



Hydrology of the Horseshoe Crab Nesting Grounds at Pahang Coast, Malaysia

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ABSTRACT

Selected hydrological parameters at the nesting grounds of horseshoe crabs along the Pahang coast were determined. A complete year data from March 2010 to February 2011 was collected using advanced multiparameter meter model Hanna HI 9828 probe. The surface water temperature varied from 29.06°C - 19.36°C at Balok and 29.1°C-15.08°C at Pekan station with mean annual temperature of 24.08±2.91°C at Balok and 22.97±3.26°C at Pekan. Surface water salinity varied from 30.11ppt-2.09ppt at Balok and 33.45ppt – 3.38ppt at Pekan station with mean annual salinity of 17.9±10.63ppt at Balok and 20.5±10.04ppt at Pekan coastal waters. Seasonal fluctuation in the pH of the water varied from 8.65-6.61 at Balok station and 8.18-6.12 at Pekan station. Throughout the sampling period the coastal waters of both the sampling sites were well mixed. The Dissolved oxygen (DO) in the water varied from 7.75-4.21ml/L at Balok and 7.75ppt – 3.06ml/L at Pekan station with mean annual DO of 6.24±0.95ml/L at Balok and 5.76±1.08ml/L at Pekan coastal waters. Physicochemical parameters showed apparent seasonal fluctuation in the salinity of the surface water was observed in both the sampling stations ($P < 0.05$) while other parameters such as temperature, pH and dissolved oxygen did not vary significantly.

Key words: Physicochemical parameters, Salinity, pH, temperature, DO and horseshoe crab nesting ground.

INTRODUCTION

In recent years, there has been much emphasis on better understanding the physicochemical parameters of coastal marine waters especially variability that effect the basic biology, physiology and ecology of many benthic

and pelagic organisms. In this respect, coastal environments are one of the most productive areas in the world that point out the necessity of having a proper knowledge regarding their benthic productivity and the environmental parameters influencing their subsistence. Several studies have been addressed on the influence of various

physicochemical parameters on the distribution and diversity of benthic community around the world (Alongi, 1990; Mazzola *et al.*, 1999; Rodrigues *et al.*, 2001; Ogunwenmo *et al.*, 2004; Yap *et al.*, 2003). Due to their high sensitivity towards the fluctuation of various hydrological parameters, they are being considered as effective tools for detecting alterations in aquatic ecosystems (Pinder *et al.* 1987). In view of this, all physicochemical parameters are the indicators of the existing water quality of the aquatic water body that would help in enriching the macrobenthic community in that habitat (Kröncke, 2006; Sivasdas *et al.*, 2011). There are number of studies were carried out to check the hydrology and its influence on the macrobenthic community in the coastal waters (Morton and Wu, 1975; Ogunwenmo *et al.*, 2004; Gosain *et al.*, 2006). However, published information on the major physicochemical parameters in the horseshoe crab nesting ground is still scanty. Hence, present study was aimed to determine the four major physicochemical parameters such as temperature, salinity, pH and dissolved oxygen in the surface waters of the horseshoe crab nesting grounds.

MATERIAL AND METHOS

Study area description and water sampling

Balok (Lat3°56.194' N, Long103°22.608' E) and Tanjung Gosong (Pekan: Lat3°36.181' N, Long103°23.946' E) in the Pahang state of East coast of Malaysia were observed to be the nesting grounds of Horseshoe crabs (Zaleha *et al.*, 2010). Adult horseshoe crabs migrate from the off shore continental shelf to spawn on intertidal sandy (*T.gigas*) and mud sandy beaches and mangrove area (*C. rotundicauda*) at every full and new moon at these sampling locations (Figure 1). Meteorological data showed that high rainfall during monsoon season (November to January). Twenty years (1968 to 1987) of accumulated data obtained from the Malaysian Meteorological Department (MMD)

showed that the monsoon seasons with strong winds and long frequency periods with mean annual rainfall of 3064mm occurred from November to January. Meanwhile the non-monsoon seasons with low rainfall occurred during April, May and June.

Field observation unveiled the intensive fishery activities along the sampling locations besides industrial activities and human inhabitation processes which could possibly introduce considerable level of contaminants into the water body. The physicochemical parameters such as temperature, salinity, pH and Dissolved oxygen were measured using the handheld multiparameter meter model Hanna HI 9828.

RESULTS

Temperature

The surface water temperature at the nesting grounds of horseshoe crabs along the Pahang coast was recorded. The surface water temperature varied from 29.06°C - 19.36°C at Balok and 29.1°C – 15.08°C at Pekan station with mean annual temperature of 24.08±2.91°C at Balok and 22.97±3.26°C at Pekan. Maximum and minimum temperature was recorded during full moon days of October 2010 (Non monsoon) and January 2011 (Monsoon) respectively at Balok station while Pekan station had maximum and minimum temperature during full moon days of March 2010 (Non monsoon) and December 2010 (Monsoon) respectively. The average surface water temperature during non-monsoon and monsoon season at Balok station was 26.13±2.23°C and 22.15±2.26°C respectively while it was 24.53±2.39°C and 19.84±2.44°C at Pekan station respectively. There was no significant variation in temperature was observed between two seasonal periods ($P > 0.05$). Monthly variation in the temperature is graphically depicted in Figure 2.

Salinity

Surface water salinity varied from 30.11ppt – 2.09ppt at Balok and 33.45ppt – 3.38ppt at Pekan station with mean annual salinity of 17.9±10.63ppt at Balok and 20.5±10.04ppt at Pekan coastal waters. The maximum and minimum salinity was recorded during full moon days of March 2010 (non-monsoon) new moon days of January 2011 (monsoon) at Balok station while Pekan station had maximum and minimum salinity during full moon days of March 2010 (Non monsoon) and February 2011 (Monsoon) respectively. The average surface water salinity during Non monsoon and Monsoon season at Balok station was 24.88±4.12ppt and 3.96±1.01ppt respectively while it was 27.08±3.56ppt and

7.34±2.71ppt at Pekan station respectively. There was a significant variation in salinity was observed between two seasonal period ($P < 0.05$) while it was non-significant during lunar cycle. Monthly variation in the salinity is graphically depicted in Figure 3.

pH

Seasonal fluctuation in the pH of the water varied from 8.65-6.61 at Balok station and 8.18-6.12 at Pekan station. The maximum and minimum water pH was recorded during full moon days of March 2010 new moon days of July 2010 (non-

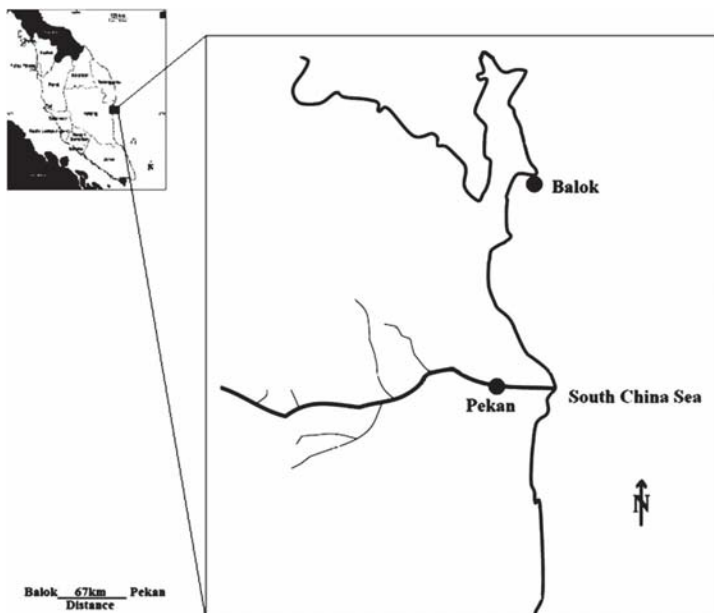


Fig. 1: Location of the sampling area

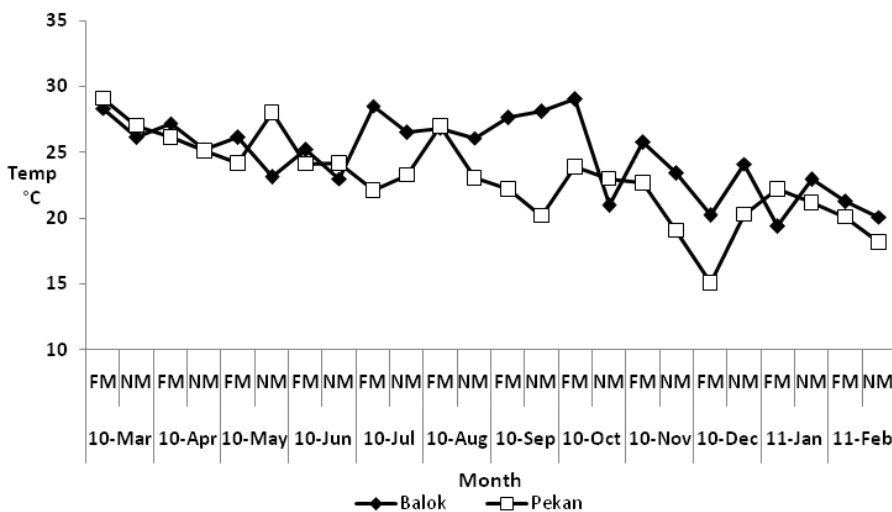


Fig. 2: Monthly variation in surface water temperature at the nesting grounds of horseshoe crabs along the Pahang coast. Note: FM = Full moon time; NM = New moon time

monsoon) at Balok station while Pekan station had maximum and minimum pH during full moon days of May 2010 (non-monsoon) and July 2001 (non-monsoon) respectively. The average water pH during non-monsoon and monsoon season at both the sampling sites (Balok = 7.57 ± 0.65 ; Pekan = 7.26 ± 0.69) did not influenced significantly by seasonal or lunar cycle. Monthly variation in the surface water pH is graphically represented in Figure 4.

Dissolved Oxygen

Throughout the sampling period the coastal waters of both the sampling sites were well mixed. The Dissolved oxygen (DO) in the water varied from 7.75 – 4.21ml/L at Balok and 7.75ppt –

3.06ml/L at Pekan station with mean annual DO of 6.24 ± 0.95 ml/L at Balok and 5.76 ± 1.08 ml/L at Pekan coastal waters. The maximum and minimum DO was recorded during full moon days of December 2010 (monsoon) and July 2010 (non-monsoon) at Balok station respectively while Pekan station had maximum and minimum salinity during new moon days of February 2011 (Monsoon) and April 2010 (Non monsoon) respectively. The average DO level during non-monsoon and monsoon season at Balok station was 6.02 ± 1.01 ml/L and 6.69 ± 0.65 ml/L respectively while it was 5.51 ± 1.08 ml/L and 6.26 ± 0.95 ml/L at Pekan station respectively. Monthly variation in the surface water pH is graphically represented in Figure 5.

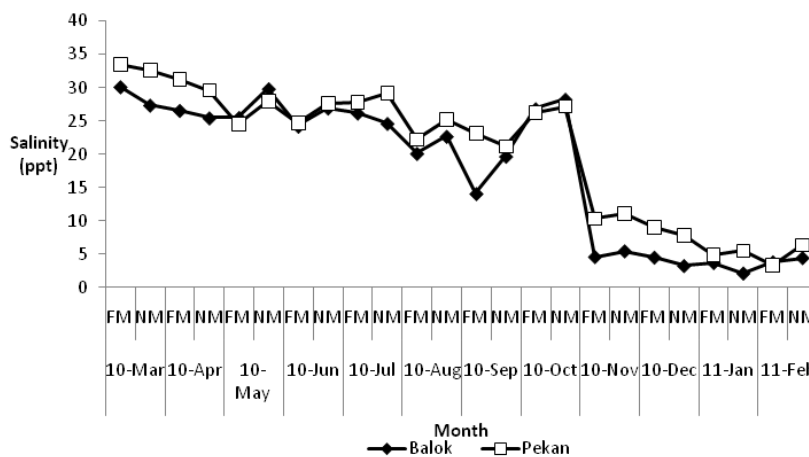


Fig. 3: Monthly variation in surface water salinity (ppt) at the nesting grounds of horseshoe crabs along the Pahang coast. Note: FM = Full moon time; NM = New moon time

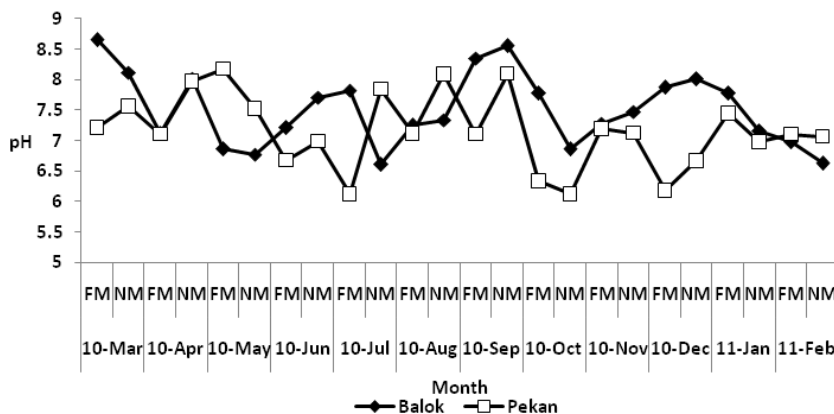


Fig. 4: Monthly variation in surface water pH at the nesting grounds of horseshoe crabs along the Pahang coast. Note: FM = Full moon time; NM = New moon time.

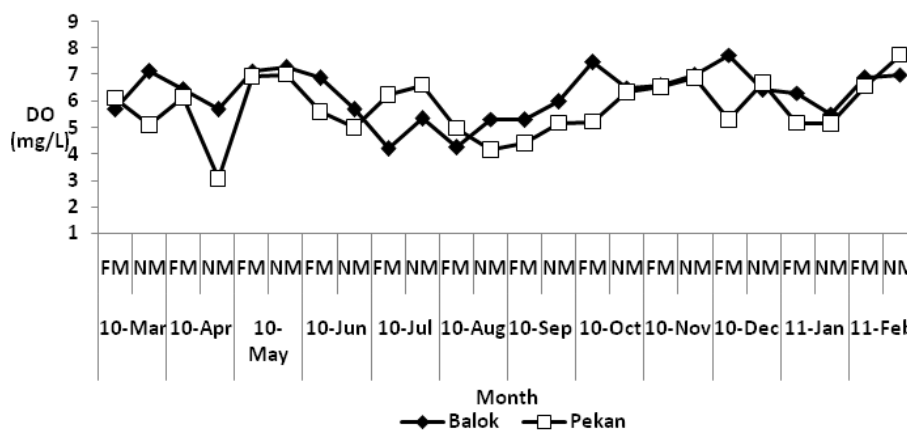


Fig. 5: Monthly variation in surface water dissolved oxygen (mg/L) level at the nesting grounds of horseshoe crabs along the Pahang coast. Note: FM = Full moon time; NM = New moon time

DISCUSSION

Apparent seasonal fluctuation in the salinity of the surface water was observed in both the sampling stations ($P < 0.05$) while other parameters such as temperature, pH and dissolved oxygen did not vary significantly. This observation was primarily due to the high fresh water inflow into the coastal waters during monsoonal seasons (Bachelet *et al.*, 2000). It is also to be noted that salinity is a main limiting factor determining the diversity and distribution of sensitive macrobenthic community along the coastal environment (Lamptey and Armah, 2008). Team, (1995) recorded an average salinity of the open sea that generally varies between 33 and 38, whereas for the coastal waters of Hong Kong, a salinity range of 20 to 30 has been reported by Hill, (2005). The two study beaches are typically of oceanic (Balok) and estuarine (Pekan) with wide ranges of salinity being recorded during the study period. The low salinities recorded at the two study sites, particularly during monsoon season, indicated that they are under the profound diluting effect of freshwater emanating from the adjacent coastal regions. It was also noted that the macrobenthic fauna of tropical sedimentary systems experiences fluctuations in salinity and sediment erosion leading to wide variations in species diversity (Alongi, 1989; Mistri *et al.*, 2000). The observed drastic reduction in the salinity during monsoonal

season probably washed away the stenohaline macrobenthic forms along the sampling stations during monsoonal period. Similar observation was also reported in previous studies (Alongi, 1990; Lomas *et al.*, 2005; Hagberg and Tunberg, 2000; Liang *et al.*, 2002). Various studies has been carried out to determine the influence of various physicochemical parameters on the egg hatching success and larval survival of horseshoe crabs (Ehlinger and Tankersley, 2003; Chatterji *et al.*, 2004; Zaleha *et al.*, 2011) where they unanimously observed the significant influence of coastal water salinity over the hatching success and faster development of horseshoe crab larval stages. It was also noted that the optimum salinity level of 30ppt would initiate/induce early hatching of horseshoe crab (*T.gigas*) eggs as early as 26 days after fertilization (Zaleha *et al.*, 2011). However, Ehlinger and Tankersley (2004) has found that embryo in the eggs of *L. polyphemus* could develop into larval stage and successfully hatch in salinities between 10 and 70 ppt. This might probably due to the nature of the embryonic membrane of horseshoe crab (*L. polyphemus*) eggs which is highly permeable to water, chloride and sodium ions proved the ability of *Limulus* to change it permeability when maintained at low salinity medium.

Temperature values were high and remained relatively stable throughout the sampling

period with annual mean temperature of $24.08 \pm 2.91^\circ\text{C}$ at Balok and $22.97 \pm 3.26^\circ\text{C}$ at Pekan. Since significant changes in the water quality parameters directly influence the community structure of macrobenthic invertebrate, surface water temperature probably had no effect on the distribution of benthos in this study. This observation agrees with those of previous work by (Kumar, 2005) who reported that the distribution, abundance, productions and population dynamics of benthic macro invertebrates in Lagos Lagoon and harbor were not affected by water temperature. Besides this fact, Shuster, (1950) reported that seasonal pattern in temperature seems to govern the breeding activity of local horseshoe crabs as spawning pairs have been observed swimming towards the breeding beaches during spring and summer when water temperatures $>20^\circ\text{C}$. Spawning of horseshoe crabs in other areas, for example, *Limulus polyphemus* in Delaware Bay, USA, also occurs during the warmer months from May to August with a peak in June (Shuster, 1950). It was also observed that surface water temperatures recorded at both the sampling stations, in general, corresponded closely to ambient air temperatures with a difference of -1°C only and such a pattern of co-variation is common for coastal waters of Hong Kong (Chiu, 1998), Singapore (Janet, 1993) and Indian ocean basin (Gosain *et al.*, 2006)

The pH of oceanic water is generally maintained within a narrow range from 8.0 to 8.3 (Hill, 2005) whereas in estuaries, pH is determined by the degree of seawater mixing with freshwater and values of between 6.9 and 7.8 are common (Team, 1995). pH levels in coastal waters are, moreover, not only affected by freshwater inputs, but also by algal photosynthesis (Bowmer and Muirhead, 1987) and organic decomposition (Albrecht *et al.*, 1997). Active algal growth uses up carbon dioxide while organic decomposition releases carbon dioxide and both cause a shift in the carbonate-carbon dioxide equilibrium leading, respectively, to an increase and a decline in pH. The conducive pH and high dissolved oxygen level in the sampling area might be due to the constant mixing of water body by the wave action (Balok station) and wage current process (Pekan station) besides the turbulence from atmospheric winds interaction.

In general, dissolved oxygen level was maximum in surface waters, particularly coastal waters. The surface water of marine and estuarine readily permits oxygen enrichment through atmospheric exchange, and sufficient light can penetrate surface waters to allow the oxygen-releasing process of photosynthesis to occur (CCME, 1999). In the euphotic zone (where light intensity is sufficient to photosynthetic processes), photosynthesis may exceed respiration and there is net production of oxygen; below the euphotic zone, a net consumption of oxygen occurs (EOS, 2003). In deeper waters, especially where light is scarce, oxygen is consumed by bacteria during decomposition of organic matter. In these cases, oxygen concentration can be reduced to negligible levels and lead to anoxic. The areas where there are significant low levels of dissolved oxygen are restricted circulation, abundant organic matter, industrial discharge, sewage etc. Oxygen depletion occurs in deep marine waters with thermal or salinity stratification occur (Prashant *et al.*, 2009). Monitoring dissolved oxygen will provide indication of water quality in coastal areas. Anoxic (no oxygen) and hypoxic (very low oxygen) events, and dissolved oxygen were used as a tool in ecosystem integrity and quality (Rosenberg *et al.*, 2002). Most aquatic animals need optimum oxygen to survive and with the exception of air, breathing animals will use oxygen dissolved in water. But the macrobenthic community primarily depends on the dissolved oxygen in water. Dissolved oxygen depletion has shown lethal effects on physiological and behavioral changes in variety of organisms (Halim *et al.*, 2007). If prolonged changes occur in dissolved oxygen levels in coastal waters, modification can also be expected in the local biotic community structure. Species intolerant of depressed oxygen will either die or try to avoid the environment, while, tolerant species will survive in low dissolved oxygen levels. In view of this, present result proved that the water in selected sampling sites are well mixed and thus exhibited high Dissolved oxygen levels.

The Relationships between Physicochemical Parameters

The physicochemical characteristics of the coastal waters fluctuate continuously due to the introduction of various domestic and industrial discharges. These parameters have direct and

indirect influence on the distribution and seasonal fluctuation of macrobenthic community composition and its diversity (ESL/RPI/GLDD, 2004). There is, however, paucity of quantitative data on both macrobenthic fauna and abiotic factors in coastal waters of Malaysia. It was also evident from previous studies that the various physicochemical parameters interact with each other and ultimately influences the community composition of sensitive organisms in the coastal habitats (Rosenberg *et al.*, 2002; Prashant *et al.*, 2009). Hence the relationship between various physicochemical parameters observed during Monsoon and Non-monsoon seasons were analyzed in this study. The observed high salinity and dissolved oxygen during monsoonal season in Balok station might be primarily due to

the high inflow of fresh waters during monsoon season and constant mixing of nutrients into the coastal region triggered the reduction in salinity with increasing dissolved oxygen content during monsoonal season in Balok station. Similar observation was reported by Penjan *et al.*, (1999) who observed positive influence of salinity and dissolved oxygen in Mekong Delta, Vietnam during monsoonal cycle. Overall, the interdependency of selected physicochemical parameters proved the unique nature of coastal waters at the nesting grounds of horseshoe crabs where the interactions between the parameters are non-significant due to the constant influx of various anthropogenic inputs (Penn and Brockmann, 1994; Brockmann, 2002; Chabot *et al.*, 2008).

REFERENCES

1. Albrecht, S. L., Baune, H. L. M., Rasmussen, P. E., & Douglas, J. C. L., Light fraction soil organic matter in long-term agroecosystems. *Columbia Basin Agricultural Research Annual Report*, **977**: 38-42 (1997).
2. Alongi, D. M., Ecology of tropical soft-bottom benthos: a review with emphasis on emerging concepts. *Revista de Biologia Tropical*, **37**: 85-100 (1989).
3. Alongi, D. M., The ecology of tropical soft-bottom benthic ecosystems. *Oceanography and Marine Biology Annual Review* **28**: 381-496 (1990).
4. Bachelet, G., de Montaudouin, X., Auby, I., & Labourg, P.-J., Seasonal changes in macrophyte and macrozoobenthos assemblages in three coastal lagoons under varying degrees of eutrophication. *ICES Journal of Marine Science: Journal du Conseil*, **57**(5): 1495-1506 (2000).
5. Bowmer, K. H., & Muirhead, W. A., Inhibition of algal photosynthesis to control pH and reduce ammonia volatilization from rice floodwater. *Nutrient Cycling in Agroecosystems*, **13**(1): 13-29 (1987).
6. Brockmann, H. J., An experimental approach to altering mating tactics in male horseshoe crabs (*Limulus polyphemus*). *Behavioral Ecology*, **13**(2): 232-238 (2002).
7. CCME., *Canadian Water Quality Guidelines for the protection of aquatic life: Dissolved Oxygen (Marine) Publication* (1299). Canadian Council of Ministers of the Environment (1999).
8. Chabot, C. C., Skinner, S. J., & Watson, W. H., Rhythms of Locomotion Expressed by *Limulus polyphemus*, the American Horseshoe Crab: I. Synchronization by Artificial Tides. *The Biological Bulletin*, **215**(1): 34-45 (2008).
9. Chatterji, A., Kotnala, S., & Mathew, R., Effect of salinity on larval growth of horseshoe crab, *Tachypleus gigas* (Muller). *Current science*, **87**: 248-249 (2004).
10. Chiu, H. M. C., Hydrography and pollution of five shores in Hong Kong. In B. Morton (Ed.), *Asian Marine Biology* **15**: 35-71: Hong Kong University Press (1998).
11. Ehlinger, G. S., & Tankersley, R. A., Larval hatching in the horseshoe crab, *Limulus polyphemus*: facilitation by environmental cues. *Journal of Experimental Marine Biology and Ecology*, **292**(2): 199-212 (2003).
12. EOS., Transaction American Geophysical Union: Trends in Marine Dissolved Oxygen: Implications for ocean circulation changes and the carbon budget., **84**: 197-204 (2003).
13. ESL/RPI/GLDD., *Environmental monitoring reports (2001- 2004) of the Keta Sea*

- Defence Project Works (KSDPW) - Technical Report*. Environmental Protection Agency (2004).
14. Gosain, A. K., Sandhya, R., & Debajit, B., Climate change impact assessment on hydrology on Indian river basins. *Current science*, **90**: 346-353 (2006).
 15. Hagberg, J., & Tunberg, B. G., Studies on the Covariation between Physical Factors and the Long-Term Variation of the Marine Soft Bottom Macrofauna in Western Sweden. *Estuarine, Coastal and Shelf Science*, **50**(3): 373-385 (2000).
 16. Halim, A. M. A., Khair, A. E. M., Fahmy, M. A., & Shridah, M. A. (2007). Environmental Assessment on the Aqaba Gulf coastal waters, Egypt. *Egyptian Journal of Aquatic Research*, **33**, 1-14.
 17. Hill, M. N., *The Composition of Sea-water Comparative and Descriptive Oceanography*: Harvard University Press (2005).
 18. Janet E, N., Remote sensing of water quality in the Singapore-Johor-Riau growth triangle. *Remote Sensing of Environment*, **43**(2): 139-148 (1993).
 19. Kröncke, I., Structure and function of macrofaunal communities influenced by hydrodynamically controlled food availability in the Wadden Sea, the open North Sea, and the Deep-sea. A synopsis. *Marine Biodiversity*, **36**(2): 123-164 (2006).
 20. Kumar, A., *Fundamentals of Limnology*: APH Publishing Corporation (2005).
 21. Lamptey, E., & Armah, A., Factors Affecting Macrobenthic Fauna in a Tropical Hypersaline Coastal Lagoon in Ghana, West Africa. *Estuaries and Coasts*, **31**(5): 1006-1019 (2008).
 22. Liang, S. H., Shieh, B. S., & Fu, Y. S., A structural equation model for physicochemical variables of water benthic invertebrates and feeding activity of water birds in the Sitsao wetlands of Southern Taiwan. *Zoological Studies*, **41**: 441-451 (2002).
 23. Lomas, J. G. d., Corzo, A., García, C. M., & Bergeijk, S. A. V., Microbenthos in a hypersaline tidal lagoon: factors affecting microhabitat, community structure and mass exchange at the sediment-water interface. *Aquatic Microbial Ecology*, **38**(1): 53-69 (2005).
 24. Mazzola, A., Mirto, S., & Danovaro, R., Initial Fish-Farm Impact on Meiofaunal Assemblages in Coastal Sediments of the Western Mediterranean. *Marine Pollution Bulletin*, **38**(12): 1126-1133 (1999).
 25. Mistri, M., Fano, E. A., Rossi, G., Caselli, K., & Rossi, R., Variability in Macrobenthos Communities in the Valli di Comacchio, Northern Italy, a Hypereutrophized Lagoonal Ecosystem. *Estuarine, Coastal and Shelf Science*, **51**(5): 599-611 (2000).
 26. Morton, B., & Wu, S. S., The hydrology of the coastal waters of Hong Kong. *Environmental Research*, **10**(3): 319-347 (1975).
 27. Ogunwenmo, Christiana, A., & Osuala, I. A., Physico-chemical parameters and macrobenthos of an estuarine creek and an artificial pond in Lagos, southwestern Nigeria. *acta SATECH*, **1**(2): 128-132 (2004).
 28. Penjan, R. A., Siriporn, P., Natinee, S., & Somboon, S., Temperature, Salinity, Dissolved Oxygen and Water Masses of Vietnamese Waters. Proceedings of the SEAFDEC Seminar on Fishery Resources in the South China Sea, Area IV : Vietnamese Waters. 346-355 (1999).
 29. Penn, D., & Brock, H. J., Nest-Site Selection in the Horseshoe Crab, *Limulus polyphemus*. *Biological Bulletin*, **187**(3): 373-384 (1994).
 30. Pinder, L. C. V., Ladel, M., Gledhill, T., Bass, J. A. M., & Matthews, A. M., Biological surveillance of water quality- 1. A comparison of macroinvertebrate surveillance methods in relation to assessment of water quality, in a chalk stream. *Archiv für Hydrobiologie*, **109**: 207-226 (1987).
 31. Prashant, J. J., Tahir, A. A., & Saad, N. K., Assessment of dissolved oxygen in coastal waters of Benghazi, Libya. *Journal of Black Sea/Mediterranean Environment*, **15**: 135-156 (2009).
 32. Rodrigues, N., Sharma, R., & Nagender Nath, B., Impact of benthic disturbance on megafauna in Central Indian Basin. *Deep Sea Research Part II: Topical Studies in Oceanography*, **48**(16), 3411-3426 (2001).
 33. Rosenberg, R., Agrenius, S., Hellman, B.,

- Nilsson, H. C., & Norling, K., Recovery of marine benthic habitats and fauna in a Swedish fjord following improved oxygen conditions. *Marine Ecology Progress Series*, **234**: 43-53 (2002).
34. Shuster, C. N., Observations on the natural history of the American horseshoe crab, *Limulus polyphemus*. *Woods Hole Oceanographic Institution Contribution*, **564**: 18-23 (1950).
35. Sivadas, S., Ingole, B., & Nanajkar, M., Temporal variability of macrofauna from a disturbed habitat in Zuari estuary, west coast of India. *Environmental Monitoring and Assessment*, **173**(1), 65-78 (2011).
36. Team, O. U. O. C., *Seawater: its composition, properties and behaviour*. Butterworth-Heinemann (1995).
37. Yap, C., Rahim, I. K. A., Ismail, A., & Tan, S. G., Species Diversity of Macrobenthic Invertebrates in the Semenyih River, Selangor, Peninsular Malaysia. *Pertanika Journal of Tropical Agricultural Science*, **26**(2): 139-146 (2003).
38. Zaleha, K., Hazwani, I., Siti Hamidah, H., Kamaruzzaman, B. Y., & Jalal, K. C. A., Effect of Salinity on the Egg Hatching and Early Larvae of Horseshoe Crab *Tachypleus gigas* (Muller, 1785) in Laboratory Culture. *Journal of Applied Sciences* (2011).
39. Zaleha, K., Kamaruzzaman, B. Y., John, B. A., & Ong, M. C., Cd, Cu and Pb Concentration Levels in Horseshoe Crab Nesting Grounds of Pahang Coast, Malaysia. *Journal of Biological Sciences*, **10**(8), 790-794 (2010).