Spawning and Nesting Behaviour of *Tachypleus gigas* along the East Coast of Peninsular Malaysia

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Abstract

Present study was aimed to address the spawning and nesting behaviour of Malaysian horseshoe crab (*Tachypleus gigas*) in observed nesting grounds of East coast of Peninsular Malaysia during monsoonal period. Field sampling was carried out in every full and new moon days between September and December 2009 in Balok (Lat3°56.194’ N, Long103°22.608’ E) and Pekan (Lat3°36.181’ N, Long103°23.946’ E) nesting grounds. A total of 26 nests were found throughout the sampling period (Balok = 4 nests [~15%]; Pekan = 22 nests [~85%]) with higher percentage nesting was noted in November (N = 11 nests [~50%]) in Pekan. No nests were found in Balok station in all sampling months except during September (4 nests). It was also noted that the horseshoe crab preferred nesting during full moon time compared to new moon period. Mean depth × width of the *T.gigas* nests were 19.96±5.6×10.72±2.1cm. Number of eggs and larval counting in each nest were also recorded together with hydrographic and sedimentological parameters of the nesting grounds. This study clearly showed negative influence of monsoonal cycle on the nest preference and spawning behaviour of *T.gigas*. Long term monitoring studies would enable us to understand the number of shore reaching horseshoe crabs which is an essential source for the wild population.

Keywords: Nesting ground, Balok and Pekan, Horseshoe crab, Population ecology

1. Introduction

An important biomedical application to detect/quantify bacterial endotoxin (lipopolysaccharide) using a specialized cell compound from horseshoe crab blood lead to greater exploitation of their natural population worldwide (John et al., 2011). At present, horseshoe crabs are the sole source of those compounds technically known as *Limulus/Tachypleus* Amebocyte Lysate (LAL/TAL) which contributes billion dollar ($) profit annually. Number of studies were attempted to explore their ecological status and biological applications of horseshoe crab especially on American species (*Limulus polyphemus*) (ASMFC, 1998; Shuster et al., 2003; Botton & Shuster, 2004; Ehlinger and Tankersley, 2004; Shuster, 2004; Tanacredi et al., 2009; Botton and Itow, 2009) and Asian conspecifics (Chiu et al., 1999; Chiu and Morton, 2003; John et al., 2010; Zaleha et al., 2010; Akbar John et al., 2011a & b; John et al., 2011; 2012a & b; Kamaruzzaman et al., 2011a & b). However, the ecophysiological status of Malaysian horseshoe crab (*Tachypleus gigas*) is still limited (Akbar John et al., 2011 and 2012a).

It is well documented that the nesting and spawning behaviour of horseshoe crab is directly related to the nature of nesting grounds (Botton et al., 1988). Wyse, (1971) has found the chemoreceptor in horseshoe crab tail helps them in differentiating the nursery/nesting beaches and thus Botton et al., (1988) concluded that horseshoe crabs are capable of discriminating between beaches of different geochemical regimes. The reproductive behaviour of horseshoe crabs is observed to be periodic and well synchronized with the tides (Gretchen and Richard 2006). During high tide, horseshoe crabs migrates towards the nesting beaches at every full and new moon days (especially at night time) to spawn. During this migration, the male crabs are tenaciously attached to the female opisthosoma using modified, claw-like pedipalps (Brockmann and Penn, 1992). Female crabs dig nests in the swash zone to deposit their eggs in clusters which contains approximately 2000 eggs (Jackson et al., 2007). The development of nested eggs in to trilobite larvae is greatly influenced by external environmental conditions.

In Malaysia, the nesting of horseshoe crab observed throughout the year especially in Balok and Pekan (East coast of Peninsular Malaysia) with the peak mating season during warmer months June and July (Kamaruzzaman et al., 2010). Few recent studies on the ecophysiology of the horseshoe crab nesting grounds in Malaysia are discussed in literatures (John et al., 2011 & 2012a). However, the detail on the nesting and spawning of *T.gigas* in Malaysian coastline is less explored. Present study was aimed to provide the spawning and nesting behaviour of Malaysian horseshoe crab (*T.gigas*) in the observed nesting grounds of East coast of Peninsular Malaysia and to provide the hydrological and sedimentological parameters of the selected nesting grounds.

2. Materials and Methods

2.1 Sampling area and sampling design

The sampling was carried out in every full and new moon days at two different stations Balok and Pekan (East coast of Peninsular Malaysia) which were observed to be the nesting grounds of horseshoe crabs (Zaleha et al., 2010) (Figure 1). Locations of nests were identified in both the nesting grounds between September 2009 and December 2009 (Monsoon season). The nests were excavated using the scope until the fertilized eggs and larvae observed, the diameter and depth of the nests were recorded using measuring tape. Number of fertilized eggs and developing larvae in each nest was counted and sediment samples were collected in replicates. The eggs and larvae were placed back in the nests and packed loosely with the surrounding sand. The number of old and new nests were also recorded based on the nest content. The nests which comprised of mixture of eggs, early trilobite and trilobite larvae were classified as the old nest while the nests contain only fertilized eggs were categorized as new nests. Total Organic Matter (TOM) and Particle Size Analysis were carried out using dry sieve method. Hydrological parameters such as surface water salinity, temperature and Dissolved Oxygen level were recorded in every full and new moon days using YSI multi probe Hanna HI 9828.

2.2 Sediment characteristics analysis

Dry sieving method was carried out to analyze the particle size of sediments. The sediments were left air-dried for a week and 100g of samples was weighed for the coarse size analysis. The samples were transferred on a mechanical shaker using a several series of mesh opening for grade classification of particle size. The samples were allowed to stand for 15 minutes in mechanical shaker and the samples of each individual sieve were weighed (Incera et al., 2003). Sediment samples were graded and type of sediments was classified and sorted using standard method of Wentworth *et al*. (1992) and Briggs (1977). The following formulas were used to calculate mean sediment size and sediment sorting values;

Mean sediment size

$$\textbf{Mean Sediment Size } (X\ ø) = \sum fm/n$$

where,

'f' = percentage weight of each grade of particle size

'm' = median of each particle size in ø

and 'n' = total number of samples in 100 where f is in percentage form

Sediment sorting value was calculated using the following equation

$$\mathbf{Sd}\ ø = \sqrt{\sum \boldsymbol{f} \frac{(\boldsymbol{m} - \boldsymbol{x}ø)^2}{\mathbf{100}}}$$

where,

'm' = median of each particle size in ø

'$\mathcal{X}$ø' = Mean sediment size

2.3 Total organic matter (ash- free dry weight method)

Lenore et al., (1999) method was adopted to determine Total Organic Matter. 50 grams of sediment was weighed and oven dried at 60°C for 24 hours. Total organic matter content was determined as the loss in weight of sediment, dried at 60°C to constant weight after combustion at 475° C for two hours.

2.4 Data analysis

Kruskall-wallis non-parametric test was used to determine the difference in horseshoe crab nest distribution between two beaches. Pearson correlation analysis was performed to check the influence of hydrographic and sedimentological parameters over the number of horseshoe crab nests and egg counts in both the sampling beaches. All statistical tests were performed using SPSS 17v.

3. Results

A total of 26 nests were found in Balok and Pekan nesting grounds during the monsoon period (Sep-Dec) comprising of 22 nests in Pekan (84.6%) and 4 nests in Balok (15.39%). Highest number of nests was found during November (N =11) followed by 7 nests in October in Pekan. No nests were observed in Balok station during the sampling months except in September with 4 nests during new-moon period. In general, highest number of nests was recorded in new-moon period compared to full moon. Distribution of old and new nests was recorded to be 42.31% and 57.69% respectively. Significant difference in the distribution of horseshoe crab nests were observed between two nursery grounds ($P < 0.01$) while it was non-significant within a beach ($P > 0.05$).

Number of fertilized eggs, early trilobite and hatched trilobite larvae in each nest varied from 0-331, 0-350 and 0-391 respectively in Pekan station. Nests in Balok consisted only of fertilized eggs (N = 376-407) and no larvae was found during the study period. Highest number of eggs, were found in Balok nests (N = 407) while the highest number of early trilobite (N =350) and trilobite (N = 391) were observed in Pekan station. *Tavhypleus gigas* preferred to lay eggs at depth varied from 5 to 15cm with mean depth of 10.73cm ±2.13 while the width varied between 8 and 28cm (Table 1). The average distance between the nests with high and low tide points were 2.6±1.6m and 9.46±3.19m respectively.

3.1 Hydrographic parameters

Highest salinity was recorded during October (25.5 ppt) in Pekan and September (22.3 ppt) in Balok while the lowest salinity was noted during November (1.62 ppt) in Pekan and October (0.66 ppt) in Balok. In general, surface water temperature was >20°C in both the stations. Highest surface water temperature was noted during December (32.6°C) in Pekan and September (32.23°C) in Balok while the lowest temperature was noted during November in both the sampling stations with the values of 25.67°C and 27.66°C respectively. Throughout the sampling period, the dissolved oxygen level in the surface water was lower in both the stations varied between 3.23-4.63mg/l in Pekan and 0.79-5.62 in Balok station (Table 2). Comparison of hydrographic parameters between two sampling sites showed no significant difference ($P > 0.05$).

3.2 Sediment characteristics

Percentage of total organic content was higher in Balok station compared to Pekan. Significant difference in total organic content was noted between two sampling stations ($P < 0.001$). Highest percentage of total organic matter (TOM) was recorded during December in Balok (0.76%) while it was 0.5% in Pekan station during November.

Lowest TOM was noted during September in both the stations with the percentage values of 0.04% and 0.3% in Pekan and Balok respectively.

Over all, mean sediment size varied between 0.38 to 0.8Ø in Pekan and 1.86-2.57Ø in Balok while sediment sorting value varied from 0.82 to1.04Ø in Pekan and 0.46-1.1Ø in Balok station. Mean sediment grain size analysis showed the presence of coarser sand in Pekan and medium sand in Balok station during September-November. However, grain size analysis indicated the prevalance of very coarse sand and finer sand particles in Pekan and Balok beaches respectively. ANOVA test showed significant variations in mean sediment size between the beaches ($P < 0.001$) while no significant variation in mean sediment size was observed between months. Sediment sorting values showed the nature of sand in both the beaches were of poorly sorted during September. During October and November, the nesting beaches constituted by moderately sorted sediments except in Balok station which consisted of poorly sorted sediment during October. No significant difference was noted in sediment sorting values between sampling stations during different months ($P > 0.05$) (Table 3).

3.3 Pearson correlation analysis

Pearson correlation analysis showed the significant influence of dissolved oxygen level and total organic content on the distribution of eggs count and number of horseshoe crab nests in both the sampling stations ($P < 0.05$). Interestingly, both the parameters were positively correlated with nesting and egg counts. However, other parameters had no significant influence on the eggs count and nest numbers along the nesting beaches (Table 4).

4. Discussion

Present study revealed less preference of horseshoe crabs in nesting eggs during monsoon period. Early studies on American horseshoe crab showed *Limulus polyphemus* prefers to nest during the warmer months from May to August with a peak in June (Sokoloff, 1975). Recent studies along the Pahang coast by Akbar John (2011a & 2012) proved that *T.gigas* also tend to prefer warmer months for nesting with the peak mating season on June-August. Throughout the sampling period, the number of eggs, larval stages in each nests showed significant number variations within and among different months. Similar observation was made by number of researchers (Cohen and Brockmann, 1983; Brockmann, 1996) who claimed that there is a significant variation in the quantities of eggs being laid by females during different tides across the mating seasons.

Nests in Pekan station had mix proportion of eggs, early trilobite and hatched trilobite larvae which might be due to the combined influence of various hydrological parameters that play a key role in development of nested eggs (Mikkelsen, 1988). Newly laid eggs are sticky and adhere to each other in a form of clumped balls which eventually hatch out as trilobite larvae after 28 days and enter the surrounding water during high tide time (Penn and Brockmann, 1994). Among all the nest content, only a single nest consisted of blackened eggs which might probably be due to the increased hydrogen sulfide content. The finding of both old and new nests in the nursery beaches with no significant difference in numbers ($P > 0.05$) could indicate the more frequent of horseshoe crab to land on Pekan shore than Balok.

Physicochemical parameters showed no significant difference within and among the sampling stations. The surface water temperature was observed to be $>20^{0}$C in both the station which is suitable for spawning activity of horseshoe crab (Atlantic States Marine Fisheries Commision 1998). Jegla and Costlow (1982) also stated that temperature also affects the rate of embryonic development and the duration of post-hatch intermolt stages, with the optimal temperature for development ranging from 25 °C to 30 °C. Pearson correlation analysis showed no significant influence of salinity and pH on the number of eggs in the nesting grounds. This observation was well corresponded with *in vitro* studies on the salinity tolerance of conspecific species *L.polyphemus* (Shuster, 1982). In addition, Zaleha *et al.* (2011) found that when eggs were incubated at temperature of 26-29^{o}C, they could hatch optimally at a wide range of salinity which is between 25 to 35 ppt. In contrast to other environmental parameters, dissolved oxygen level showed strong correlation with the number of nests at both the stations ($P < 0.05$). Chiu and Morton (2003) noted that high oxygen levels in water would help in creating well oxygenated micro habitat for developing eggs.

The present finding proves the success of egg hatching and survival of larval stages are not limited by major physicochemical parameters such as salinity, pH and dissolved oxygen level in the nesting grounds. Strong correlation was noted between total organic matter and number of eggs in nesting grounds. Lesser organic content in Balok could have promoted more nesting during September. Allochthonous inputs in the sheltered beach such as Balok comes from the fishing activity and domestic waste from the nearby fishing villages. This increases the organic content on the beach particularly during monsoon months of October to December due to high accumulation of sedimentary organic matter (Incera et al. 2003).

Mean sediment size showed the presence of medium sand particles in Balok and coarse sand in Pekan. The horseshoe crab seems to prefer the coarse sand compared to medium sand in which the number of horseshoe crab nests was found much higher at Pekan compared to Balok. Ellingsen, (2002) showed that sediment grain size determines the drainage of a beach, which greatly affects the interstitial oxygen content. The Balok beach sediments were fine to medium sand grained, thus had poor drainage compared to Pekan sediments. Jackson et al. (2007) noted on the importance of grain size to determine the hatching and development of horseshoe crab larvae where beside pebble fraction, finer sediment may increase egg survival. Nevertheless, fine grained sediments in Balok while having greater surface areas for water retention, could promote microbial growth due to the heavy human activities in the area. This further depleted interstitial oxygen, increases hydrogen sulphide, and lowers redox levels. This might be the reasons why Balok is less preferred by horseshoe crab as compared to Pekan. Significant different of grain size at these two locations indicated that the location received different wave with Pekan site exposed to stronger current. This could also explain the higher and more stable dissolved oxygen concentration in Pekan than Balok which in turn could attract nesting behavior of horseshoe crab in the area. Stronger wave also promote inundating of eggs thus increase hatching success (Ehlinger and Tankersley, 2003).

5. Conclusion

A total of 26 nests were found throughout the sampling period in two nursery ground beaches constituting high preference of nesting in Pekan compared to Balok station during monsoon period. Hydrographic and sedimentological parameters had no significant influence on number of nests and egg counts in both the sampling beaches except dissolved oxygen level and TOC. The dwindling population size of horseshoe crab in Malaysian coast line in recent years might probably be due to various anthropogenic activities in the nesting beaches which eventually alter the nursery ground quality. Long term monitoring studies would enhance our understanding on horseshoe crab spawning and breeding behaviour at their nesting grounds.

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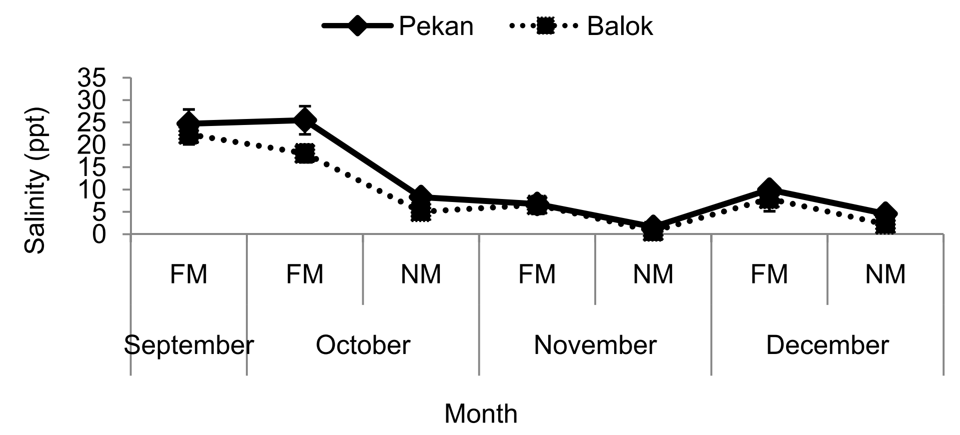
Table 1. Number of horseshoe crab nests found in the nesting grounds (Balok and Pekan) during the sampling months and the number of fertilized eggs, pretrilobite and trilobite larvae observed in each nests

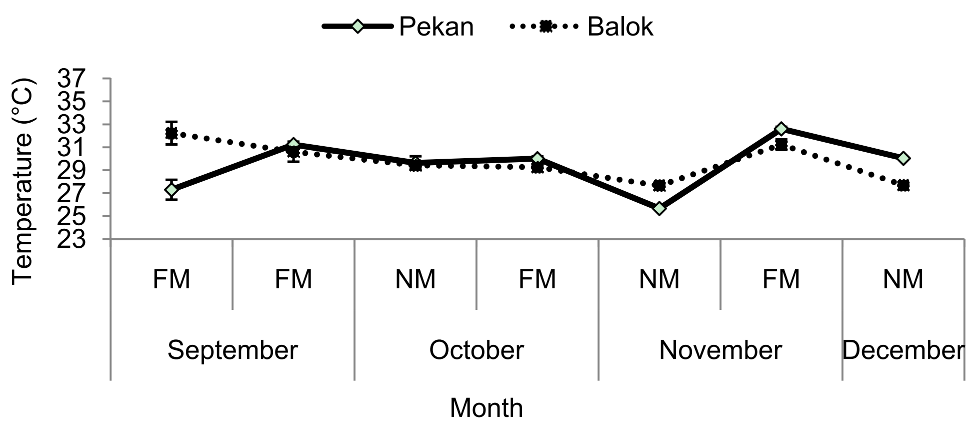
Months	Locations	Nest Number	Number of Eggs	Number of Pretrilobite larvae	Number of Hatch trilobite larvae	Total	Nest width (cm)	Nest depth (cm)
September	Pekan	1	0	350	0	350	20	11
	Balok	1	376	0	0	376	17	12
		2	407	0	0	407	19	12
		3	400	0	0	400	26	12
		4	367	0	0	367	24	15
October	Pekan	1	87	28	14	129	23	12
		2	216	0	0	216	17	12
		3	164	0	0	164	20	8
		4	191	0	0	191	9	9
		5	69	0	0	69	28	10
		6	331	0	0	331	24	9
		7	44	0	0	44	27	5
	Balok	0	0	0	0	0	0	0
November	Pekan	1	123	121	3	247	15	9
		2	326	0	0	326	25	11
		3	156	0	0	156	28	15
		4	0	0	391	391	24	10
		5	1	0	51	52	18	9
		6	1	0	1	2	18	10
		7	105	0	0	105	23	11
		8	203	0	0	203	23	13
		9	0	22	109	131	23	10
		10	0	0	9	9	18	10
		11	300	0	9	309	8	9
	Balok	0	0	0	0	0	0	0
December	Pekan	1	12	0	1	13	11	11
		2	12	7	288	307	15	11
		3	16	7	78	101	16	13
	Balok	0	0	0	0	0	0	0

Table 2. Variation in the major hydrographic parameters observed at the surface waters of nursery sites

Salinity



Temperature



Dissolved oxygen

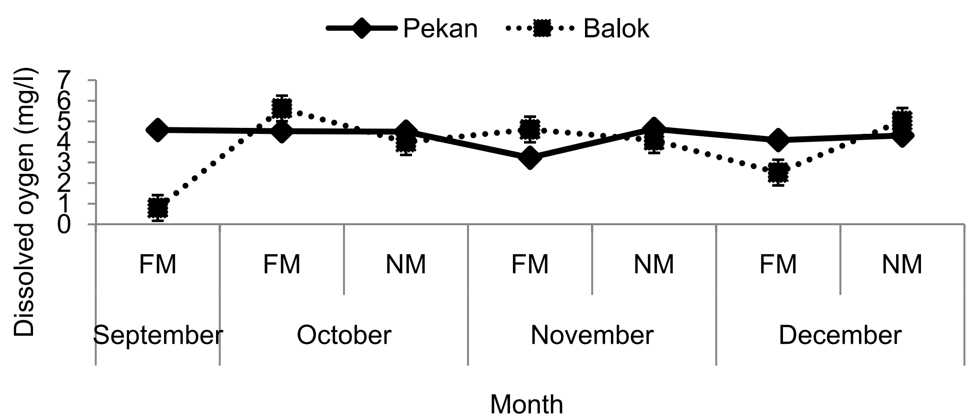


Table 3. Summary of surface sediment characteristics of the horseshoe crab nursery beaches during monsoon season. Values are presented in means

Months	Parameters	Pekan		Balok	
		NM	FM	NM	FM
September	Mean(Ø)	-	0.77	-	1.95
	Sediment Sorting value	-	1.03	-	1.02
	TOM (%)	-	0.04	-	0.30
October	Mean (Ø)	0.59	0.74	2.24	1.86
	Sediment Sorting value	1.04	0.88	0.68	1.1
	TOM (%)	0.36	0.31	0.74	0.64
November	Mean(Ø)	0.74	0.8	2.48	2.32
	Sediment Sorting value	0.83	0.82	0.62	0.8
	TOM (%)	0.50	0.44	0.66	0.70
December	Mean(Ø)	0.67	0.38	2.43	2.57
	Sediment Sorting value	0.93	0.86	0.46	0.54
	TOM (%)	0.34	0.36	0.76	0.68

FM – full moon period; NM – New-moon period; TOM – Total Organic Matter.

Table 4. Summary of Pearson's Correlation Analysis on shows the influence of various parameters over the number of horseshoe crab nests and egg counts in Balok and Pekan nursery grounds respectively

Parameters	Sampling stations	Number of horseshoe crab nests		Eggs count	
		Correlation value	Significant value (P)	Correlation value	Significant value (P)
Salinity	Balok	0.723	0.066	0.824	0.074
	Pekan	-0.085	0.857	-0.091	0.762
Temperature	Balok	0.637	0.124	0.591	0.212
	Pekan	-0.149	0.749	-0.258	0.675
Dissolved oxygen	Balok	0.822*	0.023	0.798*	0.025
	Pekan	-0.31	0.511	0.437	0.598
Mean sediment size	Balok	-0.516	0.236	-0.487	0.312
	Pekan	0.519	0.233	0.453	0.345
Sediment sorting value	Balok	0.512	0.251	0.623	0.211
	Pekan	-0.579	0.173	-0.654	0.468
Total organic content	Balok	-0.954*	0.001	-0.948*	0.001
	Pekan	0.482	0.301	0.657	0.575

* Correlation significant at the 0.05 level (2-tailed).

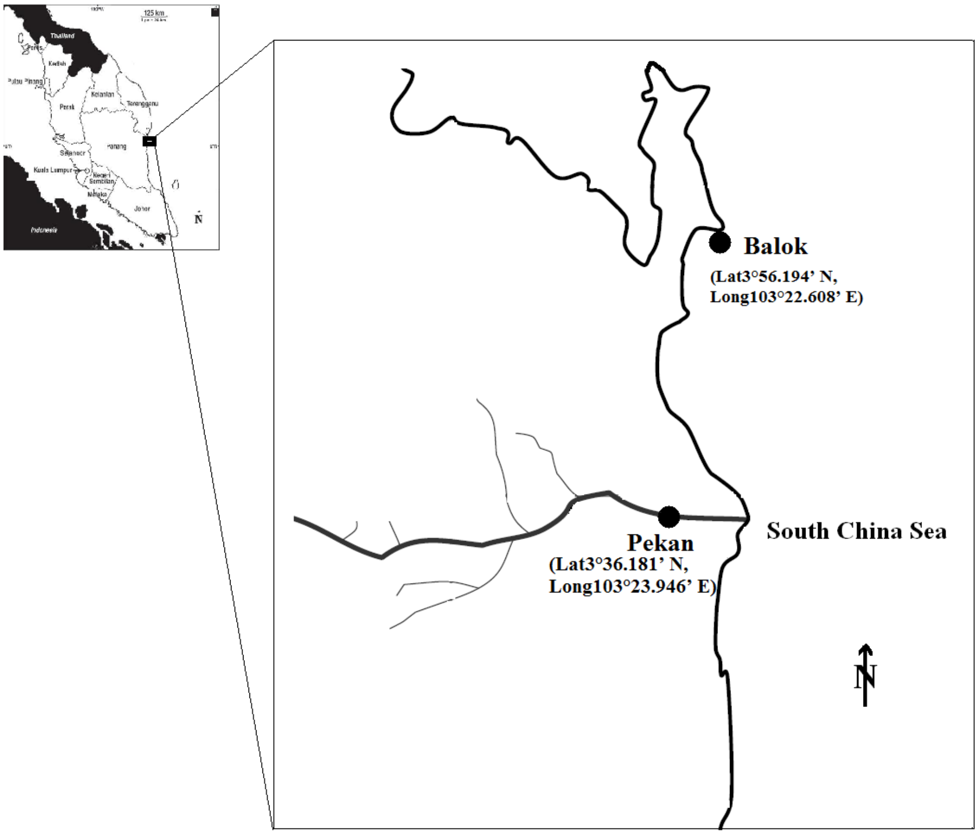


Figure 1. Location of the sampling sites along the East coast of Peninsular Malaysia