SELECTED WATER QUALITY PARAMETERS, ITS RELATION WITH ZOOPLANKTON COMMUNITY AND FATTY ACID CONTENT AT JELUTONG, PULAU JEREJAK AND KUALA JURU

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PARAMETER KUALITI AIR TERPILIH, HUBUNGANNYA DENGAN KOMUNITI DAN KANDUNGAN ASID LEMAK ZOOPLANKTON DI JELUTONG, PULAU JEREJAK DAN KUALA JURU

ABSTRAK

Kajian ini telah dijalankan untuk menentukan ciri-ciri kualiti air di perairan sekitar Pulau Pinang dan hubungannya dengan komuniti zooplankton serta kandungan asid lemak. Tiga stesen persampelan telah dipilih iaitu Kuala Juru (Stesen Juru), Jelutong (Stesen Jelutong) dan Pulau Jerejak (Stesen Jerejak). Persampelan air dan zooplankton telah dilakukan sebanyak lima kali bermula Julai 2009 sehingga April 2011. Parameter fiziko-kimia dalam kajian ini termasuk suhu, kemasinan, pH, oksigen terlarut (DO), permintaan oksigen biokimia (BOD), klorofil-*a*, ammonia-nitrogen dan nitrit-nitrogen. Persampelan zooplankton dilakukan secara menunda net plankton (WP-2). Sejumlah 8 filum dan 37 genus zooplankton telah direkodkan di ketiga-tiga lokasi persampelan tesebut. Filum Arthropoda mendominasi kesemua lokasi dengan kelimpahan relatif 78.80%, di mana Copepoda adalah yang paling dominan. Filum lain seperti Chordata (9.10%), Cyliophora (6.12%), Actinopoda (2.08%), Rotifera (2.57%), Annelida (0.63%), Cnidaria (0.51%), dan Chaetognatha (0.19%) merupakan komposisi yang kecil dalam keseluruhan taburan. Stesen Juru yang mengalami impak aktiviti manusia yang tinggi merekodkan kelimpahan relatif dan Stesen Jerejak (stesen kawalan dan dianggap mengalami kesan aktiviti antropogenik yang rendah). merekodkan kelimpahan relatif terendah. Kepelbagaian zooplankton adalah rendah di semua stesen yang mungkin disebabkan oleh kelimpahan taxa dominan yang tinggi. Fatty acid methyl esters (FAMEs)

dominan yang dikesan dalam plankton terdiri daripada Asid Lemak Tepu; SAFA (C16:0, C14:0 and C18:0), Asid Lemak Mono Tak Tepu; MUFA (C16:0, C14:0 and C18:0), Asid Lemak Poli Tak Tepu; PUFA (C18:2n6c and C20:3n6) dan Asid Lemak Sangat Tak Tepu; HUFA (C22:6n3 atau DHA). Profil kandungan asid lemak mungkin dipengaruhi oleh cara permakanan zooplankton, kepekatan nutrien dan suhu. Dalam kajian ini, walaupun Stesen Juru dianggap mengalami impak aktiviti antropogenik yang tinggi, tetapi kualiti air beransur pulih. Walaupun Stesen Jelutong dan Stesen Jerejak masing-masing mengalami impak yang sederhana dan minima, kedua-dua lokasi itu perlu diberi perhatian oleh pihak berkuasa dan juga orang awam pada masa akan datang. Komuniti zooplankton juga dipengaruhi oleh ketersediaan makanan (fitoplankton) dan aktiviti antropogenik. Walaubagaimanapun, kepekatan kandungan asid lemak tidak hanya dipengaruhi oleh aktiviti antropogenik, tetapi juga kebolehan toleransi oleh sesetengah spesis plankton.

SELECTED WATER QUALITY PARAMETERS, ITS RELATION WITH ZOOPLANKTON COMMUNITY AND FATTY ACID CONTENT AT JELUTONG, PULAU JEREJAK AND KUALA JURU

ABSTRACT

This study was carried out to determine the water quality characteristics of coastal waters around Penang island, and its relation with zooplankton community and fatty acid content. Three sampling stations were selected, namely Kuala Juru (Juru Station), Jelutong (Jelutong Station) and Pulau Jerejak (Jerejak Station). Water and zooplankton samples were collected on five sampling occasions from July 2009 until April 2011. Physico-chemical parameters measured in this study were temperature, salinity, pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), chlorophyll-*a*, ammoniacal-nitrogen and nitrite-nitrogen. Zooplankton samples were collected by horizontal towing with plankton net (WP-2). A total of 8 phyla and 37 zooplankton genera were recorded at those three sampling locations. Phylum Arthropoda dominated the whole locations with 78.80% of relative abundance, where Copepoda was the most abundant. Other phyla which were Chordata (9.10%), Cyliophora (6.12%), Actinopoda (2.08%), Rotifera (2.57%), Annelida (0.63%), Cnidaria (0.51%), and Chaetognatha (0.19%) were accounted in small abundance. Juru Station, which is highly impacted by human activities, had the highest relative abundance and Jerejak Station (control station and considered to have low impact by anthropogenic activities) had the lowest relative abundance. Zooplankton diversity was quite low at all stations, might be due to large abundance of dominant taxa. The dominant fatty acid methyl esters (FAMEs) detected on

plankton consisted of SAFA (C16:0, C14:0 and C18:0), MUFA (C16:0, C14:0 and C18:0), PUFA (C18:2n6c and C20:3n6) and HUFA (C22:6n3 or DHA). The profile of fatty acid content might be affected by grazing behavior of zooplankton, nutrients concentration and temperature. In the present study, although Juru Station was considered as highly impacted by anthropogenic activities, but the water quality is improving. Although Jelutong Station and Jerejak Station had medium and minimal impact, respectively, both locations need attention by authorities and public in future. Zooplankton community was influenced by food availability (phytoplankton) and anthropogenic activities. Fatty acid concentrations however not only impacted by anthropogenic activities, but also depend on the tolerance ability of certain plankton species.

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

The Straits of Malacca has a variety of tropical coastal species and ecosystem, due to its strategic location. It is also known that the Straits of Malacca used to be a home for dugongs, with crystal clear waters and seagrass beds for foods. Many large rivers discharge into Straits of Malacca gives it the characteristics of estuarine environment which rich in primary productivity and biodiversity.

Its ecosystems and habitats offered many valuable goods and services that are consumed and manipulated by human systems. This brings undesirable consequences such as the generation of waste products that are ultimately returned to the natural ecosystems thereby causing habitat destruction and other side effects. According to Integrated Coastal Zone Management (ICZM, 1998), the coastal zone ecosystem is being exploited for various development projects which result in deterioration of habitats and resources. Deforestation, eutrophication and urban pollution are major human impacts that affect the coastal water quality (Pinturier-Geiss *et al*., 2002).

Water quality is the physical, chemical and biological characteristics of a water body. It is used by reference to a set of standards against which compliance can be assessed. The complexity of water quality as a subject is reflected in the many types of measurements of water quality indicators. Some of the simple measurements such as temperature, pH, dissolved oxygen, and conductivity can be made in direct contact with the water source. More complex measurements that must be made in a laboratory setting require water samples to be collected, preserved, and analyzed at another location. Phytoplankton and zooplankton are reported to make excellent water quality indicators as they are sensitive to changes in the environment (Case *et al*., 2008).

The rapid changes in the natural environment in Malaysia were mainly driven by the continued eco-social growth and industrialization, and these changes have resulted in serious negative impacts on the coastal area. The coastal zone of Malaysia experiences intense human activities, where a large percentage of the population, ports, industries, tourism, and sewage discharge result in many conflicting human activities in that region. This wide range of activities contributed to the release of contaminants to the coastal zone which occurs due to man-made sources, and to the natural (physical, chemical, biological and geological) processes (Al-Gahwari, 2003).

Rapid development in Malaysia has resulted in degradation of river ecosystems, due to excessive waste disposal into the rivers. About three-quarters of the pollution entering the ocean is known to have come from human activities from land (Weber, 1993). Carpenter *et al.,* (1998) mentioned that rivers and coastal environment play an important role in assimilating and carrying wastewater from industrial sites and runoff from agricultural farms, roadways and streets. These wastes will contribute to river and marine pollution. Chan (2013) stated that the garbage thrown into the river by public may also contribute to marine pollution, as the river water will flow directly to the sea. On top of that, marine species will be endangered as a result of polluted marine environment (Mansoor and Safia, 2007).

Penang coastal areas have been affected by tremendous major human activities such as urbanization, ecotourism, agriculture, and fisheries. The Department of Environment reported that the marine water quality in Penang is poor and most parameters measured do not meet the Interim Marine Water Quality Standard for Malaysia (DOE, 1998). It is clear that the beaches are not clean anymore except for those which have been utilized for tourists' attraction. Al-Gahwari (2003) stated that the levels of water quality in rivers and coastal waters of Penang have declined. As a result, most of the coastal waters in Penang are not suitable for swimming. This is due to high bacterial levels and high turbidity of the water.

The alterations of marine water quality, focusing on Penang coastal areas in this study, may influence the lipids and fatty acid content in plankton. Lipids are a heterogeneous group of molecules which involves in many vital functions in aquatic organisms (Napolitano *et al*., 1997). Besides that, they also play a role as energy storage for animals and plants. Lipids are important as one of the sources of essential nutrient, vitamins, and act as chemical messenger (Napolitano *et al*., 1997). Fatty acids are a major component of lipid. They are essential component of all living organisms and cell membrane lipids. Fatty acid components of marine lipids are present in a great structural variety, incorporation with the variety of biological diversity of marine life (Napolitano *et al*., 1997).

The characteristic of fatty acid which can be oxidized from monounsaturated fatty alcohols into monounsaturated fatty acids of equal chain length by fish that feed on zooplankton is useful as tracers in food web study (Olsen *et al*., 1993). In addition, Graeve *et al*. (1994) mentioned that the fatty acid composition integrates dietary information over a time scale of weeks to months, thus provides medium to long term information on the pelagic system's trophic interactions (Peters *et al*., 2004).

This present research focused on the assessment of water quality and its effects on the diversity and abundance of zooplankton as well as on their fatty acid contents. In this study, emphasis is placed on zooplankton because it constitutes the foundation of the food web in the aquatic ecosystem (after phytoplankton). Zooplankton represent one of the most direct and profound responses to pollution entering the coastal area. According to Qingyun *et al.* (2007), planktonic organisms show complex and sensitive responses towards changing water quality through the changes in numbers of community levels of the organization. In this case, plankton is considered as a good indicator of water quality. In aquatic environment, zooplankton are found to be globally recognized as pollution indicator organisms besides phytoplankton (Yakubu *et al*., 2000).

Plankton abundance and species composition are sensitive to environmental changes; thus these factors can be used as environmental indicators (APHA, 1992). On top of that, the zooplankton groups' diversity is higher in marine environment compared with the fresh waters. This research was focusing on the assessment of water quality and its effects on the diversity and abundance of zooplankton as well as on their fatty acid contents.

Three stations were selected in this study; Kuala Juru, Jelutong and Jerejak to measure the effects of different types of pollutant sources to the coastal waters. Kuala Juru which is situated near the industrial sites would has been affected by pollutants from the factories. Jelutong which is near the rubbish dumpsite might be affected by the pollutants from the dumpsite itself. Jerejak which is less impacted is considered as the control station in this study.

1.2 OBJECTIVES

(i) To determine the coastal water quality at three (3) locations around Penang Island;

(ii) To assess zooplankton abundance and diversity in the coastal waters around Penang Island;

(iii) To determine fatty acid content of zooplankton samples and how it relates to species dominance; and water quality

CHAPTER 2

LITERATURE REVIEW

Seawater and fresh water are among the most important coastal resources which are utilized as primary resources by many coastal development sectors (Ozhan, 2003). In addition, coastal water is also one of the most sensitive and vulnerable resources, receiving tremendous amount of negative impacts from anthropogenic activities. These impacts may contribute to the degradation of coastal water quality. Deterioration of coastal water quality has been among the earliest coastal concerns which have attracted the initiation of serious management efforts in many locations that have become early examples of coastal management programme.

Coastal water quality can affect the abundance and diversity of the planktonic organisms, including zooplankton. Zooplankton are a group of organisms that lives suspended in the water column. It forms a very important part of the coastal water community. It has very limited mobility and depends largely on the movement via convection or wind induced currents. In almost every habitat (pelagic) of the coastal ecosystem, thousands of these organisms can be found, and due to their small size and simplicity, they are usually capable of occupying large expanses of water and multiplying at an exponential rate. Zooplankton are also known as indicators of aquatic degradation which as a group, they have worldwide distribution; and the species composition as well as community structure are sensitive to environmental condition changes, nutrient enrichment and different levels of pollutions (Arimoro, 2010).

The fatty acid content in zooplankton may act as biomarker and also as water quality indicator. Fatty acid can be described as a carboxylic acid with a long un-branched aliphatic tail (chain), which is either saturated or unsaturated. Carboxylic acids as short as butyric acid (4 carbon atoms) are considered to be fatty acids, while fatty acids derived from natural fats and oils may be assumed to have at least 8 carbons. Most of the natural fatty acids have an even number of carbon atoms, because their biosynthesis involves acetyl-CoA, a coenzyme carrying a two-carbon-atom group (Budge, 2001).

Essential compounds such as essential fatty acids are important and are required for organisms' growth. These compounds cannot be synthesized by many organisms and have to be supplied through their diet. As quoted by Kainz *et al.* (2004), phosphorus (Sterner and Elser, 2002) and essential fatty acid (Arts and Wainmann, 1999) have been identified as two key nutrients that influence food quality, and affect the somatic growth and reproduction of zooplankton. For example, polyunsaturated fatty acids (PUFAs) are required for cell membrane structure maintenance, and are important molecular components in the eyes and brains of the higher trophic organisms in the aquatic and terrestrial ecosystems (Teece *et al.* 2004).

Kainz (2004) reported that in the aquatic environment, primary producers which are mainly phytoplankton, synthesize the essential fatty acid which then be consumed by herbivores and other predators. The essential fatty acid is important in the diets of predators as it cannot be synthesized *de novo* in their bodies. Thus, they have to get it directly from the primary producers or other organisms before them in the food chain. The growth rate of the organisms of higher trophic level might also be affected by the changes of fatty acid content at the lower base of the food chain.

2.1 Coastal Water Quality

Marine pollution can be defined as the introduction of substances or energy that changes the quality of the sea water or affects the physical, chemical, or biological environment into the ocean by humans (Garrison, 2005). Anthropogenic activities are the major causes of the decline in coastal water quality, which in recent years has become a global issue because of its effects on ecosystem functioning (Marshall *et al.,* 2006).

The quality of water is very critical in maintaining a balanced environment. A balanced environment means that the stability and equilibrium among its components are achieved. The human and engineering operations release wastes that may interfere with the quality of water when they decay or dissolve in it (Ntengwe, 2006). Erosion, weathering processes, and leaching from lands produce a variety of contaminants in natural waters but such contaminants can only be reduced by normal water and wastewater treatment processes so that their presence in the particular water source may not limit their use (Nemerow, 1985).

According to DOE in 1998, samples collected from the coastal waters of Malaysia have shown high concentration of oil, grease, and heavy metals. Heavy exploitation, especially in West Coast of Peninsular Malaysia, together with water pollution from coastal development and land reclamation have resulted in the destruction of spawning and nursery grounds and caused changes in the composition of biota in the ecosystem.

Penang's coastal environment is made up of both natural ecosystems and human systems (ICZM, 1998). While Penang's coastal ecosystems still offer potential for economic and social development, there are presently many issues including pollution that might threaten the sustainable use of coastal resources and also contradictory with National and State policies.

The Integrated Coastal Zone Management (ICZM, 1998) reported that the habitats and the ecosystems of Penang's shore are facing some threats. Marine water quality in Penang also has been exceeding the Interim Marine Water Quality Standard in terms of oil and grease, concentration of suspended solids, *E. coli*, heavy metals concentration such as mercury, and lead, to name a few.

Tan (1985) has mentioned that the sewage discharge in Jelutong, Penang has been achieved about 23 million liters of raw sewage per day. The domestic discharges might be the main contributors to the degradation of coastal water quality, which can be indicated by high bacterial count, high biochemical oxygen demand, high suspended solids, low oxygen levels, and high nutrient concentration.

As mentioned in Penang State Government report in 1997, industrial wastes give impacts on coastal waters; for example turbidity. The untreated sewage or treated sewage discharge with high loadings can be assumed as the most common sources of contamination which result in the degradation of water quality due to high organic and bacterial loads (DOE, 1999). Aquaculture also gives impact on coastal waters. According to Al-Gahwari (2003), aquaculture if conducted on large scale at a single site, can cause eutrophication, reduces visual quality, and also interfering with other uses of the sea. The impacts usually involve reduction in dissolved oxygen and increase of ammonia concentration (Fridley, 1995).

2.2 Physico-chemical parameters

2.2.1 Dissolved oxygen

Dissolved oxygen (DO) can be defined as a measurement of the amount of oxygen that is measured in milligrams or millimeters dissolved in one liter of water (Prashant *et al.*, 2009). Smith (2004) mentioned that DO in warmer water has lower saturation point compared with in cooler water. Besides, water with higher velocity can hold more DO than slower moving water The DO content in natural waters can be affected by salinity, pressure, photosynthetic activity, temperature and turbulence. In the tropics, the rate of decomposition is high at the bottom of a water body; hence the production of oxygen through photosynthesis at the surface, is less than oxygen consumption (Makhlough, 2008).

2.2.2 Temperature

Heat from atmosphere, sediments and terrestrial may influence the temperature of the coastal waters. Heat enters the sea water through the surface, and then the water is heated by radiation from the sun and by the conduction from the atmosphere. Large sea surface heating and weak surface wind resulted in higher temperature at sea surface (Yanagi et al., 2001). Human activities such as direct discharge of hot effluents into the ocean can thus contribute to the raising of temperature of water. Temperature can affect the dissolved oxygen content of the sea (Boyd, 1990), where high temperature of sea water hold much lower level of dissolved oxygen.

2.2.3 pH

pH of water is the amount of hydrogen ions (H^+) that is present in water. The water is considered pure when it reaches the pH 7, which means it is neutral. This happens when each water molecule dissociates then yield one hydrogen ion (H^+) and one hydroxyl ion (OH^-). The present of carbonic acids (H_2CO_3) from rain, organic acids and mineral acids from decompositions and acidic salts in water induces the acidity of the water (Ahmad and Ahmad, 1994). Besides, dimethylsulphide released by bacteria and algae can react actively with water, thus produce hydrogen ions which then lowers the pH of water (Chien, 2008).

2.2.4 Biological Oxygen Demand (BOD)

According to ReVelle and ReVelle (1988), biological oxygen demand (BOD) is the amount of oxygen that would be consumed if all the organic materials in one litre of water were oxidized by bacteria and protozoa. Organic materials are the rich food supplies for bacteria which occur naturally in water. Those organic matters then will be decomposed by the bacteria. When this happens, much of the oxygen present in the water will be used by the aerobic bacteria, robbing other aquatic organisms of the oxygen that they need for living. When BOD levels are high, the dissolved oxygen (DO) may decreases as the oxygen has been used during the respiration of the organic materials. The great reduction of oxygen may threaten the lives of other aquatic organism, and causes high level of BOD.

2.2.5 Salinity

Salinity is the quantity or concentration of inorganic solid which is dissolved in water. The ocean’s salinity varies depending on factors such as evaporation, precipitation, rainfall and also freshwater runoff from continents. Most of the dissolved solids in seawater are salts, which are separated into ions, and sodium and chloride are the most abundant (Garrison, 2005).

2.2.6 Turbidity

Turbidity in water indicates the presence of suspended matter that scatters and absorbs the light. In nature, the majority of suspended matter is usually inorganic, but sometimes consists of organic substances. However, the variety of sources, size and character of the suspended matter means that the measurement of turbidity also gives the indication of pollution. The measurement also considered important as it can describe how the water looks to the observer. For example, the water may have low concentration of suspended solids, but if the solids are colloidal, the turbidity may be high (Pallan, 2010).

2.3 Nutrients

Nutrients are essential for phytoplankton to reproduce, survive and grow, but the excessive input of nutrients into water increase the algal growth which contribute to eutrophication (Schindler, 2006).

2.3.1 Ammonia-nitrogen (NH_3-N) and nitrite-nitrogen (NO_2-N)

According to Cech (2003), ammonia (NH_3) can be described as inorganic substance in water column and soil, which is released by decaying plant tissue and animal waste. With low dissolved oxygen in water, the soil bacteria; *Nitrosomonas* will oxidize the ammonia to nitrite ($N0_2$). Nitrification occurred where nitrite is oxidized to nitrate (NO_3) by *Nitrobacter* bacteria (Cech, 2003). When ammonia level reaches 0.1 mg/L, the surface water is considered polluted, while if the ammonia level increases to 0.2 mg/L, the water body is in high toxicity, thus considered to be unsafe for aquatic life

2.3.2 Chlorophyll-*a*

The measurement of chlorophyll is important to determine the algae biomass, thus important in monitoring water quality. Chlorophyll is a green substance in cell pigments in almost all plants, algae, and also cyanobacteria. Chlorophyll allows plants to absorb sun light in the blue and red portions of the electromagnetic spectrum. Chlorophyll *a* is one of the major pigments in plants; it has very strong absorbance bands in the spectrum's visible regions. The natural fluorescence of chlorophyll *a* which is stimulated by the sun is known to be a good detector of phytoplankton (Fischer and Kronfeld, 1990). The accurate estimation of chlorophyll *a* is important in monitoring the health of ecosystem (Asadpour *et al*., 2011). The increasing concentration of nutrient loads in seawater reflects by the chlorophyll *a* concentration, which can have severe damaging effects on the marine environment (Jamshidi and Bakar, 2011).

2.4 Zooplankton

Zooplankton are organisms which can float and drift following the water movement (Arvin, 1978). Planktonic animals are usually transparent, but are little colored due to pigmentation on particular organs. Planktonic animals are generally small; the majority of the organisms have dimensions of the order of centimeters or millimeters (Garrison, 2005). The freshwater forms of zooplankton are smaller in size than of marine counterparts, as the marine zooplankton are represented by vast animal phyla. This includes various protozoa, rotifera, cladocera, copepoda, ostracoda and also meroplankton such as insect larvae (Parsons, 1980).

They are classified by size or developmental stage, as shown in Table 2.1.

Zooplankton Categories	Size
Picoplankton	Less than 2 μm
Nanoplankton	2 - 20 μm
Microplankton	20 - 200 μm
Mesoplankton	0.2 - 2 mm
Macroplankton	2 - 20 cm
Megaplankton	0ver 200 mm

Table 2.1 Zooplankton categories according to size (MarineBio.org, 2012)

Classification according to the developmental stage consists of meroplankton and holoplankton. Meroplankton are larvae that eventually change into worms, mollusks, crustaceans, coral, fishes, echinoderms or insects during adults. Holoplankton will remain as plankton for their entire life cycle. They include; pteropods, chaetognaths, larvaceans, siphonophores and copepods (MarineBio.org, 2012). The most dominant zooplankton groups are the copepods, which are crustaceans, the group that also includes crabs, lobsters, and shrimps (Garrison, 2005).

Zooplankton are heterotrophic organisms feeding on the primary producers, phytoplankton (Garrison, 2005). Zooplankton constitute the foundation of the food web, after the phytoplankton in aquatic ecosystems. Zooplankton are used as biological indicators of water quality, pollution levels and eutrophication (Sibel, 2006). Zooplankton are also found to have greater species diversity compared to phytoplankton, which the composition varies strongly with seasonal production of meroplankton. The distribution of zooplankton within coastal water bodies might be controlled by resources and predation (Pinnel-Alloul et al., 1996).

2.5 Zooplankton composition and abundance

A slight change in the concentration and composition of zooplankton might show a subtle change of environment. Zooplankton are highly responsive to factors such as nutrient levels, temperatures, food availability, pollution, light intensities, predation, pH levels and heavy metals. Nitrogen and phosphorus are types of nutrients that will affect the prey of zooplankton which are most commonly consist of algae, protozoa, and bacteria. This is indirectly affecting the survival of zooplankton. Besides playing an important role in the marine food chain, the diversity of species, the abundance of communities and the amount of biomass of zooplankton can be used to determine the health of an ecosystem (MarineBio.org, 2012).

The most dominant zooplankton species are the copepods, accounting for about 70% of the total individuals (Garrison, 2005). Aminu and Ahmed (2000) also mentioned that copepods dominate the zooplankton community in most aquatic environments. Several factors can affect the abundance, diversity and distribution of plankton in an ecosystem, which has detrimental effects on the rest of the ecosystem. Zooplankton biomass, abundance and species diversity are useful for determining the conditions of the aquatic environment (MBO, 2007). Planktonic organisms can exhibit sensitive responses to the environmental stimuli, which can be shown by the changes of individuals, population and community levels (Qingyun *et. al.*, 2007).

Zooplankton is one of the most important sources of food for many species (Guy, 1992), thus, they are important constituent in aquatic food web. In light of this, starvation will occur if there is a lack of plankton in a particular environment. Higher temperature increase growth and multiplication of the zooplankton. The amount of available nutrients in the environment also plays a part in the distribution and density of zooplankton. High density of zooplankton can be attributed to the wastes accumulation at a particular area, which may then enhance the growth of phytoplankton that supports the zooplankton community (Davies and Otene, 2009).

According to Din (1993) based on his research around Langkawi Island, the diversity, abundance, and distribution of zooplankton may be influenced by physical parameters such as temperature and salinity; both are negatively correlated to zooplankton biomass. As with almost all marine environments, the study also found copepods to be the most abundant group. Plankton functions might be influenced by pollutants as the pollutants mostly occur on the surface. Plankton communities were found to exhibit cyclical stability, which means that their compositions varied depending on light, temperature and availability of nutrient (Noble *et al*., 2003). Thus, it can be considered that the factors that affect the zooplankton composition and abundance including environmental factors such as temperature, pH, nutrient availability, dissolved oxygen, total suspended solid (TSS), and also the predatory factors.

2.6 The fatty acid

Fatty acid is an organic compound consisting of a hydrocarbon and a terminal carboxyl group. Chain length ranges from one hydrogen atom to nearly 30 carbon atoms. Long-chain fatty acids (more than 8-10 carbon atoms) most commonly occur as constituents of certain lipids, notably glycerides, phospholipids, sterols and waxes which are esterified with alcohol. These long-chain fatty acids generally have an even number of carbon atoms which means that unbranched chains predominate over the branched chains (Daintith, 2004).

Fatty acids may be saturated with no double bond (palmitic acid), or unsaturated with a double bond (oleic acid), or two or more double bonds in which case they are called polyunsaturated fatty acid (linoleic acid). The physical properties of fatty acid are determined by the chain length, degree of unsaturation, and also chain branching. Short-chain acids are pungent liquids, and soluble in water. As the chain length increased, melting points are raised and water solubility decreases. Unsaturation and chain branching tend to lower the melting points (Daintith, 2004).

2.7 The fatty acids and zooplankton

Lipids are important in the marine environment, constituting a significant part of the total carbon flux through the trophic levels. According to Serrazanetti (1994), lipid constituents in organisms of Adriatic Sea provides information on the distribution of these compounds in the trophic chain, and also can acts as indicator of eutrophication. Lipids are known to be used widely in biogeochemical and ecological studies. They can be used to assess the health of ecosystems by measuring the degree to which they have been influenced by terrestrial and anthropogenic inputs. They also can be used to determine the biogenic material production of dietary value to marine organisms. Lipids are also act as a solvent and absorption carrier for organic contaminants. Thus, they can be drivers of pollutant bioaccumulation (Parrish, 2013). Fatty acids constitute the main part of the lipids in aquatic organisms, and many of the universally important fatty acids are only synthesized *de novo* by phytoplankton, which then be transferred to zooplankton and other consumers (Neal, *et al*., 1986).

Analysis of lipids and fatty acids has been possible since the 1950s, following the introduction of thin-layer chromatographic and gas-chromatographic methods (Reuss and Poulsen, 2002). The use of fatty acid composition as a reliable method for tracing the food source through multiple food web linkages has been applied by the conservative transfer of tracer fatty acids from phytoplankton to copepods. A useful biomarker must be synthesized at low trophic levels and remain unchanged when transferred to higher trophic levels (Napolitano *et al*., 1997).

Fatty acids are particularly useful biomarkers since they are essential components of all living cells and display a high structural diversity with higher level taxonomic specificity. Besides, gas chromatography (GC) particularly have led to the use of lipids such as fatty acids, sterols and hydrocarbons as important tools to explain about trophic relationships, and also in the detection of sources of organic matter in seawaters and sediments (Napolitano *et al.*, 1997). Fatty acids can be used to distinguish bacteria, phytoplankton classes and zooplankton orders in marine samples (Bergé and Barnathan, 2005). The long-chain polyunsaturated fatty acids are especially useful, since these fatty acids cannot be synthesized *de novo* in sufficient amounts by the predators.

The fatty acid composition in plankton not only depends on the species composition, but also on environmental factors. Zulikha in 2006 reported that the environmental factors such as light, temperature and nutrient availability, and also the physiological stage of the plankton also influence the fatty acid composition. They also mentioned that the environmental temperature is one of the factors which responsible for the differences in fatty acid level between marine and fresh water animals. Mayzaud *et al.,* (1990) reported that the fatty acid compositions of phytoplankton populations were subjected to nutrient supply.

According to Lee *et al.,* (2006), the principal storage lipids in marine organisms are tryacylglycerols (TAG) and wax esters (WE), in which a sufficient amount of those compounds is important to ensure the survival of individuals during the non-reproductive season. Various studies of the biochemical composition of calanoid copepods have shown that lipids, especially wax ester components provide important energy stores for many species of organisms (Sargent and Handerson, 1986).

In another study, Tucker (1992) reported that zooplankton is rich in the essential fatty acid (EFA), which are docosahexacnoic acid (DHA) and also eicosapentaenoic acid (EPA). Zooplankton gets its essential fatty acid by ingesting phytoplankton which is the producer in aquatic environments. The high abundance of EPA which its lipid name is 20:5 (n-3), is reported to be a characteristic for diatoms, but not suitable to be considered as a trophic marker because it is also a part of biomembranes in all organisms (Hagen et al., 1995).

In recent years, several studies on zooplankton lipids and models of simplified food chains in the natural environment have been carried out. An early study of a natural temperate plankton community was provided by Jeffries (1970). Since the 1980s, studies on natural plankton communities have been performed in different parts of the world including the temperate, Arctic and Antarctic regions (Reuss and Poulsen, 2002). The differences in fatty acid content and composition within the zooplankton community may be associated with the organism’s trophic position (Budge *et al*., 2001).

According to Kaitaranta (1986), the available information concerning the lipid and fatty acid compositions in both zooplankton and phytoplankton is mostly based on well-characterized natural population, as well as on composite samples with few dominant species. Another study done by Norrbin (1990) showed that lipid storage energy might be influenced by their body sizes. It is assumed that the smaller the species, the faster the depletion of the reserves as their higher metabolic rate and the storage space limitation, compared to the larger size zooplankton.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Sites

3.1.1 General Information about Penang Environment

Penang Island is situated on the northwestern coast of Peninsular Malaysia, located between latitudes 5º 7' N and 5º 35' N and longitudes 100º 9' E and 100º 32' E. It is bounded to the North and East by the State of Kedah, to the South by the State of Perak and to the West by the Straits of Malacca and Sumatra, Indonesia. Penang State is made up of two separate physical entities which are Penang Island that covers an area of 293 square kilometers and Seberang Perai on the mainland covers an area of 738 square kilometers (ICZM, 1998).

The climate of Penang is influenced by minor monsoonal characteristics, while the climate of Peninsular Malaysia is characterized by tropical monsoons, high temperature and humidity, and heavy rainfall. Mean air temperature in Penang is 27°C throughout the year (Chan, 1991). Penang Island is mostly dominated by granite rocks in the hill and towards the coast. Penang also has two types of beaches; sandy beaches which are dominated by mangrove-fringed mud flats, and also rocky beaches which are limited areas (Al-Gahwari, 2003).

3.1.2 Coastal Areas of Penang

The Penang coastal areas have been under pressure from sea transportation, oil industries, fisheries and also from sewerage system that carry raw sewage and then discharge it into estuary. This caused damage on environment as well as the degradation of coastal water quality (Syahreza *et al*., 2011).

Three sampling locations namely Kuala Juru (N 05 186', E 100 24.114'), Jelutong (N 05 23.176', E 100 19.293'), and Pulau Jerejak (N 05 18.067, E 100 18.702) were identified for this study. These locations were chosen based on the different anthropogenic activities on shore, and were visited on five sampling occasions on July 2009, January, April, and June 2010, and April 2011.

3.1.3 Kuala Juru

Kuala Juru is in the vicinity of the Prai Industrial Zone and has been receiving tremendous amount of organic wastes through Sungai Juru, Seberang Perai. Previous studies (Yap and Tan, 2008; Lim and Kiu, 1995) reported that, besides domestic and industrial wastes, Juru River Basin is polluted by discharges from the pig farms nearby. Total length of Sungai Juru is estimated to be about 15.62 km. Three major tributaries which discharge into the Kuala Juru, then to the Straits of Malacca are Sungai Ara, Sungai Kilang Ubi and Sungai Pasir, including a drain; Parit 4. Sungai Juru has been also identified as one of the most polluted rivers in Malaysia, and also considered as a ‘dying river’ because the catchments are made up of industrial and residential area. In 2006, total population within the catchments is estimated to be 362 400 (Toriman *et al*., 2011).

3.1.4 Jelutong

Jelutong on the other hand is associated with urban activities; the selected location has also been receiving substantial amounts of organic wastes, particularly in the form of urban sewage.

3.1.5 Pulau Jerejak

Pulau Jerejak is a 362 hectare island which located off the southeastern tip of Penang. It was formerly the main leper asylum for The Straits Settlements (1868), Quarantine Station (1875) and penal colony (1969). Jerejak Station is considered least polluted in comparison with the other two stations and as such can be regarded as a control station.