UNIVERSITI PUTRA MALAYSIA

ISOLATION AND CHARACTERISATION OF MICROPLASTICS IN WHITECHEEK SHARK (Carcharhinus dussumieri) AND PALE-EDGED STINGRAY (Dasyatis zugei) COLLECTED FROM MALAYSIA WATER

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BY,

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Thesis submitted infulfillmentt of the requirement for the degree of Bachelor Science (Environmental and Occupational Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

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ABSTRACT

ISOLATION AND CHARACTERISATION OF MICROPLASTICS IN WHITECHEEK SHARK (*Carcharhinus dussumieri*) AND PALE-EDGED STINGRAY (*Dasyatis zugei*) COLLECTED FROM MALAYSIA WATER

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Introduction: Nearly 150 million tons of plastic debris present in the ocean and are estimated to increase in the future. Microplastics were found in various marine organisms such as zooplankton, mussels, clams and fish.There were many research regarding ingestion of microplastics in fish but no study was done on Malaysian marine vertebrate organisms. **Objectives:** To compare microplastics (MPs) loads between whitecheek shark (*Carcharhinus dussumieri*) and pale-edged stingray (*Dasyatis zugei*). **Methodology:** Fish dissection conducted to isolated internal organ. Intestinal organ for each sample were digested in 10% potassium hydroxide (KOH). The digested solution then vacuum filtered using 149 µm filter paper. The filter paper had been soaks with sodium iodide and centrifuge for density separation. Then, the supernatant filtered using 8 µm filter paper and being observed using microscope to find MPs and determine it morphology. For characterization steps, micro-Raman microscopy used to identify plastics polymer composition and additives composition. **Results and Discussion**: Only an additive particle (phthalocyanine) found in this study. There were no significant different in all parameters (plastic polymer, morphology and additives) between two species. A factor possible no MPs found in this study due to *Carcharhinus dussumieri* and *Dasyatis zugei* had excreted MPs or due it feeding behaviour. Another possible factor is the technical limitation because 149µm filter membrane used in filtering the digested intestinal material. There were possible loss MPs sized less than 149 µm. There also possibility of no MPs pollution at study area. **Conclusion:** For now, *C. dussumieri and D. zugei* from Endau-Rompin coastal area not polluted with microplastics. So, *the C. dussumieri* and *D. zugei* from Malaysian water probably safe to be consumed by human or to use in other by-products.

Keywords: Microplastics, Isolation, Shark, Ray, Raman Spectroscopy.

ABSTRAK

PENGASINGAN DAN PENCIRIAN MICROPLASTIK DALAM YU MULUT BESAR (*Carcharhinus dussumieri*) DAN PARI KETUKA (*Dasyatis zugei*) DIKUMPULKAN DARI PERAIRAN MALAYSIA

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Pengenalan: Hampir 150 juta tan plastik di dalam lautan dan dianggarkan meningkat pada masa hadapan. Microplastik ditemui dalam pelbagai organisma laut seperti zooplankton, kupang, kerang dan ikan.Telah banyak penyelidikan mengenai microplastik didalam ikan tetapi tiada kajian yang telah dilakukan pada organisma laut bertulang belakang di Malaysia. **Objektif**: Untuk membandingkan kandungan microplastik antara Yu mulut besar (*Carcharhinus dussumieri*) dan Pari Ketuka (*Dasyatis zugei*). **Metodologi**: Ikan dibedah untuk mendapatkan organ-organ dalaman. Organ-organ dalam untuk setiap sampel telah dicerna dalam 10% kalium. Selepas pencernaan, organ-organ tercerna divakum dan ditapis menggunakan kertas turas 149μm. Kertas turas kemudian direndam dengan natrium iodida dan hempar untuk pemisahan ketumpatan. Larutan tersebut kemudian akan ditapis menggunakan kertas turas 8μm dan dengan menggunakan mikroskop untuk melihat morfologi mikroplastik yang dijumpai. Untuk langkah pencirian, mikro-Raman spetroskopik digunakan untuk mengenal pasti komposisi polimer plastik dan komposisi bahan tambahan. **Keputusan dan Perbincangan:** Hanya satu zarah bahan tambahan iaitu phthalocyanine dijumpai di kajian ini. Tidak ada perbezaan yang signifikan dalam semua parameter (polimer plastik, morfologi dan bahan tambahan) untuk kedua-dua spesies. Faktor penyebab tiada mikroplastik ditemui kerana *Carcharhinus dussumieri* dan *Dasyatis zugei* telah mengkumuhkan mikroplastik atau tabiat pemakanan. Satu lagi faktor mikroplastik tidak dijumpai di dalam kajian ini kerana had teknikal kerana menggunakan kertas turas 49μm digunakan dalam penapisan bahan usus dicerna. Terdapat kemungkinan kehilangan mikroplasrik bersaiz kurang dari 149 μm. Terdapat juga kemungkinan tiada pencemaran microplastics di kawasan kajian. **Kesimpulan**: Buat masa ini, *C. dussumieri* dan *D. zugei* dari kawasan pantai Endau-Rompin tidak tercemar dengan microplastik. Jadi, *C. dussumieri* dan *D. zugei* dari perairan Malaysia mungkin selamat untuk dimakan oleh manusia atau untuk digunakan dalam produk ikan.

Kata kunci: Microplastik, Pengasingan, Yu, Pari, Raman spektroskopi.

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LIST OF ABBREVIATIONS

KOH	Potassium hydroxide
LDPE	Low-density polyethylene
MPMA	Malaysian Plastics Manufacturer Association
MPs	Microplastics
NaI	Sodium iodide
PE	Polyethylene
PP	Polypropylene
PVC	Polyvinyl chloride
PS	Polystyrene
PUR	Polyurethanes
PET	Polyethylene terephthalate
PCBs	polychlorinated biphenyls

CHAPTER 1

INTRODUCTION

1.1 Background

Plastics are synthetic or semi-synthetic organic polymer that had been used in various ways such as textile industries, packaging and wide range of applications in any field related (Helmenstine, 2014; PlasticsEurope, 2014). Due to plastics' characteristics which is inexpensive, high durability and a versatile material, plastics have become an important part of our modern life. Andrady & Neal (2009) and Lechner et al. (2014) estimated that the global plastic production had increased from 1.7 million tonnes per year (1950s) to nearly 300 million tonnes per year in 2013. Knight (2012) mentioned that plastic debris is now present in the ocean and it was estimated that 150 million tonnes of the plastic debris had polluted the ocean and the amount keep increasing yearly. Reach et al. (2014) claimed that the plastic pollution in the ocean and the sea was caused by the plastic debris that originated from land which was carried by the streams and rivers due to urban runoff. For example, the sources of plastics pollution in the sea and ocean are gear lost such as fishing net and trawl due to fishing activities (Tschernij & Larsson, 2003) and accidental dumping of marine traffic (Moore, 2008).

In Malaysia, it was estimated that average growth of plastics manufacturing over 11 years is 15% (National Solid Waste Management Department, 2011). Plastics manufacturing also provide supply to certain demands such as automotive components, construction material and houseware product (TATA Strategic Management Group, 2014). Malaysian Plastics Manufacturers Association (2010) reported that about 2 million tonnes of resins in the plastics industry are produced in Malaysia every year. Frankson (2015) reported that Malaysia was listed in the top 20 country which had a problem regarding the mass of mismanaged plastic waste in 2010. These plastic debris was not only found in larger forms (macroplastics), but also in smaller forms that are known as the microplastics (MPs), which is defined as plastics particles sized between 1-1000 μm (Karami et al., 2016). Microplastics are usually found in the form of bead (de Sáet al., 2015; Lusher et al., 2012), film (Lusher et al., 2013; Mathalon & Hill, 2014; Davidson & Dudas, 2016), foam (Lusher et al., 2016: Rochman et al., 2015), and fiber (Li et al., 2015; Davidson & Dudas, 2016), fragments (von Moos et al., 2012; Li et al., 2015; Davidson & Dudas, 2016). Previous studies also showed that the microplastics can bioaccumulate inside the marine organism such fish (Foekema et al., 2013), bivalves (Li et al., 2015) and zooplanktons (Desforges et al., 2015). Microplastics can transfer accidentally from lower trophic level to higher trophic level. A study showed that a crab consumed mussels or clams contaminated with MPs may become an evidence of transfer of MPs from lower trophic level to higher trophic level and possibly to human too (Farell & Nelson, 2013; Watts et al., 2014).

1.2 Problem Statement

In Malaysia, fish is one of the main sources of protein. In 2006, Malaysian per capita consumption of fish was 150.4 g per day (Food and Agriculture Organization (FAO) UN Fisheries & Aquaculture, 2009). The number of shark which had been caught had increased from 4,140 tonnes in 1990 and had reached the highest catch of 8,695 tonnes in 2003. Next, National Plan of Action for the Management of Fishing Capacity in Malaysia had reported increment of rays caught; from 6,000 tonnes to 19,253 tonnes in 2003 (Department of Fishery Malaysia, 2006). Both whitecheeck shark (*Carcharhinus dussumieri*) and pale-edged stingray (*Dasyatis zugei*) known as predators and a keystone species in the marine ecosystem. Both species were available in Western Malaysia sea region.

The cleaning process of fish by fishmonger such as cleaning of the fish gut had become the concern of this study. There are some cases where the cleaning process of the internal organs of fish were incomplete and improperly cleanse. This process can contributed to the transfer of microplastics from the internal organ into the tissue of the fish (Moss et al., 2012) and causing possible consumption of microplastics to human. However, the effect is still a question left unanswered as there are limited publications on these. Globally, there are many research were conducted regarding the ingestion of microplastics by marine organisms, but no study was conducted in Malaysia especially in marine vertebrate organisms.

1.3 Study Justification

This is the first study being conducted to investigate microplastics contamination on *Carcharhinus dussumieri* and *Dasyatis zugei* (marine vertebrates). Shark and stingray have been generally used as a vertebrate model to assess the quality of aquatic systems as the bio-indicators of environmental contamination. Therefore, the finding may be useful to generate baseline data for future research.

1.4 Objectives

1.4.1 General Objective

To investigate microplastics loads in Whitecheek shark (*Carcharhinus dussumieri*) and Pale-edged stingray (*Dasyatis zugei*).

1.4.2 Specific Objectives

1.4.2.1 To compare the morphology of the isolated particles in the internal organs between *Carcharhinus dussumieri* and *Dasyatis zugei*.

1.4.2.2 To compare the number of plastics polymer composition of the isolated particles in the internal organs between *Carcharhinus dussumieri* and *Dasyatis zugei*.

1.4.2.3 To compare the number of isolated additive particles in the internal organs between *Carcharhinus dussumieri* and *Dasyatis zugei*.

1.5 Hypothesis

1.5.1 There is a significant difference in the morphology of isolated particles in the internal organs between *Carcharhinus dussumieri* and *Dasyatis zugei.*

1.5.2 There is a significant difference on the number of isolated plastic polymer particles between *Carcharhinus dussumieri* and *Dasyatis zugei.*

1.5.3 There is a significant difference on the number of isolated additive particles between *Carcharhinus dussumieri* and *Dasyatis zugei*

1.6 Conceptual framework

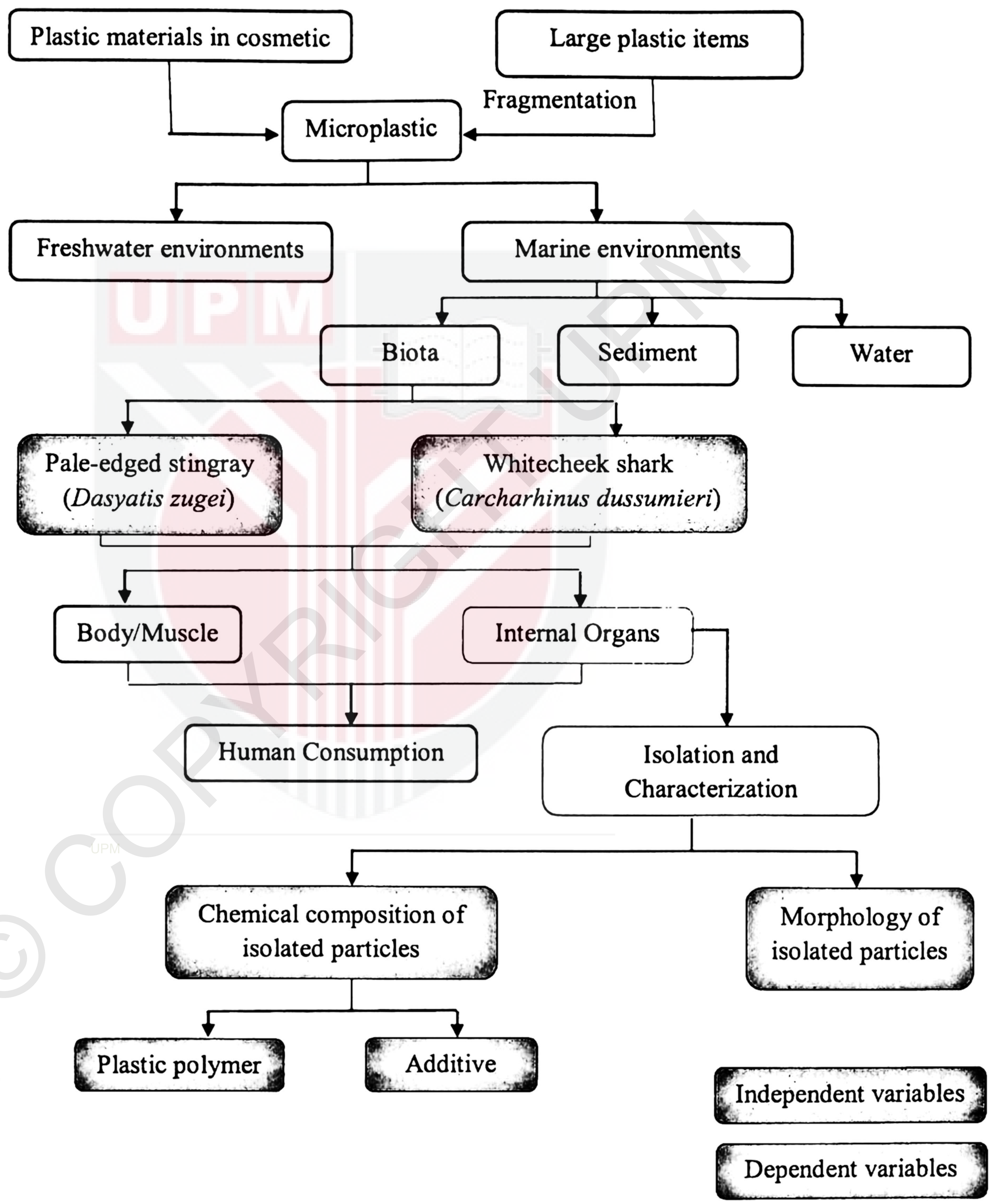

Figure 1.1: Conceptual framework on microplastics pathways from environment to human.

CHAPTER 2

LITERATURE REVIEW

2.1 Microplastics (MPs)

Microplastics (MPs) are the plastic particles with sized between 1-1000 μm (Karami et al., 2016). Microplastics presence in marine environment had increased in number due to degradation of a single plastic debris to millions particles of MPs (Cozar et al., 2014). There were two types of MPs which was differentiated based on their origin or source (Wright et al., 2013; Duis & Coors, 2016).

First, the primary MPs enter the environment through direct input in the form of micro-sized particles such as microbeads in the facial cleansers and clothing fiber that were able to pass through the wastewater treatment and ended up into the sea and ocean (Cole et al., 2011; Law & Thompson, 2014). Microbeads are excessively used in toothpaste, cosmetic product and personal health care product as an exfoliating agent (Danny, 2015). The function of microbeads in cosmetic and personal health care is to absorb excessive oil and remove dead skin. However, this ability is a concern as it able to absorb environmental pollutant such as persistent organic pollutant (Ng & Obbard, 2016). Till now, only a few countries such as Canada, United Kingdom, Ireland and United State of America had banned the use of microbeads in cosmetic products.

Next type is secondary MPs. This type of microplastics originated from large plastic items such as plastic packaging, balloon, fishing and aquaculture gears which entered to the environment through runoff, rivers, tides, winds or disaster events then goes under environmental degradation into smaller particles involved ultraviolet (UV) ray exposure (Andrady, 2011; Rios et al., 2007). In land, the degradation of large plastics items in to smaller plastics were faster compared to in the water. The fragmentation of plastics is slower in the sea due to the reduced ultra violet exposure and low water temperature (Barnes et al., 2009; Ryan et al., 2009).

Based on the previous study, there are five major forms of microplastics found in environment; bead (Davidson & Dudas, 2016; de Sá et al., 2015; Lusher et al., 2012), film (Lusher et al., 2013; Mathalon and Hill, 2014), foam (Lusher et al., 2016: Rochman et al., 2015), fiber (Davidson & Dudas, 2016; Li et al., 2015) and fragment (Davidson & Dudas, 2016; Li et al., 2015; von Moos et al., 2012).

2.1.1 Distribution of Microplastics (MPs)

Now, microplastics had been found around the globe. Cozar et al. (2014) had estimated that 7000 to 35,000 tonnes of plastic is floating on the open sea. Microplastics had been found at Northern Pacific Gyre (Rios et al., 2010), surface waters (Cozar et al., 2014) and shorelines (Brown et al., 2011).Various studies had shown that a few countries at Southeast Asia marine ecosystem had been contaminated with MPs. Ng & Obbard (2006) mentioned that microplastics had been found in Singapore's coastal marine environment. Meanwhile, a study conducted by Cordova & Wahyudi (2016) showed that MPs had found in the deep sea sediment of South-western Sumatran waters.

In Malaysia, MPs had been found in both studies conducted in West and East Malaysia's marine invertebrate and environment (Yusof et al., 2016; Noik, 2016). Although there are a few studies conducted in Malaysia shoreline and marine organism such as corneous ark (*Scapharca cornea*) but there is no study had been conducted on MPs contamination to the vertebrate marine organisms.

2.1.2 Microplastics in marine organisms.

Microplastics not only found in marine environment but also had been found in marine biota. Various studies concluded that MPs can be ingested by marine biota such as seals (Bravo Rebolledo et al., 2013), zooplankton (Desforges et al., 2015), mussels (Browne et al., 2008), clams (Davidson & Dudas, 2016), shellfish (Browne et al., 2008) and fish (Lusher et al., 2012; Foekema et al., 2013) which can transfer to human through consumption of certain marine organisms. The previous study conducted by Li et al. (2015) showed that about 60% of fingerprint oyster (*Alectryonella plicatula*) were present with MPs. Microplastics also found in higher frequency about 33% in cod fish from the English Channel (Edwin et al., 2016). In Malaysia, a study showed that there was existence of MPs in *Scapharca cornea* conducted in Terengganu (Yusof et al., 2016). In a study conducted by Alomar & Deudero (2017) had showed that MPs had been ingested by sharks (*Galeus melastomus*). Microplastics had also been found in Manta rays due to its feeding behaviour (Mattison, 2017).

2.1.3 Effects of microplastics

Microplastics can cause chemical and physical effect to organisms especially when they ingested MPs. Microplastics might become a health concern since MPs are persistent and have the abilities to absorb a persistent organic pollutant and act as a vector or transfer medium for the toxic substance to marine organisms such as pesticides and polychlorinated biphenyls (PCBs) (Engler, 2012; Ng & Obbard, 2016). Microplastics have a larger surface area with properties of hydrophobic that make them to easily attracted to waterborne contaminants that may cause toxicity to organisms (van Franeker et al., 2011; Bakir et al., 2012). A study conducted by Karami et al. (2016) showed that even the virgin low-density polyethylene (LDPE) fragments can cause toxicity and give the adverse impact of phenanthrene (Phe) to *C. gariepinus*.

Microplastics can transport to higher trophic organisms such as human (Farell & Nelson, 2013). Watts et al. (2014) stated that MPs can be transferred from mussels to crabs through food chain which increases the concern for MPs to reach higher trophic levels.

2.1.4 Bioavaibility of microplastics

Wright et al. (2013) and Desforges et al. (2015) mentioned that bioavailability of marine organisms are one of the primary environmental risks that associated with MPs. Four factors that affected the bioavaibility of MPs claimed by Wright et al. (2013) were size, abundance, colour and density. Microplastics were known as smaller size plastic which make it easily digested by lower trophic levels of the marine organism. Continuous fragmentation of large plastics items are causing the abundance of plastics particles that are available for marine organism ingestion (Browne et al., 2007, 2008; Thompson et al., 2009). The colour of microplastics playing an important role that make it bioavailable to marine organisms. For example, a study conducted by Carpenter et al. (1972), fish from the Niantic Bay area, New England had consumed only opaque, white polystyrene spherules. The density of MPs determined the organism that consumed microplastics at different water column level. For example, planktivores, filter feeders and suspension feeders living at higher water column level mostly consumed low-density plastics, such as polyethylene (PE) on the surface water.

2.1.5 Trophic transfer of microplastics

Several studies conducted to show how MPs can be transferred in a food web chain. In the lower trophic level, zooplankton had consumed MPs that were available in the marine environment as food (Desforges et al., 2015). A study conducted by Setälä et al. (2014) showed that zooplankton was able to consume MPs and can be transferred into planktonic food web (zooplankton to mysid shrimp). A study conducted by Watts et al. (2014) showed that common shore crab (*Carcinus maenas*) had consumed mussel (*Mytilus edulis*) that's contaminated with MPs. Surprisingly, after consuming their prey, the MPs still remain in the common shore crab's tissue and internal organs (Farrell & Nelson, 2013; Watts et al., 2014). This is an evidence that MPs can be transferred from lower trophic organisms to higher trophic organism.

Small organism such as small fish and prawn (Devriese et al., 2015) usually consumed zooplankton as their diet. Both marine organisms can incidentally consume MPs as they confused or mistaken MPs with their food (zooplankton) (Desforges et al., 2015). Consumption of zooplankton contaminated with MPs among small fish and prawn is another possible pathway of MPs transfer to higher tropic level. Small fish, crab, and shrimp usually eaten by higher trophic level organisms such shark and stingray (Bennet & Kyne, 2003; White, 2006).

Eriksson & Burton (2003) mentioned that the presence of MPs in the scat of fur seals (*Arctocephalus sp.)* possibly through consumption of lantern fish (*Electrona subaspera*) that had been contaminated with MPs. An experiment conducted by

Murray & Cowie (2011) showed that the presence of microplastics in Norway lobsters (*Nephrops norvegicus*) stomach after 24 hours fed with pieces of fish seeded with strands of polypropylene.

There is no study conducted on how MPs can be transferred to human, however, possible pathway MPs transfer of human traffic is through transfer by consuming contaminated seafood. Direct transfer to human by consuming contaminated with MPs marine organisms such as fish, mussel and crabs, and lobster. A previous study conducted by Van Cauwenberghe & Janssen (2014) showed that bivalves cultured for human consumption contaminated with MPs. A study conducted by Tanaka & Takada (2016) showed that presence of MPs in digestive system of Japanese anchovy which is a concern as anchovy usually consumed together with their digestive system. Estimated that worldwide consumption of fish and seafood such as shellfish is 137 grams per day (Food and Agriculture Organization (FAO) UN Fisheries & Aquaculture, 2016) in which consuming fish and seafood is a possible major route for trophic transfer of MPs to human.

2.2 Plastic polymer

Generally, plastics are man-made polymers from the polymerisation of monomers obtained from crude oil, natural gas and some from charcoal or latex from trees. PlasticsEurope (2013) mention that six most common plastic polymers produced worldwide in 2012 were full name (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), polyurethanes (PUR) and polyethylene terephthalate (PET). Polyethylene is plastic polymer that made up from natural gasses such as methane that been modified by polymerisation process. Globally, Piringer and Baner (2008) estimated that 80 million tonnes PE produced per year. Polyethylene came in low and high-density form know as high-density polyethylene (HDPE) and low-density polyethylene (LDPE). Polypropylene is a thermoplastic polymer used in several applications including packaging and labeling, textiles, plastic parts and reusable containers of various types. In 2013, the global market for PP estimated up to 55 million tonnes (Kuehner, n.d).

The PVC had been used to produce pipe, as the electrical insulator on electrical cable, clothing, and furniture (PlasticEurope, n.d). Polystyrene is a synthetic aromatic polymer made from the monomer styrene. Polystyrene also became an issue to environmentalist as this polymer degrade slowly when it released to environment and abundant in Pacific Ocean (Kwon et al., 2014). Polyurethanes were invented by Professor Dr. Otto Bayer in the 1930s. Globally, Avar (2008) estimated that 12 million metric tonnes raw PUR used in 2007 and will increase 5% every year. Polyethylene terephthalate consists of ethylene glycol and dimethyl terephthalate. More than 60% world's production of PET used for synthetic fibers and bottle production accounting for about 30% of global demand (Ji, 2003).

2.3 Plastics additives

Additives are complex blend of material added to plastic polymer in a way to improve the performance and characteristics of plastic function. Additives had to change the basics polymer into useful, safer, tougher and colourful plastics (Blackwell Plastics, 2010). Some of the additives that are used in the industry are flame retardant, plasticisers, colourants, lubricants and flow promoters.

2.3.1 Flame Retardant

The function of flame retardant added in plastics production is to prevent ignition or spread of flame in plastic material. Flame retardants are added to plastics to meet fire safety standards either by mandatory regulation or standard set by a company itself to show their product quality. Plastics that are used in critical construction, electrical and transport applications usually added with flame retardant. Usually, chemicals such as antimony trioxide and chlorinated paraffin were added in polyethylene and similar resins as flame retardants (Blackwell Plastics, 2010; Law et al., 2003).

2.3.2 Plasticisers

Plasticiser was added to change physical properties of the product such as to increase softness and flexibility and to decrease the cold flex temperature. Plasticisers also added to lower the melt viscosity. A variety of polymers such as polyvinyl acetate, acrylic polymers, cellulose acetate and PVC were added to the plastics as plasticisers. (Blackwell Plastics, 2010; Law et al., 2003).

2.3.3 Colourants

Colorant used as additives to add colour and patterns to plastics products (Blackwell Plastics, 2010). Basically, four methods used for colouring polymers such as surface coating, surface dyeing, an introduction of colour-forming groups into the polymer molecules and mass colouration. Colorants were divided into two classes (insoluble colorants (pigments) and soluble colorants (dyestuffs) (Law et al., 2003). One of the most used colourants is phthalocyanine blue due to heat resistancy when exposed to high temperature up to 260°C during process to become other products and due to high tint strength (Charvat, 2005).

2.3.4 Lubricants and flow promoter

Lubricants are used to improve the processability of plastics by increasing the flowability, especially moulding process. Internal lubricants is function to lower the viscosity and heat dissipation which improved the melt flow of material (Blackwell Plastics, 2010). Lubricant added in plastics polymer to reduce friction of moulding and end products when contacts different or same material compositions. Graphite and molybdenum disulphide used in nylons and other thermoplastics used in gear and bearing applications (Law et al., 2003).

2.4 Shark and stingray

2.4.1 Whitecheeck shark (*Carcharhinus dussumieri*)

Whitecheeck shark (*Carcharhinus dussumieri*) is small and common inshore shark that usually found on continental shelves and inshore slopes around islands. This shark widely distributed on Indo-West Pacific sea distribution in coastal waters down to 170 m (Bennet & Kyne, 2003). *Carcharhinus dussumieri* also found at other country coastal water such as Singapore, Thailand and Japan (Compagno, 2006).

Sometimes whitecheeck shark (*Carcharhinus dussumieri*) is mistaken with the blackspot shark (*Carcharhinus sealei*) due to the similar body characteristic. The *Carcharhinus dussumieri* diet mostly based on the fish. This species also included the octopus, squid, and various crustaceans including crabs in their diet (Bennet & Kyne, 2003). The size of matured male of this species can reached up to 74 cm and matured female is 71 cm. The size of young *Carcharhinus dussumieri* is usually 38 cm.

This species usually marketed for human consumption. The shark meat usually utilised as fresh meat and some were processed as salted fish. In Mukah (Sarawak, Malaysia), the sharks are caught mainly by gill nets and shark meat usually eaten raw. The small, unused discarded part and cartilage of *Carcharhinus dussumieri* were non-edible and unsuitable for human consumption are sold to fish mill factories for fertilizers and animal feeder (Bennet & Kyne, 2003; Department of Fishery Malaysia, 2006).

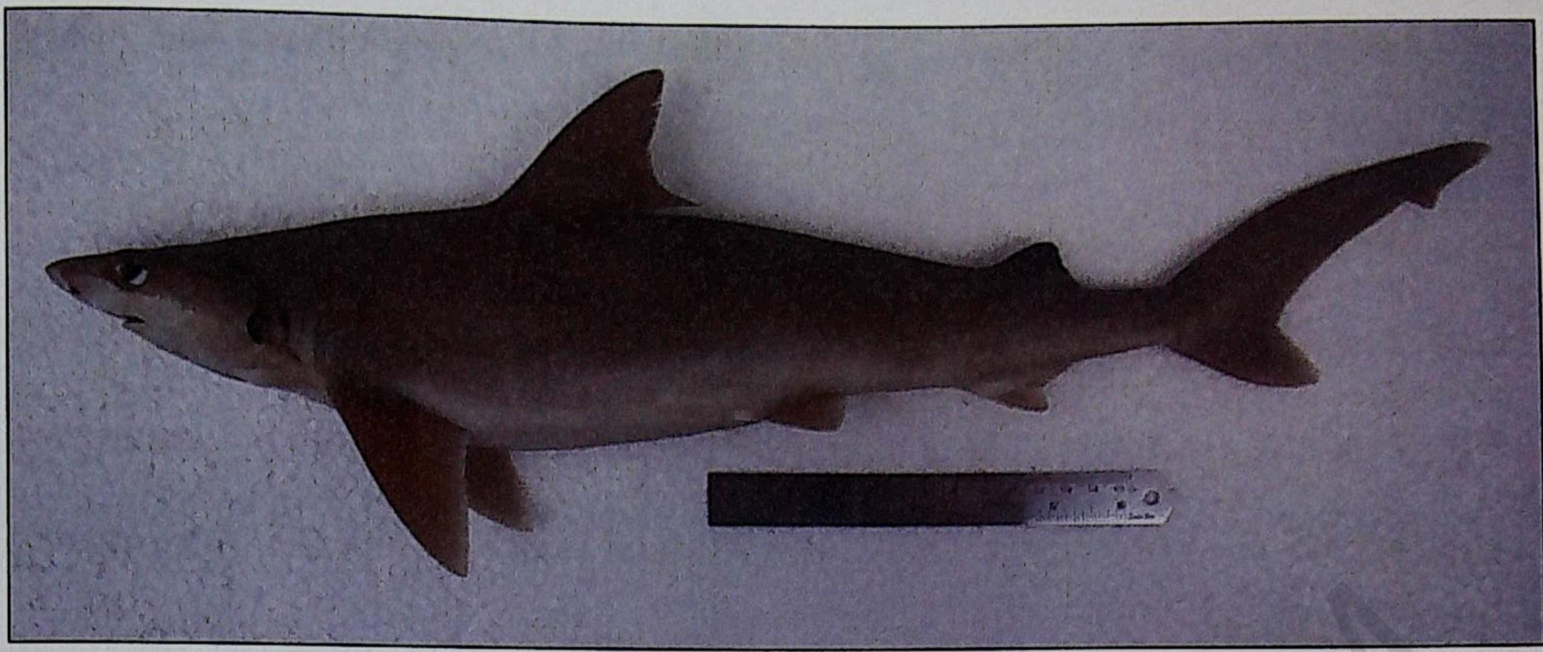

Figure 2.1: Whitecheeck shark (*Carcharhinus dussumieri*).

2.4.2 Pale-edged stingray (*Dasyati zugei*)

Pale-edged stingray (*Dasyati zugei*) habitat is at the inner continental shelf (water under 100 m deep) which favouring shallower, over flat and sandy substratum. The range distribution of pale-edged stingray extended from subcontinent eastward to Java and Borneo. This species also found in Japan, Malaysia, Myanmar, Philippines, Sri Lanka, Taiwan, China and Thailand and Vietnam (White, 2006).

In a study conducted by White (2006) mentioned that the diet of *Dasyati zugei* mainly based on bottom-dwelling crustaceans, certain prawns, and small prey such as small fishes. *Dasyati zugei* same like other stingray species, in which *Dasyati zugei* is aplacental viviparous. Initially, the young *Dasyati zugei* sustained by yolk and then the mother secreted the histotroph that later consumed by the stingray babies. Usually, the females give birth 1 to 3 stingray babies at a time. Sexually matured male usually sized at 18 cm (disc width) and females at 19 cm.

Dasyati zugei usually landed in large quantities as by-catch. This species usually caught incidentally in the bottom trawl and trammel fisheries. Although most *Dasyati zugei* landed were retained for human consumption, the value of this stingray is limited due to small size. *Dasyati zugei* meat usually consumed fresh either cooked or smoked and also can be salted (Department of Fishery Malaysia, 2006; White, 2006).

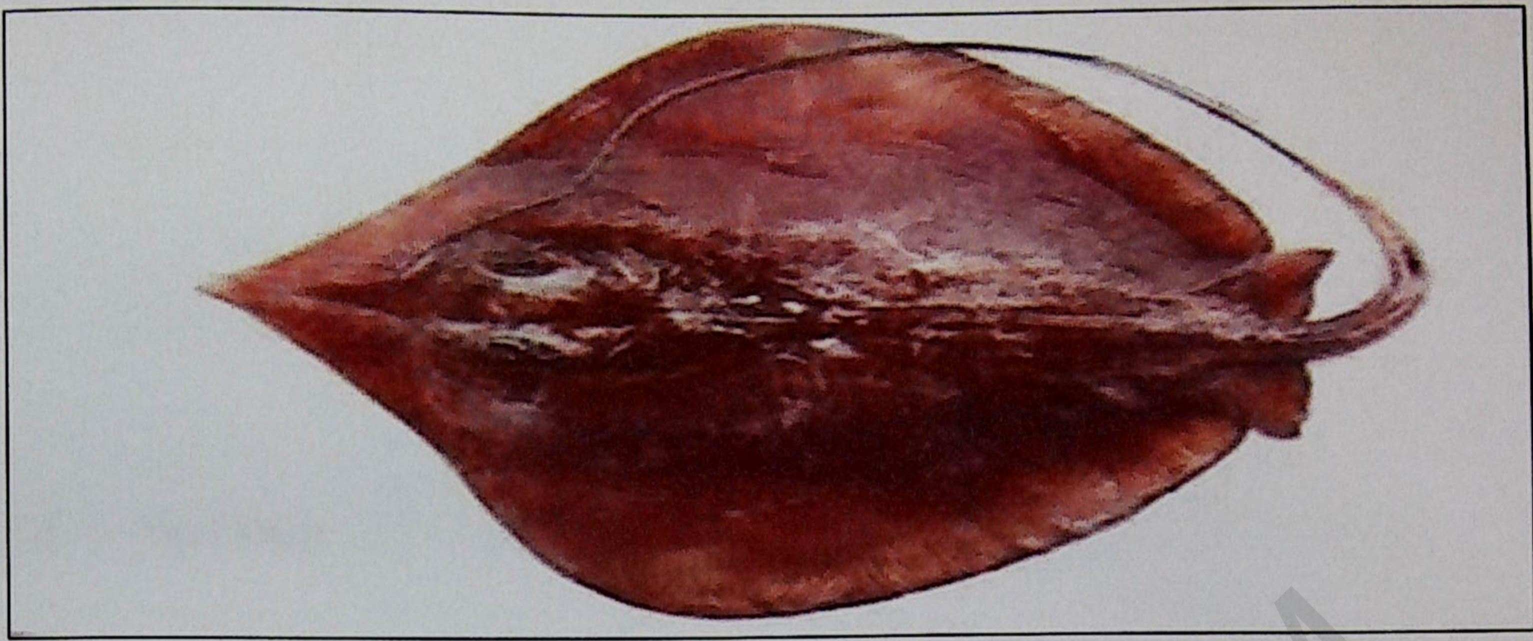

Figure 2.2: Pale-edged stingray (*Dasyati zugei*).

CHAPTER 3

MATERIALS AND METHODS

3.1 Specimen

This study was conducted by using Whitecheeck shark (*Carcharhinus dussumieri*) and Pale-edged stingray (*Dasyatis zugei*). All of the specimen collected by the fisherman up to 50 kilometers from Endau-Rompin, Pahang Darul Makmur fishing landing area in South China Sea region. Both *Carcharhinus dussumieri* (n=11) and *Dasyatis zugei* (n=15) randomly sampled with the total of 26 sample collected and stored in ice box then transferred into the freezer until the isolation and characterisation step conducted.

3.2 Specimen sampling area

Endau-Rompin coastal area is located in two different states of Malaysia between Pahang and Johor which connected with the South China Sea. The distance between from shore to catching zone is up to 50 km. This study chosen due to near recreational area (Tioman Island) and high fishing activity area.

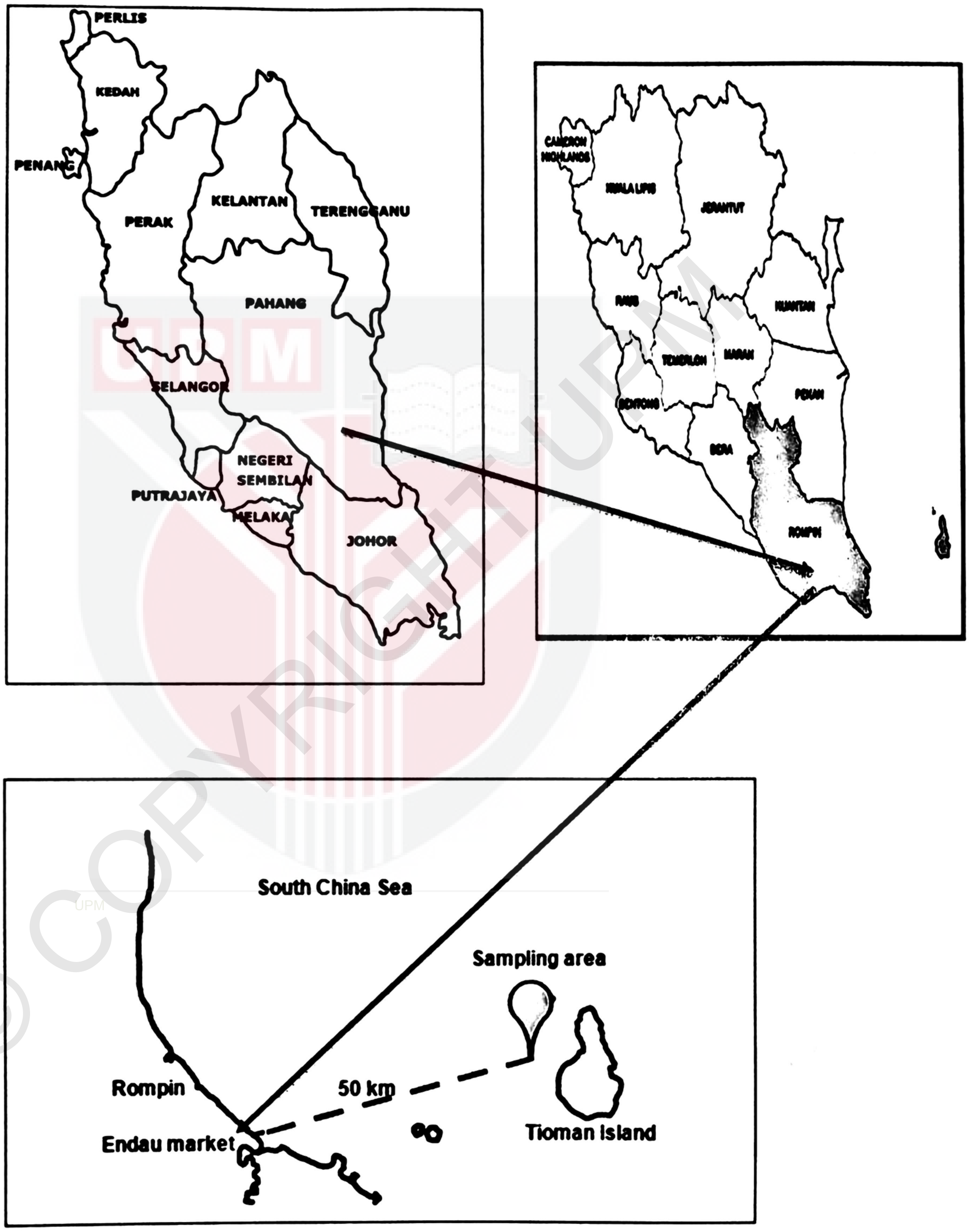

Figure 3.1: Location sample collected at Endau-Rompin coastal.

3.3 Chemicals and materials

3.3.1 Chemicals

Sodium iodide (NaI), potassium hydroxide (KOH) and ethanol 95% were supplied by R&M Chemicals (UK).

3.3.2 Filter membranes

Filter membranes No. 540 (hardened ashless, pore size 8 µm) and GF/D microfiber filter papers (pore size 2.7 µm) were purchased from Whatman and the 149 µm filter membranes were supplied by Spectrum Laboratories (USA).

3.3.3 Instruments or equipment

The following instruments and equipment were used during the study. 250 mL DURAN glass bottles were supplied by Schott (Germany). The vacuum pump (Gast vacuum pump, DOA-P504-BN, USA) was connected to filter funnel manifold (Pall Corporation, USA). Microscope (Motiz SMZ-140 Stereomicroscope) was supplied by Motic (China). Camera apparatus used was AxioCam, ERc 5S (Germany). Micro-Raman spectroscopy used was Horiba LabRam HR Evolution.

3.4 Chemical and solution preparation.

Seven hundred mL of ethanol 95% (R&M Chemicals, UK) was added with 300 mL ultrafine-purified water to produced 1000 mL of 70% ethanol. Ethanol 70% were used to clean apparatus and working area in this study.

The ratio to produce potassium hydroxide 10% solution is 1:10 in which 200 g potassium hydroxide (R&M Chemicals, UK) added in a beaker. After that then the ultrafine-purified distilled water added until reach the 2000 mL.

A 100 mL sodium iodide (NaI) 4.4M solution prepared by adding 65.95 g of NaI powder (R&M Chemicals, UK) into 50 mL beaker and diluted using ultrafine-purified water. Next, the solution was pour in 100 mL conical flask and ultrafine-purified added in until 100 mL.

3.5 Sample dissection

First, the both shark and stingray had been weigh using an analytical scale and the length of the specimen measured using a ruler. Before the dissection process started, the sample were rinsed with 95% ethanol then ultra-purified distilled water to prevent contamination from the environment. All dissection apparatus set such as scalpel, scissor and forceps were washed with 70% ethanol. After that, a scalpel used to make a slit cut on specimen anal and then scissor used to make an opening on the specimen abdomen. The internal organs such as kidney, liver, lung, stomach and intestine were taken and put into 250 mL DURAN glass bottle (Schott, Germany). Next, potassium hydroxide 10% solution was added into the bottle at proportion of 1 gram of internal organs: 10 mL potassium hydroxide 10% solution. The dissection process was conducted in the laminar cabinet as precaution step from exposure environmental contamination.

3.6 Isolation and identification of microplastics

The extraction of MPs from the digestive system was performed according to the protocol of Karami et al. (2016). All internal organs for each sample were digested in 10% potassium hydroxide (KOH) at 40 °C for 72 h. Digestion where considered complete when it is clear and no visible viscera or intestinal material was remained. The digested solution then vacuum using filter membrane pore size 149 μm (Spectrum Laboratories, USA) by Gast vacuum pump (DOA-P504-BN, USA). The filter paper then had to be soaked with sodium iodide 4.4 M for density separation. The sample was centrifuged for 2 min at 500 rpm. Then the supernatant filtered using Whatman filter membrane No. 540 (hardened ashless, pore size 8 μm) and microplastics-like particles morphology were observe using Motic SMZ-140 Stereomicroscope (Motic, China) and the selected particles were photographed using a camera apparatus (AxioCam, ERc 5S, Germany).

The morphology of microplastics were divided into 5 different type based on certain characteristics such as foam is lightweight particles with spongy texture. Second, fragments is jagged and irregular shape particles which often have an uneven surface. Third, fibers or filaments known with thin, straight and cylindrical particles. Fourth, films characteristics is thin plane of flimsy particles. Lastly, beads characteristic is rounded particles (Free et al., 2014).

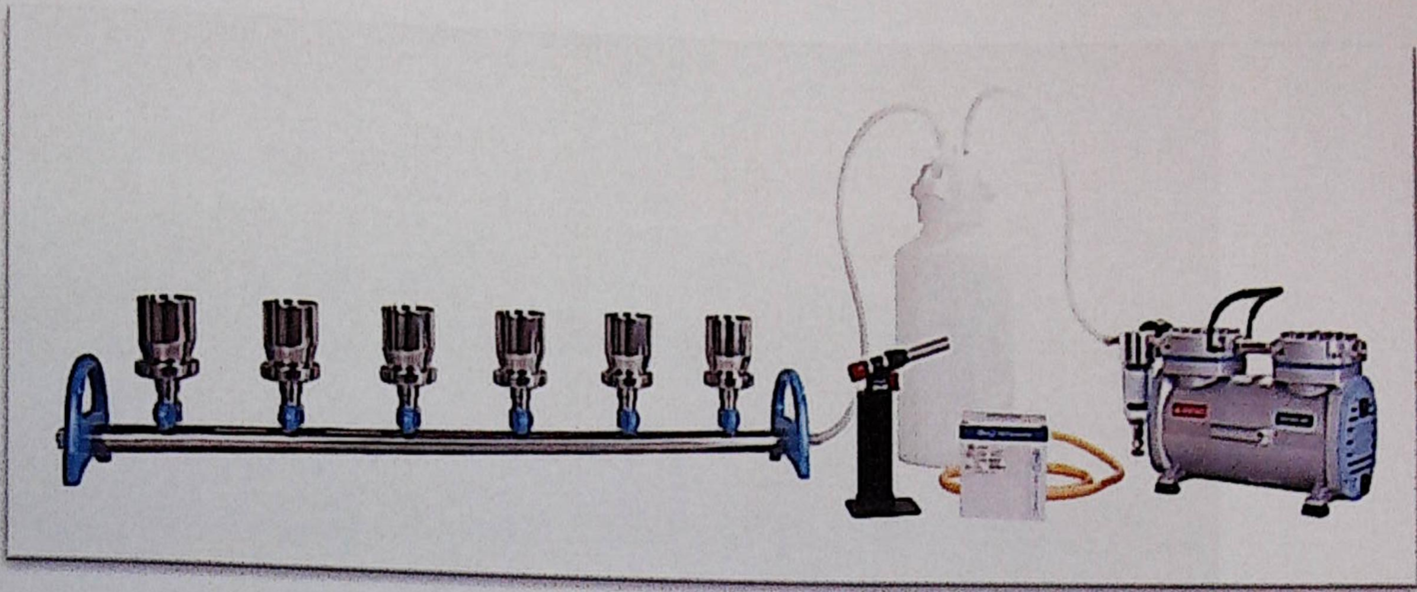

Figure 3.2: Multiple vacuum filtration

3.7 Characterisation of microplastics

Microplastics obtained from the extraction were undergoing for characterisation process to identify MPs chemical composition (polymer type and additive). Raman spectrometer (Horiba LabRam HR Evolution) equipped with a Single Mode Open Beam laser Diode (Innovative Photonic Solutions) used to confirm the microplastics chemical composition. Micro-Raman spectroscopy (Figure 3.3) is a spectroscopic technique used to observe vibrational, rotational, and other low-frequency modes in a system. The sample particle was shot under the beam of laser with wavelength of 785 μm and producing the spectrum graph which the data were compared with polymers spectral library for the plastics identification

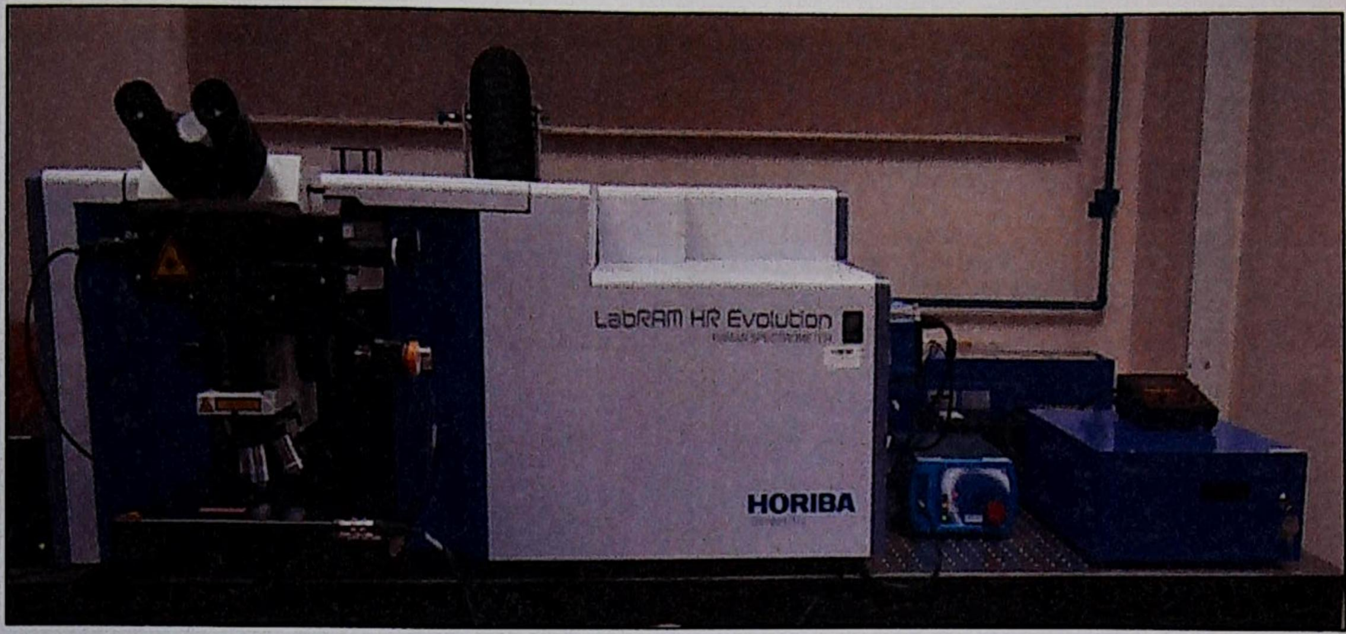

Figure 3.3: Raman spectrometer (Horiba LabRam HR Evolution).

3.8 Statistical analysis

The data analyse using SPSS software version 22 Statistical Package for Social Science) and descriptive statistics be computed by the Statistic software (V.10, Analytical software, USA). T-test (parametric) or Mann-Whitney U test (nonparametric) used to compare MP loads between *Carcharhinus dussumieri* and *Dasyatis zugei* for microplastic morphology and chemical composition (polymer and additive). The results with $p < 0.05$ was considered statistically significant.

3.9 Procedural blank and contamination prevention

In this study, to prevent contamination, all procedure in this study conducted in horizontal laminar flow cabinet. Before experiment was conducted, the working area was cleaned by mopping the floor and the surface of working area was cleaned thoroughly using 70% ethanol. Cotton lab coat, nitrile glove and face masks were worn for entire experiment process. Then, all the solvent such as ethanol were filtered with filter paper with 2.7 µm pore size filter paper. All the glassware used in this experiment was washed with a commercial dishwashing liquid, then rinsed with ultrafine-purified distilled water, and finally rinsed with ethanol and dried in an oven. Last but not least, the procedure blank without tissues sample were included together for the entire experiment.

3.10 Ethics statement

This research study is exempted from the Ethics Committee of Universiti Putra Malaysia due to justification of no involvement of human subject.

CHAPTER 4

RESULT

After characterisation of particles found in both *Carcharhinus dussumieri* and *Dasyatis zugei*. Figure 4.1 showed that only an additive (phthalocyanine) particle found in one of *Carcharhinus dussumieri* replicate (9.1%). The additive found had been confirmed as phthalocyanine. The phthalocyanine morphology type found is fragment. The statistical analysis had been ran on morphology and additives using Man-Whitney U test as data was not normally distributed. In Figure 4.2, there is no particle found in all (100%) *Dasyatis zugei* replicate.

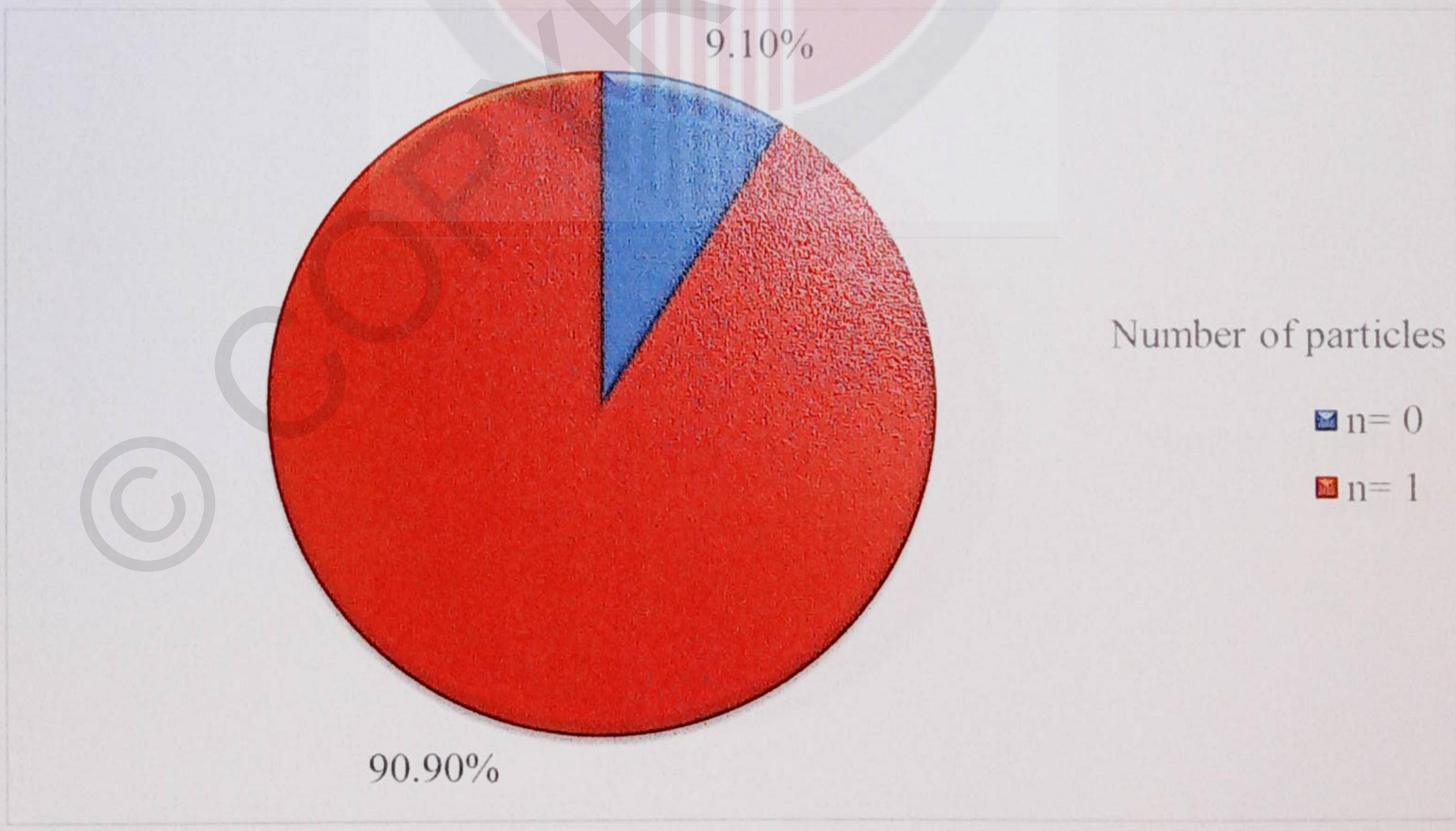

Figure 4.1: The percentage of additive (phthalocyanine) in Whitecheeck shark (*Carcharhinus dussumieri*).

Figure 4.2: A particle (phthalocyanine) found in a sample of *Carcharhinus dussumieri*.

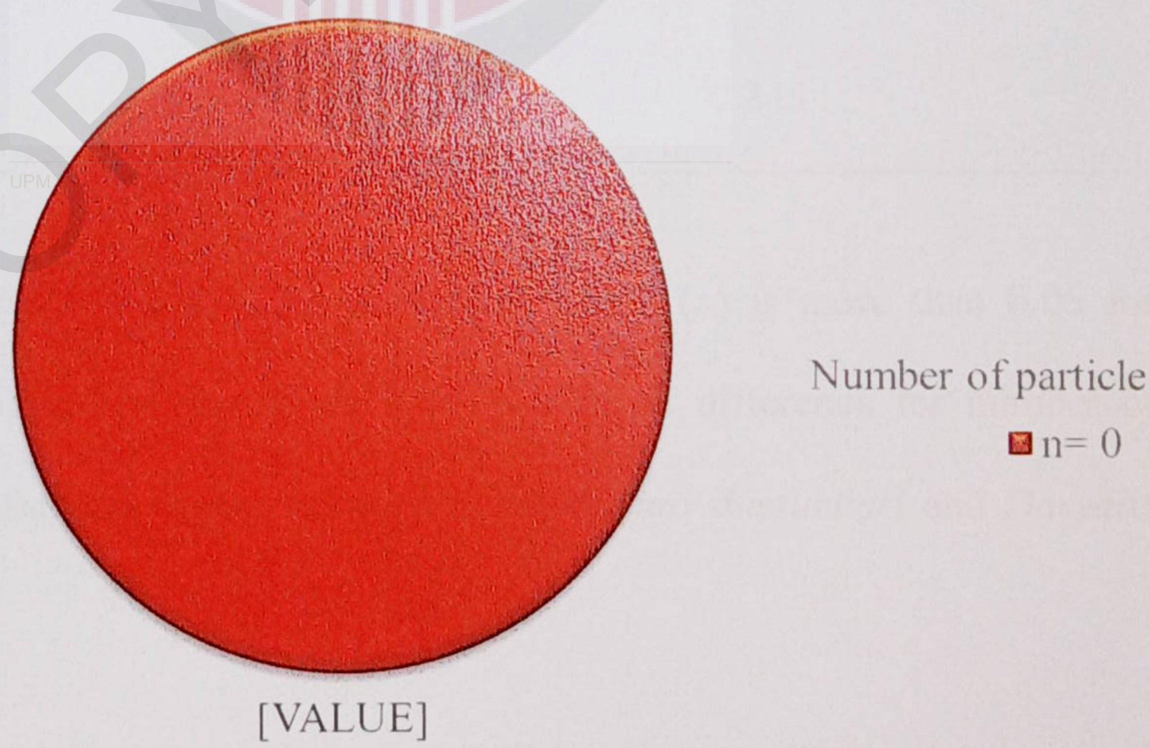

Figure 4.3: The percentage of additive (phthalocyanine) in Pale-edged stingray (*Dasyatis zugei*).

Table 4.1: Comparison of particle morphology between *Carcharhinus dussumieri* and *Dasyatis zugei*

Variable	***z*-value**	***p*-value**
Morphology		
Fragment	-1.168	0.243

From the result table 4.1, the probability value (*p*) is more than 0.05 for particle morphology (fragment), so the result is no significant different for morphology isolated particle between *Carcharhinus dussumieri* and *Dasyatis zugei*.

Table 4.2: Comparison of additive between *Carcharhinus dussumieri* and *Dasyatis zugei*

Variable	***z*-value**	***p*-value**
Additives		
Phthalocyanine	- 1.168	0.243

From the result table 4.2, the probability value (*p*) is more than 0.05 for additive (phthalocyanine), so the result is no significant difference for number of additves isolated in internal organs between *Carcharhinus dussumieri* and *Dasyatis zugei*.

CHAPTER 5

DISCUSSION

Microplastics (MPs) had been found alot around the worldwide marine environment. There is even a study conducted nearby Malaysia; for example, Ng & Obbard (2006) showed the prevalence of microplastics in Singapore coastal water and two studies conducted in Malaysia. One study conducted on the presence of micro debris on Sarawak beach and another study conducted on the presence of microplastics in corneous ark (*Scapharca cornea*) at Terengganu (Yusof et al., 2016). The Endau-Rompin costal area is near to the recreational area such as Tioman island and one of the active fisheries activities zone where the previous study showed that both factors can contribute to marine debris pollution (Miljødirektoratet, 2014; Tschernij & Larsson, 2003). Although the previous study had shown presence of microplastics near to the study location and is an available source of plastics pollution, but the findings for this study showed that no significant difference in microplastics variables such as plastics polymer composition, morphology and additives between *Carcharhinus dussumieri* and *Dasyatis zugei* as there was only a particle found in this study.

The presence of phthalocyanine from isolated a particle in *Carcharhinus dussumieri* internal organs sample was possible evidence of presence of microplastics in marine organisms. The phthalocyanine is usually used as synthetic pigment in the ceramics, textile and excessively in plastics industry (Charvat, 2005).

However, the presence of phthalocyanine with strong Raman signal make the identification of the plastic particle composition difficult (Karami et al., 2017). Karami et al. (2017) mentioned that the strong Raman signal from the pigment could alter the polymer spectra and hinder comparisons with the reference library. There is also a possibility that phthalocyanine found is not microplastics from marine debris pollution but originated from other contamination such as corroded paint because phthalocyanine used as colourant for the industrial paint (Lomax, 2005). However, the particle found not had similar mechanical properties with paint particles such as brittleness when tested during optical examination (Imhof et al. 2016).

The absent of microplastics particle found in this study is could be due to the technical limitation because 149 μm filter membrane was used during filteration of the digested intestinal material. There were possible loss of MPs sized less than 149 μm because a study conducted by Foekema et al. (2013) had found microplastics particle with a size of 0.04 mm (40 μm). Another factor that contributed to the absent of microplastics in the study was due to eating behavior of *Carcharhinus dussumieri* and *Dasyatis zugei*. Both species catching their food by hunting and attack the prey compared to the filter feeder fish in which they hunt their predator by straining suspended matter and food particles from water by passing the water over a specialized filtering structure which easy consumed of microplastics that suspended in marine environment (Garrison, 2013).

Another possible factor can contribute to loss of microplastics is both *Carcharhinus dussumieri* and *Dasyatis zugei* is because both sampled species had excreted their waste matter causing loss of microplastics during excretion. Both *Carcharhinus dussumieri* and *Dasyatis zugei* living at different coastal zone level area. *Carcharhinus dussumieri* is a pelagic organism type which living near to shore or coastal surface but *Dasyatis zugei* is demersal organism type that lives at the seabed. Although living in different sea zone level, the similarity for both species habit is that they live near to shallow water area known as continental shelf which had less than 100 meter depths (Pinnet, 2003). Similarities for both species habitat at shelf sea is evidence for absent of microplastics at Endau-Rompin coastal as this study had used the different type of organism aquatic layer (pelagic and demersal zone) to find the presence of microplastics that occupy different niches. The hypothesis aligns with the facts that microplastics polymer have a different density from low density (PE and PP) to high density (PVC, PUR, PS and PET) plastics polymer and will present at different coastal water column. If MPs are present at the pelagic or demersal zone, thus, MPs should also been found in the *Carcharhinus dussumieri* or *Dasyatis zugei*. Although presence of possible microplastics source is due to tourism and recreational activities, the MPs are not found probably because less or no pollution occurred in study area (Derraik, 2002).

Microplastics need be concerned as they can cause various impacts either to animal or human. A previous study showed that microplastics can end up to human through trophic transfer. Microplastics can transported to higher trophic organisms such as human (Farell & Nelson, 2013). Watts et al. (2014) stated that MPs can be transferred from mussels to crabs through food chain which increases the concern for MPs to reach higher tropic levels. For example, small fish or crabs had consumed MPs as their food. Then small fish or crabs polluted with MPs eaten by the larger predator such as *Carcharhinus dussumieri* and *Dasyatis zugei* and MPS were remain in the intestine (Farell, 2013). Microplastics can be transferred either directly or indirectly to human from the unthroughly cleaning process of the fish internal organs. Usually, consumer did not clean the fish guts and internal organs properly or directly smoked or salted the shark and rays. In Mukah (Sarawak, Malaysia), shark meat usually eaten raw. Another possible pathway of transfer of MPs from lower to higher trophic level is through the fish meal or fertilizer. The small, unused discarded part and cartilage of *Carcharhinus dussumieri* were non-edible and unsuitable for consumption are sold to fish mill factories for fertilizers (Department of Fishery Malaysia, 2006). Fish usually used as a formulation for livestock feeder. The fish meal made up from the 'trash fish' meaning fish cannot be consumed by human due to small in size, rotten fish or unused fish parts. If MPs is present in the 'trash fish', the MPs can pass through to another animal that fed on fish meal. Then, human will the eat livestock had polluted with MPs.

The whole sharks and rays had been utilised fully from their meat as a source of protein for human consumption and the inedible part had been processed to other products. Vannuccini (1999) mentioned that sharks and rays also had been used as by-products such as cosmetics product, liver oil and fishmeal. Shark and stingray originated from the Endau-Rompin coastal area are safe to make as food supply and fish meal as the coastal not polluted with microplastics. So, there is no probability of trophic transfer of microplastics from sharks and stingrays product to human.

Although there is possible transfer of MPs to human, the effect either physically or chemically of the microplastics to human is difficult to measure. Previous study showed that microplastics can absorb the toxic pollutant such as persistent organic pollutants (POPs), drugs, and pesticide (Engler, 2012; Ng & Obbard, 2016). Bakir et al. (2014) study showed that microplastics also able to released POPs under the simulated stomach condition. Karami et al. (2017) mentioned that route of contaminants transfer to human highly occur in the food and water compared through MPs due to small in size.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Although the previous study had shown the presence of microplastics in the ocean, the microplastics had not found in *Carcharhinus dussumieri* and *Dasyatis zugei*. For now, *Carcharhinus dussumieri* and *Dasyatis zugei* from Endau-Rompin coastal area not polluted with microplastics. So, the *Carcharhinus dussumieri* and *Dasyatis zugei* from Malaysian water is safe to be consumed by human or to be used in other by-products. There is possible that other organisms in that area were contaminated.

6.2 Recommendation

For future research, it is recommended to use different pore size of filter membrane which is less than 149 μm to make sure no loss of microplastics during the isolation process. Next, for future study, the researcher should conduct experiment using the filter feeding marine organisms as sample due to their eating habits and probably consume the microplastics directly. Lastly, next experiment should include analysis on the edible tissue such as muscle.

REFERENCES

Adams, L. S., Peace, C., Petersen, G., Wiggins, P., & Leary, M. (2006). Environmental Issues with Materials in Green Buildings, (October).

Andrady, A.L. and Neal, M.A. (2009). Applications and societal benefits of plastics. *Philosophical Transactions of the Royal Society B: Biological Science*, *364*, pp. 1977-1984.

Andrady, A.L. (2011) Microplastics in the marine environment. *Marine Pollution Bulletin, 62*, pp. 1596–1605.

Alomar, C., & Deudero, S. (2017). Evidence of microplastic ingestion in the shark Galeus melastomus Rafinesque, 1810 in the continental shelf off the western Mediterranean Sea. *Environmental Pollution, 223*, 223-229. doi:10.1016/j.envpol.2017.01.015

Avar, G. (October 2008). Polyurethanes (PU). *Kunststoffe international (10/2008):* 123–127.

Bakir, A., Rowland, S. J. and Thompson, R. C. (2012).Competitive sorption of persistent organic pollutants onto microplastics in the marine environment. *Marine Pollution Bulletin*, *64*, pp. 2782-2789.

Bakir, A., Rowland, S. J. & Thompson, R. C.(2014). Enhanced desorption of persistent organic pollutants from microplastics under simulated physiological conditions. *Environmental Pollution 185*, pp. 16–23

Baumann, E. (1872). "Ueber einige Vinylverbindungen" (On some vinyl compounds), *Annalen der Chemie und Pharmacie*, *163* : pp. 308–322.

Barnes, D. K. A., Galgani, F., Thompson, R. C. and Barlaz, M. (2009).Accumulation and fragmentation of plastic debris in global environments. *Philosophical Transactions of the Royal Society B: Biological Science*, *364* (1526), pp. 1985−1998.

Bennett, M.B. & Kyne, P.M. (SSG Australia & Oceania Regional Workshop, March 2003). 2003. *Carcharhinus dussumieri.* The IUCN Red List of Threatened Species2003:e.T41734A10550671.http://dx.doi.org/10.2305/IUCN.UK.2003. RLTS.T41734A10550671.en. Downloaded on 01 May 2017.

Besseling, E.; Wegner, A.; Foekema, E. M.; van den Heuvel- Greve, M. J.; Koelmans, A. A. (2013). Effects of microplastic on fitness and PCB bioaccumulation by the lugworm Arenicola marina (L.). *Environmental Science &Technology*, *47* (1), pp. 593−600.

Blackwell Plastics. (2010). *Plastics additives*. Retrieved April 03, 2017, from http://www.blackwellplastics.com/additives.html

Bravo Rebolledo, E. L., van Franeker, J. A., Jansen, O. E., & Brasseur, S. M. (2013). Plastic ingestion by harbour seals (Phoca vitulina) in The Netherlands. *Marine Pollution Bulletin*, *67*(1), pp. 200–202.

Browne, M. A., Dissanayake, A., Galloway, T. S., Lowe, D.M. and Thompson, R. C. (2008).Ingested Microscopic Plastic Translocates to the Circulatory System of the Mussel, *Mytilus edulis* (L.). *Environmental Science & Technology, 42*, pp. 5026–5031.

Carpenter, E.J., Anderson, S.J., Harvey, G.R., Miklas, H.P., Peck, B.B., 1972. Polystyrene spherules in coastal waters. *Science 178* (4062), 749e750.

Charvat, R. (2005). *Coloring of Plastics: Fundamentals*. Hoboken, New Jersey: John Wiley & Sons.

Cole, M., Lindeque, P., Halsband, C. and Galloway, T.S. (2011) Microplastics as contaminants in the marine environment: a review. *Marine Pollution Bulletin* , *62*, pp. 2588–2597.

Cole, M. and Galloway T.S. (2015).Ingestion of nanoplastics and microplastics by Pacific oyster larvae. *Environmental Science & Technology*, *49* (24), pp. 14625–14632

Cole, M., Lindeque, P., Fileman, E., Halsband, C. and Galloway, T. S. (2015).The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus. Environmental Science & Technolog.*, *49* (2), pp. 1130–1137

Compagno, L.J.V. (2006). Whitecheek shark (*Carcharhinus dussumieri*). Retrieved April 28, 2017, From http://species-identification.org/species.php

Cordova, M.R. and Wahyudo A.J. (2016). Microplastic in the deep-sea sediment of Southwestern Sumatran waters. Marine Research in Indonesia *41*(1)

Cozar, A., Echevarria, F., Gonzalez-Gordillo, J.I., Irigoien, X., Ubeda, B., Hernandez- Leon, S., Palma, A.T., Navarro, S., García-de-Lomas, J., Ruiz, A., Fernandez-de- Puelles, M. L. and Duarte, C. M. (2014). Plastic debris in the open sea. Proceedings of the National Academy of Sciences U. S. A 111 (28), 10239-10244.

Danny, L. (2015). "Five Things to Know About Congress' Vote to Ban Microbeads". *Smithsonian magazine*. Retrieved 05 June 2017.

Davidson, K., & Dudas, S. E. (2016). Microplastic Ingestion by Wild and Cultured Manila Clams (Venerupis philippinarum) from Baynes Sound, British Columbia. Archives of Environmental Contamination and Toxicology, *71*(2), pp. 147-156. doi:10.1007/s00244-016-0286-4

Duis, K. and Coors, A. (2016). Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. *Environment Science Europe* , *28*(2)

Department of Fishery Malaysia, Malaysia national plan of action for the conservation and management of shark (Malaysia NPOA-shark). (2006). Kuala Terengganu: Departmen Penyelidikan dan Pengurusan Sumber Perikanan Marin.

Derraik, J. G. B. (2002) The pollution of the marine environment by plastic debris: a review. *Marine Pollution Bulletin*, *44* (9), pp. 842−852.

de Sá,L.C.,Luís,L.G.,Guilhermino,L.,2015.Effects of microplastics on juveniles of the common goby(*Pomatoschistus microps*): confusion with prey, reduction of the predatory performance and efficiency, and possible influence of develop-mental conditions. *Environmental Pollution*, *196*, pp. 359–362.

Desforges, J. W., Galbraith, M. and Ross, P. S. (2015). Ingestion of microplastics by zooplankton in the Northeast Pacific Ocean. Environmental Contamination & Toxicology.

Devriese, L. I., van der Meulen, M. D., Maes, T., Bekaert, K., Paul-Pont, I., Fr??re, L., … Vethaak, A. D. (2015). Microplastic contamination in brown shrimp (Crangon crangon, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area. *Marine Pollution Bulletin*, *98*(1–2), 179–187. https://doi.org/10.1016/j.marpolbul.2015.06.051

Endo, S., Takizawa, R., Okuda, K., Takada, H., Chiba, K., Kanehiro, H., Ogi, H., Yamashita, R., Date, T., 2005. Concentration of polychlorinated biphenyls (PCBs) in beached resin pellets: variability among individual particles and regional differences. *Marine Pollution Bulletin*

Engler, R. E. (2012). The complex interaction between marine debris and toxic chemicals in the ocean. *Environmental Science & Technology*. *46*, pp. 12302-12315.

Eriksson, C., & Burton, H. (2003). Origins and Biological Accumulation of Small Plastic Particles in Fur Seals from Macquarie Island. *AMBIO: A Journal of the Human Environment*, *32*(6), 380–384. https://doi.org/10.1579/0044-7447-32.6.380

Farrell, P. and Nelson, K. (2013). Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). *Environmental Pollution*, *177*, pp. 1-3

Foekema, E. M., Murk, A. T., Franeker, J. A. V. and Koelmans, A. (2013). Plastic In North Sea. Environmental Science & Technology.

Foekema, E. M., De Gruijter, C., Mergia, M. T., van Franeker, J. A., Murk, A. J., & Koelmans, A. a. (2013). Plastic in North Sea Fish. *Environmental Science & Technology*, *47*(15), 130711150255009. https://doi.org/10.1021/es400931b

Food and Agriculture Organization (FAO) UN Fisheries & Aquaculture. (2009). Statistics and main indicators FAO Fisheries statistics Marine sub-sector. Retrieved April 30, 2017, from http://www.fao.org/fishery/facp/MYS/en

Food and Agriculture Organization (FAO) UN Fisheries & Aquaculture. (2016). Global per capita fish consumption rises above 20 kilograms a year. Retrieved June 05, 2017, from http://www.fao.org/news/story/en/item/421871/icode/

Frankson, S. (2015). 8 Million Tons of Ocean Plastic Could be Prevented – The Plastic Bank. Retrieved October 10, 2016, from http://plasticbank.org/preventocean-plastic/

Free, C. M., Jensen, O. P., Mason, S. A., Eriksen, M., Williamson, N. J., & Boldgiv, B. (2014). High-levels of microplastic pollution in a large, remote, mountain lake. *Marine Pollution Bulletin*, 85(1), pp. 156–163. https://doi.org/10.1016/j.marpolbul.2014.06.001

Garrison, T. (2013). *Oceanography: an invitation to marine science*.

Helmenstine, A. M. (2014). What Is Plastic? Retrieved October 8, 2016, from http://chemistry.about.com/od/polymers/f/What-Is-Plastic.htm

Hoff, R and Mathers, RT. (2010). "Chapter 10. Review of Phillips Chromium Catalyst for Ethylene Polymerization". In Hoff, Ray; Mathers, Robert T. *Handbook of Transition Metal Polymerization Catalysts*. John Wiley & Sons.

Ikhwanuddin M., and Ihwan M. Z. (2014).Present Distribution of Pedunculate Barnacle of Wild Mud Crab Scylla Olivacea from Setiu Wetland, Terengganu Coastal Water, Malaysia. Poultry Fish Wildlife Science 2.2

Jackson, G.D., Buxton, N. G. and George, M. J. (2000).Diet of the southern opah *Lampris immaculatus* on the Patagonian Shelf; the significance of the squid *Moroteuthis ingens* and anthropogenic plastic. *Marine Ecology Programme*. Ser., *206* , pp. 261–271

Ji, L. N. (2013). "Study on Preparation Process and Properties of Polyethylene Terephthalate (PET)". *Applied Mechanics and Materials*. 312: 406–410.

Karami, A., Romano, N., Galloway, T. and Hamzah, H., (2016). Virgin microplastics cause toxicity and modulate the impacts of phenanthrene on biomarker responses in African catfish *(Clarias gariepinus*). *Environmental Research*, *151*, pp.58-70.

Karami, A., Golieskardi, A., Choo, CK., Romanoc, N., Ho, YB and Salamatinia, B., (2016) A high-performance protocol for extraction of microplastics in fish. *Science of the Total Environment*, pp.10

Karami, A., Golieskardi, A., Keong Choo, C., Larat, V., Galloway, T. S., & Salamatinia, B. (2017). The presence of microplastics in commercial salts different countries. *Scientific Reports*, *7*(April), 46173. https://doi.org/10.1038/srep46173

Knight, G. Plastic Pollution. Chicago, Ill.: Heinemann Library, 2012.

Kuehner, M. (n.d.). Market Study: Polypropylene (3rd edition). Retrieved April 03, 2017, from http://www.ceresana.com/en/market-studies/plastics/polypropylene/

Kwon, BG; et al. (May 2014). "Regional distribution of styrene analogues generated from polystyrene degradation along the coastlines of the North-East Pacific Ocean and Hawaii". *Environmental Pollution*. *188*, pp. 45–49.

Law, C., Whish, R., Bailey, D., Enforcement, T., Courts, E. U., Commission, T. E., Oft, T. (2003). Preface to the seventh edition, (April), 1–1174.

Law, K. L., & Thompson, R. C. (2014). Microplastics in the seas. *Science,345*(6193), 144-145. doi:10.1126/science.1254065

Lechner, A., Keckeis, H., Lumesberger-Loisl, F., Zens, B., Krusch, R., Tritthart, M., Glas, M. and Schludermann, E. (2014). The Danube so colourful: a potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. *Environmental Pollution*, *188*, pp. 177-181

Lomax, S. Q. (2005). Phthalocyanine and quinacridone pigments: their history, properties and use. *Studies in* Conservation, 50(sup1), 19–29. H ttps://doi.org/10.1179/sic.2005.50.Supplement-1.1

Lusher, A., McHugh, M. and Thompson, R. (2012). Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Marine Pollution Bulletin 67*, pp. 94–99

Lusher, A. L., McHugh, M. and Thompson R. C. (2013).Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Marine Pollution Bulletin*, *67* (1), pp. 94–99

Lusher, A. L., Welden, N. A., Sobral, P., & Cole, M. (2016). Sampling, isolating and identifying microplastics ingested by fish and invertebrates. *Analytical Methods*.

Li, J., Yang, D., Li, L., Jaben, K., and Shi, H. (2015). Microplastics in commercial bivalves from China. *Environmental Pollution*, *207*, pp. 190-195

Malaysian Plastics Manufacturer Association (MPMA). (2010). Malaysian Plastics Industry Directory 2009/2010.

Mathalon, A.,Hill,P.,2014.Microplastic fibers in the intertidal ecosystem surounding. Halifax Harbor, Nova Scotia .*Marine Pollution Bulletin*, *81*, pp. 69–79.

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., Kaminuma, T., 2001. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. *Environmental Science & Technology*, *35*(2), pp. 318– http://dx.doi.org/10.1021/es0010498.

Mattison, C. (2017). Manta Rays and Microplastics. Retrieved June 05, 2017, from https://www.mission-blue.org/2017/02/manta-rays-and-microplastics/

Moore, C.J. (2008). Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. *Environmental Research, 108*, pp. 131-139.

Miljødirektoratet), N. E. A. (2014). Sources of microplastic pollution to the marine environment Project report. https://doi.org/M-321|2015

Murray, M., Cowie, P.R., 2011. Plastic contamination in the decapod crustacean Nephrops norvegicus (Linnaeus, 1758). *Marine Pollution Bulletin, 62* (6), 1207–1217. http://dx.doi.org/10.1016/j.marpolbul.2011.03.032

National Solid Waste Management Department. (2011). A Study on Plastic Management In Peninsular Malaysia, (December), 282.

The National Association for PET Container Resources (NAPCOR). (2015). What is PET? Retrieved April 03, 2017, from http://www.napcor.com/PET/whatispet.html

Ng, K., & Obbard, J. (2006). Prevalence of microplastics in Singapore's coastal marine environment. *Marine Pollution Bulletin*, *52*(7), pp. 761-767. doi:10.1016/j.marpolbul.2005.11.017xx

Noik, V. J. (2014, Dec 8-9). The occurrence of plastic microdebris on the beaches of Santubong and Trombol in Kuching, Sarawak, Malaysia. Paper presented at International Conference on Agriculture, Biology and Environmental Sciences, Bali. doi:10.17758/IAAST.A1214071

NPCS Board of Consultants & Engineers (2014) Chapter 6, p. 56 in *Disposable Products Manufacturing Handbook*, NIIR Project Consultancy Services, Delhi

Oehlmann, J., Schulte-Oehlmann, U., Kloas, W., Jagnytsch, O., Lutz, I., Kusk, K.O., Wollenberger, L., Santos, E.M., Paull, G.C., Van Look, K.J., Tyler, C.R., 2009. A critical analysis of the biological impacts of plasticizers on wildlife.*Philosophical Transactions of the Royal Society B: Biological*

PlasticEurope. What is plastic? (2014). Retrieved October 09, 2016, from http://www.plasticseurope.org/what-is-plastic.aspx

PlasticEurope. (2015). Plastics – the Facts 2014 / 2015 An analysis of European plastics production , demand and waste data, 1–34.

PlasticEurope. (n.d.). Types and categories of plastics. Retrieved April 03, 2017, from http://www.plasticseurope.org/what-is-plastic/types-of-plastics-11148.asp

PlasticEurope. Plastics-The Facts 2013: An analysis of European latest plasticsproduction, demand and waste data. *Page* 1–40 (PlasticEurope, 2013). At (http://www.mendeley.com/catalog/plasticsthe-facts-2013-analysis-european-latestplastics-production-demand-waste-data/)

Pinet, Paul R. (2003). *Invitation to Oceanography*. Boston: Jones & Bartlett Learning. ISBN 978-0-7637-2136-7. Retrieved 04 April 2017

Piringer, OG and Baner, AL. (2008). *Plastic Packaging: Interactions with Food and Pharmaceuticals (2nd ed.)*. Wiley-VCH. ISBN 978-3-527-31455-3.

Rech, S., Macaya-Caquilp_an, V., Pantoja, J.F., Rivadeneira, M.M., Jofre Madariaga, D., and Thiel, M. (2014). Rivers as a source of marine litter e a study from the SE Pacific. *Marine Pollution Bulletin*, *82*, pp. 66-75.

Ryan, P. G., Moore, C. J., van Franeker, J. A., and Moloney, C. L. (2009). Monitoring the abundance of plastic debris in the marine environment. *Philosophical Transactions of the Royal Society B: Biological Science*, *364* (1526), pp. 1999–2012.

Rios L.M., Moore C., Jones P.R. (2007) Persistent organic pollutants carried by synthetic polymers in the ocean environment. *Marine Pollution Bulletin*, *54*, pp. 1230–1237.

Setälä, O., Fleming-Lehtinen, V., & Lehtiniemi, M. (2014). Ingestion and transfer of microplastics in the planktonic food web. *Environmental Pollution*, *185*, 77–83. https://doi.org/10.1016/j.envpol.2013.10.013

Tanaka, K., & Takada, H. (2016). Microplastic fragments and microbeads in digestive tracts of planktivorous fish from urban coastal waters. *Scientific Reports*, *6*(1), 34351. https://doi.org/10.1038/srep34351

TATA Strategic Management Group. (2014). Plasticulture and Food Processing 2014. https://doi.org/2014

Teuten, E.L., Rowland, S.J., Galloway, T.S., Thompson, R.C., 2007. Potential for plastics to transport hydrophobic contaminants. *Environmental Science & Technology*, *41* (22), pp. 7759–7764. http://dx.doi.org/10.1021/es071737s.

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364* (1526), pp. 2027–2045

Tschernij, V. and Larsson, P.O., (2003). Ghost fishing by lost cod gill nets in the Baltic Sea. *Fisheries Research* , *64*, pp. 151-162.

UNEP. (2009) Marine Debris: A Global Challenge. UNEP, Nairobi (232 pp)

Vangheluwe, P. (n.d.). Polyethylene (PE). Retrieved April 03, 2017, from http://www.plasticseurope.org/what-is-plastic/types-of-plastics-11148/polyolefins/polyethylene.aspx

Van Cauwenberghe, L., & Janssen, C. R. (2014). Microplastics in bivalves cultured for human consumption. *Environmental Pollution*, *193*, 65–70. https://doi.org/10.1016/j.envpol.2014.06.010

Van Franeker, J. A. et al. (2011).Monitoring plastic ingestion by the northern fulmar Fulmarus glacialis in the North Sea. *Environmental Pollution 159*, pp. 2609–2615

Van Franeker, J. A. and K.L. Law., K. L. (2015) Seabirds, gyres and global trends in plastic pollution. *Environmental Pollution* , *203*, pp. 89–96

Vannuccini, S. (1999). *Shark Utilization, Marketing and Trade* (FAO FISHERIES TECHNICAL PAPER 389). Retrieved from http://www.fao.org/docrep/005/x3690e/x3690e00.htm

von Moos,N.,Burkhardt-Holm,P.,Köhler,A.,2012.Uptake and effects of microplastics on cells and tissue of the blue mussel Mytilus edulis L. after an experimental exposure. *Environmental Science & Technology 46*, pp. 11327–11335.

Watts, A. J. R., Lewis, C., Goodhead, R. M., Beckett, S.J., Moger, J., Tyler, C. R., Galloway, T. S. (2014). Uptake and retention of microplastics by the shore crab *Carcinus manenas*. *Environmental Science & Technology*, *48* (1), pp. 8823-8830.

White, W.T. (2006). "*Dasyatis zugei*". *IUCN Red List of Threatened Species. Version 2008*. International Union for Conservation of Nature. Retrieved April 29, 2017.

Wright, S.L., Thompson, R.C., Galloway, T.S. (2013). The physical impacts of microplastics on marine organisms: a review. *Environment Pollution. 178*, pp. 483-492.

Yusof, S. I., Alyza Azzura, A., Shaakirah, A. S., Sabiqah T. A., and Siti Aishah. (2016). Microplastics Ingestion by *Scapharca Cornea* At Setiu Wetland, Terengganu, Malaysia". *Middle-East Journal of Scientific Research*, *24* (6), pp. 2129-2136.

APPENDICES

Appendix B

Length and weight of samples

SHARK	WEIGHT (g)	LENGTH (cm)
1	346	43
2	273	39.4
3	288	38
4	276	39.5
5	264	38.5
MEAN (SD)	289.4 (32.8) g	39.68 (1.96) cm

STINGRAY	WEIGHT (g)	LENGTH BODY ONLY (cm)	LENGTH BODY + TAIL (cm)
1	181	19	42.33
2	239	21	49.5
3	207	19.3	42
4	170	18	37.3
5	167	19	45.4
MEAN (SD)	192.8 (30.25) g	19.26 (1.1) cm	43.3 (4.5) cm