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## Population structure of *Tachypleus tridentatus* (Chelicerata: Merostomata) at a nursery beach in Puerto Princesa City, Palawan, Philippines

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### Abstract

This study aimed to determine the population structure of juvenile *Tachypleus tridentatus*, one of two species of horseshoe crabs found in Palawan, at Aventura Beach, Puerto Princesa City, Palawan. Surveys to assess the population in terms of size, density, size-frequency, age structure, sex ratio and distribution were conducted from June to October 2002. The habitat was mapped and physical/chemical parameters taken to further describe it. The position of population members was plotted on the map. Individuals were measured, marked, sexed and released. Prosoma width was measured for living individuals and exuviae. Postembryonic stages were identified based on histograms with different interval size. The normal distribution of the animals per instar stage was tested with the Kolmogorov–Smirnov test. After the 5-month survey a total of 125 juveniles was recorded and 40% of them were recaptured. Population size was estimated to be 150 individuals. Seven postembryonic stages were identified based on their prosoma width. Male: female ratio was 1:1.8 and the age of the first five postembryonic stages ranged from 5.4 months to 17.6 months. The population density was 1.47 individuals per 100 m<sup>2</sup>. The majority of the animals of all postembryonic stages were found between 0 and 50 m from the mean high tide level. Further studies on the population structure of *T. tridentatus* with regards to the adult population are seen as a necessity.

**Keywords:** Horseshoe crab, mark–recapture, Merostomata, nursery, Philippines, population structure, *Tachypleus tridentatus*

### Introduction

Horseshoe crabs survived without remarkable changes of features from their ancestors in previous geologic ages (Buchsbaum et al. 1987). Four extant species representing three genera currently exist in the world, one on the east coast of North America and three in Asia (Sekiguchi 1988a). The American species is *Limulus polyphemus* (Linnaeus, 1758), and *Tachypleus tridentatus* (Leach, 1819), *T. gigas* (Mueller, 1785) and *Carcinoscorpius rotundicauda* (Latreille, 1801) occur in Asia.

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Of the three Asian species two are reported for the Philippines, *T. tridentatus* and *C. rotundicauda* (Waterman 1958). Sekiguchi (1988a) recorded both species for Palawan. A recent survey from northern to central Palawan though could only confirm the presence of *T. tridentatus* (Schoppe 2002).

Horseshoe crab populations are rapidly declining around the world as reports from the USA (Botton and Haskin 1984; Bell and Henderson 1993), Japan (Itow 1998), Hong Kong (Morton 1999), Taiwan (Chen et al. 2002), Singapore (Hong 2004, citing personal communication with Sivasothi), Thailand (Sekiguchi 1989) and the Philippines (Schoppe 2002) have shown. Among the causes blamed for the decline are reproductive failure due to pollution (Itow 1998), habitat destruction (Itow et al. 1991), extensive reclamation and revetment operations in coastal areas (Shinohara 1989; Itow 1998; Chen et al. 2002), and fishing in combination with beach pollution and habitat destruction (Morton 1999; Zhou and Morton 2004). In Palawan habitat conversion, destruction, and fishing activities were identified as the major threats (Schoppe 2002).

Though decreases in numbers of the three Asian species are conspicuous all over their range of distribution they are still only listed under the 'data deficiency' category of IUCN (2003).

The biology and ecology of horseshoe crabs had not been studied in the Philippines until recently. Schoppe (2001) reported on the cultural significance of *T. tridentatus* for people from Palawan. A year later the first data on the distribution of the species in Palawan and the Philippines in general were published (Schoppe 2002). Kaiser (2002) was the first to look into the ecology and morphology of *T. tridentatus* from Palawan. She established striking differences in the postembryonic development of *T. tridentatus* in the tropics in comparison with results from subtropical regions.

The continuous reduction of appropriate breeding habitats in Palawan demand more studies on the species. In order to prevent further losses, the ecology and biology of the tropical *T. tridentatus* has to be understood as a basic requirement for proper management.

## Study area

The study was conducted at Aventura Beach (named after a beach resort), Banciao Banciao, Puerto Princesa City, Palawan at 09°43.804'N, 118°46.370'E, facing the Sulu Sea (Figure 1). The study area covered approximately 20,000 m<sup>2</sup> which extend from the resort's beach to a nearby pier in the north and to Canigaran Beach in the south. Scarce mangrove vegetation and dense seagrass beds characterize the area. The substratum ranges from rocky to sandy-muddy.

## Methodology

For the general description of the area in terms of substratum, and presence of seagrass and mangroves, and the recording of the capture locations, the area was mapped and measured. The habitat was further described in terms of grain size, soil and water salinity, dissolved oxygen and temperature. The grain size was characterized after the Wentworth grade scale (English et al. 1997), salinity was measured with an Atago hand refractometer, dissolved oxygen with a WTW TA 197-OXI electrode, and temperature with an ordinary mercury thermometer.

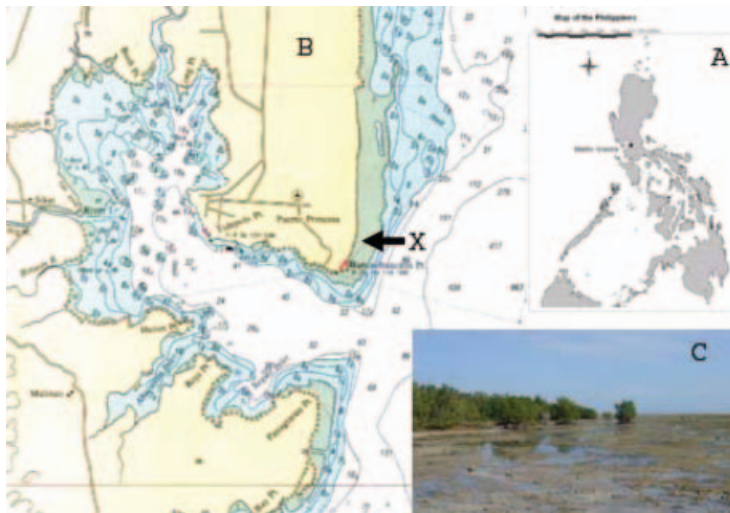


Figure 1. Study area. (A) Map of the Philippines; (B) map of Puerto Bay wherein the study site is indicated (X); (C) nursery habitat of the study site.

The population survey was conducted for five consecutive months, from June to October 2002. The intertidal area was searched for individuals and exuviae by systematically walking in parallel lines. Animals were detected through sight or touch. Surveys were conducted for an average of 6 days per month (4 h per day) during the daytime low tide.

The population size of *T. tridentatus* was assessed using the mark-recapture method. The method allows the calculation of the total number of individuals (abundance) based on the ratio of known (marked) to unknown (new) individuals (Begon 1979). All animals caught in the area were marked by cutting the tips of the opisthosomal spines following a coding system and released.

The horseshoe crab population size ( $N$ ) was estimated after Schumacher and Eschmeyer as given in Krebs (1989).

$$N = \frac{\sum_{t=1}^S (C_t \times M_t^2)}{\sum_{t=1}^S (R_t \times M_t)}$$

The computation of the variance follows the formula:

$$\text{Variance } \frac{1}{N} = \frac{\sum (R_t^2 / C_t) - (\sum R_t \times M_t)^2 / \sum (C_t \times M_t^2)}{s - 2}$$

The standard error is given by:

$$\text{Standard error } \frac{1}{N} = \sqrt{\frac{\text{Variance } (1/N)}{\sum (C_t \times M_t^2)}}$$

where  $C_t$ =total number of individuals caught on day  $t$ ;  $R_t$ =number of marked individuals caught on day  $t$ ;  $M_t$ =number of marked individuals before the sampling on day  $t$ .

Population density (individuals per 100 m<sup>2</sup>) was determined based on the estimated population size per area inhabited.

The prosoma width (PW) of all individuals and exuviae was measured. The prosoma widths were sorted from the smallest to the largest. The size–frequency distribution was then expressed in histograms with different interval sizes (0.1, 0.2, 0.3 cm). The relative number of individuals per postembryonic stage (PES) served to assess the age structure of the population. Sex was identified based on the size and shape of the genital papillae as described in Yamasaki et al. (1988). The ratio of male to female individuals was determined for each size group and for the whole population.

Microcal origin 4.1 was used to derive the mean, SD, SE, Student's *t* test, and frequency distribution. Analysis of normal distribution through the Kolmogorov–Smirnov test was done using SPSS 9.0 for Windows.

## Results

### *Habitat*

The assessment of the habitat for factors that might affect the existence of horseshoe crabs revealed that 10,200 m<sup>2</sup> are composed of sandy-muddy substratum while the remaining 9800 m<sup>2</sup> are covered by seagrasses, rocky substrata and patches of mangroves. The seagrass beds are inhabited by many invertebrates and hence attract sea birds and people. The people collect invertebrates either for commercial or subsistence purposes. Fish corrals and fences to collect milkfish fry are also abundant in the area.

The average temperature measured was  $29.7 \pm 0.35^\circ\text{C}$ . The dissolved oxygen had an average of  $8.8 \pm 0.17 \text{ mg l}^{-1}$ . Water salinity had a mean of  $34.7 \pm 1.0$  and salinity of the interstitial water had an average of  $37.1 \pm 1.05$  ppt. The area is dominated by medium fine sand (42.2%), followed by very fine sand (27.0%), fine sand (10.2%), coarse sand (7.7%), mud (5.0%), very coarse sand (4.7%), and gravel (3.2%).

### *Population size*

A total of 125 juvenile *T. tridentatus* were found during the 5-month survey. No adult individuals were found. Forty per cent of the individuals were recaptured, 26 once, 12 twice, six three times, five four times, and one individual was recaptured five times. The analysis of the mark–recapture data revealed an estimated population size of 150 juvenile *T. tridentatus*, hence the actual survey covered 83% of the total juvenile population.

### *Population density*

Juveniles of *T. tridentatus* inhabited 10,200 m<sup>2</sup> of the study area while the remaining 9800 m<sup>2</sup> are covered by seagrass and avoided by the animals. Accordingly, the density was computed to be 1.47 individuals per 100 m<sup>2</sup> or 0.75 individuals per 100 m<sup>2</sup> if the entire area, including seagrass beds, is considered.

### *Size structure of postembryonic stages*

The prosoma width of a total of 179 juvenile individuals and exuviae was measured. The smallest juvenile had a prosoma width of 2.97 cm while the largest animal had a prosoma width of 18.3 cm. In 42.5% of all animals the prosoma width was smaller than or equal to 9 cm and only 4.47% had a prosoma width of 13.11 cm or more.

The size–frequency histogram is multimodal showing different size clusters which differed in abundance (Figure 2). Each cluster represents a postembryonic stage. The identification of the PES is best done if the different cohorts are graphically split. By presenting the smallest animals (PW 2.97–5.1 cm) at 0.1 intervals, two postembryonic stages can be identified (Figure 3). The smallest animals, which represent one group, were named postembryonic stage ‘A’ since it was not yet known which PES these individuals

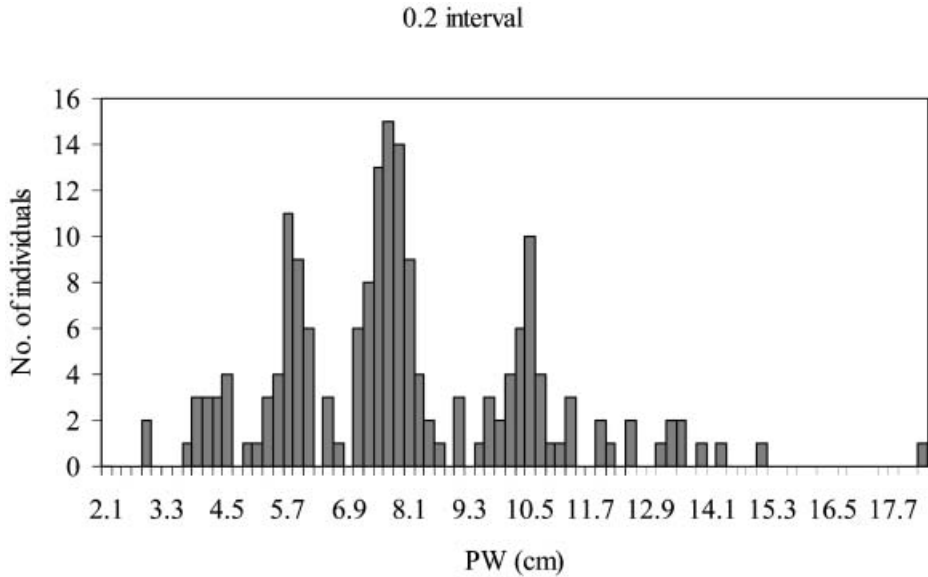


Figure 2. Size–frequency distribution of all individuals and exuviae using a 0.2-cm interval size.

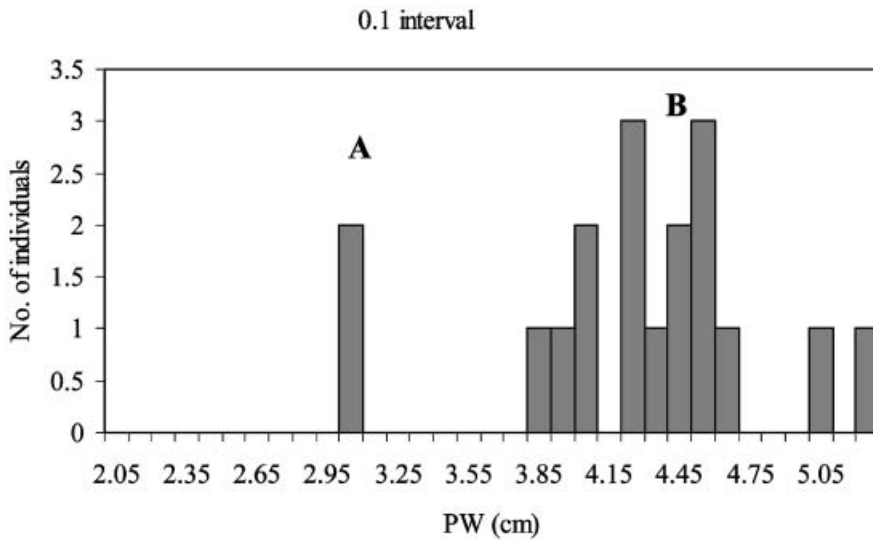


Figure 3. Size–frequency distribution of the smaller individuals using a 0.1-cm interval size.

really represented. The following size clusters were called correspondingly B, C, D, E, F, and G.

A 0.1 cm interval size was also used for the animals with PW 5.32–12.57 cm (Figure 4). Based on this, another three postembryonic stages (C–E) were identified (Figure 4). To clearly identify the larger juveniles (PW 13.11–18.30 cm) an interval size of 0.3 cm was needed (Figure 5). This revealed two more postembryonic stages (F and G). Since only one animal was found to represent ‘G’ this postembryonic stage was not considered in the following analysis.

Optically, a normal distribution was recognizable for a total of five postembryonic stages. The established size ranges of the postembryonic stages were tested with the

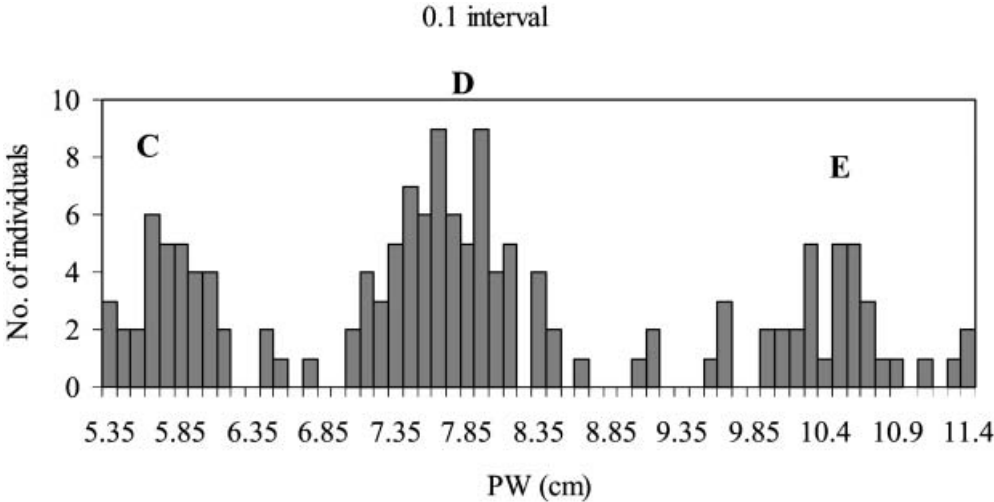


Figure 4. Size–frequency distribution of the medium-sized individuals using a 0.1-cm interval size.

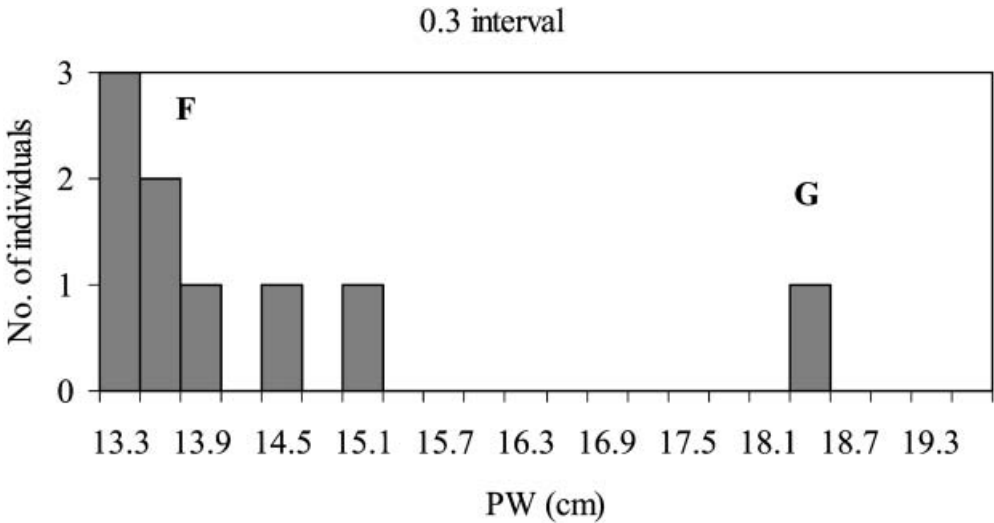


Figure 5. Size–frequency distribution of the largest individuals using a 0.3-cm interval size.

Kolmogorov–Smirnov test for normal distribution. The resulting levels of significance ( $P$ ), which in all cases exceeded 0.05 and the critical value (KS-Z) confirmed the optical impression of normal distribution (Table I).

#### Age structure

No exuvia of a previously marked individual was found; hence the exact age of a certain postembryonic stage remained unknown. But since the different PESs represent age classes it was possible to identify the age structure of the population (Figure 6). It was found that most individuals belonged to the postembryonic stages B–E. The highest number of individuals belonged to PES D (33.6%), PES E (26.4%) and PES C (23.2%) (Figure 6). Postembryonic stage B was still relatively common (11.2%), while the other postembryonic stages (F, G and A) had only very few individuals.

#### Sex ratio

Basis for the sex ratio were 47 females and 26 males. Fifty-two individuals—mainly during the first month of sampling—were not sexed. Females were generally more abundant than males (Figure 7). The ratio of male to female individuals was 1:1.8.

Table I. Prosoma width (PW) of postembryonic stages (PES).

PES	A	B	C	D	E	F
Mean PW (cm)	2.98	4.32	5.86	7.78	10.63	13.75
SD	0.01	0.40	0.33	0.45	0.73	0.64
SE	0.01	0.10	0.05	0.05	0.12	0.23
Min PW (cm)	2.97	3.77	5.33	7.01	9.52	13.11
Max PW (cm)	2.99	5.19	6.74	9.19	12.58	15.01
$n$	2	15	37	76	40	8
KS-Z	0.368	0.666	0.698	0.701	1.171	0.609
$P$	0.999	0.767	0.715	0.710	0.129	0.852

KS-Z, critical value of the Kolmogorov–Smirnov test;  $P$ , level of significance.

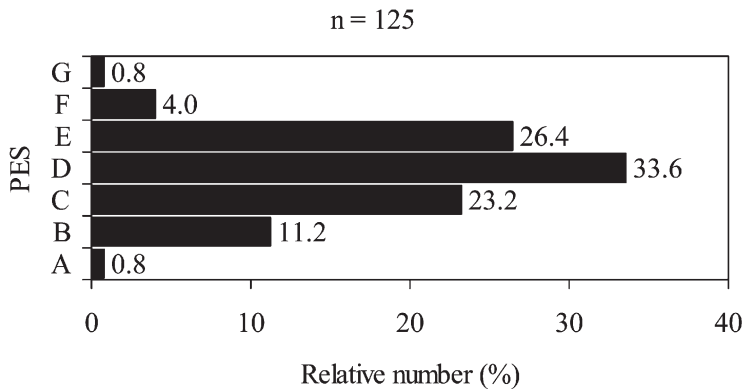


Figure 6. Age structure of juvenile population.

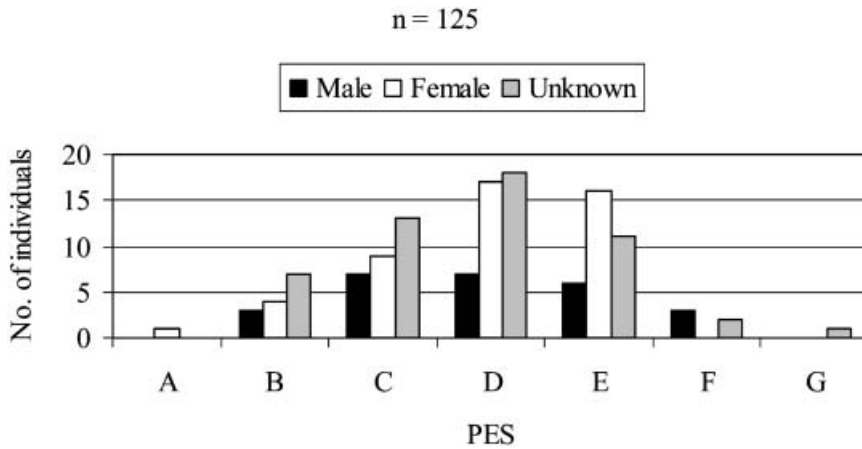


Figure 7. Number of male, female and individuals for which sex was not identified per postembryonic stage.

### Distribution

To determine whether the juveniles occupy with increasing size different areas in the intertidal, the place and the postembryonic stage of each collected individual was plotted on a prepared map. The analysis of these data revealed that the majority of all individuals from all postembryonic stages were found between 0 and 50 m from the mean high tide level. There was no spatial distribution pattern within these 50 m for the different postembryonic stages.

### Discussion

Most of the discussion will be based on a study conducted by Kaiser from May to December 2001 at BM Beach, a few kilometres north of Aventura Beach. Kaiser's (2002) study is so far the only comparable study that investigated a tropical population of *T. tridentatus*.

The values obtained for the physical and chemical water and soil parameters are typical for intertidal areas devoid of pollution (compare Chiu and Morton 1999). The values also meet the requirements of the species as indicated by other studies (Chiu and Morton 1999; Kaiser 2002). The daily fisheries activities and recently observed (S. Schoppe, personal observation) sand quarrying disturb the habitat as well as the juveniles.

The densities of 0.75 and 1.47 individuals per 100 m<sup>2</sup> obtained for the whole study area and for the suitable substrata, respectively, are very similar to those obtained by Kaiser (2002) at a nearby nursery beach. Kaiser (2002) computed an overall density of 0.6 individuals per 100 m<sup>2</sup> and a density of 2.0 individuals per 100 m<sup>2</sup> for the suitable substrata. There are no other comparable data for the species from other tropical areas. Based on data generated by Hong (2004) for juvenile *C. rotundicauda* from Singapore it was calculated that this species occurs in densities of about 2.05 individuals per 100 m<sup>2</sup>. This density was considered high (Hong 2004). To determine whether the number of juvenile *T. tridentatus* in Aventura Beach is high enough to sustain the population remains to be studied. The present data are important baseline data for a status evaluation. It is expected that the continuously increasing fishery activities in the area are posing a serious threat to the remaining population.

The present study is the second to generate data on the size of a juvenile population. Kaiser used the same method to estimate the population size. She estimated a total of 298 individuals inhabiting an area of about 70,000 m<sup>2</sup>. The Aventura population was comparably smaller (125 individuals), however, the habitat was also smaller (20,000 m<sup>2</sup>). Considering the smaller size of the area in Aventura Beach, results from both sites are quite similar. Kaiser found 262 individuals of which 88% were recaptured within 8 months. The present study was conducted for 5 months only and it generated a similarly high recapture rate (83%). The high recapture rate in both studies indicates that the search method is very effective and that 5 months suffice for the recapture method. This is an important aspect to be considered in future studies.

The fact that the entire population was composed of only juveniles identifies the study area as a nursery beach of *T. tridentatus*. Size-frequency cohorts of juveniles differed in abundance reflecting one or a combination of the following: (1) different magnitude of recruitment from year to year; (2) different magnitude of mortality; (3) gradual movement of larger individuals to deeper waters. The absence of adults can be explained by the fact that they occupy deeper waters. They are only found near the beach during the breeding season (Sekiguchi 1988b).

The size range of the animals that were encountered (2.97–18.3 cm) indicates that not all postembryonic stages were found. Kaiser (2002) found individuals between 1.4 and 36.9 cm in PW whereas those larger than 22 cm were adults. The reason why no individuals smaller than 2.9 cm were found in Aventura is expected to be related to the fact that very small juveniles are much more vulnerable to predation and mortality caused by fishing activities than larger ones.

Gotto and Hattori (1927), based on *T. tridentatus* from the wild, identified a total of 14 stages, wherein the last two represent adult male and female individuals, respectively. Sekiguchi et al. (1988), on the other hand, identified a total of 17 postembryonic stages based on laboratory studies. Their last two stages represent adult male and female individuals, respectively. Both studies were conducted with *T. tridentatus* from subtropical Japan. Kaiser (2002) compared her data with the above and found significant agreement with Gotto and Hattori's data. Hence, the present study used Kaiser's data to identify the postembryonic stages found at Aventura. The comparison revealed that the smallest animals found (PES A) actually are already in their sixth postembryonic stage. The consecutive stages were identified as postembryonic stages 7–12, whereas PES 12 represents the last juvenile stage (Table II).

The comparison with Kaiser (2002) also permitted age classes to be assigned to the postembryonic stages (Table II). Based on this the PES 6 (A) is about 5.4 months old, PES 7 (B) is 7 months, PES 8 (C) is 9 months, PES 9 (D) is 12.6 months, and PES 10 (E) is 17.6 months old. The population was dominated by juveniles between 9 and 17.6 months of age.

The present study provides the first published data on the sex ratio of juveniles. Under the local circumstances where adult females are an appreciated by-catch in fishing (Schoppe 2001), a ratio in favour of females is advantageous. Therefore it would be interesting to assess the sex ratio of adults at the same site.

The assessment of the distribution of the animals within the intertidal area revealed that the majority inhabited the first 50 m from the mean high tide level. Several factors may be influential in this: (1) according to Gotto and Hattori (1927) the larvae settle in the direct vicinity of the nest, which is close to the shore; (2) the juveniles seem to avoid the dense seagrass beds that cover the seaward part of the study area; and (3) human disturbances are

Table II. Comparison of postembryonic sizes with those of Kaiser (2002).

This study		Kaiser (2002)		
PES name	PW (cm)	PW (cm)	Age (d) at time of moult into the	
			PES	PES
A	2.97–2.99	1.4–1.44	3	37
		1.65–1.99	4	67
		2.24–2.84	5	107
		2.90–3.67	6	163
B	3.77–5.19	3.94–5.10	7	219
C	5.33–6.74	5.26–6.96	8	279
D	7.01–9.19	7.02–9.42	9	380
E	9.52–12.58	9.6–12.65	10	530
F	13.11–15.01	12.8–16.45	11	
G	18.3	17.13–21.12	12	
		21.66–28.9	13	
		29.2–36.9	14	

higher at the seaward area. The occurrence of most of the individuals at the landward part of the intertidal seems to be further related to the substratum. The juveniles prefer sandy-muddy substrata where they can bury themselves before the incoming high tide. The lower intertidal is mainly covered by seagrasses hence the animals have difficulties digging into the substratum and crossing the seagrass.

The fact that only one large juvenile (PES 12) was found confirms the nursery function of the area and indicates that larger juveniles have already migrated to deeper waters. In fact, several authors noted that juveniles move with increasing size gradually towards deeper water (Shuster 1979; Rudloe 1981; Kaiser 2002).

Acknowledgement

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