2	21.4	35.6	27.9	31.2	10.9	26.3	10.5	14.8	13.1	17.9	23.6	33.2	0.51	2.67
6	59.1	63.3	29.3	37.1	28.8	35.2	18.4	27.3	10.9	15.9	15.7	19.9	29.8	26.5	1.13	3.38
7	57.7	65.2	31.0	33.3	32.0	31.1	24.9	25.8	13.1	14.9	16.0	18.1	27.1	35.9	1.03	2.15
8	55.5	67.2	27.0	32.9	28.5	31.9	15.6	27.9	11.6	13.8	14.0	18.1	28.5	37.9	0.62	2.46
9	54.0	67.4	27.9	33.1	28.0	30.9	15.9	27.2	12.0	13.9	13.7	18.4	26.1	33.9	0.82	2.97
10	52.7	56.2	26.3	30.3	27.3	18.9	14.3	22.4	12.0	14.9	13.5	16.0	26.4	29.5	0.51	1.85
11	53.2	69.2	28.5	35.9	29.5	32.2	20.1	27.9	12.5	15.4	14.5	18.7	24.0	36.7	0.72	2.97
12	45.5	59.9	21.3	30.5	27.8	30.3	11.0	25.1	10.3	13.0	13.0	16.8	24.2	28.9	0.72	1.85
13	47.2	59.4	26.2	29.6	24.8	28.8	21.2	18.0	10.2	11.6	13.9	15.9	23.2	29.8	0.51	1.36
14	51.2	57.2	25.1	31.0	24.2	32.0	20.3	17.9	10.7	13.1	13.5	16.0	16.9	27.1	0.62	1.33
15	49.9	-	27.0	-	24.3	-	20.8	-	11.1	-	15.0	-	24.8	-	0.92	-
16	55.2	-	27.5	-	18.9	-	20.4	-	11.2	-	13.5	-	28.0	-	0.41	-
17	50.6	-	31.1	-	30.2	-	27.4	-	12.9	-	18.1	-	19.1	-	0.92	-
Semporna																
1	47.5	58.2	24.3	33.9	21.1	27.8	13.7	22.8	10.6	12.9	13.4	16.7	23.8	27.3	0.33	3.00
2	46.9	58.8	25.8	36.6	23.8	24.2	18.8	21.8	10.8	11.9	14.2	15.1	23.9	30.1	0.72	0.99
3	50.5	57.2	25.1	25.4	24.8	22.8	14.0	21.5	11.1	11.8	13.0	14.9	25.3	29.6	0.41	0.93
4	51.3	54.5	24.3	27.1	23.8	25.9	13.8	21.4	10.5	12.1	12.9	15.8	28.1	25.6	0.41	1.35
5	26.8	55.4	23.8	28.0	15.8	24.9	6.8	21.9	6.6	12.0	10.0	15.2	13.4	29.8	0.21	1.14
6	40.9	53.4	22.2	25.2	20.8	23.9	12.1	21.1	10.1	11.1	12.1	14.9	20.7	29.6	0.31	0.93
7	47.2	55.9	22.8	29.3	20.8	23.8	12.7	21.7	10.1	12.2	12.1	16.1	26.1	21.3	0.21	2.69
8	48.5	56.2	24.5	28.2	21.5	22.9	13.7	23.1	10.8	11.9	13.6	15.7	24.0	31.3	0.31	1.35
9	46.3	45.2	23.0	27.3	23.5	24.4	18.9	22.5	10.1	12.1	13.1	15.4	23.1	17.3	0.62	1.14
10	50.8	56.9	25.4	29.8	25.0	26.9	14.3	21.8	11.1	12.9	13.0	16.1	25.4	29.2	0.72	1.35
11	30.0	52.3	14.5	27.8	17.0	23.2	7.3	22.3	7.2	12.4	11.0	15.4	15.5	26.1	0.41	0.93
12	42.9	53.9	22.7	26.3	20.5	23.6	11.5	23.6	11.2	11.4	12.0	14.9	20.2	26.5	0.32	1.24
13	48.2	56.1	23.0	28.9	21.0	23.8	12.5	23.4	10.5	12.2	12.2	16.1	25.2	28.7	0.31	1.97
14	-	44.2	-	25.9	-	24.8	-	20.6	-	11.2	-	13.9	-	19.5	-	1.86
15	-	53.7	-	28.2	-	24.9	-	21.9	-	12.1	-	15.0	-	27.2	-	1.04
16	-	51.4	-	26.0	-	24.1	-	14.9	-	11.1	-	14.0	-	25.8	-	1.14
17	-	51.0	-	25.6	-	24.0	-	14.6	-	11.0	-	13.9	-	25.4	-	1.04
18	-	51.5	-	25.0	-	24.0	-	13.0	-	12.0	-	12.9	-	26.5	-	0.93

Note: Abbreviated morphometric indicators are TL = total length, CL = carapace length, PW = prosoma width, PL = prosoma length, OL = opisthosoma length, IOL = interorbit length, TEL = telson length and BW = body weight. Hyphens (-) indicate absence of samples to match either male or female *T. tridentatus* counts.

Table 2. Size classification of *Tachypleus tridentatus* using descriptive statistics

Criteria	Kudat			Kunak			Semporna		
	Negative	Symmetrical	Positive	Negative	Symmetrical	Positive	Negative	Symmetrical	Positive
Male									
TL	2	9	2	3	12	2	2	11	0
CL	2	9	2	2	13	2	1	12	0
PW	3	8	2	3	13	1	2	9	2
PL	2	8	3	2	13	2	2	9	2
OL	1	10	2	4	9	4	2	11	0
IOL	1	10	2	1	13	3	2	10	1
TEL	1	9	3	2	12	3	2	10	1
BW	3	8	2	5	10	2	2	8	3
Female									
TL	3	14	4	2	10	2	2	14	2
CL	3	14	4	4	6	4	0	16	2
PW	2	15	4	1	12	1	2	13	3
PL	3	14	4	2	12	0	3	15	0
OL	3	15	3	3	10	1	4	12	2
IOL	3	13	5	3	8	3	4	10	4
TEL	3	14	4	2	10	2	3	14	1
BW	4	12	5	2	11	1	0	16	2

Note: Abbreviated morphometric indicators are TL = total length, CL = carapace length, PW = prosoma width, PL = prosoma length, OL = opisthosoma length, IOL = interorbit length, TEL = telson length and BW = body weight.

Table 3. Applied statistics using linear equation and regression values for *Tachypleus tridentatus* size classification

Criteria	Kudat			Kunak			Semporna		
	Negative	Symmetrical	Positive	Negative	Symmetrical	Positive	Negative	Symmetrical	Positive
Male									
PL	y = 1.6761x (r^2=0.766)			y = 1.6375x (r^2=0.833)			y = 1.4011x (r^2=0.678)		
	13	0	0	15	2	0	6	7	0
TEL	y = 2.8669x (r^2=0.787)			y = 2.0755x (r^2=0.707)			y = 2.4764x (r^2=0.729)		
	13	0	0	17	0	0	13	0	0
BW	y = 0.0887x (r^2=0.704)			y = 0.0613x (r^2=0.700)			y = 0.045x (r^2=0.654)		
	0	0	13	0	0	17	0	0	13
Female									
IOL	y = 1.2044x (r^2=0.693)			y = 1.8079x (r^2=0.719)			y = 1.1985x (r^2=0.734)		
	16	5	0	14	0	0	18	0	0
TEL	y = 2.1222x (r^2=0.690)			y = 3.2825x (r^2=0.727)			y = 2.1021x (r^2=0.724)		
	21	0	0	14	0	0	18	0	0
BW	y = 0.1642x (r^2=0.682)			y = 0.2327x (r^2=0.566)			y = 0.1056x (r^2=0.573)		
	0	0	21	0	0	14	0	0	18

Note: Equations presented as y = mx + c represents linear relationships whereas value in brackets (r^2) is the regression variance. The horseshoe crab, *Tachypleus tridentatus* size is classified into negative (unusual), symmetrical and positive (unusual) representations.

directly measuring the crabs using morphometric keys (carapace, prosoma and total length) so that allometric (weight-length and length-length) relationships can be produced. With inconsistent sample size that reached a maximum of 43 pairs, the growth of T. tridentatus is thought to remain uniform (Tan & Jardine, 2019; Hu et al., 2018; Kaiser & Schoppe, 2018) because an isometric growth, also achieved with T. gigas (Zauki et al., 2019b; Jawahir et al., 2017; Chaimongkol &

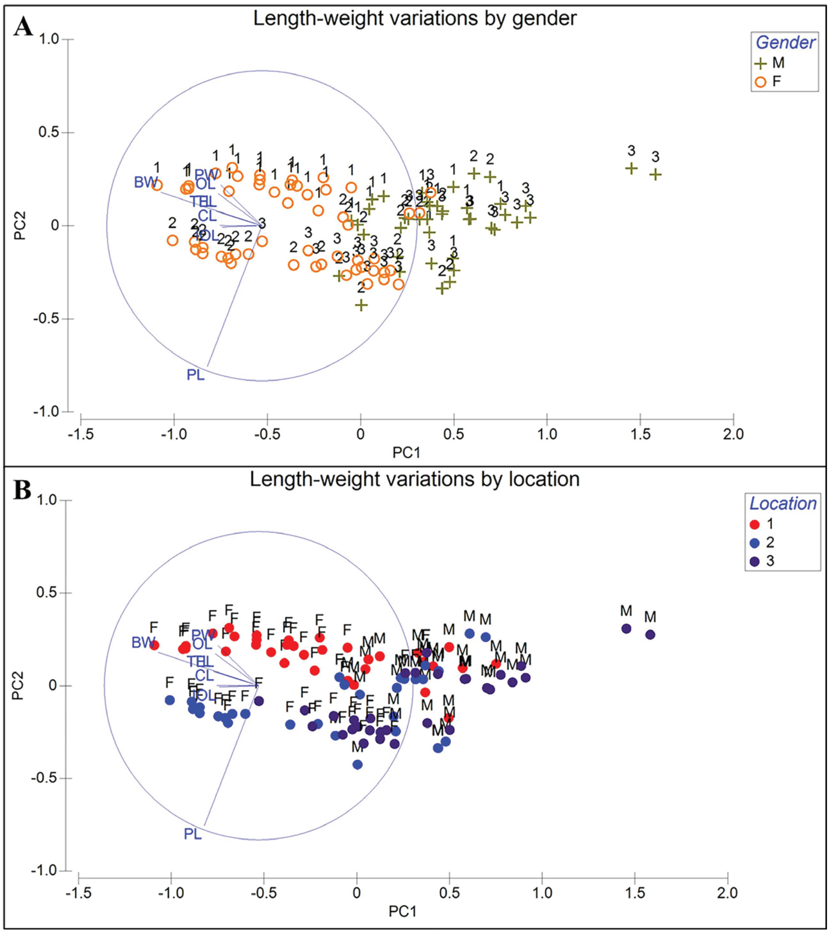

Figure 3. Principal component for *Tachypleus tridentatus* by (A) gender and (B) location (Kudat, Kunak and Semporna) in Sabah. Abbreviations for M = male and F = female whereas numbers are used to indicate the horseshoe crab population origin such as 1 = Kudat, 2 = Kunak and 3 = Semporna

Intanai, 2018; Martinez et al., 2018; Nelson, et al., 2016) would mean, the environment is healthy and that food resources are sufficient. When the conventional length-weight relationship is employed on samples from Kudat, Kunak and Semporna in the present study, it is learnt that morphometric values that were benchmarked on existing size range for *T. tridentatus* were producing a negative size and positive weight range which was never achieved previously. However, the findings remain doubtful as the entire sample pool was indicated with that size-weight classification.

Thus, using applied statistics (with square root transformation ($\sqrt{Xi}$), stepwise and principal component analysis, the *T. tridentatus* from Kudat were classified with 'symmetrical' sizing whereas it was negative for the crabs that originate from Kunak and Semporna. Comparative morphological sizing between *T. tridentatus* from less disturbed areas in Taiwan [2], Malaysia (Manca et al., 2017; Mohamad et al., 2016), China (Hu et al., 2015) and Korea (Yang & Ko, 2015) consider the crabs from Kudat with symmetrical total length (male: 50–65 cm; female: 60–80 cm), prosoma width (male: 25–30 cm; female: 30–40 cm), prosoma length (male: 20–30 cm; female: 30–40 cm) and weight (male: 600–1500 g; female: 1300–3700 g) while demarcating them with symmetrical weight and length. Meanwhile, the *T. tridentatus* from Papar, Sandakan and Tawau (Sabah, Borneo) have a short telson, the crabs from Kunak possessed wide (width) prosoma and those from Semporna exhibit an immature range of size (23–41% smaller).

Generally, horseshoe crabs develop and feed in nursery grounds within a nativity range (Brockmann et al., 1994; Vijayakumar et al., 2000; Brockmann, 2002; Zaleha et al., 2012; Smith et al., 2013; John et al., 2018), while their spawning and feeding activities in the estuary is a homing attraction. Thus, the populations do not mix unless they were intentionally introduced after becoming bycatch. The knowledge on *T. tridentatus* evolution with wide prosoma and diminished sexually mature sizes is yet to emerge. We are certain that morphological sizing is a strategy adopted by horseshoe crabs after learning from their environment. Presently, *T. gigas* and *C. rotundicauda* are known to change spawning locations on the shore, constructs burrows with different depths and stocks the nest differently (28–303 eggs) in every season (Kwan et al., 2018, 2016; Pati et al., 2020).The fisheries practices, residential inhabitancies and mangrove forest clearing over many generations in Kunak and Semporna (Harun et al., 2019; Khandaker et al., 2019; Bakker et al., 2017; Vasquez et al., 2017; Mitra et al., 2016; Vasquez et al., 2015; Muda, 2010) may have influence on the bodies of *T. tridentatus*. Clearly, the environment conditions *vis-à-vis* shortage of sustenance are responsible for diversified morphometric sizing in horseshoe crabs (Bergland et al., 2019; Godfray et al., 2019; Pinsky, 2019) which means, the accurate size classifications for *T. tridentatus* offer a baseline correction that indicates unusual growth (smaller built) among the sexually mature crabs.

CONCLUSIONS

Horseshoe crabs like *T. tridentatus* have previously been studied for the length and weight relationships but unfortunately, the number of morphological indicators was lesser (4–6) and the morphometric values were not binomial. This study provides a comparison between the raw data treated with descriptive statistics and novel technique where the transformed data is merged with applied statistics. With the *T. tridentatus* morphology indicators limited to prosoma, interorbital and telson, it was found that the crabs from Kudat, Kunak and Semporna had negative sizing for length and positive sizing for their weight. With applied statistics, the accuracy was improved because the *T. tridentatus* from Kudat had symmetrical sizing for length, whereas the crabs from Kunak and Semporna exhibited negative sizing for length. Though increasing the sample size to 200 crabs per species is challenging in Malaysia, it is possible that applied statistics will change the selection of morphometric indicators and enhance the accuracy of size classifications. As of now, the prosoma length, telson length and weigh of the crabs from Kudat are validated baseline morphometric values that can be used as control group in other studies.

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