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Journal of Threatened Taxa

Building evidence for conservation globally

www.threatenedtaxa.org

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

ARTICLE

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26 December 2018 | Vol. 10 | No. 15 | Pages: 12916–12932
10.11609/jott.4125.10.15.12916-12932



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ISSN 0974-7907 (Online)
ISSN 0974-7893 (Print)

Journal of Threatened Taxa | www.threatenedtaxa.org | 26 December 2018 | 10(15): 12916–12932

POSTEMBRYONIC DEVELOPMENT OF THE TRI-SPINE HORSESHOE CRAB *TACHYPLEUS TRIDENTATUS* (MEROSTOMATA: XIPHOSURA) IN A NURSERY HABITAT IN THE PHILIPPINES

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Abstract Populations of the Tri-spine Horseshoe Crab *Tachypleus tridentatus* have dramatically decreased over their distribution range and conservation efforts are now crucial. The implementation of appropriate management strategies and stock assessment rely on accurate growth-rate estimates. The postembryonic development of the species in the tropics, however, is not elucidated. To provide the information needed to assess the demographics of juvenile populations and to judge the status of *T. tridentatus* in the Philippines, we conducted a mark-recapture experiment in a nursery habitat on Palawan Island. The results obtained during the 10-month period provide the first consecutive data on the stepwise growth of the species in the Philippines and the first near comprehensive dataset collected within a single population of juveniles in the tropics. By analyzing size-frequency (prosomal width) distributions of 853 individuals and by using 94 juveniles that molted during the study, 13 molt stages were differentiated. Based on the intermolt periods of six instars, we estimated the growth curve of *T. tridentatus* following two models (non-linear and power function). The data support the assumption that growth continues year-round in the tropics and also indicate that the average age of mature male and female *T. tridentatus* in the Philippines ranges from three to four years. The agreement with a field study in Japan suggests that 14 postembryonic stages may be characteristic for the development of natural populations throughout the range of the species. Though more data are needed to validate these results, the study provides a sound baseline for future studies in the tropics.

Keywords: Tri-spine Horseshoe Crab, juveniles, development stages, intertidal zone, morphometry, allometry, size-age relationship, growth curve.

Abbreviations: AA - Distance between the anal angles; BL - Body length (PL+OL+TL); BM beach - Bernardo Marcelo Beach (study site); CL - Carapace length (PL+OL); DS - Development stage; Eyes - Distance between the compound eyes; IMP - Intermolt period; OL - Opisthosomal length; OW1–3 - Opisthosomal width 1–3; PES - Postembryonic stage; PL - Prosomal length; PW - Prosomal width; SPSS - Statistical Package for the Social Sciences; TL - Telson length; **Statistics:** CI - 95% confidence interval; df - Degrees of freedom; M - Mean value; Min / Max - Minimum and maximum values; n - Sample number; p - Significance level; r - Pearson's correlation coefficient; SD - Standard deviation; SE - Standard error of the mean; T - Statistics of the one-sample t test; U - Statistics of the Mann-Whitney U test; W - Statistics of the Shapiro-Wilk Test; Z - Statistics of the Kolmogorov-Smirnov test.

DOI: <https://doi.org/10.11609/jott.4125.10.15.12916-12932>

Editor: Ruth H. Carmichael, University of South Alabama, Dauphin Island, USA.

Date of publication: 26 December 2018 (online & print)

Manuscript details: Ms # 4125 | Received 14 March 2018 | Final received 03 August 2018 | Finally accepted 02 December 2018

Citation: Kaiser, D. & S. Schoppe (2018). Postembryonic development of the Tri-spine Horseshoe Crab *Tachypleus tridentatus* (Merostomata: Xiphosura) in a nursery habitat in the Philippines. *Journal of Threatened Taxa* 10(15): 12916–12932; <https://doi.org/10.11609/jott.4125.10.15.12916-12932>

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Funding: Mainly self-funded. The 'Studienstiftung des Deutschen Volkes' provided a scholarship for the first author in 2001.

Competing interests: The authors declare no competing interests.

For Author Details and Author Contribution see end of this article.

Acknowledgements: We are especially grateful for the cooperation of the Western Philippine University in Puerto Princesa City on Palawan, Philippines. We extend our special thanks to Prof. Günter Arlt from the University of Rostock, Germany. We further thank Sarah Barredo for her contribution to the collection of juveniles in 2017. The authors are much obliged to the two anonymous reviewers for their constructive comments that considerably improved the manuscript.

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INTRODUCTION

The Tri-spine Horseshoe Crab *Tachypleus tridentatus* (Leach, 1819) (Xiphosura: Chelicerata) is the largest of four extant species of ancient marine arthropods, the origin of which can be traced back to 445 million years (Shuster et al. 2003; Rudkin et al. 2008). The information about horseshoe crabs in the Philippines is especially scarce. Waterman (1958) recorded *T. tridentatus* and *Carcinoscorpius rotundicauda* (Latreille, 1802) for western and southern Philippines, respectively; based on a picture, both species were listed as occurring in the Province of Palawan (Sekiguchi 1988). The first survey conducted from northern to central Palawan confirmed the presence of *T. tridentatus* in the area (Schoppe 2002).

Recent harvest pressures and habitat loss prompted the need for management actions to protect horseshoe crabs (Berkson et al. 2009). Although still listed in the Data Deficient category by the IUCN (2017), observations in Taiwan, Japan, Hong Kong, Singapore, Malaysia, Borneo, and Thailand indicate that populations of *T. tridentatus* have dramatically decreased (Itow 1993; Hsieh & Chen 2009, 2015; Chen et al. 2015; Kwan et al. 2016; Lee & Morton 2016; Wada et al. 2016; Manca et al. 2017). Unlike other regions in Asia, in the Philippines, there is no commercial exploitation of *T. tridentatus* for the production of amebocyte lysate or food, but habitat loss due to land reclamation, sea sand mining, and coastal development are destroying its natural breeding beaches and nursery habitats. As a result, *T. tridentatus* faces a high risk of extinction without management efforts to conserve its viable populations and habitats (Schoppe 2002).

The implementation of effective management strategies and stock assessment relies on accurate estimations of growth rates (size at age, size at maturity, and age at first capture), size distributions, and population size (Froese et al. 2008; Chang et al. 2012; Cunningham & Darnell 2015; Cao et al. 2016). A lot of effort was made during the last few decades to understand the growth biology of horseshoe crabs, including laboratory studies on the influence of environment factors such as water temperature and salinity (Jegla & Costlow 1982; Chen et al. 2010; Zaleha et al. 2011), pH (Tanacredi & Portilla 2015), sediment type (Hong et al. 2009; Hieb et al. 2015), tank size (Hieb et al. 2015; Chen et al. 2016), and food quantity and quality (Carmichael et al. 2009; Schreiberman & Zarnoch 2009; Hu et al. 2013). Due to the lack of calcified structures persisting through the molt, however, the age of the different instars was not

unambiguously determined for any horseshoe crab species (Carmichael et al. 2003; Chen et al. 2010).

Specifications for *T. tridentatus* vary strongly in the literature, although there is a consensus that the females molt once more than the males to reach maturity. Based on laboratory studies, Sekiguchi et al. (1988) calculated that the females spent 14 years to molt 16 times before attaining maturity, while Chen et al. (2010) estimated that the females mature in stage 15 after four years when reared in warm water. Goto & Hattori (1929) identified 14 postembryonic stages by measuring individuals in their natural habitats in Japan. Kawahara (1984), on the other hand, suggested that the females in Japan molt 15 times in 10 years to reach maturity, while Asano (1942, cited in Lee & Morton 2005) estimated that they molt 18 times in 16 years. To our knowledge, the postembryonic development of *T. tridentatus* in their tropical environment is not yet determined, but laboratory studies indicate that the growth of juveniles could continue throughout the year when the temperature is greater than 28°C (Lee & Morton 2005; Chen et al. 2010). Most studies concerning the life history of *T. tridentatus* are from Japan, China, or Hong Kong — countries where ecdysis and spawning appear to stop during colder seasons (for instance, Sekiguchi et al. 1988; Chiu & Morton 2004; Zhou & Morton 2004; Lee & Morton 2005; Hu et al. 2009, 2015; Kwan 2015; Kwan et al. 2015) — while fewer studies were conducted in the tropics (Robert et al. 2014; Mohamad et al. 2016; Manca et al. 2017; Mashar et al. 2017).

In this study, we characterized the postembryonic development of *T. tridentatus* in a nursery habitat on Palawan to provide sound baseline data needed for conservation, particularly in southeastern Asia (Berkson et al. 2009; Shuster & Sekiguchi 2009). In 2001, the population in the study site was estimated to comprise of 298 individuals with a male-to-female ratio of 1.2:1 (Kaiser 2002). The main objectives of this study were 1) to identify the number of instars until maturity, 2) to describe the post-embryonic growth patterns, and 3) to estimate the size-age relationship of *T. tridentatus* in the Philippines. These data represent the first dataset of this type for the tropics.

MATERIALS AND METHODS

Study site

The growth of juvenile *T. tridentatus* was studied in a nursery habitat located close to the Puerto Princesa City on the eastern coast of Palawan in the Philippines

(9.762°N & 118.772°E) (Fig. 1). The study region is characterized by a rainy season that lasts from June to November and a dry season from December to May (PAGASA 2017). The annual rainfall averages at 1,684mm, while the mean annual temperature is 27.2°C. The area has a mixed semidiurnal tidal cycle (National Ocean Service 2008); the tidal range during spring tide may rise up to 1.9m. The mean water temperature in the study area is 29°C and 31°C during high and low tides, respectively (Table 1). The average pH ranges between 7.6 and 8 and the mean salinity between 30 and 31‰ (Table 1). The study site reported upon herein is the Bernardo Marcelo Beach (hereinafter BM beach) (Image 1), a 130m-wide and 600m-long seagrass meadow. About 65% of the sampling area (50,700m²) is covered with seagrass, while the rest comprises sandy patches. The mean grain size is 0.103±0.02 mm (n = 33). It comprises a zone between the mean low and mean high water levels and the upper sub-tidal range. The local residents use the northern part of the beach for recreation in the weekends. Shellfish are gathered and fishermen position bottom-set gillnets seaward of the sampling area in a seagrass meadow without larger sand patches and an adjacent riff (about 600m wide).

Sampling strategy (capture-mark-recapture)

The juvenile population of *T. tridentatus* at BM beach was assessed during daytime at low tide from May to December 2001 for 84 days and between April and May 2017 for 23 days. Following Rudloe (1983), the assessment started about two to three hours before the lowest water level was reached and lasted for four to five hours. By walking barefoot in a zigzag course, the



Image 1. The study site BM beach at low tide

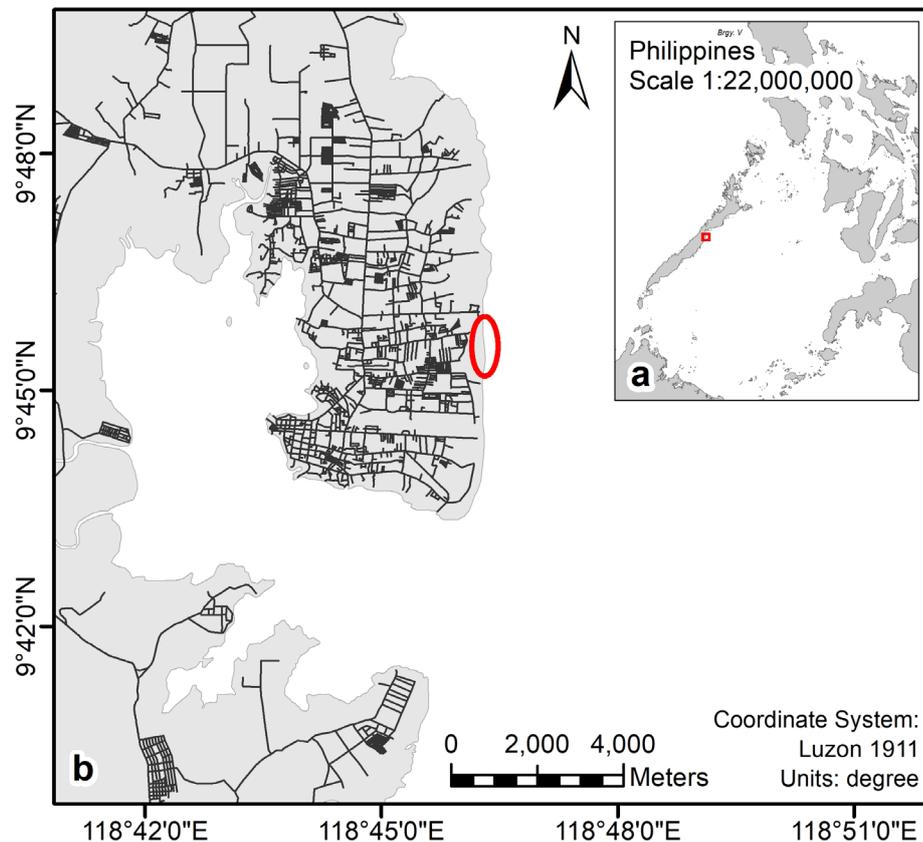


Figure 1. The geographic location of the study site.
 a - Puerto Princesa City on the eastern coast of Palawan in the Philippines;
 b - Bernardo Marcelo Beach

surface of the entire sampling area was systematically searched for juveniles and exuviae. The majority of juveniles were found with the help of their feeding trails and mostly in the sandy-muddy substrate; some larger individuals were sensed by foot. Additionally, several adult horseshoe crabs were handed over by fishermen and found at the market; they were measured as well.

Morphometric parameters (prosomal length, opisthosomal length, telson length, prosomal width, opisthosomal widths 1–3, the distance between the anal angles, the distance between the compound eyes) were measured to the nearest 0.1mm using a Vernier caliper (Fig. 2). Sex was identified based on the size and shape of the genital papillae (Bonaventura et al. 1982). Superglue was used to fix a number on the prosoma of each individual. For identification after ecdysis, the lateral mobile spines of the opisthosoma were shortened

(Kawahara 1982) following a coding system (Appendix 1). The identification mark of the adults was modified following Sokoloff (1978). All juveniles were released where they were found. The individuals found during or shortly after ecdysis were kept under an ambient temperature of 25–32 °C in a tank with air supply and the water of BM beach; they were measured and marked when the carapace had sufficiently hardened. A few individuals supplied by fishermen or shellfish gatherers were marked and released at BM beach.

Data analyses

Unless otherwise stated, the significance threshold was set to $\alpha = 0.05$ and the significance was tested at a two-tailed level. Statistical Package for the Social Sciences (SPSS), version 15.0 (SPSS, Inc., Chicago) and Microsoft Excel 2010 were used to carry out the

Table 1. Mean values (M) ± standard deviation (SD) of the hydrographic conditions prevailing in the nursery habitat of Bernardo Marcelo Beach on Palawan, with sample size (n) and minimum and maximum values (Range)

Tide		Temperature (°C)	pH	Salinity (‰)	Dissolved oxygen (%)
High	Mean ± SD	29.2 ± 1.2	7.6 ± 0.05	31.0 ± 0.82	71.5 ± 34.3
	Range	26.5–32.0	7.5–7.7	29.0–32.4	25.8–111.9
	n	50	17	59	35
Low	Mean ± SD	31.1 ± 0.9	8.0 ± 0.15	30.0 ± 1.05	94.2 ± 45.3
	Range	29–32.5 ¹	7.8–8.3	27.4–32.0	48.3–228.0
	n	54	10	67	46

¹ On hot days, the water temperature during low tide was up to 41°C.

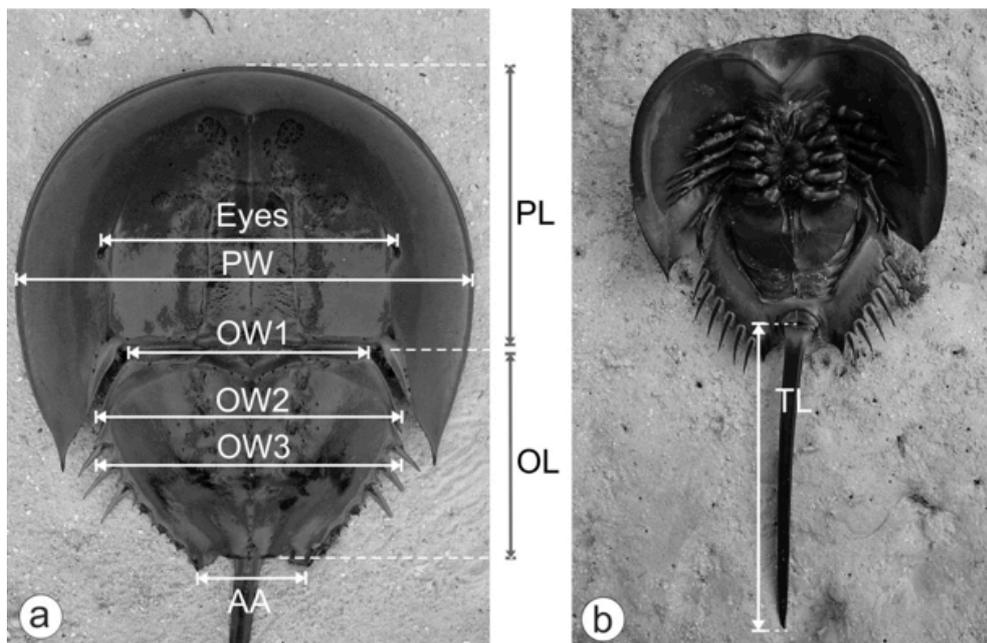


Figure 2. Measurements taken. a - prosomal length (PL), opisthosomal length (OL), prosomal width (PW), opisthosomal width (OW1–3), and the distance between the anal angles (AA) and between the compound eyes (Eyes); b - telson length (TL). The carapace length (CL) equals PL+OL; the body length (BL) equals PL+OL+TL

statistical tests.

Cohort demarcation

The prosomal width (PW) was used to mark the instar stages that characterize the postembryonic development of *T. tridentatus* on Palawan. The PW of all individuals and exuviae were grouped into specified size intervals and size-frequency histograms were plotted. The resulting histograms were multimodal and indicated different cohorts or development stages (DS) that differed in abundance. The visual cohort demarcations were adjusted by comparing the data with 94 individuals that molted during the study period or whose individual exuviae was measured (hereinafter referred to as molting individuals). The measured adults allowed the defining of the size limits for the cohort of sexually mature males and sub-adult females, as well as the stage of sexually mature females.

The resulting size ranges of the DS were ascertained by conducting a modal progression analysis of the size-frequency distributions with the Fish Stock Assessment Tool, FISAT II (FAO 2006–2017, <http://www.fao.org/>). FISAT II applies the maximum likelihood concept to separate the normally distributed components of the data, thus allowing the demarcation of the cohorts from the multimodal distributions. Thereafter, a one-sample t test was used to compare the means calculated in FISAT with the DS defined by the visual demarcation and the molting individuals.

Body measures

The Shapiro-Wilk test ($n \leq 50$) and the Kolmogorov-Smirnov test (with Lilliefors correction) ($n > 50$) were used to examine whether the established size ranges and the body parameters were normally distributed. The homogeneity of variances was tested using the Levene's test (Field 2009). Because some data showed a non-normal distribution when differentiating the juveniles' sex, the non-parametric Kruskal-Wallis test was used to compare the body measures of females and males. Subsequently, pair-wise comparisons for each DS were performed using the Mann-Whitney procedure. A Bonferroni correction for multiple comparisons was applied to the significance level (differences were regarded as significant at $\alpha < 0.006$). The Mann-Whitney procedure was further used to compare various morphometric ratios between juvenile and adult *T. tridentatus* (males, females, and individuals with unknown sex were pooled). A one-sample t test was used to compare the carapace lengths per DS with the mean values reported by Goto & Hattori (1929).

Hiatt growth model

According to Hiatt (1948), a linear growth model can be used to fit the post- and pre-molt size of crustaceans and this model was applied to assess horseshoe crab growth (Carmichael et al. 2003; Hu et al. 2013). The model describes the growth under natural conditions as $PW_{n+1} = a + b PW_n$, where PW_n is the pre-molt PW at instar n and PW_{n+1} is the post-molt PW at instar $n+1$. The y-intercept indicates whether the size increment increases ($a < 0$) or decreases ($a > 0$) with an increase in the size of the animals or whether it stays constant during the development ($a = 0$). Slope b represents the growth coefficient, allowing conclusions on the variations between the size increments of the consecutive molts. The Hiatt growth model was applied first to illustrate the size increment observed for the 94 molting individuals. Additionally, the model was applied based on the data of the cohort demarcation by using the average PW of the instars. A Student's t test was used to compare the slopes of the regression lines in the two Hiatt diagrams to allow conclusions to be drawn regarding the demarcation of the DS identified at BM beach.

Allometric growth

The allometric growth of each morphometric parameter (y) was expressed as a power function of the PW (x) with the equation $y = a x^b$ (Fuiman 1983). The relative growth coefficient was estimated by linear regression of the log-transformed allometric growth curve of the type $\log y = \log a + b \log x$, where a is the intercept and b the growth coefficient (Gould 1966). The growth coefficient identifies whether the growth pattern indicates isometric growth ($b = 1$ for the lengths or widths and $b = 3$ for weight), positive allometry ($b > 1$ for lengths or widths and $b > 3$ for weight), or negative allometry ($b < 1$ for lengths or widths and $b < 3$ for weight). If the value 1.0 (or 3.0 in the case of the weight) is outside the 95% confidence interval of b , the difference is regarded as significant. A Student's t test was used to compare the slopes of the regression lines to assess whether males and females differed significantly. To illustrate a specific characteristic of the growth of the horseshoe crabs' telson, a linear regression for the relation between the PW and the telson length (TL) was again carried out graphically.

Growth curve

The first postembryonic stage (PES), the trilobite larvae, was not found during the study. The PW of the trilobite larvae was estimated following the Dyar's rule ($\ln PW = a PES - b$), which was already applied

by Waterman (1954). A cubic regression was used to describe the growth curve of PES 1–9. Chang et al. (2012) compared the fit of different models ranging from simple equations to models describing continuous and discontinuous growth for two lobster species and two crab species. They concluded that the non-linear model applied by Castro (1992) was the best model to quantify and predict the relationship between the pre-molt length and the intermolt period (IMP) for the selected crustaceans, although they suggested that different models should be used to reduce the uncertainty in model selection. Based on the IMPs observed at BM beach, two methods were applied to predict the growth curve and estimate the age of *T. tridentatus* at sexual maturity. The non-linear model describes the IMP as a function of the average carapace length (CL) per instar stage, $IMP \text{ (days)} = a + b CL^c$ (see Castro 1992; Chang et al. 2012). The second method describes the age as a power function of the PES, $age \text{ (months)} = a PES^b$.

RESULTS

Cohort demarcation

The PWs of the juveniles at BM beach ranged from 0.9cm to 21.1cm. The largest mature female had a PW of 36.9cm. Excluding the animals exhibiting

physical injuries on the prosoma, 853 PWs were used in demarcating the growth stages (Table 2). Owing to the natural growth variability of individuals, the size ranges of the DS increase with increasing age. The cohorts were, therefore, classified with frequency distributions of increasing interval widths. Five DS were identified in the histogram, presenting the smallest animals at 0.05cm intervals (Fig. 3a). The cohort with a PW ≤ 1.1cm was termed DS A, with subsequent cohorts named in alphabetical order. It has to be stressed that DS A does not represent PES 1, the trilobite larvae. Trilobite larvae were not found during the study.

The DS F–I were depicted most clearly with an interval size of 0.2cm (Fig. 3b). For larger juveniles and adults, an interval size of 0.5cm was the best, but the number of measurements did not allow a clear demarcation between the biggest juvenile stage (DS K) and the adult stages (DS L–M) (Fig. 3c). The difference between DS J and the cohort of sexually mature males and sub-adult females (DS L) then revealed the DS K. Because the size limits of this cohort could not be unequivocally validated with our data, DS K was not included in the following statistical analyses. The mean PWs identified in FISAT did not differ significantly from the PWs defined by visual demarcation (one-sample t test, $p > 0.05$), thereby confirming the cohort demarcation (Table 2, Fig. 4).

Table 2. Mean (M_{PW} in cm) with standard error (SE) and the minimum (Min) and maximum (Max) prosomal width of the development stages (DS) A–M found at the study site on Palawan, along with sample number (n), test statistic of the Shapiro–Wilk Test (W for $n \leq 50$), the Kolmogorov–Smirnov test (Z for $n > 50$), and associated p-values. Further shown are the results of the modal progression analyses in FISAT II: the mean prosomal width ($M2_{PW}$ in cm), standard deviation (SD_{M2}), the statistics of the one-sample t test (T^a), and the asymptotic significance (two-tailed p^a)

DS	n	M_{PW} (cm)	SE	Min	Max	W	Z	p	FISAT II			
									$M2_{PW}$ (cm)	SD_{M2}	T^a	p^a
A	12	0.98	0.02	0.90	1.10	0.96	-	0.717	0.99	0.06	-0.57	0.581
B	36	1.30	0.01	1.18	1.44	0.95	-	0.113	1.31	0.07	-0.65	0.523
C	46	1.82	0.01	1.58	1.99	0.97	-	0.221	1.84	0.09	-1.23	0.225
D	54	2.48	0.02	2.22	2.84	-	0.09	0.200	2.49	0.12	-0.61	0.544
E	69	3.34	0.02	2.90	3.70	-	0.08	0.200	3.35	0.21	-0.21	0.832
F	102	4.40	0.03	3.90	5.10	-	0.09	0.200	4.44	0.25	-1.58	0.118
G	143	5.96	0.04	5.20	7.00	-	0.06	0.066	5.96	0.45	0.08	0.939
H	159	8.23	0.04	7.02	9.42	-	0.04	0.200	8.28	0.63	-1.04	0.301
I	143	11.11	0.06	9.60	12.70	-	0.06	0.200	11.19	0.79	-1.18	0.239
J	26	14.00	0.19	12.80	16.45	0.94	-	0.100	14.11	0.87	-0.58	0.569
K	13	18.84	0.36	17.13	21.12	0.91	-	0.197	19.13	2.21	-0.79	0.443
L	34	25.40	0.29	21.66	28.90	0.98	-	0.618	25.61	1.33	-0.74	0.465
M	15	32.38	0.68	29.20	36.90	0.90	-	0.065	30.84	3.59	2.26	0.040

^aA one-sample t test was used to compare $M2_{PW}$ with the PWs measured per DS.

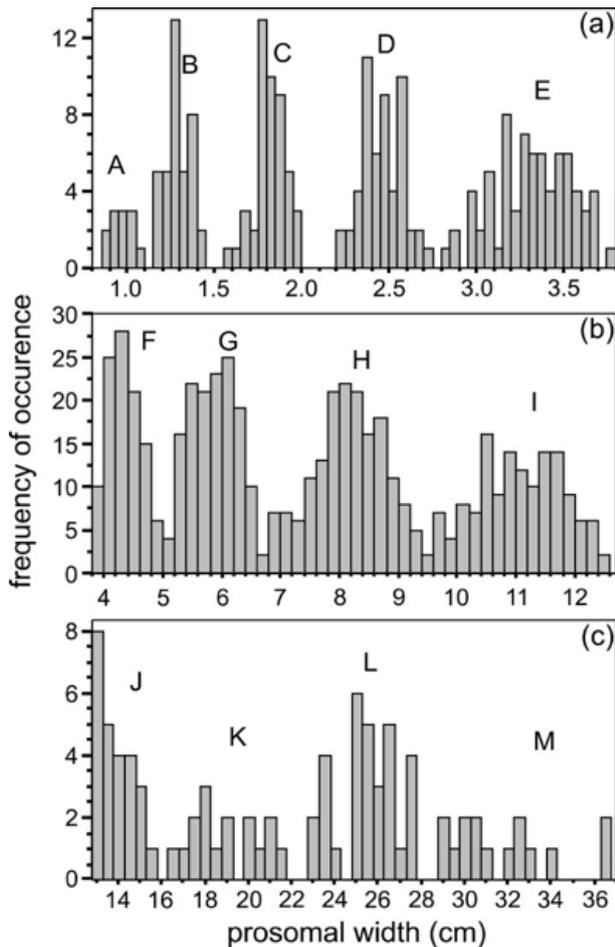


Figure 3. Size-frequency histograms a - with an interval size of 0.05cm, indicating the development stages A–E; b - an interval size of 0.2 cm, indicating the development stages F–I; c - an interval size of 0.5cm, indicating the development stages J–M

Morphology of *T. tridentatus* on Palawan

Before reaching sexual maturity, almost no morphometric differences could be observed between the sexes (Kruskal-Wallis test, $p > 0.05$). An exception was DS H, where males and females differed in all body parameters (Mann-Whitney U test with Bonferroni correction, $p < 0.006$). Individuals whose sex was not determined, 330 of 804 juveniles and exuviae, were not included in the analyses. The DS A–B were not considered as they were too small for sex determination.

The morphometric parameters of the instars A–M in Tables 3–4 were pooled for both sexes, including the exuviae but excluding individuals with injuries in the relevant body parts. Most of the body parameters in Table 3 showed a normal distribution. The comparison of various morphometric ratios between juveniles and adults (pooled male and female data) with the Mann-Whitney procedure revealed highly significant

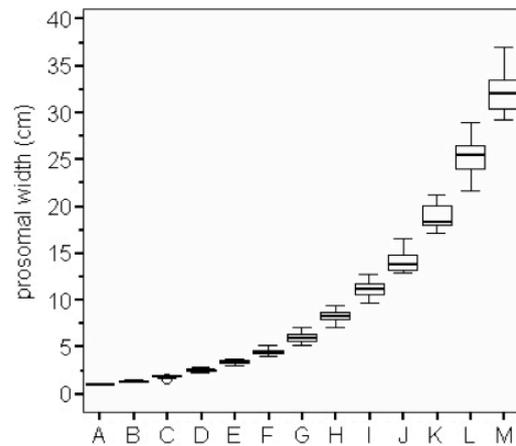


Figure 4. Box plot illustrating the median, interquartile range, extreme values (whisker), and outliers (°) of the prosomal width for each development stage found at Bernardo Marcelo Beach, Philippines

differences for the ratios PW/OW2, PL/OL, OW2/OL, CL/PW, and CL/TL (Appendix 2). The Eyes/PW and PW/PL ratios were statistically insignificant at the 95% probability level (Appendix 2).

Growth analyses
Hiatt growth model

The regressions of the relation between the pre- and post-molt PWs of *T. tridentatus* on Palawan support the linear correlation predicted by Hiatt (1948). The slopes of the Hiatt growth equations describing the relation between the pre- and post-molt PWs of 94 molting individuals ($PW_{n+1} = 0.23 + 1.27 PW_n$, $r^2 = 0.9955$; Appendix Fig. A1) and those of the DS resulting from the cohort demarcation ($PW_{n+1} = 0.26 + 1.27 PW_n$, $r^2 = 0.9993$; Appendix Fig. A2) were statistically indistinguishable ($t_{102} = 1.927$, $p > 0.05$), thereby confirming the identified DS. The positive constant in the Hiatt equations indicated that the percentage of growth decreased with increasing body size. The mean increase in the size of the PW of the molting individuals was between 34.7% and 33.0% in the initial stages of their development (DS A–G), while those from DS H and I showed average increases of 29.2% and 27.9%, respectively. A strongly reduced growth was observed in animals exhibiting serious physical injuries; less serious damages usually had no or little effect on the growth increment during ecdysis (results not shown).

Allometric growth

The growth of the PL, the OL, and the TL (hence the CL and the BL) throughout the 11 instar stages (DS C–M)

Table 3. Mean body parameters (M in cm) ± standard error (SE) of the development stages (DS) A–M found at the study site on Palawan. The parameters are prosomal length (PL), opisthosomal length (OL), telson length (TL), total body length (BL), opisthosomal width 1–3 (OW1–3), wet weight, and distance between the compound eyes (Eyes)

	PL (cm)	OL (cm)	TL (cm)	BL (cm)	OW1 (cm)	OW2 (cm)	OW3 (cm)	Wet weight (g)	Eyes (cm)
DS	M ±SE	M ±SE							
A	0.58 ±0.01	0.43±0.02	0.35 ±0.02	1.35 ±0.04	0.59 ±0.02	0.78 ±0.01	0.75 ±0.01	0.07 ±0.00	0.58 ±0.02
B	0.80 ±0.01	0.53 ±0.01	0.86 ±0.01	2.21 ±0.02	0.74 ±0.01	1.03 ±0.01	1.03 ±0.01	0.18 ±0.01	0.77 ±0.01
C	1.03 ±0.01	0.76 ±0.01	1.45 ±0.01	3.24 ±0.03	0.99 ±0.02	1.41 ±0.01	1.41 ±0.01	0.52 ±0.02	1.03 ±0.01
D	1.35 ±0.01	1.03 ±0.01	2.23 ±0.02	4.58 ±0.03	1.25 ±0.02	1.90 ±0.01	1.91 ±0.01	1.12 ±0.04	1.35 ±0.01
E	1.79 ±0.02	1.41 ±0.01	3.22 ±0.03	6.45 ±0.05	1.60 ±0.01	2.51 ±0.02	2.54 ±0.02	2.67 ±0.08	1.79 ±0.01
F	2.44 ±0.02	1.86 ±0.01	4.48 ±0.04	8.81 ±0.06	2.23 ±0.02	3.27 ±0.02	3.40 ±0.02	6.03 ±0.14	2.35 ±0.02
G	3.40 ±0.02	2.56 ±0.02	6.16 ±0.05	12.09 ±0.07	3.03 ±0.03	4.28 ±0.03	4.54 ±0.03	15.45 ±0.31	3.22 ±0.02
H	4.50 ±0.02	3.57 ±0.02	8.84 ±0.07	16.99 ±0.10	3.96 ±0.03	5.65 ±0.03	6.15 ±0.04	35.45 ±1.00	4.40 ±0.03
I	6.10 ±0.04	4.90 ±0.03	12.35 ±0.11	23.28 ±0.18	5.21 ±0.03	7.34 ±0.04	8.19 ±0.05	100.77 ±2.18	5.95 ±0.04
J	7.75 ±0.13	6.21 ±0.12	15.18 ±0.38	28.75 ±0.66	6.52 ±0.07	8.59 ±0.10	9.74 ±0.12	181.06 ±6.83	7.42 ±0.13
K	10.34 ±0.23	8.71 ±0.21	21.76 ±0.42	40.69 ±0.86	8.72 ±0.21	11.60 ±0.21	12.96 ±0.21	471.10 ±33.91	10.04 ±0.20
L	13.75 ±0.21	11.58 ±0.15	28.73 ±0.37	54.11 ±0.68	11.76 ±0.20	15.26 ±0.17	17.01 ±0.28	1302.7 ±76.88	13.54 ±0.47
M	18.46 ±0.43	14.80 ±0.33	33.76 ±0.78	66.77 ±1.37	14.28 ±0.30	18.41 ±0.48	21.24 ±0.41	2215.1 ±96.83	17.44 ±0.47

Table 4. Sample size (n) and the average increase in size (in %) of the mean body parameters shown in Table 3 for the development stages (DS) A–M compared to the DS before ecdysis. The parameters are prosomal length (PL), opisthosomal length (OL), telson length (TL), total body length (BL), opisthosomal width 1–3 (OW1–3), wet weight, and distance between the compound eyes (Eyes)

	PL	OL	TL	BL	OW1	OW2	OW3	Wet weight	Eyes
DS	n (%-incr)	n (%-incr)							
A	10 (-)	10 (-)	12 (-)	9	8 (-)	6 (-)	6 (-)	4 (-)	7 (-)
B	31 (39.2)	25 (21.8)	32 (145.5)	20 (64.1)	35 (25.7)	24 (32.0)	24 (37.6)	11 (169.4)	33 (32.9)
C	36 (28.1)	45 (43.9)	41 (69.4)	33 (46.3)	46 (32.4)	42 (36.8)	41 (36.6)	24 (183.9)	43 (32.9)
D	49 (31.7)	47 (35.2)	44 (53.8)	37 (41.5)	51 (26.4)	49 (34.4)	49 (35.3)	39 (117.7)	51 (31.0)
E	65 (32.7)	64 (37.1)	52 (44.5)	50 (40.7)	57 (28.2)	60 (32.3)	57 (32.8)	56 (137.8)	61 (32.9)
F	94 (36.2)	95 (32.5)	75 (39.2)	70 (36.6)	94 (39.6)	91 (30.2)	88 (33.9)	77 (125.5)	90 (31.3)
G	125 (39.1)	132 (37.6)	114 (37.6)	101 (37.2)	116 (35.7)	115 (30.7)	114 (33.4)	91 (156.4)	114 (36.9)
H	143 (32.3)	147 (39.3)	127 (43.4)	112 (40.6)	143 (30.9)	138 (32.0)	139 (35.6)	55 (129.4)	149 (36.7)
I	135 (35.7)	141 (37.5)	132 (39.7)	121 (37.0)	134 (31.4)	139 (29.9)	137 (33.2)	116 (184.3)	143 (35.1)
J	22 (26.9)	25 (26.7)	21 (22.9)	17 (23.5)	23 (25.1)	22 (17.0)	22 (19.0)	17 (79.7)	26 (24.7)
K	12 (33.4)	12 (40.2)	10 (43.3)	10 (41.5)	11 (33.9)	12 (35.0)	11 (33.1)	10 (160.2)	12 (35.3)
L	27 (33.0)	27 (33.0)	26 (32.0)	25 (33.0)	22 (34.7)	28 (31.6)	22 (31.2)	15 (176.5)	28 (34.8)
M	12 (34.3)	13 (27.8)	10 (17.5)	9 (23.4)	7 (21.5)	13 (20.6)	9 (24.9)	7 (70.0)	13 (28.8)

was positively allometric with the PW in both sexes, except for the PL of males that grew isometrically with the PW (Table 5). The growth of the OW2–3 and the AA in both sexes and of the OW1 and the eyes of males were negatively allometric with the PW (Table 5). Except for the TL, however, the deviation from isometric growth was small in all cases of allometry. The differences between the sexes were mostly small or insignificant; the

greatest difference lay in the PLs and TLs (Table 5). The increase in wet weight was isometric with the PW (Table 5). The increase in wet weight and the growth of the PL, however, was negatively allometric when data were pooled for males, females, and individuals with unknown sex (Appendix 3). The telson growth had three distinct growth curves (triphasic), including juveniles of the instar stages DS A–C, juveniles of DS D–K, and adults (Fig. 5).

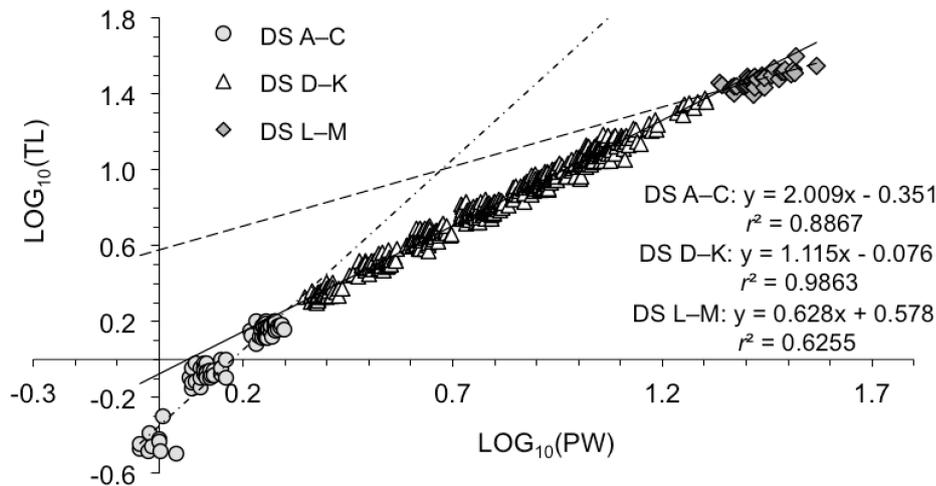


Figure 5. The relationship between the log-transformed telson length (TL) and the log-transformed prosomal width (PW) pooled over both sexes, including the juveniles with unknown sex. Three growth phases were detected throughout the 13 instars (DS A–M)

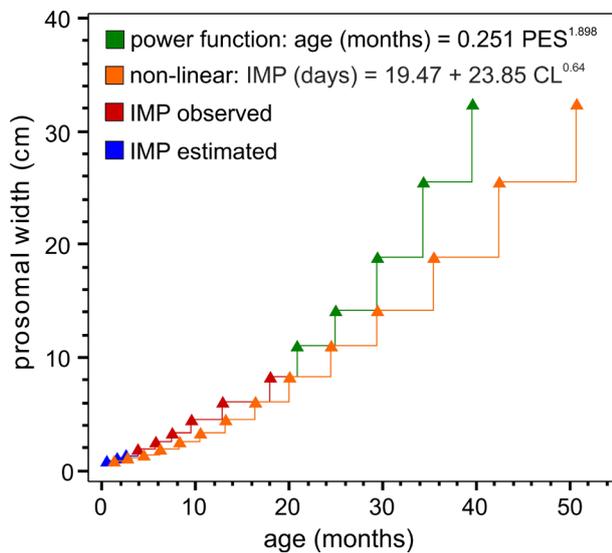


Figure 6. Growth curves predicted for *Tachypleus tridentatus* on Palawan, illustrating the relationship between the average prosomal width measured per postembryonic stage (PES) and the age (months). Two methods were used — a non-linear equation for the intermolt period (IMP) and the average carapace length (CL) — to calculate the growth curve of PES 1–14 ($r^2 = 0.9015$) and a power function to predict the curve of PES 10–14 ($r^2 = 0.9901$)

Growth curve describing the postembryonic development in the Philippines

Molting frequency: The time between two molts was observed for 20 juveniles of six consecutive stages (Table 6). The IMPs slowly increased with an increase in the size of the animals. The time between hatching and entering DS C was not identified during the study. Hence, the exact age of the cohorts at BM beach could not be determined, though we know that when juveniles

entered DS I, they were older than 436 days (Table 6).

Prosomal width of the trilobite larvae: A statistically significant match was found for the carapace lengths of DS C, E, L and the lengths measured in the respective PES of Goto & Hattori (1929), as well as for the mature females without considering the instar stage (Table 6). Based on the average PWs of PES 2–11 (DS A–J) and PES 13–14 (DS L–M), the Dyar’s rule ($\text{LN PW} = 0.294 \text{ PES} - 0.578$, $r^2 = 0.9993$) was used to estimate the width of PES 1 with 0.75cm. On an average, the carapace length of *T. tridentatus* on Palawan was 1.67% smaller than the PW (all measures). Animals with a PW of 0.75cm would, therefore, have a carapace length of 0.74cm (Table 6).

Ages of the postembryonic stages: Observations indicated that the periods slowly increased with the increasing size of the animals (Table 6). With an estimated average IMP of 14 days for the trilobite larvae and of 30 days each for PES 2–3, the presumed age of *T. tridentatus* in PES 9 (PW 8.23cm) would be 17 months. The cubic equation $\text{PW} = 0.622 + 0.216 t + 0.026 t^2 - 0.001 t^3$ ($r^2 = 0.9995$) described the postembryonic period of PES 1–9 (Appendix Fig. A3). Based on the observed IMPs of PES 4–9 (DS C–H), two methods were used to predict the age at which *T. tridentatus* attained sexual maturity in the Philippines. The non-linear model for the relationship between the IMP and the average carapace length (CL) per instar stage, $\text{IMP (days)} = 19.47 + 23.85 \text{ CL}^{0.64}$ ($r^2 = 0.9015$), and the power function, $\text{age (months)} = 0.251 \text{ PES}^{1.898}$ ($r^2 = 0.9901$). The resultant growth curves, illustrating the size-age relationship, suggested that the mean ages of mature male and female *T. tridentatus* in the Philippines are 2.7–3.5 and 3.1–4.2 years, respectively (Fig. 6).

Table 5. Allometric relationships between the prosomal width (PW in cm) and various morphometric parameters (y) based on the log-transformed equation with the intercept a, the growth coefficient b with 95% confidence interval (in bold indicates allometry), Pearson's correlation coefficient r, and the number of cases n. The parameters are prosomal length (PL), opisthosomal length (OL), telson length (TL), carapace length (CL), body length (BL), opisthosomal width 1–3 (OW1–3), the distance between the anal angles (AA) and between the compound eyes (Eyes), and the weight. The statistics of the Student's t test (T) and the asymptotic significance (two-tailed) (p) indicate whether males (m) and females (f) differ significantly

x = PW (cm)			allometric growth: $\log y = \log a + b \log x$				Sex difference	
Sex	y	n	a	b	95% CI _b	r	T	p
m	weight (g)	224	0.07	2.99	2.956 / 3.016	0.997	1.899	0.058
f	weight (g)	190	0.07	3.02	2.990 / 3.055	0.997		
m	PL (cm)	269	0.56	0.99	0.982 / 1.003	0.996	4.662	< 0.001
f	PL (cm)	211	0.53	1.02	1.014 / 1.035	0.997		
m	OL (cm)	274	0.40	1.04	1.030 / 1.048	0.997	1.158	0.248
f	OL (cm)	218	0.40	1.05	1.037 / 1.053	0.998		
m	TL (cm)	244	0.90	1.08	1.066 / 1.098	0.993	-9.447	< 0.001
f	TL (cm)	181	0.87	1.09	1.073 / 1.108	0.994		
m	CL (cm)	264	0.96	1.01	1.006 / 1.022	0.998	13.751	< 0.001
f	CL (cm)	206	0.92	1.03	1.026 / 1.041	0.999		
m	BL (cm)	225	1.84	1.05	1.041 / 1.062	0.997	1.709	0.088
f	BL (cm)	167	1.80	1.06	1.049 / 1.071	0.998		
m	OW1 (cm)	268	0.53	0.96	0.940 / 0.972	0.991	2.684	0.008
f	OW1 (cm)	209	0.49	0.99	0.970 / 1.001	0.993		
m	OW2 (cm)	268	0.89	0.88	0.873 / 0.888	0.998	1.442	0.150
f	OW2 (cm)	215	0.87	0.89	0.879 / 0.897	0.997		
m	OW3 (cm)	260	0.84	0.94	0.932 / 0.948	0.998	2.090	0.037
f	OW3 (cm)	209	0.83	0.95	0.939 / 0.958	0.997		
m	AA (cm)	266	0.32	0.95	0.939 / 0.966	0.993	0.452	0.652
f	AA (cm)	206	0.32	0.96	0.943 / 0.973	0.994		
m	Eyes (cm)	282	0.55	0.99	0.983 / 0.998	0.998	1.333	0.183
f	Eyes (cm)	222	0.54	1.00	0.989 / 1.006	0.998		

DISCUSSION

The presented results provide the first consecutive data on the stepwise growth of *T. tridentatus* in the Philippines. By covering all stages except the increase from the trilobite stage, our study provides the first nearly comprehensive dataset collected within a single population of juveniles in the tropics. We found that 14 instars characterize the postembryonic development of the species in the Philippines. The similarities between the stages identified on Palawan and those of *T. tridentatus* in a nursery habitat in Japan suggest that these 14 stages may be characteristic of natural populations throughout the species distribution range. Our findings further support the assumption that growth continues year-round in the tropics and suggest that the average age of mature male and female *T. tridentatus* in the Philippines ranges from three to four years.

Postembryonic stages and development time

The number of postembryonic stages reported for *T. tridentatus* in the literature varies strongly and there is a relative paucity of consecutive growth data from wild populations (Goto & Hattori 1929). The information on development time is largely based on laboratory studies (Sekiguchi et al. 1988; Lee & Morton 2005; Chen et al. 2010). To assign the stage and age of the individuals collected in nursery habitats in Hong Kong (Chiu & Morton 2004; Kwan 2015; Kwan et al. 2016) and southern China (Hu et al. 2009, 2015), recent studies applied the size-age relationship established by Sekiguchi et al. (1988) while rearing artificially fertilized eggs in the laboratory.

The demarcation of cohorts was confirmed in FiSAT and by comparing the slopes of the Hiatt growth equations for molting animals and the cohorts (Figs. A1–A2, Table 2). The similarity between the 13 DS identified

Table 6. Comparison between the mean carapace lengths (CL in cm) of the instar stages (DS) on Palawan and the postembryonic stages (PES) in a nursery habitat in Japan (Goto & Hattori 1929), indicating the two-tailed significance (p) and the statistics of the one-sample t test (T, df). Further shown are the mean prosomal widths (PW in cm) and the intermolt periods (IMP in days) ± standard deviation (SD) on Palawan, and the laboratory studies by Sekiguchi et al. (1988) and Chen et al. (2010)

Goto & Hattori		<i>Tachypleus tridentatus</i> on Palawan						Sekiguchi et al.		Chen et al.		
PES	CL (cm)	DS	CL (cm) ± SD	p	T (df)	PW (cm)	IMP (days) ± SD (n)	PES	PW (cm)	IMP (days)	PW (cm)	IMP (days)
1	0.74	–	0.74 ^b			0.75 ^b		1	0.6	hibern.	0.63	53
2	0.88	A	1.01 ± 0.08	0.001	4.784 (8)	0.98		2	0.78	21	0.89	81
3	1.26	B	1.33 ± 0.06	< 0.001	5.630 (21)	1.30		3	1.07	60	1.23	21
4	1.76	C	1.78 ± 0.09	0.120	1.595 (35)	1.82	40 ± 4.8 (4)	4	1.33	hibern.	1.64	44
5	2.30	D	2.36 ± 0.08	< 0.001	5.486 (44)	2.48	56 ± 9.3 (5)	5	1.75	60–80	2.20	35
6	3.15	E	3.19 ± 0.18	0.096	1.691 (61)	3.34	56 ± 6.8 (6)	6	2.22	364	2.89	56
7	4.18	F	4.30 ± 0.26	< 0.001	4.522 (92)	4.40	60 ± 0 (3)	7	2.80	364	3.83	46
8	5.56	G	5.93 ± 0.37	< 0.001	11.089 (118)	5.96	101 (1)	8	3.73	364	4.98	50
9	7.42	H	8.07 ± 0.48	< 0.001	15.865 (138)	8.23	150 (1)	9	4.27	364	6.57	78
10	9.64	I	11.00 ± 0.81	< 0.001	19.444 (132)	11.11		10	4.84	364	9.10 ^d	104
11	12.92	J	13.94 ± 1.17	0.001	4.059 (21)	14.00		11	7.00 ^c	364	12.20 ^d	111
12	17.27	K	19.05 ± 1.47	0.002	4.188 (11)	18.84		12	9.00 ^c	364	16.30 ^d	132
13	25.60	L	25.33 ± 1.78	0.525	-0.793 (26)	25.40		13	11.50 ^c	364	21.80 ^d	159
14	27.42	M	33.45 ± 2.36	< 0.001	8.853 (11)	32.38		14	14.70 ^c	364	29.10 ^d	189
								15	18.80 ^c	364	38.90 ^d	228
m ^a	25.47	m ^a	25.31 ± 2.08	0.795	-0.263 (27)	25.24		16	24.00 ^c	364		
f ^a	32.27	f ^a	33.24 ± 2.85	0.264	1.177 (11)	31.57		17	30.80 ^c			

^a Adult males (m) and females (f) without allocation to the PES.

^b PW predicted following the Dyar's rule. On an average, the CL is 1.67% smaller than the PW; the predicted CL is hence 0.74cm.

^c Sekiguchi et al. (1988) calculated the PW of PES 11–17 with a constant rate of 1.28 (Hibernation: hibern).

^d Chen et al. (2010) predicted the PW with the stepwise growth equation, age (months) = 3.092 e^{0.183 PES} (r² = 0.9959).

at BM beach and the 13 PES (PES 2–14) considered by Goto & Hattori (1929), which were comparable in size, indicated that all expected life stages except the trilobite larvae (PES 1) were found in the present study (Table 6). Furthermore, the average carapace length estimated for the trilobite larvae equaled the length measured by Goto & Hattori (1929). As also reported by the authors, small but mature females were present in the PES 13 (DS L) and large males in the PES 14 (DS M). These findings explain why the two studies differed significantly in terms of the carapace length of PES 14, while the comparison between the adult females (without considering the instar stage) showed insignificant results.

Differences, however, can be noted compared to the findings reported from rearing experiments, especially those by Sekiguchi et al. (1988) (Table 6). The last animal used in the study by Sekiguchi et al. (1988) died in the PES 10 with a PW of 4.84cm; animals with a comparable PW on Palawan were in PES 7. By calculating the subsequent PWs with a constant rate of 1.28, the authors concluded that sexually mature females were in PES 17 (Table

6). As already reported for *T. tridentatus* (Waterman 1954; Kawahara 1982) and the Atlantic Horseshoe Crab *Limulus polyphemus* (Linnaeus, 1758) (Carmichael et al. 2003), both the relative increase in size of the molting individuals and the positive constant in the Hiatt equations indicated that the percentage growth on Palawan decreased with an increase in body size (Figs. A1–A2 in Appendix), a rate of 1.28 being observed in the upper size range. An approximate agreement, therefore, was only found between PES 10–14 (DS I–M) of Palawan and PES 13–17 calculated by Sekiguchi et al. (1988) (Table 6). Moreover, the IMPs differed greatly from those of the present study. Animals with a PW of 2.22cm molted only once per year, like the subsequent instars (Table 6). On the other hand, animals with a comparable size on Palawan molted on an average after 56 days; the subsequent stages also showed a much faster development. The observed inverse relationship between size and molt frequency (Table 6) supports the findings reported for horseshoe crabs (Waterman 1954; Carmichael et al. 2003; Chen et al. 2010) as well as many

crustacean species (Kurata 1962; Caddy 1987; Chang et al. 2012).

Considering the high water temperatures in the study region throughout the year and the increasing length of the IMPs, we assumed that the trilobite larvae in the Philippines molt after two weeks and the PES 2–3 after 30 days. Following our estimations, the mean age of juveniles entering PES 10 (mean PW of 11.1cm) was 14.5 months (Fig. 6). The observed IMPs and our age estimations for adults were similar to the four years estimated by Chen et al. (2010), although an additional stage was observed in their experiment (Table 6). The authors reared juvenile *T. tridentatus* under conditions better comparable to those prevailing at BM beach. The temperature was 28–30 °C, seawater salinity was 30‰, water flow was maintained, more substrate was provided for digging, and the tank was much larger than that in Japan. The least time to hatch and greatest survival of the Asian species were observed in water temperatures of around 29°C (Carmichael & Brush 2012), and Chen et al. (2004) suggested 28–31 °C as the optimal seawater temperature for the year-round growth of the juvenile *T. tridentatus*. These observations were in line with the results of Yeh (1999, cited in Chen et al. 2010) and Lee & Morton (2005), who reported that ecdysis in *T. tridentatus* continues when the temperature remains at >28°C but stops at <22°C. Our findings support the assumption that growth continues year-round in the tropics and suggest that *T. tridentatus* in the Philippines attain sexual maturity at the age of three to four years. The similarity with the instars from the Inland Sea in Japan (Goto & Hattori 1929), where the water temperature drops down to 13°C in winter, suggests that 14 stages may be characteristic of the postembryonic development of natural populations throughout the distribution range of *T. tridentatus*. Temperature seems to have less influence on the molt increment than on the IMP. To confirm these findings, however, further investigations should be carried out in habitats located at the limits of the distribution range of the species.

In light of the fact that the growth increments per molt of *T. tridentatus* in the two nursery habitats were comparable, it is likely that the caging operations are responsible for the deviations observed in the laboratory studies. Significantly reduced growth increments at each molt and longer IMPs in the laboratory were observed in horseshoe crabs (Carmichael & Brush 2012) and various crustacean species (e.g., Hiatt 1948; Harms et al. 1994; Bonilla-Gómez et al. 2013). The effects of the holding time at the laboratory and the space available in the holding tank were reported (e.g., González-Gurriarán

et al. 1998). The development of *L. polyphemus* in the laboratory was considerably slower than that in their natural habitat — the postembryonic growth time was nearly halved when the embryonic development took place in nature (Jegla & Costlow 1982). As shown for horseshoe crabs (Carmichael & Brush 2012; Hu et al. 2013) and several crustacean species (Hartnoll 2001; Chang et al. 2012), the IMP increased and the rate of increase in size decreased when the quantity or quality of food in culture was suboptimal. The diet of horseshoe crabs in nature is broad and highly mixed and they move up the food webs as they age and grow (Carmichael et al. 2004; Zhou & Morton 2004). The most commonly offered diet in culture was brine shrimp (*Artemia* spp.) or dietary supplements that were not part of their known natural diet (see Carmichael & Brush 2012). Moreover, recent studies with horseshoe crabs show the importance of the sediment type for growth and survival (Hong et al. 2009; Hieb et al. 2015), while others found shorter hatching times and higher molting frequency and molt increments with increased water circulation or increased dissolved oxygen concentrations (Carmichael & Brush 2012). The relationships with environmental factors and the potential interactions among different variables were not resolved (Carmichael & Brush 2012). These reports, however, illustrate that variations in the growth rate can be considerable, depending on the abiotic and biotic parameters, which might explain the large differences observed with the number of stages and the size-age relationship reported by Sekiguchi (1988) and the comparable development time, with only one additional instar stage reported by Chen et al. (2010). These findings may further imply the similarity between BM beach and the nursery habitat of Goto & Hattori (1929) in terms of environment conditions (apart from water temperature) and quality of food supply. Further studies are needed to characterize the habitat of different populations and to identify the key drivers for growth and survival in nature and in rearing experiments.

Growth pattern

Our finding that the relationship between the pre- and post-molt PWs of juvenile horseshoe crabs could be fitted with the Hiatt growth model is consistent with the results of previous studies (Carmichael et al. 2003; Hu et al. 2015). Moreover, the Hiatt equations were almost identical to the equation for a juvenile population assessed at a nursery habitat in southern China (Hu et al. 2015). As also reported by Sekiguchi et al. (1988), three distinct growth curves described the relative

growth of the TL and the PW throughout the 13 stages. Accordingly, Chen et al. (2010) reported two phases for the nine juvenile stages considered by them. Deviations from isometric growth in the other body parts were fairly small on Palawan — the shape of the horseshoe crab at different development stages was therefore similar. Isometric growth was reported by Chen et al. (2010) for most of the body parts of the juveniles in their study. Lee & Morton (2005), in contrast, reported a positive growth allometry for the weight of juveniles collected in their natural habitat but reared in the laboratory. On Palawan, significant differences between juveniles and adults were indicated in most of the analysed body ratios. Most findings agree with those of Chiu & Morton (2003), although they reported insignificant differences for the CL/PW and CL/TL ratios. Several studies (for instance, Yamasaki et al. 1988; Chiu & Morton 2003; Mohamad et al. 2016) revealed that morphometric parameters, body ratios, and the allometric relationships of adult and juvenile *T. tridentatus* differed significantly between populations and geographic regions, a characteristic that might explain the differences between the studies. The differences might also be due to the different size ranges considered and whether or not sex was differentiated.

Limitations

The absence of the trilobite larvae might suggest that no recent spawning activity occurred at BM beach. Because the subsequent stages were present and the smallest instar stages are difficult to detect, however, they may have been simply overlooked. The newly hatched larvae do not need to feed because they can subsist on the yolk of the embryo (Botton et al. 1992). The low occurrence of PES 12 (DS K) at BM beach supports earlier observations that larger juveniles were moving further down in the intertidal zone, with subadults at the seaward limit (Rudloe 1981; Kaiser 2002; Hu et al. 2009; Morton & Lee 2011). It might also imply a greater mortality of juveniles in PES 12, though laboratory studies revealed a decrease in mortality rates with increasing age (Carmichael & Brush 2012).

It was recognized that mark-recapture experiments were often the only method to validate the growth of natural populations (González-Gurriarán et al. 1998; Lee & Morton 2005; Hu et al. 2015), but several authors emphasized that caution should be used when extrapolating from tagged animals to natural populations (Kurata 1962; Caddy 1987). Although handling during measuring and marking may have disturbed the juveniles in the present study, we doubt that cutting the mobile spines for identification would have harmed the animals

to an extent that their growth was no longer natural — we observed that animals with injuries showed lower rather than higher molt increments. Given a recapture rate of 57% in 2001 (Kaiser 2002), the labels glued on the prosoma also seemed to pose no adverse impacts on health.

CONCLUSIONS

Determining when a horseshoe crab reaches sexual maturity is of extreme importance for interpreting the population dynamics to enable their management and conservation (Carmichael et al. 2015). By presenting the first consecutive data on the postembryonic development of *T. tridentatus* in the Philippines, the present study adds to the fragmentary background knowledge about the species in southeastern Asia and provides the information needed to assess the demographics of juvenile populations and judge the status of *T. tridentatus* in the Philippines. With populations having decreased dramatically over their distribution range, it is clear that these data are now in demand. Because of the small number of IMPs observed and the absence of the trilobite larvae, however, more data are needed to validate the present findings. The relative paucity of consecutive growth data from wild populations and the lack of comparable data for the tropics made it difficult to judge the universal applicability of the dataset. Nevertheless, the reported results provide sound quantitative and qualitative baseline data for future assessment and monitoring studies in the tropics. To define a wider range of applicability, future investigations should aim to determine the size-age relationship of natural *T. tridentatus* populations from different geographic regions, preferably in habitats located at the limits of the species distribution range. These studies should also characterize the abiotic and biotic parameters of habitats because the causes of variable growth and survival rates have important implications for conservation and aquaculture efforts that are aimed at restoring depleted populations of horseshoe crabs.

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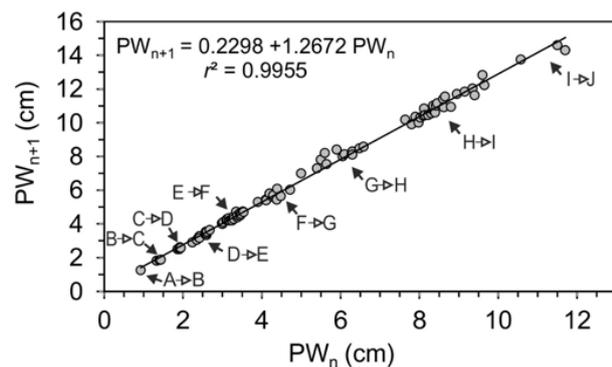
Zhou, H. & B. Morton (2004). The diets of juvenile horseshoe crabs, *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* (Xiphosura), from nursery beaches proposed for conservation in Hong Kong. *Journal of Natural History* 38(15): 1915–1925; <https://doi.org/10.1080/0022293031000155377>

Appendix 1. Coding system used for cutting the lateral mobile spines of the opisthosoma

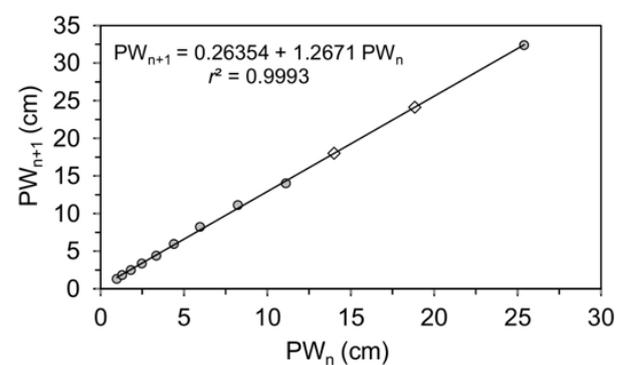
Spines left	Value	Spines right	Value
L1	1	R1	40
L2	2	R2	70
L3	4	R3	80
L4	7	R4	90
L5	10	R5	100
L6	20	R6	200

Appendix 2. Means (M) ± standard error (SE), minimum and maximum values, and sample number (n) of various morphometric ratios in juveniles and adults (males, females, and individuals with unknown sex are pooled for comparison). The statistics of the Mann-Whitney test (U) and the asymptotic significance (two-tailed) (p) indicate whether juveniles and adults differ significantly. The parameters are prosomal width (PW), prosomal length (PL), opisthosomal length (OL), telson length (TL), carapace length (CL), opisthosomal width 2 (OW2), and distance between the compound eyes (Eyes)

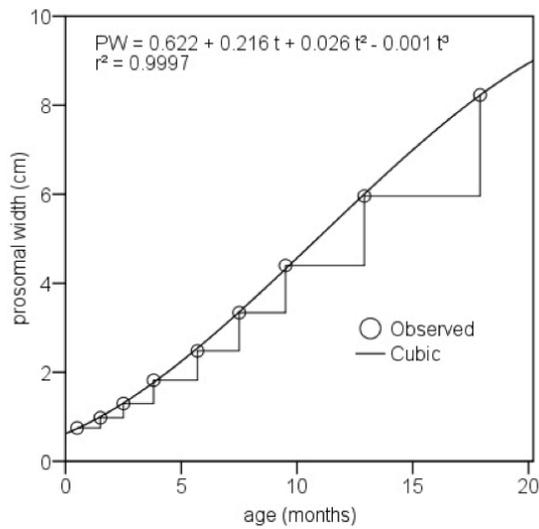
Body ratio	Life stage	n	M ± SE	Min–Max	U	p
PW/OW2	Juvenile	698	1.41 ± 0.004	1.14–1.75	1844.5	< 0.0001
	Adult	39	1.64 ± 0.03	0.91–1.90		
Eyes/PW	Juvenile	729	0.54 ± 0.001	0.45–0.71	12830.5	> 0.05
	Adult	41	0.53 ± 0.004	0.49–0.60		
PL/OL	Juvenile	694	1.30 ± 0.004	1.07–2.00	5575.5	< 0.0001
	Adult	39	1.20 ± 0.01	1.08–1.30		
PW/PL	Juvenile	722	1.81 ± 0.004	1.45–2.07	13232.5	> 0.05
	Adult	39	1.83 ± 0.02	1.66–2.10		
OW2/OL	Juvenile	664	1.66 ± 0.007	1.26–2.67	324.5	< 0.0001
	Adult	40	1.30 ± 0.01	0.97–1.43		
CL/PW	Juvenile	770	0.93 ± 0.006	0.33–1.13	10309.5	< 0.001
	Adult	40	0.99 ± 0.02	0.43–1.13		
CL/TL	Juvenile	643	0.97 ± 0.01	0.35–4.38	8261.5	< 0.01
	Adult	35	0.89 ± 0.02	0.36–1.05		



Appendix Figure A1. The Hiatt diagram illustrates the relationship between the prosomal widths before (PW_n) and after ecdysis (PW_{n+1}) for 94 animals that molted during the study period



Appendix Figure A2. The Hiatt diagram illustrates the relationship between the average prosomal widths before (PW_n) and after ecdysis (PW_{n+1}) for the development stages identified by means of the size-frequency histograms (circles). Since the limits of DS K could not be unequivocally validated with our own data, the two rhombs were calculated with the regression equation, which was determined by the values of the circles



Appendix Figure A3. The stepwise growth of the postembryonic stages (PES) 1–9 of *Tachypleus tridentatus* on Palawan described by a cubic regression equation. The development time for PES 1–3 was estimated (see text)

Appendix 3. Allometric relationships between the prosomal width (PW in cm) and various morphometric parameters (y) based on the log-transformed equation with the intercept a, the growth coefficient $b \pm$ standard error (SE) and 95% confidence interval (in bold indicates allometry), Pearson's correlation coefficient r, and the number of cases n (pooled over males, females, and individuals with unknown sex). The parameters are prosomal length (PL), opisthosomal length (OL), telson length (TL), carapace length (CL), body length (BL), opisthosomal width 1–3 (OW1–3), the distance between the anal angles (AA) and between the compound eyes (eyes), and the weight

x = PW (cm)		Allometric growth curve: $\log y = \log a + b \log x$			
y	n	a	$b \pm$ SE	95% CI _b	r
weight (g)	522	0.08	2.97 ± 0.009	2.954 / 2.990	0.997
PL (cm)	761	0.57	0.98 ± 0.003	0.979 / 0.990	0.997
OL (cm)	782	0.40	1.04 ± 0.003	1.031 / 1.041	0.998
TL (cm)	697	0.70	1.20 ± 0.007	1.182 / 1.209	0.989
CL (cm)	731	0.96	1.01 ± 0.002	1.006 / 1.014	0.998
BL (cm)	614	1.70	1.09 ± 0.003	1.081 / 1.094	0.997
OW1 (cm)	747	0.55	0.94 ± 0.004	0.928 / 0.945	0.992
OW2 (cm)	739	0.84	0.90 ± 0.002	0.896 / 0.904	0.998
OW3 (cm)	719	0.81	0.96 ± 0.002	0.953 / 0.962	0.998
AA (cm)	734	0.30	0.99 ± 0.004	0.986 / 1.003	0.993
eyes (cm)	770	0.57	0.98 ± 0.002	0.971 / 0.980	0.998

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Author Contribution: SS and DK designed the study. DK acquired and analyzed the data and prepared the manuscript. SS was the supervisor of DK in 2001 and contributed to a similar extent to the development of the manuscript.





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ISSN 0974-7907 (Online); ISSN 0974-7893 (Print)

December 2018 | Vol. 10 | No. 15 | Pages: 12907–13046

Date of Publication: 26 December 2018 (Online & Print)

DOI: 10.11609/jott.2018.10.15.12907-13046

www.threatenedtaxa.org

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