



Episodic human activities and seasonal impacts on the *Tachypleus gigas* (Müller, 1785) population at Tanjung Selangor in Peninsular Malaysia



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ABSTRACT

The scientific literature on population dynamics, breeding biology and nesting behaviour of the Asian horseshoe crabs is limited and still to emerge from several locations in South and Southeast Asia. The present study (2009–2011), aimed at observing the spawning activity of *Tachypleus gigas* (Müller, 1785) in relation to local environmental conditions, was carried out from Tanjung Selangor (Sites 1–3) on the east coast of Peninsular Malaysia. Southwest (SW) monsoon (May–September) was found to be more favourable (range, 456–1309 eggs) than Inter-monsoon (IM) (April and October) (169–317 eggs), whereas no eggs at the time of Northeast (NE) monsoon (November–March). Meanwhile, *T. gigas* preferred Site-1 (located close to River Pahang mouth) for its nesting in the SW and shifted to Site-2 and/or Site-3 (located 150–200 m interior) in the IM periods. This nest shifting behaviour of *T. gigas* was primarily associated with the seasonal water current that becomes intensive during NE monsoon. However, the human activities such as sand mining (2010), jetty (2010–2011) and road/bridge (2011–present) constructions in the vicinity have affected the normal physicochemical conditions of the sediment/water. Importantly, the sediment being represented largely by medium sand with moderate sorting, symmetrical and very leptokurtic properties in 2009 was changed into coarse sand with poor sorting, fine/very fine skewed and extremely leptokurtic in 2010–11. In addition, the heavy metal contamination of Cadmium and Selenium in the sediment and Hydrogen Sulfide in the water increased considerably (2010–11). These changes, not only reduced *T. gigas* spawning activity/egg counts between 2009 and 2010 (from an average of 912 ± 690 to 360 ± 288) ($\pm SD$), but also correlated with its total absence during 2011 (PRIMER v.6). Since Tanjung Selangor beach (adjoining the Terus and Pahang Tua estuary) is one of the very few known spawning grounds for the Malaysian horseshoe crab (*T. gigas*), it is imperative to conserve and manage this area and it should be sanctioned as a 'Marine Protected Area'.

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1. Introduction

The status of the populations of the three Asian horseshoe crab species – *Tachypleus gigas* (Müller, 1795), *Tachypleus tridentatus* (Leach, 1819) and *Carcinoscorpius rotundicauda* (Latrelle, 1802), are either unknown or decreasing due to anthropogenic disturbances (e.g. overexploitation, release of untreated sewage, factory effluent,

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etc.), more than the natural causes (beach erosion) (Chatterji and Shaharom, 2009; Shin et al., 2009; Hu et al., 2010; Cartwright-Taylor et al., 2011). Among others, the physicochemical changes in the coastal environment such as beach morphology (erosion/accretion), sediment texture and water chemistry are severely affecting the horseshoe crab populations worldwide and thereby a shift or permanent loss of its spawning grounds at many places (Botton et al., 1988; Botton and Loveland, 1989; Penn and Brockman, 1994; Schrading et al., 1998; Widener and Barlow, 1999; Smith et al., 2002a; Jackson et al., 2007; Hu et al., 2010). Under the sustained human disturbances on the coastal habitats on one hand and vanishing breed/nursery grounds on the other, the long-term survival of these crabs is also a major concern to the scientists (Chen et al., 2004; Patti, 2008; Chatterji, 2013).

While a great deal of scientific works, ranging from molecular and biotechnology aspects to population dynamics, breeding biology and spawning behaviour, are available on the American horseshoe crab – *Limulus polyphemus* (Linnaeus, 1758) (Brockmann, 2002; Botton and Loveland, 2003; James-Pirri et al., 2005; Conrad et al., 2006; Schwab and Brockmann, 2007; Becker-Pauly et al., 2009; Schaller et al., 2010; Sason et al., 2012; Smith et al., 2013; Jackson et al., 2014; Beekey and Mattei, 2015); the studies pertinent to the Asian horseshoe crabs are limited (Vijayakumar et al., 2000; Iwanaga, 2002; Chatterji et al., 2004a, b; Shin et al., 2009; Hu et al., 2010; Faurby et al., 2011; Obst et al., 2012; Sahu and Dey, 2013), and still to emerge from several locations in South and Southeast Asia. In fact, the Asian horseshoe crabs are represented by 'Data Deficient' category in the IUCN Red list for threatened species (IUCN, 2014).

Though all three Asian horseshoe crabs are present in the Malaysian waters for ages, they gained considerable scientific attention only recently (Yap et al., 2008; John et al., 2010; Chatterji et al., 2008; John et al., 2011; Kamaruzzaman et al., 2011; Tan et al., 2011; Rozihan et al., 2013), and yet to receive a nationwide priority for its conservation and management. The spawning and nesting behaviour of *T. gigas* in Peninsular Malaysia was first studied by Zaleha et al. (2012). However, their study was limited to a short-term (4 months) investigation for Southwest monsoon at Balok and Pekan areas. The management efforts can be more effective if the long-term data/information on horseshoe crab's population and threats to their spawning and nursery grounds is available (Burger et al., 2002; Smith et al., 2002a, b; Chen et al., 2004). Therefore, site-specific monitoring on the environmental conditions as well as the crabs' landing during mating seasons is necessary (Jackson et al., 2007).

The present study began in 2009 with the primary goal of observing the spawning activity of *T. gigas* vis-à-vis local environmental settings at Tanjung Selangor on the east coast of Peninsular Malaysia. However, in the light of human activities such as sand mining and physical infrastructure developments (i.e. jetty and road/bridge) in the vicinity from 2010, the impact of these issues was also measured on the spawning behaviour of *T. gigas* until end of the study in 2011. The main objective is to identify the influence of local environmental conditions (e.g. sediment, water and season) on nesting sites as well as spawning behaviour of *T. gigas*. At the end, possible recommendations were offered to conserve and manage this vulnerable spawning ground of the Malaysian horseshoe crab.

2. Materials and methods

2.1. Study area

Tanjung Selangor beach, located in the State of Pahang, is one of the two known spawning grounds (another being Pantai Balok) for

T. gigas on the east coast of Peninsular Malaysia (Lat: 3° 36.152'–3° 36.206' N; Long: 103° 23.943'–103° 23.952' E) (Fig. 1A). Since Tanjung Selangor comes under the jurisdiction of Pekan town/district in Pahang, few researchers have also referred this place's name as Pekan or Tanjung Gosong in their publications (e.g. John et al., 2011, 2012; Zaleha et al., 2012). The local people gather at Tanjung Selangor beach and collect the horseshoe crabs for their fish feed preparation as well as personal consumption. The two distributaries namely, Terus and Pahang Tua, emanating from River Pahang opens into South China Sea and support the estuarine environment (Fig. 1B).

The climate of Malaysia is influenced by Northeast (NE) (November–March) and Southwest (SW) (May–September) monsoons separated by two Inter-monsoonal (IM) periods (April and October). In the case of Tanjung Selangor, the (average) temperature varies between 21 and 35 °C, and remains warm throughout the year (WU, 2014). While highest precipitation occurs with the onset of NE monsoon (232–594 mm), the lowest will be during February–April (2–13 mm). The tides, with a range of 0.5–3.5 m, are mixed in nature (NHC, 2011).

In recent years, there are considerable human activities in the form of aquaculture (including sport fishing, fish landings) and leisure boating at Tanjung Selangor. We recall that sand mining (2010), jetty (2010–2011) (Fig. 1B–D) and road/bridge construction (2011 – to present) (Fig. 1E) activities are also the prominent issues. Such developments, not to mention the additional burdens of pollution, can create changes to the local environment and thereby possible adverse effect on the subsistence dwellers. In particular, the changes in beach sediment (grain size) characteristics along with increased heavy metal contamination were evidenced in the present study.

2.2. Data collection

The present study was carried out between July 2009–April 2010 and June 2010–June 2011 (23 months). Out of 2 km sandbar at Tanjung Selangor, the nesting activity of *T. gigas* was found only at a small portion along 350 m. However, after taking into account the horseshoe crab imprints on exposed sand (in-between high and mid tide markings) and the places with regular yield of nests along 350 m, three sampling sites (Site-1: 3° 36.34' – 3° 36.36' N; Long: 103° 23.83' – 103° 23.88' E, Site-2: 3° 36.27' – 3° 36.29' N; Long: 103° 23.88' – 103° 23.91' E and, Site-3: 3° 36.14' – 3° 36.17' N; Long: 103° 23.92' – 103° 23.93' E) (located 150–200 m apart) were identified for every month's biological and environmental data collection (Fig. 1B). While the fieldwork between 2009 and 2010 was conducted two times a month, covering both full moon and new moon periods, only full moon periods were covered for 2010–2011 (due to financial limitations). Since there were no eggs found at Site 3 from June 2010, our subsequent data collection/analysis was focused on Sites 1–2. For the biological and the environmental data analysis/interpretation in this paper, we only considered the months that found with eggs and/or spawning crabs at least from one sampling site. In order to understand the impact of the local environmental changes on *T. gigas* nesting sites, we have also analysed the additional influencing factors such as heavy metals (i.e. Lead (Pb), Chromium (Cr), Selenium (Se) and Cadmium (Cd)), Total Organic Carbon (TOC), Hydrogen Sulfide (S^2), and nutrients (i.e. Nitrite (NO_2), Nitrate (NO_3) and Phosphate (PO_4)) in the sediment and the water (for 2010–2011 data).

2.2.1. Biological sampling

The sampling sites were visited at night during high tide for spawning crabs estimation and to mark the nest locations, whereas in daytime during low tide for eggs observation. At each sample

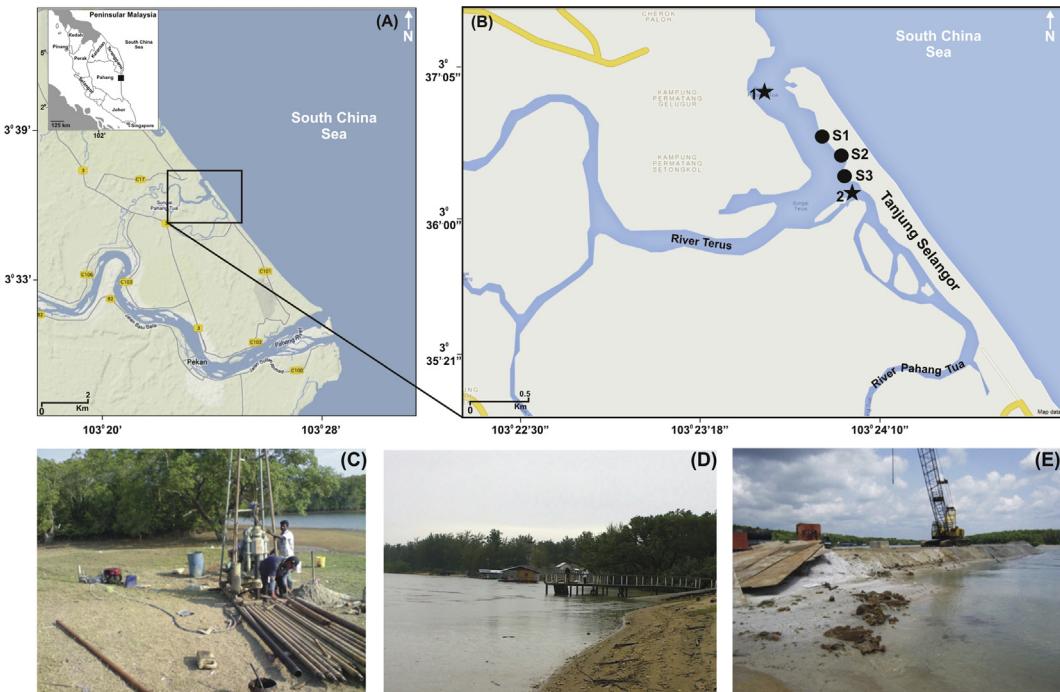


Fig. 1. (A) Location of Tanjung Selangor in the state of Pahang on the east coast of Peninsular Malaysia. The River Pahang and its distributaries namely, Terus and Pahang Tua, opening into the South China Sea are visible (box: study area); (B) Study area showing the three sampling sites (S1–3) on the sheltered beach of Tanjung Selangor. The stars named after 1 and 2 show the location of sand mining and jetty construction activities in the vicinity (Google Map data® 2013). The photographic evidences on jetty and bridge construction works at Tanjung Selangor – (C) piling work for jetty construction in 2010; (D) new jetty in 2011; (E) road/bridge construction in progress (2011 – to present).

site, the number of nests i.e., horseshoe crab imprints that contained eggs, were identified by gently removing the sediment (using plastic hand shovel). From each nest, the available egg clutches were collected into a 2 mm sieve (using hand) and cleaned with the seawater to separate them as well as remove debris/sediment particles attached. Once the total egg count was obtained, they were placed back into the same pit and covered by the sand. The number of male/female spawning crabs was obtained through counting them by sight and/or catching them with hand (after finding their air bubbles on the water surface). In addition, the mating status of the crabs was recognized as a spawning pair if one male amplexed with one female; satellite male if it is single and available close to the amplexed pair to fertilize their eggs, and solitary female if it is all alone (cf. Mattei et al., 2010).

2.2.2. Water quality

The surface water quality parameters such as temperature (°C), salinity (‰), pH and dissolved oxygen (DO) (mg l^{-1}) were measured *in situ* by using YSI 556 multi-probe sensors (YSI Inc., Yellow-Springs, OH, USA). For laboratory analyses, the (surface) water samples (about 500 ml) were collected into plastic bottles and added Hydrogen chloride (HCl) to stabilize the organic carbon (at pH 2) before keeping them in the ice box (USEPA, 2004).

At the laboratory, each water sample was filtered through a microglass filter paper (Whatman® GF/C, England) and analysed the nutrients and S² (using spectrophotometer: HACH DREL 2400, USA) (HACH, 2004), the metals (using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Varian Vista-Pro, USA)) (Guéguen et al., 2001), and the TOC (using TOC analyser: Shimadzu, TOC-VCPh, Japan) (USEPA, 2004); whereas the filter paper was used for Chlorophyll-a (Chl-a) estimation (Parsons et al., 1984).

2.2.3. Sediment characteristics

Both soil temperature (using laboratory thermometer,

sensitivity: 0.2 °C) and pH (DM-13 Takamura Electric Works, Japan) were measured *in situ* at the time of fieldwork. A transparent 3' PVC pipe of 1 m length (pre-marked up to 50 cm) was pushed into the soil and the depth above the anoxic sand layer (black in colour) was measured as moisture depth at each site. In addition, a separate portion of the sediment (100 g) from each site (using hand shovel) was collected for laboratory (i.e. grain size, metals, TOC) analyses. All the samples were subjected to oven dry for 3 days (at 45 °C) and then separated into different fractions using set of sieves (<63–4000 µm mesh size) in a mechanical sieve shaker (Retsch AS 200 Basic, Germany). The logarithmic method of moments was followed to estimate different grain size variables such as mean grain size (φ), sediment mean ($X\varphi$), sorting ($\sigma\varphi$), skewness ($SK\varphi$) and kurtosis ($K\varphi$) (Blott and Pye, 2001). For metals, the sediment fraction <63 µm was used and analysed in ICP-OES (Noriki et al., 1980). TOC was estimated with the help of TOC analyser (USEPA, 2004). In addition, to find the level of contamination in the sediment samples, both sediment enrichment factor and geo-accumulation index were calculated by following the standard equations (e.g. Khalik et al., 1997).

2.3. Statistical analyses

While the One-Way ANOVA was used to find differences in the biological and the environmental parameters tested (using OriginPro v.9.1), the Principal Component Analysis (PCA) was used to test the percentage (%) variation of the environmental parameters in relation to *T. gigas* egg counts (root-transformed data) (using PRIMER v.6) (Clarke and Gorley, 2006). In order to understand the influence of local environmental conditions on *T. gigas* nesting sites, a statistical routine called BEST – amalgamating BIO-ENV and BVSTEP procedures, in PRIMER v.6 was followed. In this context, the global BEST match test (with an option of 99 permutations) was performed to know the environmental variables that have positive

and negative impact on the nesting sites (Spearman's rank (ρ) coefficients).

3. Results

3.1. Nesting activity of *Tachypleus gigas*

The nesting activity of *T. gigas* was found only during full moon periods and took place mostly at Site-1 (Table 1A). In addition, the egg yield was higher at the time of 2009 (mean egg count, 912), whereas it decreased by 2010 (360 eggs), and completely absent in 2011. Season-wise, SW monsoon favoured highest egg production (range, 456–1309) than to IM (169–317 eggs), whereas no spawning at the time of NE monsoon (although few adult crabs were noticed). In relation to the changes in nesting places, *T. gigas* preferred Site-1 during the SW and shifted to Site-2 and/or Site-3 by the end of SW and during the IM periods. Overall, there was a considerable decrease in the number of spawning crabs (One-way ANOVA, $F = 1.1$, $P = 0.4$) as well as nests (One-way ANOVA, $F = 13.3$, $P = 0.004$) between 2009 and 2010–11 investigations (Table 1A). In total, 15 spawning pairs, 8 satellite males and 1 solitary female were found in 2009, whereas only 3 satellite males for 2010 and, 1 spawning pair and 2 satellite males for 2011.

3.2. Environmental conditions

3.2.1. Sediment

The sediment composition at the three sampling sites was represented largely by sand (>90%) with coarse to medium texture (0.2–2.0X ϕ) (Table 1B). However, the sediment fraction containing 0.250 mm grain size (representing medium sand) was higher than 0.180 mm (medium-fine sand), and 0.125 mm (fine sand). While the range of sorting (0.7–1.5 $\sigma\phi$) indicated it as a moderate to poorly sorted sediment, the skewness (0–1.9SK ϕ) and the kurtosis (2.3–7.3K ϕ) represents the area as symmetrical to very fine skewed, and very leptokurtic to extremely leptokurtic, respectively. The TOC was found to be low at all sites (0–0.4%) (Table 1B).

Between 2009 and 2010–11, medium sand, medium-fine sand and mud (i.e. silt & clay) compositions were decreased while the moisture depth increased by two folds (Table 1B) (One-way ANOVA, $F = 56$, $P < 0.0001$). The sediment representing symmetrical and very leptokurtic properties at Site-1 has been changed into fine to very fine skewed, and very leptokurtic to extremely leptokurtic. Also, Site-2 acquired the same edaphic conditions as that of Site-1 (i.e. fine to very fine skewed and very leptokurtic to extremely leptokurtic) by the end of the present study. At Site-3, increased fine sand with poor sorting and medium texture was witnessed (Table 1B).

Among the metals, the concentration of Cr (32.1–37.7 mg kg $^{-1}$) was higher than Se (9.2–16.1 mg kg $^{-1}$), Pb (7.1–11.2 mg kg $^{-1}$), and Cd (6.2–6.7 mg kg $^{-1}$) in the order (Table 1B). However, the metal induced enrichment factor (Table 2A) and the geo-accumulation index (Table 2B) indicated a severe contamination of both Cd and Se at all nesting sites.

3.2.2. Water

The surface water temperature at the nesting sites varied between 27 and 31 °C (Table 1C). However, the site-specific variations among the commonly observed parameters such as pH, salinity and DO, between 2009 and 2011, did not reveal any significant differences. In relation to the season, only salinity changes are prominent where it increased with the onset of SW monsoon (up to 32.6‰), remained low to moderate during the IM (4–15‰), and low during the NE monsoon (4‰) (Table 1C). Among the other variables tested exclusively for 2010–11 (i.e. Chl-a, TOC, NO₂, NO₃, PO₄ and S²), the

S² concentration increased significantly between 2010 and 2011 (One-way ANOVA, $F = 17.5$, $P = 0.002$). In the case of metals, the (average) concentrations of Se, Pb, Cr and Cd are found within the range of 0.1–0.6 mg l $^{-1}$ (Table 1C).

3.3. Environmental influence on the nesting sites

The PCA based on sediment observations (except metals) indicate 38% of its variance along axis-1 (eigenvalue: 4.6), whereas axis-2 showed 24% (eigenvalue: 2.8) (cumulative variation: 62%) (Fig. 2A–B). A clear-cut separation between the 2009 (sites clustered on to the positive side of both axes) and the 2010–11 findings (sites distributed in all directions) was observed. The (Spearman) correlation coefficients drawn between the soil parameters and the egg counts of *T. gigas* (through the BEST analysis) are optimized for three variables notably, sorting, kurtosis and fine sand ($\rho = 0.33$). Similarly, the water quality observations also revealed a well-marked demarcation between 2009 and 2010–11 investigations (Fig. 3A–B). While its variation along the first axis was 62% (eigenvalue: 6.2), the second axis showed 15% (eigenvalue: 1.5) (cumulative variation: 78%). The BEST analysis indicated that DO and S² are the optimal variables ($\rho = 0.24$). In the case of heavy metals, the PCA showed 71% of the total variation (eigenvalues for axes 1 and 2: 4.0 and 1.6) (Fig. 4A–B) with a best correlation ($\rho = 0.31$) of metals originated from the sediment (Cd, Pb and Se) than to the water (Cr).

4. Discussion

4.1. Tanjung Selangor as a spawning ground for *T. gigas*

Habitat requirements for the spawning activity of the horseshoe crab species are almost the same, except for *C. rotundicauda* that breeds in the muddy areas usually near mangroves (Anderson and Shuster, 2003; Lee and Morton, 2005; Seino, 2009; Cartwright-Taylor et al., 2011). The female crabs of *T. gigas*, *T. tridentatus* and *L. polyphemus* spawn along the intertidal beaches of the estuarine coasts (at 15–20 cm depth below the sand), where the eggs will be fertilized by an attached male and/or satellite male(s) (Ehlinger and Tankersley, 2003; Shuster et al., 2003; Jackson et al., 2007). In the case of east coast Peninsular Malaysia, only two beaches namely, Tanjung Selangor and Balok, are well-known for the occurrence of *T. gigas* (Chatterji et al., 2008; Tan et al., 2011; John et al., 2011, 2012; Zaleha et al., 2012). In fact, the present study also confirms Tanjung Selangor as a potential spawning ground for *T. gigas* where the adult crabs/spawning pairs were found throughout the present investigation (Table 1A). The sandy areas in-between upper and mid tidal zones are providing a suitable environment for spawning, nurture and development of the horseshoe crab eggs as also observed by the other researchers elsewhere (e.g. Jackson et al., 2007, 2008; Cartwright-Taylor et al., op. cit.; John et al., 2012; Shin et al., 2014).

Although adult horseshoe crabs have arrived mostly during mid to end of a lunar (full moon) cycle in the SW and IM seasons, their number was found to be less (except for 2009) at Tanjung Selangor (Table 1A). The satellite males observed in the present study could represent their polyandrous behaviour to maximize sperm competition (Mattei et al., 2010; Smith et al., 2013). On the other hand, overexploitation of the female crabs for fish feed preparation and edible purposes might be also a reason for more male occurrences. Similar threats to the horseshoe crabs have been reported from the other countries like Thailand, Hong Kong, India, America, etc. (Ferrari and Targett, 2003; Jackson et al., 2007; Ngy et al., 2007; Hu et al., 2010; Basudev et al., 2013; Beekey and Mattei, 2015). Therefore, the dropdown in the number of adult crabs as well as the

Table 1Biological, sediment and water quality observations from the nesting (1–3) sites of *Tachypleus gigas* at Tanjung Selangor.

2009									2010									2011									
July			August			September			October			June		July		October		March		April		May					
S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S3	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2		
(A) Biology																											
No. of nests	5	—	—	7	—	—	8	—	—	1	2	3	—	2	—	—	2	—	—	—	—	—	—	—	—	—	
No. of clutches	10	—	—	12	—	—	11	—	—	1	3	6	—	3	—	—	3	—	—	—	—	—	—	—	—	—	
No. of eggs	954	—	—	1011	—	—	1962	—	—	115	520	692	—	220	—	—	169	—	—	—	—	—	—	—	—	—	
No. of male <i>T. gigas</i>	—	—	—	—	2	15	1	—	2	—	—	3	—	3	—	—	—	—	1	—	—	1	—	—	1	—	
No. of female <i>T. gigas</i>	—	—	—	—	3	10	—	—	1	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
(B) Sediment																											
Mean (X_ϕ)	1.1	0.8	0.6	1.1	0.8	0.7	0.9	1.2	0.9	0.7	1.1	1.2	1.5	1.3	0.4	0.2	0.3	0.2	1.6	2	0.3	0.6	0.6	0.8	—	—	
Sorting (σ_ϕ)	1	1.3	0.7	1	1.2	0.7	1.3	0.9	0.9	1.5	0.9	0.9	0.8	1.1	1.1	1.1	0.8	1	1.2	0.9	0.8	0.9	0.9	0.9	1	—	
Skewness (SK_ϕ)	0.1	0.3	0.2	0	0.2	0.1	0	0	0	0.1	0	0	0.2	0.9	0.9	0.3	0.5	0.8	1.9	0.8	0.2	0.1	0.2	—	—	—	
Kurtosis (K_ϕ)	2.8	2.7	2.9	2.8	2.6	3	2.8	2.7	2.8	2.8	2.9	2.7	4	2.7	3.1	3.7	3.7	3	2.7	7.3	4.5	3.6	2.8	2.3	—	—	
Gravel (%)	4.1	5	5.2	4.2	4.3	5.8	4.3	5	5.6	5.1	5.1	5.1	0.4	1.5	5.9	11	5.7	14.3	3.1	3.7	3.1	3.4	4.7	4.8	—	—	
Sand (%)	92.5	91.8	91.8	93.2	92.3	91.1	92.1	92.6	91.3	91.2	92	92.1	98.5	97.3	93.3	88.5	94.2	85.6	96.3	95.7	96.3	95.7	94.7	94.9	—	—	
Silt & clay (%)	3.3	3.3	3.1	2.6	3.4	3.1	3.6	2.4	3.1	3.7	2.9	2.8	1	1.3	0.8	0.5	0.1	0.1	0.6	0.6	0.6	0.9	0.6	0.4	—	—	
0.125 mm (%)	2.3	1.9	2	1.6	2.1	2.8	1.9	1.9	3	2.2	2.3	1.6	9.6	8.3	2.5	2.4	0.3	1.8	2.2	1.7	0.4	0.3	0.2	0.5	—	—	
0.180 mm (%)	12.7	9.6	6.2	11.1	9.2	7	12.2	10.4	8.6	13.2	9.2	10	7.6	12.6	6.9	3.4	0.6	3.8	9.7	5.9	3.3	3.6	4.2	8.9	—	—	
0.250 mm (%)	16.9	13.5	8.2	15.6	13.5	8.2	17	15.6	8.5	14.1	12.6	17	24.7	18.3	6.7	4.4	1.1	5.7	6.8	9.9	4	7	13.4	17.5	—	—	
Moisture depth (cm)	6.8	4.3	6.9	6.6	4.7	6.7	5.6	3.8	6.4	5.4	2.7	5.9	13.4	13.2	12.8	12.4	9.7	9.8	8.1	8.8	9.4	10.7	10.3	8.3	—	—	
Cd (mg kg ⁻¹)	—	—	—	—	—	—	—	—	—	—	—	—	6.8	5.5	7.6	8.1	6.3	5.9	2.5	4.3	7.7	7.5	7.8	7.7	—	—	
Cr (mg kg ⁻¹)	—	—	—	—	—	—	—	—	—	—	—	—	32.2	32.8	35.6	33.3	46.4	45.8	33.4	38.9	28	31.4	31.6	29.2	—	—	
Pb (mg kg ⁻¹)	—	—	—	—	—	—	—	—	—	—	—	—	8.9	9.9	5.7	5.7	5.9	6.4	12.2	8.7	9.4	13.1	12.4	11.6	—	—	
Se (mg kg ⁻¹)	—	—	—	—	—	—	—	—	—	—	—	—	7.1	8.2	11.7	9.9	9.1	9	20.1	14.1	14	13.2	18.6	16.9	—	—	
TOC (%)	—	—	—	—	—	—	—	—	—	—	—	—	0.1	0.1	0.4	0.1	0.3	0.1	0.1	0	0.2	0	0.3	0.3	—	—	
(C) Water																											
Temperature (°C)	29.5	29.5	29.5	30.4	30.3	30.2	30.6	30.7	30.1	29.6	29.5	27.2	29.2	29.5	29.6	29.6	31	30.9	30.2	30.3	30	30	30.5	30.7	—	—	
pH	7.8	7.6	8.2	7.6	7.8	8.1	7.7	7.9	7.7	7.9	8.1	8.2	7.3	7	7.3	7.2	7.9	8	6.7	6.8	6.9	7	7.8	7.8	—	—	
Salinity (%)	32	31.5	30	31.5	30.6	29.8	25.5	24.9	24	14.8	15.1	15.2	32.6	31.5	32.1	32	6.6	5.8	4.3	4	4.1	3.9	14.5	13.7	—	—	
DO (mg l ⁻¹)	5.1	4.8	4.4	4.3	3.9	3.9	3.3	4.3	3.8	4.2	5.9	4.8	5.3	6.3	5.3	5.7	3.4	4	4.5	4.6	8.5	8.7	4.9	5	—	—	
NO ₂ (mg l ⁻¹)	—	—	—	—	—	—	—	—	—	—	—	—	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	—
NO ₃ (mg l ⁻¹)	—	—	—	—	—	—	—	—	—	—	—	—	1.3	1.4	1.5	1.8	0.4	0.5	1.4	1.5	1.5	2.2	0.9	1.4	—	—	
PO ₄ (mg l ⁻¹)	—	—	—	—	—	—	—	—	—	—	—	—	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.1	0.1	0.4	0.3	—	—
S ² (μg l ⁻¹)	—	—	—	—	—	—	—	—	—	—	—	—	2.7	4.3	5.3	6	13	16	30.7	45	63.7	55.3	14.9	30.7	—	—	
Chl-a (mg l ⁻¹)	—	—	—	—	—	—	—	—	—	—	—	—	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	—
Cd (mg l ⁻¹)	—	—	—	—	—	—	—	—	—	—	—	—	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	—	—
Cr (mg l ⁻¹)	—	—	—	—	—	—	—	—	—	—	—	—	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	—
Pb (mg l ⁻¹)	—	—	—	—	—	—	—	—	—	—	—	—	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	—
Se (mg l ⁻¹)	—	—	—	—	—	—	—	—	—	—	—	—	0.5	0.5	0.2	0.1	0.2	0.2	0.4	0.3	0.3	0.3	0.6	0.5	—	—	—
TOC (ppm)	—	—	—	—	—	—	—	—	—	—	—	—	3.4	8.8	4.4	8.1	8.2	3.7	2.5	6.4	1.3	4.5	2.3	5.7	—	—	—

“—” indicates no sample observed. As explained in methodology, the above table shows data for the year, months and sites (S1–S3) that were found with eggs and/or adult horseshoe crabs during the course of investigation (for complete monthly details please vide the [supplementary material](#)). All observations in the table were found during full moon periods only.

Table 2

Metal induced Enrichment factor (EF) and Index of geo-accumulation values at the nesting (1–3) sites of *Tachypleus gigas* in Tanjung Selangor.

	2010				2011							
	June		July		October		March		April		May	
	S1	S3	S1	S2								
(A) Enrichment factor												
Cadmium (Cd)	246.9 ^E	279.1 ^E	252.5 ^E	231.6 ^E	296.6 ^E	288.9 ^E	222.6 ^E	102.7 ^E	285.7 ^E	355.8 ^E	284.3 ^E	316.7 ^E
Chromium (Cr)	4.3 ^M	3.8 ^M	3.0 ^M	3.1 ^M	6.2 ^S	6.4 ^S	5.5 ^S	3.9 ^M	2.9 ^M	4.3 ^M	3.3 ^M	3.4 ^M
Lead (Pb)	2.3 ^M	1.8	0.9 ^D	0.9 ^D	1.4 ^D	1.6 ^D	2.1 ^M	2.5 ^M	1.7 ^D	3.1 ^M	2.2 ^M	2.4 ^M
Selenium (Se)	265.7 ^E	210.8 ^E	221.3 ^E	254.7 ^E	303.4 ^E	315.0 ^E	491.6 ^E	592.8 ^E	372.9 ^E	450.7 ^E	481.4 ^E	498.0 ^E
(B) Index of geo-accumulation												
Cadmium (Cd)	3 ^H	4 ^{HE}	3 ^H	2 ^{MH}	4 ^{HE}	4 ^{HE}	4 ^{HE}	4 ^{HE}				
Chromium (Cr)	-2 ^{PU}											
Lead (Pb)	-2 ^{PU}	-1 ^{PU}	-2 ^{PU}	-1 ^{PU}	-1 ^{PU}	-1 ^{PU}						
Selenium (Se)	3 ^H	3 ^H	3 ^H	4 ^{HE}	3 ^H	4 ^{HE}						

As explained in methodology, the above table shows data for the year, months and sites (S1–S3) that were found with eggs and/or adult horseshoe crabs during the course of investigation and when metals in sediments were analysed (for complete monthly details please vide the [supplementary material](#)). The contamination categories for Enrichment factor are indicated by superscripts E = extreme, M = moderate, S = significant, D = deficient to minimal and, Index of geo-accumulation are indicated by the superscripts HE = heavy to extreme, H = heavy, MH = moderate to heavy, PU = practically uncontaminated.

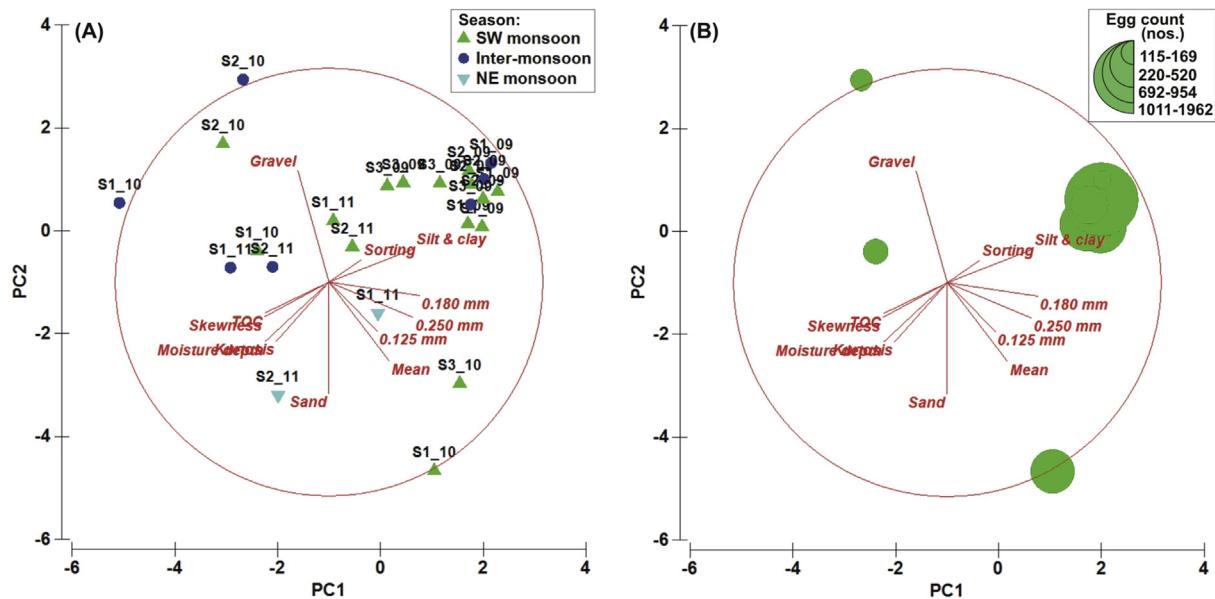


Fig. 2. Principle Component Analysis (PCA) showing sedimentological variations in relation to the nesting sites of *Tachypleus gigas* superimposed by – (A) season and, (B) egg count. The sampling sites (S1–3) and the year of observations (2009–11) are indicated side by side with an underscore. The transparent circle in each panel represents correlation circle and the orientation of the environmental (sediment) lines approximate their correlation to the ordination axes.

frequent occurrence of satellite males孤雌 females at Tanjung Selangor are also signalling other scientific concerns (in addition to overexploitation) whether this kind of situations are temporary or due to unsuitable environmental conditions. While the former concern is uncertain, the latter was evaluated in the forthcoming sections (§4.3–4.4). In fact, Brockmann and Johnson (2011) have found several unpaired male and females of *L. polyphemus* at Florida Gulf coast, but claimed it as short-term variations due to the season and the tidal height changes. However, both Mattei et al. (2010) and Beekey and Mattei (2015) revealed that an increase in the number of single crabs ashore could be a sign of very low adult density and their decreased mate finding ability. In any case, further monitoring on the spawning crab's arrival at Tanjung Selangor is necessary. Also, marking and tagging experiments (cf. Swan, 2006) would be able to identify their revisits, if any, to this area.

4.2. Spawning activity of *T. gigas* under normal conditions

The spawning activity of the horseshoe crabs had a monsoonal

influence (Smith and Michels, 2006; Moore and Perrin, 2007; Jackson et al., 2010; Brockmann and Johnson, 2011; Smith et al., 2013; Jackson et al., 2014). Since the local environmental disturbances at Tanjung Selangor (especially, sand mining, jetty and road/bridge constructions) were practically witnessed from mid 2010, the results obtained for 2009 are likely to represent the normal conditions. Under this scenario, the spawning activity of *T. gigas* was found to be associated with more seawater intrusion during the SW and the IM periods (Table 1A–B). The present observations are also in agreement with the findings of Tan et al. (2012) who have worked on the spawning populations of *T. gigas* at Balok beach.

There exists some evidence that spawning horseshoe crabs prefer moderately sorted sediments to increase the chances of their egg hatching and survival (Chiu and Morton, 2003). Among the three sediment variables that strongly associated with the biological observations (i.e. sorting, kurtosis and fine sand) (BEST analysis), only sorting ($\sigma_\phi = 0.9$ –1.0, moderately sorted) had a positive impact on the egg counts of *T. gigas* (Fig. 2A–B) (Table 1B: values correspond to the sites of eggs availability in 2009). The moderately

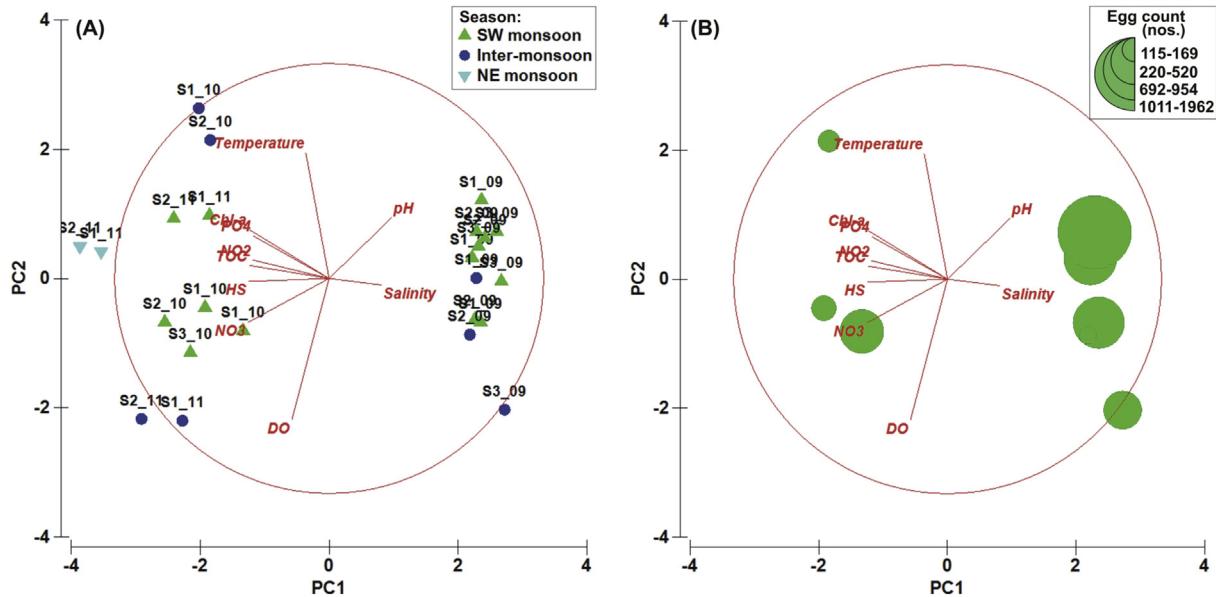


Fig. 3. Principle Component Analysis (PCA) showing water quality variations in relation to the nesting sites of *Tachypleus gigas* superimposed by – (A) season and, (B) egg count. The sampling sites (S1–3) and the year of observations (2009–11) are indicated side by side with an underscore. The transparent circle in each panel represents correlation circle and the orientation of the environmental (water) lines approximate their correlation to the ordination axes.

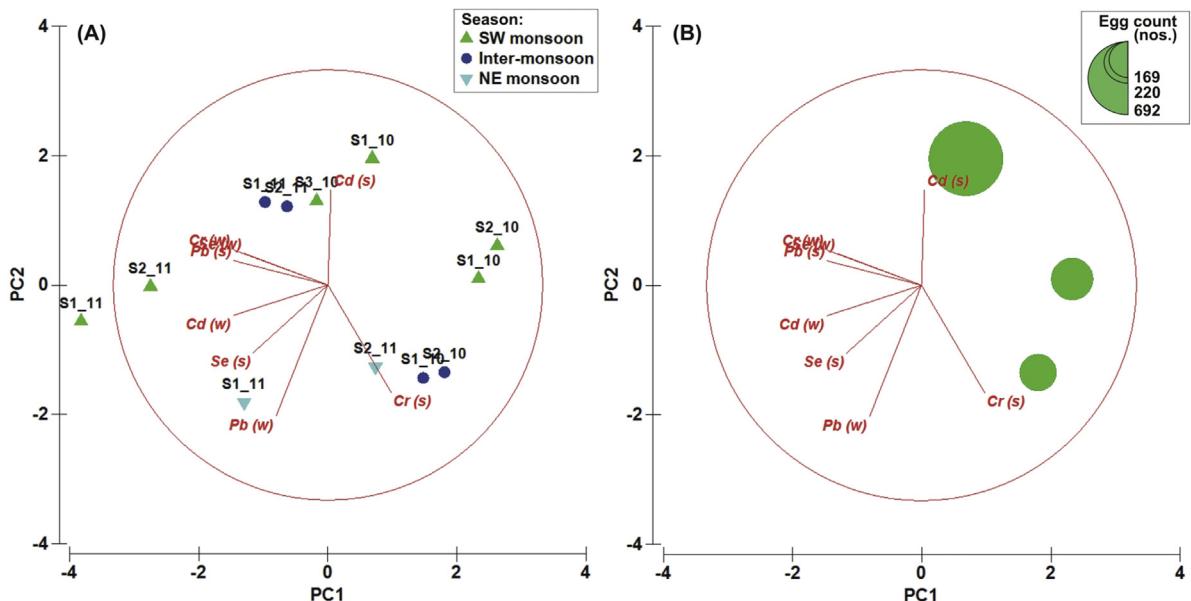


Fig. 4. Principle Component Analysis (PCA) showing heavy metal variations in relation to the nesting sites of *Tachypleus gigas* superimposed by – (A) season and, (B) egg count. The nesting sites (S1–3) and the year of observations (2010–11) are indicated side by side with an underscore. The metals detected from the water and the sediment were represented by 'w' and 's' in parentheses. The transparent circle in each panel represents correlation circle and the orientation of the metal lines approximate their correlation to the ordination axes.

sorted sediments indeed contain medium porosity and permeability that can create a well-oxygenated environment for the eggs, larvae and juveniles (John et al., 2012; Tan et al., 2012). In addition, the medium sand ($X_{\varphi} = 1.1\text{--}1.2$) with symmetrical ($SK_{\varphi} = 0\text{--}0.1$) and very leptokurtic ($K_{\varphi} = 2.7\text{--}2.9$) properties was found to be more suitable for normal spawning activity of *T. gigas* (Table 1B). Earlier, Chatterji et al. (1996) also observed the nesting preference of the Indian *T. gigas* at medium sediments. Even for the spawning activity of *L. polyphemus*, the medium sand is important (Jackson et al., 2007). The above observed edaphic conditions have favoured the Site-1 to have a maximum (40%) nesting frequency than

to the Site-2 (30%) and the Site-3 (10%). Also, the movement of fine (0.125 mm) sand from river mouth to interior estuary, especially during SW monsoon became advantageous for the Site-1 to be a most preferred location for *T. gigas* spawning (Table 1B). The horseshoe crabs do not prefer fine-grained sediment due to its greater desiccation properties (Chatterji et al., 1996). Therefore, the orientation of fine sand towards the declined egg counts in 2010 is understandable (Fig. 2A–B).

We hypothesized that the monsoonal (moderate to high) salinity played a crucial role for triggering spawning behaviour in *T. gigas*. In fact, an ambient salinity of 25–30‰ was found to be the

best for horseshoe crab's embryonic development and eggs hatching (Zaleha et al., 2011; Faizul et al., 2013). In the present study, both pH (7.6–8.2) and salinity (15–32‰) (Table 1C: values corresponds to the sites of eggs availability) were associated positively with the maximum egg counts in 2009 (Fig. 3A–B). Perhaps the low salinity and the high water current during NE monsoon must have hindered the spawning activity of *T. gigas*. Under the given set of environmental prerequisites, the statements available on the year-round spawning activity of *T. gigas* in Malaysia (e.g. Hajeb et al., 2009; Kamaruzzaman et al., 2011; John et al., 2012; Zaleha et al., 2012) are still questionable.

4.3. Spawning activity of *T. gigas* under disturbed conditions

At Tanjung Selangor, the sand mining (2010), jetty (2010–2011) and road/bridge (2011 – present) construction activities proved disruptive to the normal environmental conditions. Importantly, the altered sediment led by coarse sand ($X_\phi = 0.2\text{--}0.8$) with poor sorting ($\sigma_\phi = 1.0\text{--}1.2$), fine/very fine skewed ($SK_\phi = 0.1\text{--}0.9$) and extremely leptokurtic ($K_\phi = 3.6\text{--}7.3$) properties did not support the spawning activity of *T. gigas* (Table 1B: values correspond to the sites of nil egg counts). In contrast, Zaleha et al. (2012) found highest number of *T. gigas* nests in coarse sand and concluded that their nest/egg counts had no significant influence of water and sediment parameters. The differences encountered between present study and Zaleha et al. (2012) could be due to different time-span of observations. The negative impact of kurtosis (with raised values between 2009 and 2010–11) can be evidenced from its orientation towards the nil egg counts in 2011 (Fig. 2A–B). Site-1 and Site-2 that have shown the decreased egg counts in 2010 are also represented largely by the above (altered) sediment characteristics. However, the increased moisture depth between 2009 and 2010–11 investigations reveal no negative impact on the spawning activity of *T. gigas* (eggs found at 2.7–13.4 cm depth).

Horseshoe crabs try to avoid the areas with high Hydrogen Sulfide (Jackson et al., 2007; Ray and Clarke, 2010; Vasquez et al., 2015). In relation to the geochemical observations at Tanjung Selangor, the negative impact of S^2 on the nesting sites was clear, whereas the orientation of DO towards the nil egg counts in 2011 is rather uncertain (Fig. 3A–B). Also, the extreme loads of Cd and Se in the sediment are seemed to be of non-natural origin (Table 2A–B). The jetty construction with drilling and sand dredging operations must have unearthed these metals and spread to the nearby locations. While the orientation of Pb and Se in the sediment towards the nil egg counts in 2011 may show their possible negative impact, the presence of Cd and Cr (also in sediment) towards the declined egg counts in 2010 can confirm the continued spawning activity of *T. gigas* in metal contaminated areas (Fig. 4A–B). Similarly, the Cr in the water seems to be influential (Fig. 4A–B). In general, the horseshoe crabs can tolerate metal pollution (Wren et al., 1995; Rainbow, 1996; Botton, 2000; Burger et al., 2002), and several researchers found the accumulated metals in their body (e.g. Burger et al., 2002; Yap et al., 2008; Kamaruzzaman et al., 2011). However, metal enrichment close to the nesting sites can trigger developmental abnormalities in the embryos (Itow et al., 1998; Botton, 2000; Kamaruzzaman et al., 2011). Nevertheless, the consumption of these crabs and its eggs might pose severe health problems to the local people. Along with sediment and water related changes, the mechanical (sound) disturbances due to jetty construction must have also shown their negative impact on the spawning crab's arrival at Tanjung Selangor.

The loss of habitat leads to the loss of biodiversity. In the case of Asian horseshoe crabs, their decreased populations have already been reported from various countries including Malaysia

(Christianus and Saad, 2007; Patti, 2008; Hsieh and Chen, 2009; Shin et al., 2009; John et al., 2012; Tan et al., 2012). Even Balok – the only remaining spawning ground of *T. gigas* (east Peninsular Malaysia), is also facing several human disturbances in the recent years (John et al., 2012; Zaleha et al., 2012). Therefore, it is imperative to conserve and manage these horseshoe crab spawning areas under appropriate scientific direction. If there is any clue on the crabs' return to Tanjung Selangor observed then the local authorities (e.g. Fish Development Authority of Malaysia – LKIM, Pahang Dept. of Fisheries – DOF) should also consider the possibility of declaring this area as a 'Marine Protected Area'. The arrangement of sign boards indicating 'prohibited horseshoe crab fishing' and 'protected area' status would be able to raise awareness among the local people. Similar to the other Asian countries (e.g. India), inclusion of the horseshoe crabs in wildlife conservation act of Malaysia is highly essential. These measures can help not only to retain the suitability of Tanjung Selangor as one of the very few spawning grounds for *T. gigas* in Peninsular Malaysia, but also to receive a nationwide priority for its conservation and management.

4.4. Nesting behaviour of *T. gigas*

The horseshoe crabs prefer calm waters with low surface currents for their nesting (Smith et al., 2002a; Moore and Perrin, 2007; Schaller et al., 2010). Under the normal conditions at Tanjung Selangor, the nesting activity which took place mostly at Site-1 was shifted to Site-2 by the end of SW monsoon (i.e. September), and moved further in unto Site-3 during IM period (i.e. October) (Fig. 5). Although (South China Sea) water current at the sampling sites was found to be mild during SW monsoon (with light winds), it gradually increased by the end of (SW) monsoon and during IM (Akhir et al., 2011). While the mild water currents could facilitate fine sediment transportation from river mouth to interior estuary (as Site-1 is still a preferred place), the strong water currents are of no choice to the spawning *T. gigas* and hence went to more sheltered locations (as Sites 2 and 3 became favourable). Intense water current (accompanied by strongest winds) and rainfall along the east coast of Peninsular Malaysia can be observed every year with the onset of NE monsoon (Camerlengo and Demmler, 1997). Therefore, the nesting behaviour of *T. gigas* at Tanjung Selangor is also influenced by the seasonal water current. Previously, the linkage

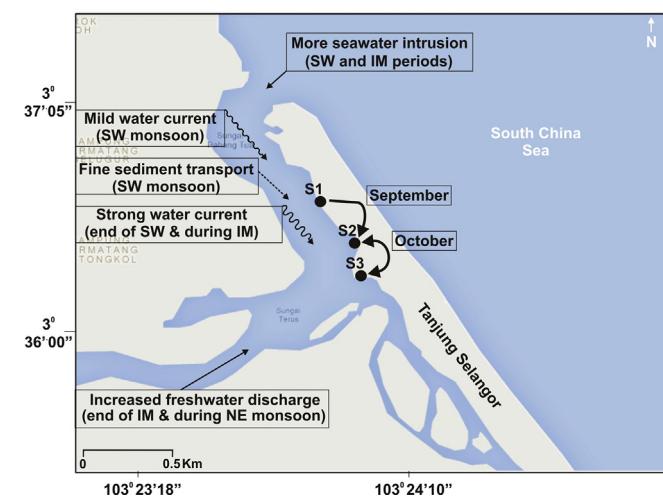


Fig. 5. Schematic representation on the nesting behaviour of *Tachypleus gigas* at Tanjung Selangor (Google Map data® 2013). The spawning site(s) selection by amplexed pairs, under normal environmental conditions, is influenced by the monsoonal (SW/IM) water current and the relevant processes (indicated in boxes).

between sediment transport (influenced by water current) and nesting behaviour of *T. gigas* in India was established by Chatterji et al. (1996). Later, Berkson and Shuster (1999) also found the impact of underwater current on spawning *L. polyphemus* at Delaware Bay. In this context, our subsequent (2010) observations have indicated the remigration of *T. gigas* to Site-1 for SW spawns and Site-2 for IM spawns (Table 1A). Despite the fact that Sites 1–3 are located only 150–200 m apart, the topography of the sandbar with bents/curves – especially at Site-2 and Site-3 (Fig. 5), are also seemed to be influential (at least to some extent) for selection of the nesting places by *T. gigas*. However, this phenomenon was entirely disturbed by the manmade activities in 2010–2011. As of now the spawning *T. gigas* abandoned Tanjung Selangor and not sure whether it would be temporary or permanent. On the other hand, there is also a possibility of having their new spawning ground(s) along the coast (cf. Chatterji et al., 2008; Chatterji and Shaharom, 2009). Therefore, further investigations on the spawning *T. gigas*' arrival at Tanjung Selangor as well as to other places, if any, along the east coast of Peninsular Malaysia are required.

5. Conclusions and recommendations

Though much of the horseshoe crab literature in Peninsular Malaysia abounds on *T. gigas*, the present study is the only comprehensive investigation with repeated coverage of the seasons and wide array of the environmental observations. Nevertheless, this is the only study that evaluated the nesting activity of *T. gigas* under normal and disturbed conditions. The important conclusions and recommendations of this study are –

1. Conditions that favour/influence spawning site selection:
 - High salinity
 - Medium sand with moderate sorting, symmetrical and very leptokurtic properties
 - Water current and fine sediment transportation
 - Beach topography
2. Changes observed in population and environmental conditions after sand mining, jetty and road/bridge constructions:
 - Decreased/nil egg counts
 - Coarse sand with poor sorting, fine/very fine skewed and extremely leptokurtic properties
 - High Hydrogen Sulfide
 - Metal induced pollution in sediment as well as water
3. Recommendations:
 - Measurements on water current/sediment transport (at river mouth and inside estuary)
 - Enact a no-harvest regulation on adult female *T. gigas*
 - To consider Tanjung Selangor as a 'Marine Protected Area'
 - Increase awareness and education of local people on horseshoe crab's significance and conservation, along with possible health risks if they were consumed.
 - Continued monitoring on the arrival of spawning *T. gigas* at Tanjung Selangor as well as other places along the east coast of Peninsular Malaysia
 - Marking and tagging experiments to find revisits of the horseshoe crabs
 - Inclusion of the horseshoe crabs in wildlife conservation act of Malaysia

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Appendix A. Supplementary material

Supplementary material related to this article can be found at <http://dx.doi.org/10.1016/j.ecss.2015.08.003>.

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