THE EFFECT OF ENVIRONMENTAL FACTORS ON INTERTIDAL MOLLUSC ASSEMBLAGES IN PENANG ISLAND, MALAYSIA

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by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATE

LIST OF ABBREVIATIONS

°C	Degree Celsius
ppt	Part per thousand
µm	Micrometer
mm	Millimeter
cm	Centimeter
m	Meter
m^2	Meter square
ml	milliliter
mg/L	Milligram per litre
%	Percent
>	More than
<	Less than

KESAN FAKTOR PERSEKITARAN TERHADAP HIMPUNAN MOLUSKA DI KAWASAN PASANG-SURUT SEKITAR PULAU PINANG, MALAYSIA

ABSTRAK

Gangguan antropogenik seperti pembangunan pantai, penambakan tanah, perikanan tradisi (seperti pengutipan moluska yang boleh dimakan; *Donax cuneatus* dan *Meretrix meretrix*) dan aktiviti pelancongan merupakan ancaman kepada moluska dalam pelbagai aspek. Kajian ini menerangkan komposisi spesies dan taburan moluska di kawasan zon pasang-surut di Pulau Pinang yang telah dipengaruhi oleh aktiviti yang berbeza di kawasan tersebut. Lima tapak kajian yang telah dipilih, iaitu Teluk Aling (TA), Pulau Betong (PB), Jelutong (JT), Teluk Kumbar (TK) dan Batu Feringghi (BF) telah dijalankan persampelan secara rawak dan tersusun dengan pengambilan sampel dari zon pasang-surut rendah, zon pasang-surut tengah dan zon pasang-surut atas dari bulan Mei 2015 sehingga Januari 2016. Sejumlah 30 spesies gastropoda dan bivalvia yang terdiri daripada 22 famili and 25 genera telah dikenalpasti. Famili Trochidae telah mencatatkan bilangan individu gastropoda tertinggi (min kelimpahan: 158.33 individu/m^3) manakala Famili Mytilidae mencatatkan bilangan individu bivalvia tertinggi (min kelimpahan: 9.56 individu/m^3). *Zeuxis* sp. dan *Diplodonta asperoides* merupakan spesies yang biasa ditemui di semua tapak kajian kecuali di BF. Kepelbagaian spesies (H'= 1.21 ± 0.22) dan kesamarataan spesies tertinggi (J' = 0.92 ± 0.10) direkodkan di TK; kekayaan spesies tertinggi (S = 6.60 ± 6.47) dicatatkan di PB; jumlah kelimpahan tertinggi (2535 individu/m^3) direkodkan di TA; dan bahan organik tertinggi (3.03% ± 0.89) dicatatkan di PB. Parameter persekitaran iaitu suhu sedimen, saliniti sedimen, bahan organik dan saiz partikel menunjukkan perbezaan signifikan di dalam kelima-lima tapak kajian (ANOVA, $p < 0.05$). Berikut merupakan nilai min bagi suhu sedimen dan saliniti sedimen yang telah ditentukan di zon pasang-surut rendah: (30.88 ± 0.37 °C; 30.07 ± 1.17 ppt), zon pasang-surut tengah: (30.55 ± 0.61 °C; 15.85 ± 1.20 ppt) zon pasang-surut atas: (30.24 ± 0.85 °C; 8.23 ± 0.84 ppt). Analisis saiz partikel mendapati kandungan pasir mengikut jenis saiz partikel tertentu pada setiap tapak kajian: sebagai contoh, TA terdiri daripada pasir halus dan pasir yang sangat halus. Hasil keputusan PERMANOVA mendedahkan bahawa setiap tapak kajian mempunyai perbezaan yang signifikan ($P = 0.001$), di mana terdapat suatu gambaran pengasingan/perbezaan spesies moluska bagi setiap zon pasang-surut dan juga semasa bulan persampelan yang ditunjukkan menggunakan plot nMDS. Contohnya, *Umbonium vestiarium* merupakan spesies yang paling dominan dan ditemui di setiap zon pasang-surut serta pada setiap bulan persampelan, dan spesies ini hanya dijumpai di TA. Analisis SIMPER mengkategorikan peratusan spesies yang menunjukkan sumbangan tertinggi dalam peratusan persamaan purata mengikut zon pasang-surut di setiap tapak kajian dan juga mendapati *U. vestiarium* menyumbang 90% spesies di TA. Hasil kajian yang meliputi perbezaan kepelbagaian dan kelimpahan spesies di kawasan zon pasang-surut Pulau Pinang boleh menjadi refleksi kepada aktiviti antropogenik. Oleh itu, polisi pengurusan lestari yang efektif bagi kawasan-kawasan zon pasang-surut adalah diperlukan untuk mengekalkan biodiversiti moluska ini.

THE EFFECT OF ENVIRONMENTAL FACTORS ON INTERTIDAL MOLLUSC ASSEMBLAGES IN PENANG ISLAND, MALAYSIA

ABSTRACT

Anthropogenic disturbances such as coastal development, land reclamation, artisanal fishing (such as collection of edible mollusc; *Donax cuneatus* and *Meretrix meretrix*) and tourism activities have been a threat to intertidal mollusc communities in various aspects. Present study describes the species composition and distribution of molluscs in intertidal region of Penang Island that was influenced by different activities occurring in the area. Five selected sites, i.e. Teluk Aling (TA), Pulau Betong (PB), Jelutong (JT), Teluk Kumbar (TK) and Batu Feringghi (BF) were randomly stratified sampled according to low-, mid-, and high zonation area from May 2015 to January 2016. A total of 30 gastropod and bivalve species belonging to 22 families and 25 genera were identified. Family Trochidae recorded the highest number of individual gastropod (mean abundance: 158.33 individuals/m^3) while Family Mytilidae recorded the highest number of individual bivalve (mean abundance: 9.56 individuals/m^3). *Zeuxis* sp. and *Diplodonta asperoides* were the most common species found at all sites except in BF. The highest species diversity ($H' = 1.21 \pm 0.22$) and species evenness ($J' = 0.92 \pm 0.10$) was recorded at TK; the highest species richness (S = 6.60 ± 6.47) was observed at PB; the highest total abundance (2535 individuals/m^3) was recorded at TA; and the highest organic matter (3.03% ± 0.89) was observed at PB. Environmental parameters, i.e. soil temperature, soil salinity, organic matter and particle size did differ significantly among the five sites (ANOVA, $p < 0.05$).

The following mean values for soil temperature and soil salinity were determined; lower zone: (30.88 ± 0.37 ºC; 30.07 ± 1.17 ppt), middle zone: (30.55 ± 0.61 ºC; 15.85 ± 1.20 ppt) upper zone: (30.24 ± 0.85 ºC; 8.23 ± 0.84 ppt). Particle size analysis discovered high content of every sand particles type at which site; for example, TA highly comprised with fine sand and very fine sand. PERMANOVA findings indicated that each of the site differ significantly (P = 0.001), where the separation of mollusc community pattern was visualized using nMDS plots for every zonation and during sampling month was differed at each site. For example, *Umbonium vestiarium* predominantly found at every zonation and observed in all sampling month, and only occurs in TA. SIMPER analysis categorized the contributing percentage of the major species according to zonation in all site, also discovered *U. vestiarium* made up to 90% of total species found at TA. Present finding of the species diversity in the intertidal region of Penang Island could be a reflection of anthropogenic activities. Therefore, an effective sustainable management policy for these intertidal areas is needed in order to maintain the mollusc biodiversity.

CHAPTER 1: INTRODUCTION

Molluscs are among the most diverse, and well-known of the invertebrate groups with the valid number of species recorded currently estimated around 45,000 to 50,000 marine, 25,000 terrestrial and 5,000 freshwater species (Rosenberg, 2014). They are soft bodied animals, often with hard shells for protection and also exhibit an enormous range in size (Tagliapietra & Sigovini, 2010). Molluscs are divided into three main groups; Gastropoda, Bivalvia and Cephalopoda and are the dominant classes of fossil and living molluscs. Gastropods and bivalves are key elements of marine ecosystems, as they are relatively sedentary thus, making them unable to avoid deteriorating water/sediment quality conditions.

The present study deals with the mollusc biodiversity influenced by environmental conditions in relation to anthropogenic activities on the intertidal region in five different sites in Penang Island. Penang Island, is one of the most touristic locations in Malaysia with many development around the coastline in relation to rapid economic and industrial growth (i.e. The Light Waterfront project, Queensbay project), which were nearby to the study sites. The present study discusses the findings of species diversity, richness and evenness of intertidal molluscs corresponds to varied environmental factors (i.e. soil temperature, soil salinity, soil pH, organic matter, and particle size that affects its distribution. Basically, appropriate temperature, salinity, pH and rainfall are a vital element in the distribution of benthic organisms (Rodrigues et al., 2016; Hernandez-Alcantara & Solis-Weiss, 1955; Beasley et al., 2005).

Intertidal region is ecologically and economically crucial both for humans and wildlife. This area is normally known as littoral zone where the area lies between the high tide and low tide. The intertidal area consists of various habitats that support many living forms (Omar et al., 2011; Terlizzi et al., 2002; Menge et al., 1986). Organisms living in an intertidal area must be able tolerant to a wide range of natural conditions as this zone is considered to be a harsh living environment due to unpredictable salinity, desiccation, temperature, currents, and predators. Hence, many organisms possess special adaptive features to overcome these challenges. For instance, mussels avoid themselves from drying by closing their shell tightly to retain moisture (Senechal-Brown & Dean, 1997).

Intertidal shorelines can be divided into three zones: lower intertidal, middle intertidal and upper intertidal. The lower intertidal remains submerged most of the time and are populated by diverse communities, including members of all the major phyla. The middle intertidal is occasionally submerged by the tides and is associated with more competition for space and there is more number of predators. In the upper intertidal, only the most resilient species can survive, since the conditions are similar to that of terrestrial areas (Spring, 2002; Senechal-Brown & Dean, 1997).

Sessile and mobile molluscs are an essential element of natural intertidal assemblages. Intertidal mollusc plays a significant role in terms of providing food sources to aquatic animals, migratory birds, and humans. They form a key link in the food chain and have a high socioeconomic value for coastal fisheries. Moreover, they function in marine ecosystems through nutrient cycling and energy flow.

In addition, intertidal zone also serves as a recreational spot for tourism. Thus, mollusc is identified as an important biological indicator in marine and estuarine environments due to their ability to assess water quality and ecological risk. Unfortunately, the diversity of intertidal mollusc seems to be declining due to the changes in water conditions resulting from human activities, pollution level, and climate change (Deepananda & Macusi, 2012; Omar et al., 2011).

Reduction in diversity is due to development of coastal area that destroys many habitats and causes many of macrobenthic fauna to be eroded. Any coastal development and reclamation projects by the shoreline usually induce high siltation and sedimentation process. This alarming trend is a cause for concern, particularly in developing tropical regions of the world such as those observed in Malaysia. At present, a majority of the coastal zones in Malaysia have been reclaimed for urban development. For example, development activities that occur in Penang Island such as construction of infrastructure like jetty, commercial and residential development (i.e. project: Koay Jetty of clan jetties 1970s, Jelutong coastal highway 1990s, Tg. Tokong and Seri Tanjung Pinang land reclamation 2000s). Moreover, one recent mixed residential and commercial development that caused sea reclamation near the Penang Bridge started in late 2008, called The Light Waterfront (Chin, 2007).

Coastal reclamation leads to many negative effects such as an increase in water turbidity, sediment deposition, disturbances to aquatic and pollutants that is generated during reclamation activities and anoxic conditions in deeper water occurs (Lu et al., 2002). Furthermore, it also weakens the growth of sessile benthic organisms (Mostafa, 2012; Lu et al., 2002). Macrobenthos provide essential information for instance, it acts as bioindicator of heavy metals (Halina et al., 2010), plays key roles in major ecological processes (Covich et al., 1999), shows rapid response to environmental changes (Venturini et al., 2004) and is an important component in socio-economic-fisheries (Deepananda & Macusi, 2012).

Therefore, analysis of macrobenthic infauna is very important in marine environmental monitoring programs (Bilyard, 1987). As stated by Chong and Chou (1992), complete benthic fauna will be depleted due to coastal reclamation. This is also supported by Ge and Jun-Yan (2011), where the number of marine species declines when the living environment has been seriously affected through reclamation activities. However, the consequence of reclamation on benthic communities is not fully known. In the present study, the distribution and abundance of mollusc was surveyed in the intertidal region involving the five sites from the north, east, south and west part of Penang Island since Penang has been actively involved in land reclamation in 2001. Generally, it is important to study the consequences of anthropogenic disturbances such as land reclamation and development as it may cause significant stress in intertidal macrobenthic communities.

1.1 Research aims

In view of the aforementioned understanding of the subject, the aims of the study is to prepare a baseline database of the mollusc assemblages in the intertidal region specifically in different study sites selected that was subjected to varied effects of environmental factors around anthropogenically influenced shores in Penang Island, which will lead a proposal of sustainable management policies for intertidal molluscs and intertidal ecosystems at present and future.

1.2 Research question

Do environmental factors variation; soil temperature, soil salinity, soil pH, organic matter and particle size in relation to anthropogenic activities on the intertidal region pose significant effects on mollusc assemblages?

1.3 Hypothesis

Postulated hypothesis are:

(a) There are significant differences in mollusc's composition and distribution at different study site corresponds accordingly with varied component of environmental factors.

(b) The effects of environmental factors on mollusc communities are proportional to the pressure made by anthropogenic activities.

1.4 Research objectives

(a) To determine the composition and abundance of intertidal molluscs around selected study sites in Penang Island

(b) To investigate mollusc assemblages in terms of spatial and temporal distribution in the intertidal zone

(c) To study the environmental factors (such as soil temperature, soil pH, soil salinity, organic matter and particle size) affecting mollusc distribution

CHAPTER 2: LITERATURE REVIEW

2.1 The Pyhlum Mollusca

The phylum Mollusca is one of the largest groups in the animal kingdom. Mollusca derives from the Latin word meaning “soft-bodied”. Molluscs are made up of animals such as squids, octopuses, bivalves, gastropods, chitons and aplacophoran. Molluscs are commonly found and their ecology is diverse, occupying aquatic and terrestrial environment (Rittschof & McClellan-Green, 2005). They colonize land, freshwater, saltwater, brackish water, sandy, rocky, muddy, coral reefs, estuaries and mangrove swamps. Molluscs are also categorized as benthic community that contribute to human diet especially bivalves and gastropods. To date, the role and contribution of molluscs is still not well known even though they can be classified as common elements of the benthic communities (Cuker, 1983).

In both bivalves and gastropods, shells are present to protect the internal parts. Bivalves have two shells (Figure 2.1) that provide protection and enable free rotation about a hinge axis while gastropods (Figure 2.2) have only one shell or some lack in shell altogether. Basically, the shells of bivalves and gastropods play similar role as they run some additional functions like support connection with locomotion and builds defenses towards environmental conditions (Thomas, 1988).

The Mollusca community structure is also very useful as model system to study environmental quality, environmental toxicology and biodiversity as they act as an effective bioindicator tool in assessing ecosystem health status (O’Connor, 2002; Monirith et al., 2003; Straw & Rittschof, 2004; Halina et al., 2010).

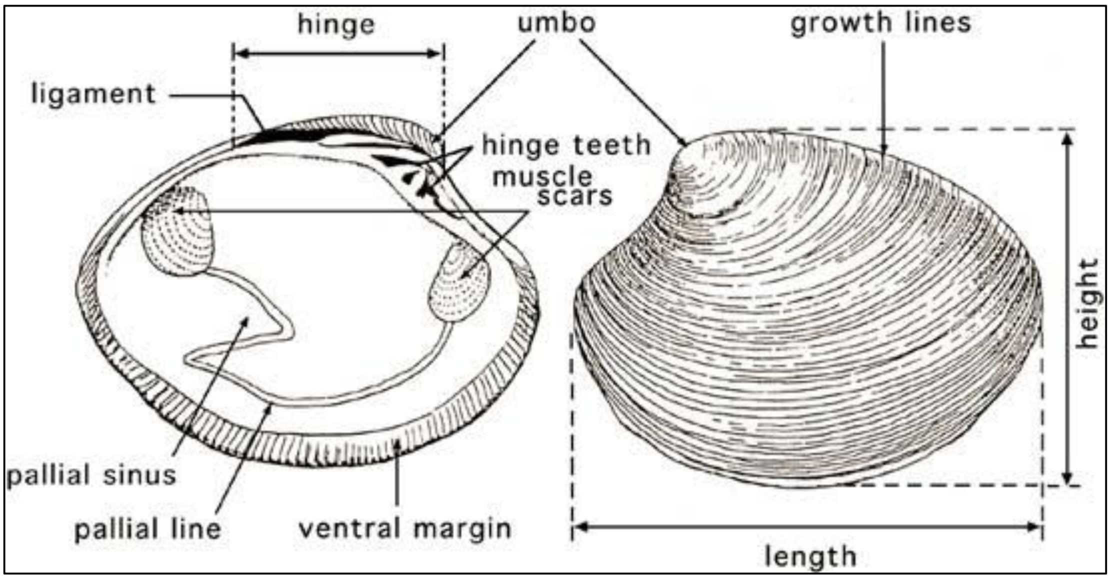

Figure 2.1: Basic morphological structure of a bivalve (source from: Cesari & Pellizzato, 1990).

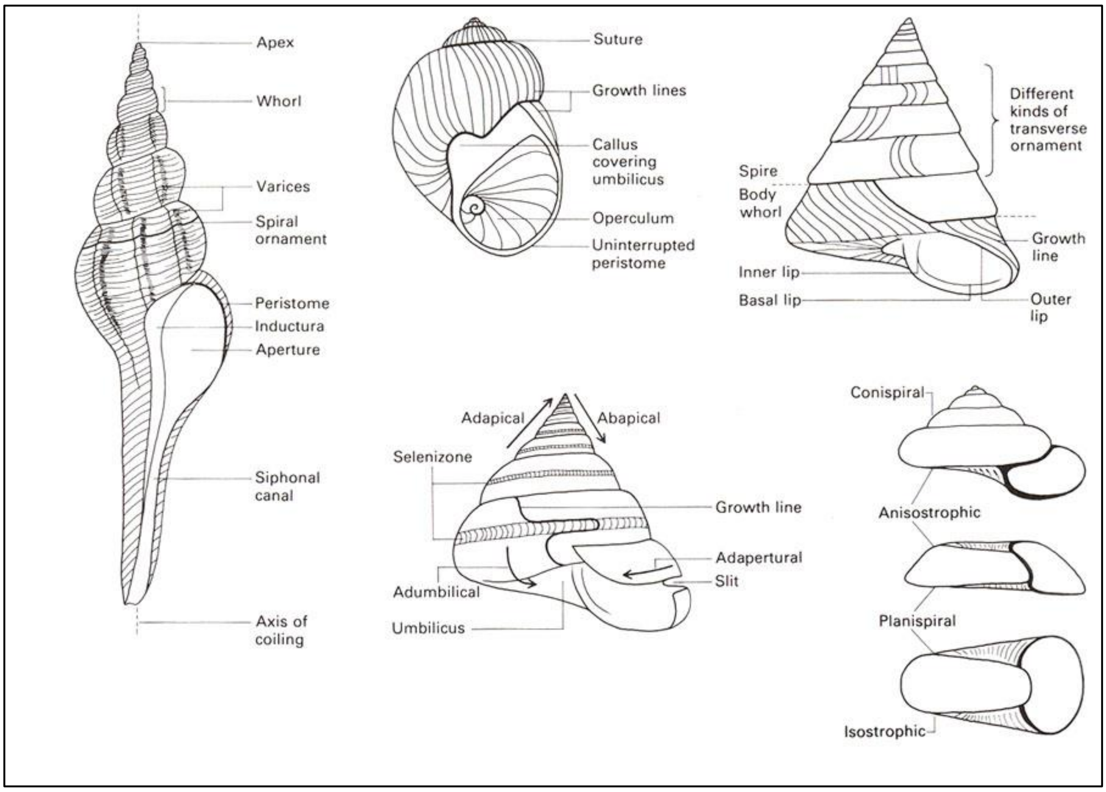

Figure 2.2: Basic morphological structure of gastropods (source from: Boardman et al., 1987).

2.2 Mollusc studies in the tropical environment

Recent studies done in tropical intertidal environment was on a marine macrogastropoda in the Northern Straits of Malacca involving three islands; Pulau Songsong, Pulau Bidan and Pulau Telor. The most dominant species found was *Morula marginatra* while Pulau Songsong had the highest species diversity among the islands. Physical characteristics of Pulau Songsong including smooth rocks and bare surface contributed to the high number of *M. marginatra* as they can easily be spotted and collected (Siti-Balkhis et al., 2014).

Study done by Cob et al. (2012) in the intertidal areas of Mersing, Johor reported Mollusca as the most dominant taxa as gastropods highly dominated the mangrove area. Phylum Mollusca comprises of *Nerita* was abundant from the middle to upper intertidal zones, as this herbivorous mollusc are considered as gregarious type which congregate in a flock with a high degree of interaction with other living animals (Tan & Clements, 2008).

Similarly, study conducted in Teluk Aling, Penang documented four higher taxa and phylum Mollusca recorded the highest abundance among Polychaeta, Crustacea and Echinodermata (Omar et al., 2011). This study also revealed mollusc species i.e. *Umbonium vestiarium* dominated the intertidal area with higher species composition of invertebrate in lower zone. Species composition is indicated by the number of species found while the species diversity takes into account number of species present and the evenness of species distribution. In addition, this study also showed higher species diversity of invertebrate in the middle zone. Findings obtained in this study was supported by Yonge (1949) who reported on bivalves stating that they are mostly found in mid to low shores where the tide frequently washes against the shore.

In addition, the mollusc assemblages in intertidal zones of Batu Ferringhi, Teluk Aling and Teluk Bahang were studied, where 10 families and 10 genera were recorded at the study sites (Ng, 2016). This study revealed the highest abundance of molluscs at lower intertidal zone compared to the other zone. The abundance of mollusc showed positive correlation with salinity of soil and organic matter content. However, negative correlation was detected between mollusc abundance and the soil pH.

One study conducted in subtidal coastal waters of Penang National Park revealed the relationship of sediment type and benthic molluscs' assemblages. Based on the findings, Nuculanidae, *Megastomia* sp., and *Timoclea* sp. was found to have a positive correlations with sandy particles (1000 µm – 250 µm). High energy habitats in this study, such as sandy areas supported a high abundance of molluscs (Alianie et al., 2013).

In a study of composition and distribution of intertidal mollusc fauna at Penang beaches, i.e. Muka Head, Teluk Bahang, Batu Feringghi, Teluk Kumbar, Tanjung Asam and Balik Pulau by Nor Haizon (1984) discovered a total of 14 families consisting of 21 species of mollusc. The families were Trochidae, Naticidae, Thaiididae, Cerithiidae, Nassariidae, Donacidae, Veneridae, Corbulidae, Mytilidae, Tellinidae and Carridae. This study indicated that the particle size distribution of sediment is one of the factors that influenced the occurrence and numerical abundance of species at all study sites. In fact, the particle size distribution of sediment was different is actually due to difference in wave action on the beaches. However, in this study, salinity was not calculated as a factor as similar readings were taken at each study site.

2.3 Ecological and commercial importance of intertidal mollusc

Intertidal mollusc has been recognized as a key element of productive marine ecosystem in intertidal ecology (Spilmont, 2013). They also play a vital role in nutrient cycling, for instance the elements C, N, P that are excreted by bivalves and regenerated from their biodeposits are subsequently recycled back to the water column to support further phytoplankton production and they are also known as important contributors of nitrogen (usually in the form of ammonium, NH_4^+) to both subtidal and intertidal systems. In addition, they area also important in detrital decomposition and as a food source for higher trophic levels (Covich et al., 1999; Venturini et al., 2004; Reiss & Kroncke, 2005; Lu, 2005; Nordhaus et al., 2009). For example, gastropods ecologically play an important role as grazers to scavengers and carnivores (Sturm et al., 2006). Mollusc shells are extremely important for maintaining the fundamental life processes classified as biogenic minerals. Intertidal mollusc are food for other fauna like demersal fish, shrimps, crabs (Ramesh et al., 1990) and as non-food resources such as shells used for ornamental purposes while operculum used for decorative or cosmetic, also as medicinal element (Raghunathan et al., 2004).

Furthermore, gastropods like snails are functional agents to structure the intertidal assemblages (Underwood et al., 1983; Magalhaes, 2000) and have relatively high biomass. They are also important in environmental monitoring and toxicology with their limited mobility, hence resistant to chemical contamination. Also, they are sensitive to deteriorating conditions, thus it is very crucial to assess the diversity and abundance of these organisms especially during any development activities because they are useful for revealing ecological states of ecosystems in response to environmental disturbances (Pires-Vanin et al., 2011: Alongi, 1990).

2.4 Factors affecting the distribution and abundance of intertidal mollusc

Biotic and abiotic elements are factors that primarily affect the distribution, zonation and community structures of sandy beaches. Biotic interactions comprises of recruitment, predation and competition for food whereas abiotic interactions such as disturbances like temperature, salinity, wave action, tidal regime, storms and fisheries (Denny & Paine, 1998). Intertidal ecology has been used as reference in determining these factors as they are widely recognized as an important littoral zone in marine ecosystem. When the physical and biological factors interact with each other, it leads to biodiverse intertidal zone.

Other aspects identified to affect intertidal molluscs and pose serious problems to the ecosystem are desiccation, freezing, overheating and wave impacts. The lower limit in distribution of intertidal molluscs is sometimes influenced by biotic interaction between competitors and natural enemies. Lower shores always provide better growth conditions and high rate of survival compared to high shores in regardless of the existence or non-existence of biological interaction (Franke & Gutow, 2004). Generally, the ability of one animal to colonize and tolerate the changing conditions in habitat will determine their abundance and species variation (Raffaelli & Hawkins, 1996).

Apart from that, mollusc community structure could be affected by anthropogenic activities. Studies on rocky intertidal shores by Vaghela (2010), stated that the degree of anthropogenic activities such as; tourism, trampling and exploitation probably altered the community composition over the time. Besides that, reduction in faunal complexity occurred in the study done by Deepananda (2008) in relation to anthropogenic activities and the author also suggested on using multiple methods to measure ecological stress. In addition, Chee and Sim (2016) reported on the effects of land reclamation in Penang towards the coastal macroinvertebrates in the reclaimed study areas, i.e. Queensbay, Lebuhraya Lim Chong Eu, North Butterworth Container Terminal and Bagan Ajam R&R. The average species diversity and evenness on reclaimed land was the lowest compared to the adjacent to reclaimed areas and less disturbed areas.

2.5 Mollusc as a bioindicator

Molluscs have been successfully used to gain information on the quality of several ecosystems and the pollution effect in their environment (Markert et al., 1999; Stoykov & Uznova, 2001). There are special features of molluscs that enable them to be known as biological indicators such as the regular accumulation of chemical contaminants in sediment during the exposure, hypoxia/anoxia caused by the degradation of organic matter, inability to safeguard itself from adverse conditions due to limited mobility and the ability for stress level detection through the taxonomic groups and functional diversity (Tagliapietra & Sigovini, 2010) based on the composition, diversity and vigour of mollusc communities that differ naturally according to the bioclimatic region, the type of ecosystem and the specific features of the habitat in which the organisms live, such as bottom water salinity, water renewal rate and sediment type.

Molluscs can also be a good indicator for heavy metal pollution (Rovere et al., 2009; Halina et al., 2010; Dauvin, 2008; Maher et al., 1997). One study conducted by Balogun et al. (2011) showed high density of *Pachymelania aurita* and *Neritina glabrata* that suggests less pollution impact at the particular station because these species were classified as pollution sensitive species. Other studies related to the pollution levels with relation to physicochemical parameters of the water; temperature and calcium concluded that mollusc population is highly affected by changes in the physicochemical characteristics (Garg et al., 2009; Udayantha & Munasinghe, 2009; Sharma et al., 2013).

Bivalve was reported to be a very good bioindicator in selenium accumulation, as it showed very accurate results compared to other organisms such as zooplankton, macroalgae and seagrass (Bhattacharya et al., 2003). For instance, a filter-feeding bivalve, *Anadara trapezia* has been proven to be an essential bioindicator fauna for selenium metal due to the ability of *A. trapezia* to retain heavy metal in its body (Chapman, 2005; Maher et al., 1997).

2.6 Anthropogenic disturbances: conversion on land use activities

Anthropogenic disturbances such as commercial fishing, coastal development, land reclamation, trampling and handling on the intertidal shore communities were the type of activities that directly and indirectly altered the environment and affecting the intertidal community assemblages. Lindegarth and Hoskin (2001) suggested an assessment of the impact of human disturbances on marine ecosystem evaluated by using macrobenthic organisms because they represent important ecological indicators. Mollusc was particularly selected as the special interest of the study as they are omnipresent, relatively sedentary and reflect site-specific conditions (Shin &

Ellingsen, 2004; Paine, 1980), as well as indicators of environmental status (Dietl et al., 2016; Paiva, 2001).

In Penang, several coastal land reclamation projects were carried out, for instance Seri Tanjung Pinang, Tanjung Tokong project which severely affected the quality of community life (Nadzhirah et al., 2015). Beach erosion and siltation takes place as dynamic equilibrium disrupted. Besides that, reclamation project on Jelutong coastal highway 1990s (Leong, 2011) had caused major environmental impacts such as loss of natural ecosystem, i.e. mangroves, fishing grounds and increase sedimentation and water pollution as marine mud and clay pushed out to sea.

Most of the reclamation projects caused negative consequence to aquatic life as heavy sedimentation occurred, which changed the environment (Leong, 2011). On the other hand, some researchers classify reclamation as physical disturbance to biological groups in marine environment as these activities impact on the growth of sessile benthic organisms (Lu et al., 2002) due to the relatively sessile habit and, thus, the incapability to avoid unfavourable conditions, macrobenthic species are sensitive indicators of changes in the marine environment caused by natural or anthropogenic disturbances.

Industrial effluent, fertilizers runoff, sewage and dredge spoils to coastal zone causes coastal pollution. This may lead to unhealthy environment that affects both humans and marine life. Anthropogenic disturbances from various activities such as tourism, exploitation, fishing pressure, reclamation areas, trampling, marine litter and other possible activities cause negative consequences in terms of growth and survival of fauna (Roy et al., 2003; Smith & Murray, 2005). Habitat fragmentation which is also known as a process of reduction in the total area of habitat is distinguished as a major path of the eroding fauna diversity (Lindenmayer & Fischer, 2013). Roy et al. (2003) through his research done along the southern California positively related anthropogenic activities to declination of gastropods species i.e. *Lottia gigantean, Fissurella volcano, Tegula aureotincta,* and *Acanthinucella spirat* at that particular area.

Similarly, Kohn and Blahm (2005), evaluating the effects of human activities along the sandy intertidal area off Western Australia revealed similar outcomes. Human influences have led to broad-scale damage of dynamic habitats that were reflected as both acute and chronic effects. There are several threats to this intertidal ecosystem including pollution, global climate change, effects of fishing, introduction of invasive species, sedimentation of coastal area and tourism.

In China, coastal reclamation has been continuously practiced due to the population pressure since 1950s in the need to support their social and economic development (Wang et al., 2014). One investigation that has been done on benthos life in Tianjin Harbor Industrial Zone revealed the effect of reclamation activities on benthos. Diversity and community structure of benthos tends to change after the reclamation that was proved by the findings on the diversity index of Shannon-Wiener of benthos that decreased from 1.28 to zero (Kunyu et al., 2010).

CHAPTER 3: MATERIALS AND METHODS

3.1 Study sites

Penang Island is a metropolitan island, an area of approximately 293 km^2 and is situated on the northwest of Peninsular Malaysia. The study sites lies within the region of north, east, south and west of Penang Island. The selection of sites was based on the different development/disturbances occurring in the area (Figure 3.1 & Table 3.1).

Table 3.1: Abbreviations, GPS coordinates and anthropogenic activities of the study sites.

Study site	**Abbreviation**	**GPS coordinate**
Teluk Aling	TA	N 05°28.0780” E 100° 12.009”
Pulau Betong	PB	N 05°18.5370” E 100° 11.619”
Jelutong	JT	N 05°22.8820” E 100° 19.090”
Teluk Kumbar	TK	N 05°16.9110” E 100° 12.721”
Batu Feringghi	BF	N 05°28.4560” E 100° 14.811”

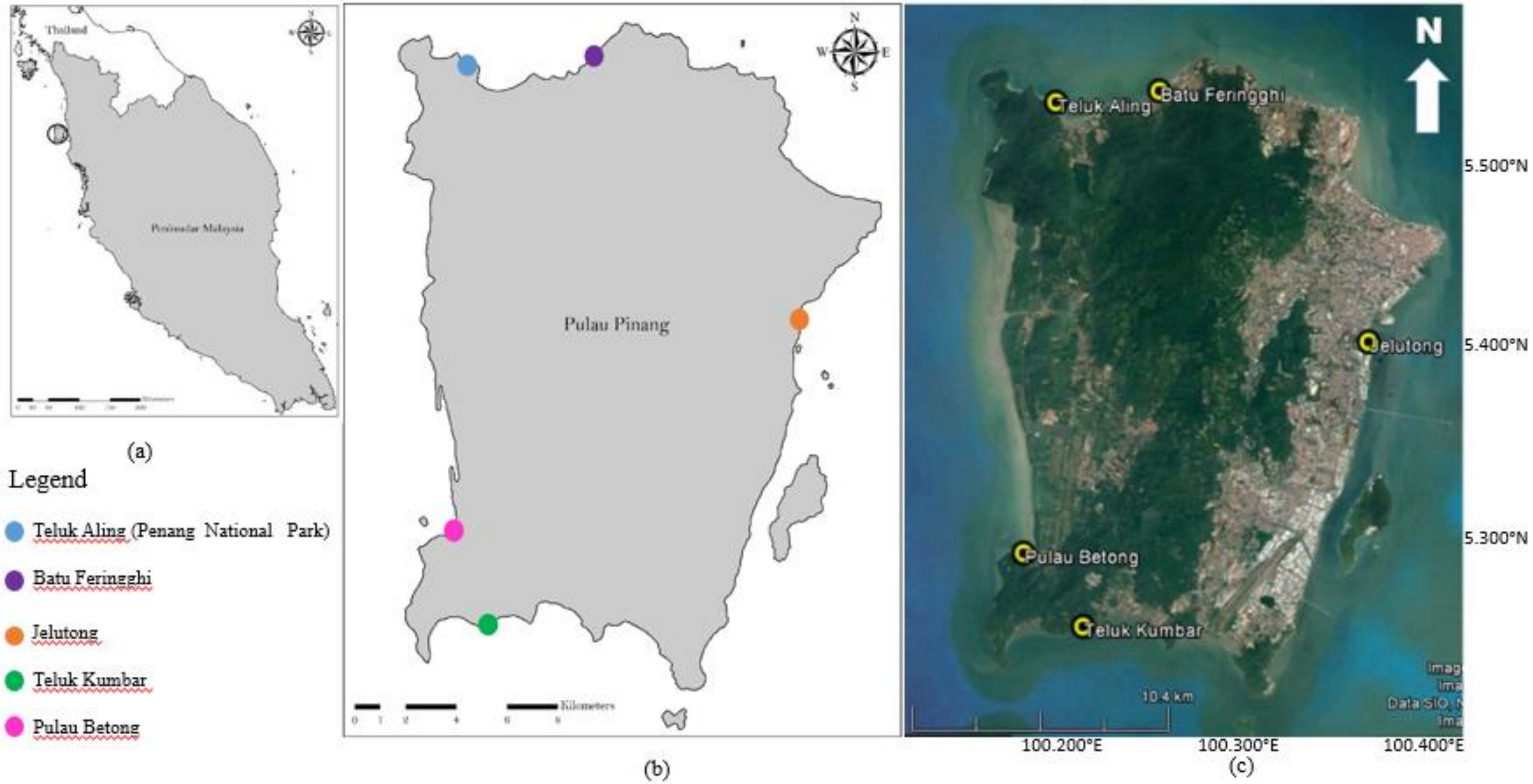

Figure 3.1: Detailed map of the study site (a) Penang, (b) study sites and (c) Google Earth view of Teluk Aling (TA), Batu Feringghi (BF), Jelutong (JT), Teluk Kumbar (TK) and Pulau Betong (PB).

3.1.1 Teluk Aling (TA)

Teluk Aling is located in the northwest coast of Penang Island in the Penang National Park. Teluk Aling is a part of Penang National Park gazetted in 2003 under the National Park Act of 1980. Teluk Aling intertidal area comprises of rocky shores and sandy beach. The intertidal distance during the low tide is 26 meter while the beach slope is 15º. Teluk Aling is highly comprises of very fine sediment texture. The Centre for Marine and Coastal Studies (CEMACS) that conducts research in coastal and marine studies is also situated here. Visitors can access this area by boat from Teluk Bahang jetty or by trekking through park trails.

3.1.2 Pulau Betong (PB)

Pulau Betong is situated in the southwest corner of Penang Island. Pulau Betong is a small fishing village that was developed at the estuary of Sungai Pulau Betong. The river flows along mangrove swamp and eventually out to the sea. Near the coastal area are several floating fish cages. Pulau Betong comprises of an extensive intertidal area of 122 meter length during low tide and with the beach gradient of 49º. Pulau Betong comprises of firm coarse sand and silt and clay sediment texture.

3.1.3 Jelutong (JT)

Jelutong is located in the eastern part of Penang Island. This region is declared as reclaimed area since 1990s when the coastal highway was built by IJM Land Berhad development. The intertidal distance during the low tide is 69 meter while the beach slope is 40º. Previously, mangroves once covered this area and reclamation work was done for the construction of residential areas around Jelutong. Hence, sea sand from other places was dumped to the reclamation area. Through personal communication with an engineer (Mr. Khan) from IJM Land Berhad, it was found that a new project of cable construction of Tenaga Nasional Berhad (TNB) was conducted along Jelutong to mainland area. This intertidal area is highly comprises of coarse sand and medium sand particles.

3.1.4 Teluk Kumbar (TK)

Teluk Kumbar is located in the southern part of Penang Island. Teluk Kumbar, with its beautiful beaches is a popular picnic spot among locals. Moreover, it also has a small fishing village for local fishermen. Few private owned chalets were built near the coast. The length of the intertidal area is 22.2 meter during low tide and with the beach gradient of 23º. Teluk Kumbar comprises of firm coarse sand and medium sand particles.

3.1.5 Batu Feringghi (BF)

Batu Feringghi is located in the northern part of Penang Island. Sandy beaches along the coastline is impacted with a variety of beach sports and water sports activities. This area is surrounded with the hotels, restaurants and souvenir shops that are concentrated near to the beach. The beach area is famous among locals as well as tourists for recreational spot. Batu Feringghi comprises of comparatively small area of intertidal of only 14 meter in length during low tide and with the beach gradient of 16º. The type of sediment characteristics in this area is firm coarse sand and medium sand particles.

3.2 Sampling activities

The sampling activities were done from May, 2015 to January, 2016 during lowest spring tide for every two months interval. The samples were collected during this period due to the greatest difference between high and low water during this tidal cycle.

3.3 Sampling design

At each study site, intertidal shores were divided into three zones along the tidal gradient. The zones were low-tidal zone, mid-tidal zone and high-tidal zone. These three zones ranged from the upper limit of splash of spray zone in high-tidal zone to 0.5 m water depth at lower low-tidal zone. For example, the lower zone is determined as the region nearest the water line during low tide, where upper zone is an adjacent to the dry zone, while middle zone is a region between the two former ones (Eliane & Cecilia, 2003). Sampling of mollusc was done using stratified random sampling method as proposed by Flores-Garza et al. (2011) and Deepananda and Udayantha (2013), in their studies. This stratified random sampling refers to samples at particular site and zonation area that have similar probability to be sampled. The samples were collected from each study site with a total of five replicates sampled from each zone as shown in Figure 3.2. The sampling number was generated from Research Randomizer software, for example a set of number containing five random number (i.e. 1, 3, 6, 7 and 9 referring to five replicate) which was used as reference to collect the sample at each zone. The process were repeated for three times to form three different sets of number.

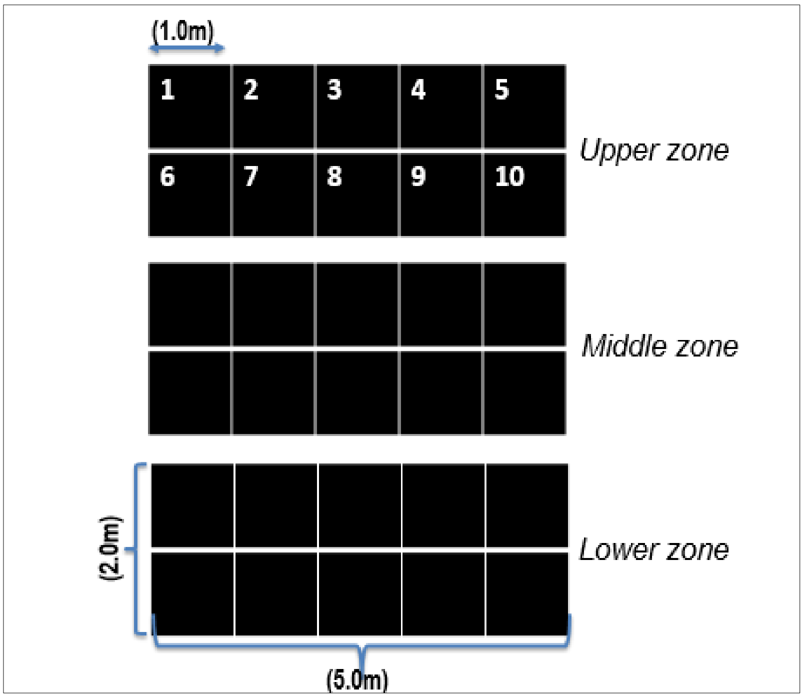

Figure 3.2: Sampling design illustration of the research study.

3.4 Sampling procedure

On the intertidal shore, a core with the volume of, $V = \pi r^2 h$ (0.003117 m^3) with dimension of 0.21 m (diameter) and 0.09 m (depth) was placed vertically on the substrate as a tool to collect the samples as it enable not only the sample from the surface of sediment but also takes into account the sample in that particular depth that might be present. A quadrate frame (1 m x 1 m = 1 m^2) was randomly placed, and a total of five quadrate of mollusc samples were taken inside the quadrate from each zonation. Hence, 15 mollusc samples were obtained from each of study site. Those samples were put into labeled bags of LZ for lower zone, MZ for middle zone and UZ for upper zone. A scoop of substrate about 100 g was also taken within the quadrate for organic matter and particle size analysis and then transferred into different bag for further analysis. The mollusc samples were sieved using a 500 µm sieve and the retained samples were put back into new plastic bag and preserved with mixed solution of (10% formalin and Rose Bengal) that was diluted with seawater. The purpose of using Rose Bengal, a known common stain for accelerating the sorting process; distinguishing molluscs from debris and differentiating live and empty molluscs.

3.5 Environmental conditions

Several physical parameters of sediment, i.e. soil temperature, soil pH and soil salinity were quantified in triplicates at each study site during the study period. The reading values of soil temperature and soil pH were recorded by placing those tools into the sand by using Fisher Scientific™ Traceable™ Digital Waterproof Thermometer and STARTER 300 Portable pH Meter (Ohaus, USA). Meanwhile, soil salinity was measured by pressing pore water from the sand and computed the reading value using electronic digital refractometer (Refractometer ATAGO-USA). In addition, beach profile was carried out to observe the gradient of the beach by using clynometer.

3.6 Laboratory analysis

3.6.1 Sorting

Subsampling methods was applied for further laboratory work of sorting and identification procedures. This methods is very useful and synonym in intertidal studies which is in the processing of the samples with large numbers of invertebrates. The *Volume-based methods* based on manual from the National Environmental Effects Monitoring (EEM) Office (2002) adopted from Wrona et al. (1982), of subsampling was selected to count the organisms found. Five subsamples were randomly picked and placed in the tubes from each replicate of the quadrate samples. Altogether, there were 25 subsamples from one zone part of lower zone, middle zone and upper zone and total of 75 subsamples from each study site. Mollusc abundance was measured using mollusc density (number of individuals per unit volume). The calculation of *Volume-based methods* is shown below:

Volume-based methods

> For example: Calculation for Species 1

> Correction Factor = total volume of sample in corer (0.003117 m^3) / (no. of tubes sorted x tube volume (0.000079 m^3)

> 5 tubes/replicates sorted

> Final count upon sorting until the 5^{th} tube recorded 18 number of individuals/m^3

> Correction factor = 0.003117m^3 / (5 x 0.000079 m^3) = 7.89

> Estimate of total number of organisms in sample = 18 x 7.89 = 142 number of individuals/m^3

3.6.2 Species identification

Once sorted, the subsamples were identified to the lowest level of taxon. The instruments used in observation and identification were dissecting microscope LEICA EZ4 and stereo microscope OLYMPUS SZX9. The picture of the organism with scale or drawing was recorded for further identification. Several aspects of mollusc sample was considered as the key to species identification, including the shell shape, shell structure, pattern and colour, shell proportions, the spire angle, aperture shape, whorls number, the type of outer lip, the present/absent of sculpture and axial, the present/absent of teeth/ridges/lamellae in aperture. The identification process of the mollusc was based on previous studies Okutani (2000); Hosseinzadeh et al. (2001); Kira (1965); Beesley et al. (1998); Cob et al. (2012); Shabdin & Rosniza (2010) and

Sim et al. (2013). In addition, the international checklists of species World Register of Marine Species (WoRMS) was used to catalogue species correctly.

3.6.3 Organic matter analysis

Sediment samples for organic content were analyzed by direct gravimetric method suggested by Bale and Kenny (2005), where 5 g of oven dried sediment was placed in a crucible and heated for 3 hours at 600 °C in a muffle furnace. The sediment sample was left to cool overnight and was reweighed again to get the actual weight loss on ignition/LOI content of dried sediment. The formula used for organic matter calculation is:

$$\text{Organic matter (\%)} = \frac{\text{Initial weight} - \text{Final weight (g)}}{\text{Initial weight (g)}} \times 100$$

3.6.4 Particle size analysis

Particle size analysis was carried out by dry sand sieving method (Holme & McIntyre, 1971; McBride, 1971; Brown & McLaachlan, 1990). A total of 25 g of dried sample was put into 250 mL beaker filled with tap water. Next, 10 mL of sodium hexametaphosphate was added and left overnight. The sample was then washed using 63 µm sieve and the sediment retained on the sieve was dried again in the oven at 105 °C and the dried sample sieved through a stack of sieves that consisted of different mesh sizes (1000 µm, 630 µm, 425 µm, 250 µm, 125 µm, 63 µm) using mechanical shaker. Classified values from the analysis were (1) coarse sand, (2) mixture of coarse and medium sand, (3) medium sand, (4) fine sand, (5) very fine sand, (6) silt and clay. In addition, the soil texture percentage were classified based on United States Department of Agriculture (USDA) (Davis & Bennett, 1927). The ternary diagram of USDA classification is shown below (Figure 3.3).