Surveillance and histopathological study of microplastics in marine fish from the gulf of Thailand

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ในปัจจุบันปัญหาไมโครพลาสติกเป็นประเด็นสำคัญที่ส่งผลกระทบต่อสิ่งแวดล้อมทั่วโลก อีกทั้งยังส่งผลเสียทั้งทางตรงและทางอ้อมต่อมนุษย์และสัตว์ การบริโภคอาหารทะเลที่ปนเปื้อนไมโครพลาสติกเป็นช่องทางการรับสัมผัสไมโครพลาสติกที่สำคัญของมนุษย์ การศึกษาในครั้งนี้ทำการเฝ้าระวังและศึกษาลักษณะการปนเปื้อนไมโครพลาสติกในปลาทะเลที่เป็นอาหารจากอ่าวไทยตอนบน ผลการศึกษาพบไมโครพลาสติกในทางเดินอาหารของปลาร้อยละ 46.86 หรือจำนวนชิ้นเฉลี่ยเท่ากับ 1.556±0.47 ชิ้นต่อตัวปลา หรือ 0.035±0.014 ชิ้นต่อกรัมของน้ำหนักตัวปลา ไม่พบไมโครพลาสติกในอวัยอื่น ๆ ได้แก่ ตับ ไต กล้ามเนื้อ และอวัยวะสืบพันธุ์ พบว่าการปนเปื้อนของไมโครพลาสติกมีความสัมพันธ์กับชนิดและระดับที่อยู่อาศัยของปลา แต่ไม่มีความสัมพันธ์กับพื้นที่แหล่งที่มาของปลา โดยพบว่าปลาพื้นน้ำมีปริมาณการปนเปื้อนไมโครพลาสติกมากกว่าปลากลางน้ำ รูปร่างและสีของไมโครพลาสติกที่พบมากที่สุดจากการศึกษาคือไมโครพลาสติกชนิดเส้นใยและสีน้ำเงินตามลำดับ ชนิดของพอลิเมอร์พลาสติกที่พบมากที่สุดคือพอลิเอสเตอร์และพอลิเอทิลีน โดยพบพอลิเอสเตอร์มากที่สุดในปลาพื้นน้ำและพบพอลิเอทิลีนมากที่สุดในปลากลางน้ำ จากการศึกษาพบว่าการพบไมโครพลาสติกในปลาในธรรมชาติไม่มีความสัมพันธ์กับรอยโรคทางจุลพยาธิวิทยา จากข้อมูลข้างต้นสามารถประเมินการรับสัมผัสไมโครพลาสติกของคนไทยผู้บริโภคปลาได้เท่ากับ 0.06 ชิ้นต่อคนต่อวันสำหรับปลาทะเลทั่วไป และ 0.095 ชิ้นต่อคนต่อวันสำหรับปลาทู อย่างไรก็ตามความเสี่ยงของการรับสัมผัสไมโครพลาสติกจากการบริโภคเนื้อปลาหรือความเสี่ยงทางอ้อมจากปลาป่นที่ทำมาจากปลากลางน้ำนั้นยังอยู่ในระดับต่ำ และการเฝ้าระวังอย่างต่อเนื่องในอนาคตยังเป็นสิ่งจำเป็นในการประเมินผลกระทบและประเมินความปลอดภัยทางอาหารทั้งต่อมนุษย์และสัตว์

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Microplastic (MP) problems have been threatened aquatic environment worldwide for decades. This study demonstrated current MP contamination characteristics in marine food fishes from the upper Gulf of Thailand. MPs were found in gastrointestinal tracts of 46.86% of fish samples, which were 1.556±0.47 pieces per fish or 0.035±0.014 pieces per gram of fish bodyweight. No plastics were detected from muscle, liver, kidney or gonad of the fishes. There was a significant relationship between MP contamination and fish species or the fish grouping. Benthic fish had a higher contamination rate than pelagic fish. Fiber-type and blue color were the most abundant MPs characteristic observed. The most common polymer was polyester, followed by polyethylene. Polyester was a dominant polymer among benthic fishes while polyethylene dominant in pelagic fishes. The contamination of MPs was not related to histopathological lesions in natural marine fishes. The expected MPs exposure of Thai marine fish consumers were 0.06 pieces per person per day for general marine fish and 0.095 pieces per person per day for Short Mackerel. The expected exposure risk from consuming fish muscle and risk from fishmeal consuming animal were still relatively low. Continuing surveillance and exposure assessment are crucial in determining MP risks to human health in near future.

Field of Study:	Veterinary Medicine	Student's Signature ....
Academic Year:	2020	Advisor's Signature ....

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Sirawich Srisiri

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CHAPTER 1
INTRODUCTION

Importance and Rationale

For decades, the issues of plastic wastes have been a threat to aquatic ecosystems and marine animals worldwide. Asian countries, including Thailand, have high rates of plastic waste production. Approximately 86% of global marine plastic debris are disposed from these countries This is because high plastic usage, high population density and waste mismanagement in these countries (Lebreton et al., 2017). Apart from large plastic debris, microplastics are recently and increasingly becoming issues in many aquatic environments. These microplastics could be either directly polluted or degraded from macro-size plastics (Jovanović, 2017). In general, the definition of microplastics can be defined as synthetic solid particles or polymeric matrix, with size ranging from 1 milimeter to 5 millimeters, of either primary or secondary manufacturing origin (Frias and Nash, 2019).

From rivers, mangrove forests, beaches to the ocean, Microplastic particles have been contaminated in various marine environment globally (Ng and Obbard, 2006; Andrady, 2011; Nor and Obbard, 2014). The sediments in the gulf of Thailand also have a moderate amount of microplastics contamination (Matsuguma et al., 2017; Wang et al., 2020). Referring to many published studies, these microplastics are small enough to be ingested by numerous marine organisms. Different prevalence of microplastic-ingested fish was reported from various locations due to many factors. At least 36.5 percent of fish samples from the English channel had microplastics in their gastrointestinal tract (Lusher et al., 2013). Higher ratios were reported from the Mediterranean sea, 58 percent of fish samples had microplastics in their stomach and intestine (Güven et al., 2017). In Malaysia, many species of fish have reportedly ingested plastic particles in ratios that differed among species, from 0 to 60 percent (Karbalaei et al., 2019). In Thailand, a report on microplastics ingestion in fish was from the lower gulf of Thailand. More than 54 percent of fish samples from Songkhla contained plastics in their gastrointestinal tract (Azad et al., 2018). The contamination of microplastics in fish occurred not only in marine fish but also in freshwater fish. 72.9% of freshwater fish from the Chi river in North-eastern Thailand found microplastics in their gastrointestinal tract (Kasamesiri and Thaimuangphol, 2020). Apart from fish, a study also reported contamination of microplastics in tissues of sessile invertebrates from the Thai eastern coast (Thushari et al., 2017).

Ingestion of microplastics can cause physical injuries or obstruction of the gastrointestinal tract in small aquatic animals (Mazurais et al., 2015; Horton et al., 2017). In larger fish, there was evidence that microplastics can be absorbed via intestinal mucosa and get into the circulatory system of the fish. Accumulation in internal organs could be microscopically observed. These pure microplastics appear to have little harm to the fish. This is because most plastic polymers are considered chemically inert and has no short-term harm to the fish (Jovanović, 2017; Jovanovic et al., 2018).

On the other hands, some plastic polymers may act as endocrine disrupting agents and cause reproductive problems in aquatic animals (Halden, 2010). From the study of Rochman et al. (2014), Fish fed with polyethylene (PE) microplastics showed some endocrine disrupting signs and abnormal germ cells proliferation after 2 months. As well as the study of Sussarellu et al. (2016), Oysters' reproductive system were also affected by polystyrene (PS) microplastic. Another reported consequence of pure microplastic ingestion is the disturbance of cholesterol and LDL levels in African catfish (*Clarius gariepinus*) (Karami et al., 2016).

In contrast to pure microplastics, plastics in the environment can be contaminated with other chemicals which is intentionally or unintentionally added to plastics, including unreacted monomers, residue catalyst, solvent or persistent organic pollutants (POPs) from the marine environment. This is because the hydrophobicity of plastic can absorb a wide range of chemical compounds found in aquatic environments. Polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) are 2 examples of POPs, which can be potentially harmed if ingested by marine organisms with high risk of accumulation in the food chain (Arthur et al., 2009; Jovanović, 2017; Niaounakis, 2017). The study of Rainieri et al.(2018) revealed that microplastics together with contaminants posed significantly greater effects on fish, including alteration of liver gene expression. It can be implied that microplastics can increase the chance of toxicity and accumulation of these POPs in fish, which could be further potentially harm to fish consumers.

Nowadays, Microplastics are now becoming issues in different environment across the globe at an accelerating rate (Andrady, 2011). These microplastics are considered an emerging threat to not only to aquatic animals, but also to human food safety and human health. Ingestion of microplastics by human can be confirmed by presence of microplastic particles in stool (Schwabl et al., 2019). Seafood consumption is potential sources of microplastics intake for humans. The meta-analysis by Cox et al. (2019) showed that seafood eaten by American people has microplastics contamination at 1.48 particles per gram. The studied also showed that American people have ingested microplastic particles from food and beverages at 106-142 particles per person per day.

For Thai people, data on microplastics contamination in seafood are still limit. Despite the fact that Thai people consumed seafood higher than global average and ranked top in Asia. In 2016, Fish and fisheries products consumed by Thai people were at the amount of 27.2 kilograms per capita, while the global average is only 19.7 per capita (FAO, 2019). Therefore, determination of microplastics contamination in Thai seafood and other seafood products are crucial data in determining risk from microplastics for Thai people.

In this study, fish species living in different oceanic zone of Northern gulf of Thailand, including pelagic and benthic zone, was selected to study the prevalence and distribution of microplastics. It could determine the current status of microplastic contamination in aquatic environment and food fish in Thailand. The amount and distribution of microplastic contaminants from fish to human in Thailand could be implied from this study. Therefore, food safety and health awareness related to plastics in human food sources could be evaluated in the future.

Apart from human food safety aspect, effects of microplastics on histopathological changes in internal organ and changes in the reproductive system from potentially endocrine disrupting effects of some plastics polymers were evaluated. This is because in spite of many experimental studies done to evaluate the effects of microplastics in fish and other animals, very few were studied about the correlation of microplastic and its health effects in animals in natural habitat. The varieties of plastic polymer types and effects of each plastic polymer can also be assessed from this study. This could be beneficial for veterinarian for evaluating effects of microplastics to fish and another aquatic animals' health.

Objectives of the study

1) To determine the prevalence and distribution of microplastics in marine fish in different oceanic zone from the gulf of Thailand
2) To evaluate relationship of histopathological changes in fish with amount and polymer types of microplastics

Keywords (English): Gulf of Thailand, Histopathology, Marine fish, Microplastic.

Keywords (Thai): อ่าวไทย จุลพยาธิวิทยา ปลาทะเล ไมโครพลาสติก

Hypothesis

- Microplastics can be detected in marine fish from the gulf of Thailand.
- Microplastic contamination is related to histopathological change in fish from the gulf of Thailand.

Advantages of Study

1. A very first study about environmental microplastics effects on fish health in Thailand. The prevalence and distribution of microplastics in fish living in different oceanic zone can be understood.

2. To understand the correlation between particular polymer types of microplastics and histopathological changes in fish, which may relate to effects of plastics on fish health and human health. The quantity and effect of microplastics consumed by human can be related to amount and effect of microplastics contained in fish tissue

3. Further studies on effects of microplastics on fish or risk on fish consumers could be investigated by using the results from this study as basic information.

CHAPTER 2
LITERATURE REVIEW

2.1 Plastics

Plastics are anthropogenic synthetic or semi-synthetic materials made from polymers. The word “polymer” is defined as “many repeating parts”, in which many single units are called “Monomer” joined together in the polymerization process to form a large repetitive molecule. These polymeric molecules have been modified with processes and additives to become moldable into various shapes and forms, in the form of “plastic”. Plastics have been commercialized since the early 1900s and became essential parts of modern human society nowadays (Shashoua, 2012; Hutley and Ouederni, 2016). Global productions of plastics were continuously increasing in a non-linear manner compared to the global population. This can be implied that global plastics consumption and plastics waste in the future will be increasing at a higher rate (Geyer et al., 2017).

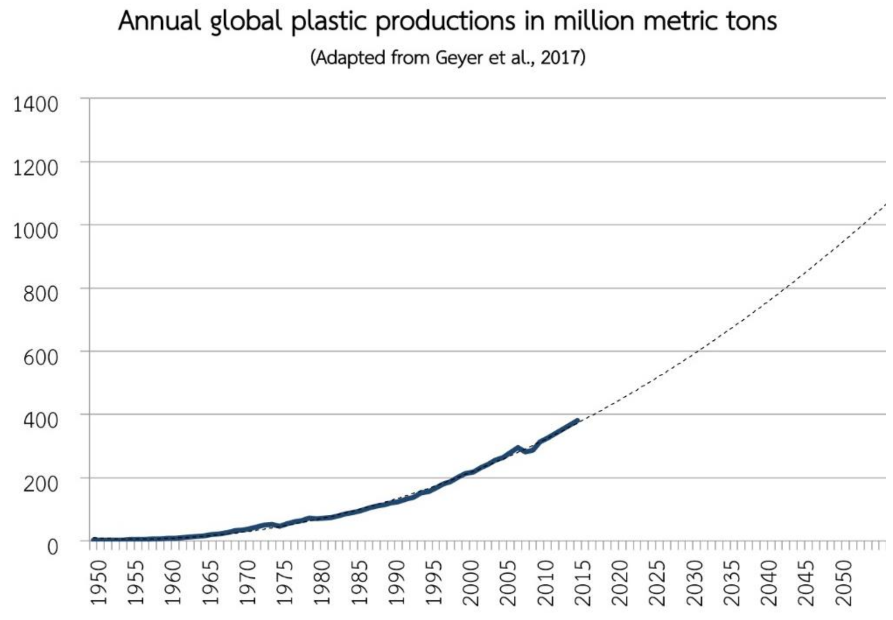


Figure 1 Global trend of plastics production, adapted from Geyer et al. (2017)

Same as other polymers, Plastic polymers are made of many repeating monomers, which are small organic molecules, becoming larger and much higher molecular weight macromolecules (Chanda and Roy, 2008). Major types of plastic polymers are made from petroleum-based products, known as polyolefin. Olefins, literally “oil-forming”, are hydrocarbon molecules that consist of at least two carbon molecules. Only small parts of plastic today are made of or partially made of natural, non-petroleum products (Hutley and Ouederni, 2016). For example, polymerization of ethylene molecules (C2H4) under the influences of heat, light, and chemical catalysts, will result in “polyethylene”. Common plastics used in daily life can be divided into 2 groups, including thermoplastics, which are plastics that soften on heating and can be molded, and thermoset plastics, which are plastics that cannot be molded on heating. Samples of common plastic polymers in daily life are including polyethylene (plastic household product, plastic bags, pipes, plastic sheets, water bottle), polypropylene (pipes, straw, bottle caps), polyvinylchloride (PVC film, pipes, toys), polystyrene (Styrofoam, cups), polyamide (Nylon rope, fishing net), polyacrylonitrile (Synthetic fibers), etc. Each plastic polymer type will have different chemical and physical properties (Chanda and Roy, 2008; GESAMP, 2015).

Virgin or pure plastic materials contain only chains of repetitive molecules of plastic monomers. However, plastics used in our daily life usually have been modified by adding plastic additives to give them stability and desirable performances. Additives can be divided into 4 groups, namely functional additives, colorants, fillers, and reinforcement additives. Functional additives were the largest and most common groups of additives used for the functional purpose of plastics, such as plasticizer, stabilizer, flame-retardant, heat-stabilizer, biocides, etc. Plasticizer, for example, chlorinated paraffin and phthalate compounds, was the most common functional additives used. They usually contained up to 10-70% w/w. The other commonly used functional additives were flame retardants, which can be around 0.7-25% w/w. Flame retardants can be divided into 3 groups, namely organic non-reactive (e.g. halogenated and phosphate esters compound), inorganic non-reactive (e.g. compound or oxide of antimony, aluminum, zinc, etc.) and reactive (e.g. bromine and/or phosphorus-containing polyols, halogenated phenols, tetrachlorophthalic anhydride, phosphonate esters, dibromoneopentyl alcohol). Less commonly functional additives that usually found in low concentration in plastics, ranging from 0.001 to 3 % w/w, were stabilizers, UV stabilizers, antioxidants, heat stabilizers, lubricants, or biocides (Hahladakis et al., 2018).

The rest 3 groups of additives were colorants, fillers, and reinforcement additives. Colorant’s additives, such as pigments or azo-colorants, were additives that give plastics their desire color and appearance. Fillers and reinforcement additives were less common, which were added for physical proposes (Hahladakis et al., 2018).

Additionally, plastics may contain contaminants that were no additives. Such contaminants were residual or unreacted monomers and oligomers. These were monomers or oligomers that did not completely react to form plastics polymers. These molecules usually quickly migrate from plastics, usually into food, beverages, or the environment (Hahladakis et al., 2018).

Despite the fact that plastics are revolutionary materials that have changed the world in many aspects, plastics have posed significant risks to the ecosystem and environment worldwide. Marine plastic debris was the most persistent and problematic group of marine debris nowadays (GESAMP, 2015). Asian countries, including Thailand, have a comparatively high rate of plastic waste production. It is estimated that 86% of global marine plastic debris comes from Asian rivers. The reasons are very high plastic consumption, high population density, and mismanagement of plastic wastes (Lebreton et al., 2017).

Large marine plastic debris poses much significant and obvious harm to aquatic lives, ranging from entanglements of aquatic animals, disturbances of fishing and maritime activity, or even visual pollution. Smaller plastic debris, due to the small size, obvious impacts were rarely demonstrated in the past. However, these small-size plastic particles, or so-called "microplastics" have been gaining more attention from the scientific community in recent days due to their potential negative effects on aquatic lives and the environment (GESAMP, 2015).

2.2 Microplastics

Microplastics are very small plastic particles in micro-sized. The scientific reports of small pieces of plastic debris in the environment can be dated the back to early 1970s, but the term "microplastics" started appearing in literature in the 1990s to 2000s (GESAMP, 2015). Since microplastics are a considerably new field of study at present, the terminology is still inconsistent. National Oceanic and Atmospheric Administration (NOAA) of the United States have defined one of the very first definitions of microplastics in 2009. They defined microplastics as plastic particles that are smaller than 5 millimeters in size, based on the fact that these size ranges of plastics can be ingested and contributing to different effects from larger plastics (Arthur et al., 2009). In 2019, two contradictory definitions of microplastics were proposed, both in January 2019. The first one was from Hartmann et al. (2019), They defined microplastic as plastic particles with a size between 1 μm to 1000 μm. They also designated other criteria of microplastics by characteristics of chemical composition, solid-state, and solubility. The second definition in 2019 was proposed by Frias and Nash (2019) They defined microplastics as follows: “Microplastics are any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 μm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water”. The latter one is more widely adopted in the field of marine pollution study. The Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) had also recommended using 5 millimeters as the upper limit for microplastics definition. This limit was mainly for monitoring proposes, due to its existing uses globally (GESAMP, 2019)

Nowadays, Microplastics are being increasingly concerned about their impact on animals and the environment. The source of microplastics in the environment can be divided into 2 types, which are microplastics from primary origin and microplastics from the secondary origin (Andrady, 2011; GESAMP, 2019).

2.2.1 Primary Microplastics

Primary microplastics are industrial microplastics or microplastics from some commercial products. These microplastics were intentionally produced in small size for certain purposes. An example of primary microplastics is scrub beads in facial cleanser. These particles are being polluted into rivers and seas globally (Andrady, 2011; GESAMP, 2019).

2.2.1 Secondary Microplastics

Secondary microplastics are microplastics that deriving from breaking down larger size plastic pieces. The breaking down process could be resulting from physical damage, sunlight, temperature, oxidation, or even some microorganisms. These factors cause the plastic to become brittle and break into smaller pieces (Andrady, 2011; GESAMP, 2019).

2.3 Fate of plastics and microplastics in the environment

When a piece of plastic debris was dumped into the environment, many factors were responsible for the fate of that plastic piece. These factors were, for instance, source of entry, quantities of debris, plastics' physical characteristics to some chemical and biological processes. The source of contamination is the most important factor that affects the fates of plastics in the environment. This is because, in a different locality, a different environmental factor will affect these plastics debris (Critchell and Lambrechts, 2016).

Most of the land plastics debris that resulting from mismanagement waste disposal ended up in the ocean. More than 90% of plastics debris input into the ocean was believed to come from rivers all over the world. Turbulent water flows and flooding can cause both buoyant and non-buoyant plastics to flow from inland rivers into the sea. It was estimated that the peak in plastics waste emissions from the river to the sea was between May to October, which was under the influences of monsoon in some particular region with high plastics waste production (Lebreton et al., 2017).

The Source of entry of plastics is also a critical factor to imply management measures on plastics contamination, both for macroplastics, primary microplastics, and secondary microplastics. The degree of plastics contamination in a certain locality can also help to investigate the source of entry (GESAMP, 2015). Effect of source of entry to the fate of plastics debris was, for example, plastics left near the coast with wind effects tend to beach up faster than those left in an open ocean. These beached up plastics might undergo a degradation process on the beach or might be re-suspended into the ocean when tides come up or the wind change direction. Knowing the source of entry can help to manage plastic problems in some situations. For instance, in the case of a plastics source that was close to the coast with wind effect, beach cleaning will be the most important protocol to imply because plastics debris will be beached shortly after getting into the sea (Critchell and Lambrechts, 2016).

Beached plastic debris might undergo degradation process on the land, which makes it become land microplastics, or being buried in the beach. Some plastics might be re-suspended into the sea. The re-suspension rate of plastics was differed between locations due to topographic factors such as local wind, tides, wave, or rain shadow (Critchell and Lambrechts, 2016). From the reasons mentioned earlier, plastics debris might undergo a degradation process far away from its sources of entry. This is why fragment-type microplastics, most of which were from the secondary origin, were found higher in a remote area rather than an urban area. On the other hand, microplastics deposition near urbanized areas tends to be primary or fiber-type microplastics, which results directly from human activities in an urbanized area (Alomar et al., 2016).

As plastic pieces got into the environment, they exposed to many environmental factors, such as UV irradiation or temperature. This debris will undergo weathering degradation and loss its mechanical integrity and become fragmented, which was significantly caused by secondary microplastics. These processes occur at a high rate especially on the beaches where weathering degradation and other environmental factors were abundant. On the contrary, plastic debris floating in the water or sinking in deep-sea sediment will have a slower rate of degradation processes. However, the exact understanding of the mechanism and dynamics of plastics weathering processes was still limit (GESAMP, 2015).

As mentioned earlier, UV irradiation was the most important factor that causes plastics degradation in the marine environment. This photo-degradation process in most plastics requires oxygen, a major pathway involved was free-radical mediated oxidation. Other pathways were including ionic radiation and hydrolysis. Some pollutants can accelerate the degradation processes of plastics, such as Carbon monoxide (CO), Sulphur Dioxide (SO_2), Nitrous oxide (NO), and Ozone (O_3). After plastics loss their integrity, discolored, and became brittle, physical forces, such as water current, wind, or even animals, took the role in breaking these plastics into small fragments (GESAMP, 2015; Hahladakis et al., 2018).

The degradation process might depend on chemical bonds between polymeric molecules. However, many factors affect the rate of the process in the environment. For instance, plastics additives, such as UV and heat stabilizers, made plastics more tolerable to such environmental factors. Other factors such as mechanical forces, temperature, salinity, or micro-fauna on plastics surface can also affect the rate of the process. Such diverse factors made it unreliable to predict the age of microplastics in the ocean (GESAMP, 2015).

Weathering processes cause several changes to plastics. First of all, oxidation processes cause polymers or additives to turn into yellowish colors because of their degradation products. These processes also added oxygenated functional groups into polymers, especially the carbonyl (>c=o) group. Polymeric chain scission and degradation of amorphous polymer can cause the microplastic to change in its crystallinity, which is how its polymeric chain was oriented. All of these processes cause plastics to change their physical properties from the original forms. Weathered processes can also change the chemical properties of the plastics. Oxidative moieties added after oxidation processes such as carbonyl group resulting in the change of FTIR curves compare to its original properties. This is the reason why FTIR curves of marine plastics debris were not always similar to the curve in the data library (Andrady, 2017).

The rates of degradation processes also depend on the location of plastics or microplastics in the environment. Beached plastics tend to degrade faster than other places because of their direct exposure to UV light, high oxygen availability, and high temperature. Plastics in the ocean or the sediment degrade slower than those on the beaches because of the lower temperature of seawater, lower UV and oxygen exposure for those in deeper water. In addition, fouling of aquatic fauna or sediment on the surface of plastics also decreases their exposure to UV light and oxygen, which can slow down the degradation processes (Andrady, 2017).

Complete degradation of plastics occurred rarely in the environment. Complete degradation means that plastic molecules were completely degraded into carbon dioxide and methane. Only some biodegradable plastics can undergo these processes in a short matter of time (GESAMP, 2015).

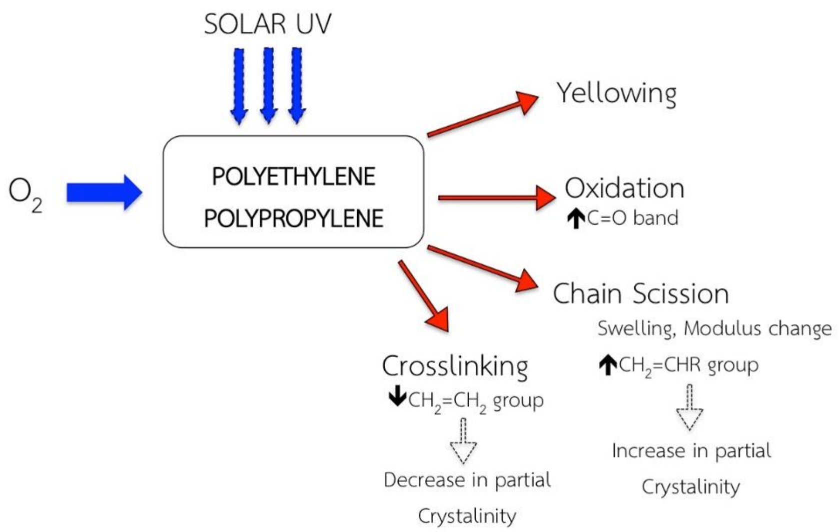


Figure 2 Effects of weathering process on plastics debris, given polyethylene and polypropylene as examples, adapted from Andrady (2017).

Few studies have studied the trend of microplastics distribution in a marine environment. Oceanography knowledge can predict the horizontal distribution and accumulation of microplastics and others plastic debris. Water currents, waves, wind, and local turbulent motion of seawater were all affecting the movement of microplastics. In the same way as large plastic debris, the source of entry of microplastics played an important role in determining the distribution pattern of microplastics. This is because the different locality has different environmental factors affecting the distribution. Accumulation of floating microplastics usually occurred in ocean gyres, where debris was retained due to convergences of ocean current (GESAMP, 2015). A computational model using hydrodynamic data can describe the occurrence of a gyre or debris accumulation zone in the ocean. There are 5 large accumulation zones throughout the world, namely Indian Gyre, North Pacific Gyre, South Pacific Gyre, North Atlantic Gyre, and South Atlantic Gyre. Smaller accumulation zone can be found in the area with a significant inland debris discharge or high population density. Noting that the Gulf of Thailand was among one of the accumulation areas (Figure 3) (Lebreton et al., 2012).

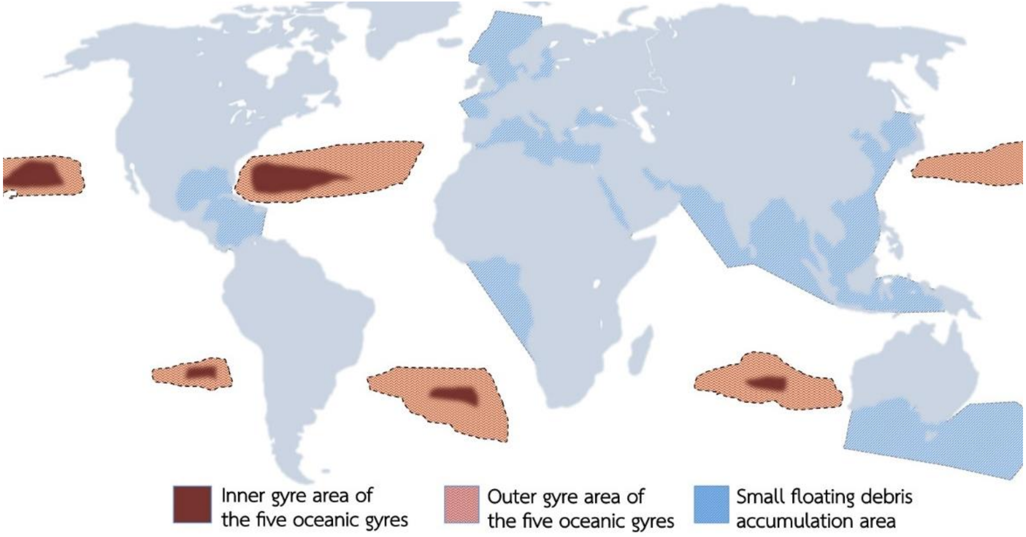


Figure 3 Global five Large Oceanic Gyres and other smaller floating debris accumulation area, adapted from Lebreton et al. (2012).

Origins of microplastics also contribute to the determination of their fate. Microplastics with primary origin or microplastics that were directly resulting from human activities usually accumulate near the urbanized areas. Fiber microplastics occurring from laundries, such as polyester, polyamides, and rayon, were examples of this group of microplastics. These fiber polymers usually quickly sank to the bottom of the sea and accumulated near their sources. On the other hand, microplastics with a secondary origin, which were mostly, fragment-type microplastics, usually found in a remote area. This is because large plastic debris was easily carried far away from its origin by the effects of wind or currents (Alomar et al., 2016; Erni-Cassola et al., 2019; Zambrano et al., 2019). Therefore, Since the degradation process of plastic debris takes a long time, the actual source of most secondary microplastics found in a certain area might be far away (Critchell and Lambrechts, 2016).

Vertical transport of microplastics in seawater usually depends on buoyancy changes of particles, which make the particle “sink”. The sinking rate can be varying from 10-150 meters per day. The sinking rate was slower in deeper seawater. After sinking into the sediment, accumulation of microplastics can occur. However, with physical forces or bioturbation processes, microplastics can be re-suspended into water columns again (GESAMP, 2015). Given this theory, all types of plastics, either low or high density, should gradually sink and be found in the same proportion throughout the water column. However, from the meta-analysis of Erni-Cassola et al. (2019), the different proportions of microplastic types were found among water depth, which means that there might be some other factors affecting the sinking rate or re-distribution rate of different plastic types. From the study, the vertical distribution of microplastic particles depended on their polymer density. Low-density plastics, for example, Polyethylene (PE), were the major type of microplastic found on surface water. Significantly lower PE particles were found in the water column or deep-sea sediments. Polypropylene (PP) contamination was also high in surface water. However, PP prevalence has no statistical difference between surface water, water column, and sediment. On the contrary, high-density plastics, such as polyamide (PA) and polyester (PET), polyvinyl chloride (PVC), or chlorinated PE, have a higher prevalence in the sediment than water column and water surface (Erni-Cassola et al., 2019). The specific gravity of each plastic polymer was shown in table 1. The tendency to float or sink certain plastic polymer in the aquatic environment can be told by comparing its specific gravity to the specific gravity of seawater (0.3). However, modifications that caused density change can alter the sinking or floating nature of that type of polymer (GESAMP, 2019).

Effects of sources and other hydrodynamic activity on the vertical movement of microplastics were evidenced in the study of Wang et al. (2020). The study revealed that the deposition of microplastics in the sediment of the Gulf of Thailand was heterogeneous. The highest degree of deposition was at the mouth of the upper gulf of Thailand, where the water area broadens and the effects of two current took place. Sediment at the mouth of Chao Phraya River also had higher microplastics deposition than the mouth of Bang Pakong River, which has markedly lower urban and industrial area compare to the Chao Phraya River. On the other hand, average microplastics deposition in the Gulf of Thailand was lower compared to the Mediterranean Sea, which is a close sea where hydrodynamic effects on microplastics were weaker than the open sea like the gulf of Thailand.

***Table** 1 Buoyancy and floating behavior of common plastic polymers in the aquatic environment, based on the density differences (Modified from the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) Report and Studies No. 99 (GESAMP, 2019)).*

Polymer	Common applications	Specific gravity	Behavior
Polystyrene (expanded)	Cool boxes, floats, cups	0.02-0.64	Float
Polypropylene	Rope, bottle caps, gear, strapping	0.90–0.92	
Polyethylene	Plastic bags, storage containers	0.91–0.95	
Styrene-butadiene (SBR)	Car tyres	0.94	
Average seawater		1.03	
Polystyrene	Utensils, containers	1.04–1.09	Sink
Polyamide or Nylon	Fishing nets, rope	1.13–1.15	
Polyacrylonitrile (acrylic)	Textiles	1.18	
Polyvinyl chloride	Thin films, drainage pipes, containers	1.16–1.30	
Polymethylacrylate	Windows (acrylic glass)	1.17-1.20	
Polyurethane	Rigid and flexible foams for insulation and furnishings	1.20	
Cellulose Acetate	Cigarette filters	1.22–1.24	
Polyethylene terephthalate (PET)	Bottles, strapping	1.34–1.39	
Polyester resin + glass fiber	Textiles, boats	>1.35	
Rayon	Textiles, sanitary products	1.50	
Polytetrafluoroethylene (PTFE)	Teflon, insulating plastics	2.2	

Marine organisms also play a role in the horizontal displacement of microplastics. These organisms can carry plastic particles away for a significant distance in the ocean. Sea birds or seals can bring these microplastics back up on land, hence complicating the microplastics distribution pattern (GESAMP, 2015).

After excreted with feces, ingested microplastics can quickly sink to the sediment together with fecal pellets. This makes vertical displacement of the particles occurred faster than those free-floating particles. Other organic matters can incorporate with the particles and consequently become marine snow, which also accelerates the sinking rate of the particle (Erni-Cassola et al., 2019).

Apart from the displacement and weathering processes that occurred on plastic particles, chemical changes can slowly occur within the plastic particles in the environment. As mentioned earlier, plastics pieces contain various additives or residues. These chemicals gradually missed or migrated from plastics from time to time, depending on their chemical properties, availability, or ambient environments. The emission can take place in all phases of a plastics life cycle, into the environment, air, water, food, or even saliva and sweat. These substances emitted from plastics were called potentially Toxic substances (PoTSs), due to their potential to cause harmful effects to an organism and the environment. Some examples of PoTSs releasing were from plastic food containers. Additives, such as Phthalates, Bisphenol A, or antioxidants can slowly release into food after plastics were in contact with food. Some kinds of food can escalate the diffusion rate of these PoTSs, for example, olive oil can increase the rate of emission of phthalates compound from PVC. Plastic oligomer or monomer can also be released from plastics into the environment. Styrene, a toxic monomer of polystyrene, can be emitted from polystyrene food containers into the food. With the help of high temperature and food fat content, the styrene emission rate can be greatly increased (Hahladakis et al., 2018).

2.4 Microplastics contamination in aquatic environment

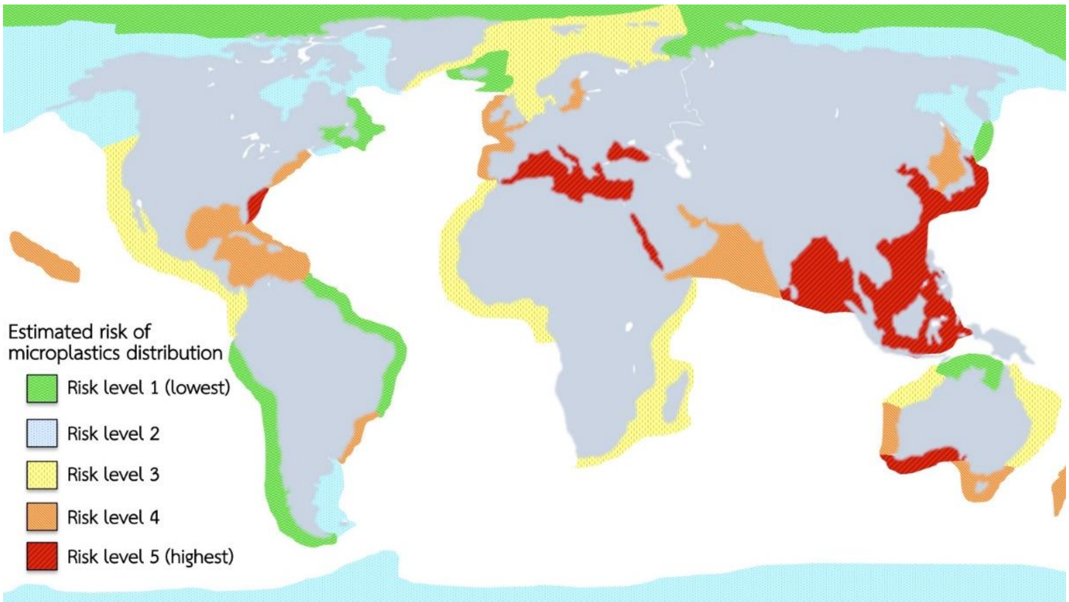


Figure 4 Estimated risk of microplastics distribution in global marine environment, based on Lebreton et al.(2012) (Graphic from GESAMP (2015))

Plastics litter problems have been existing for many decades. Among these problems, microplastics have also been contaminating many environments worldwide, especially in aquatic environments. Many reports from all over the world have confirmed the presence of microplastics in various environments, including freshwater, beaches, mangrove forest, sea sediments, seawater, and even sea ice (Ng and Obbard, 2006; Andrady, 2011; Nor and Obbard, 2014; GESAMP, 2015; Zeng, 2018; Kasamesiri and Thaimuangphol, 2020; Wang et al., 2020). After the anthropogenic occurrence, environmental and anthropogenic factors were responsible for the distribution of this plastic debris. The environmental factor may have a greater role in distributing these particles to the vast environment (Shahul Hamid et al., 2018).

The contamination degree of microplastics in the environment has differed from place to place. However, the collection and interpretation method poses difficulties in a direct comparison between places. For example, Mediterranean Sea surface water had microplastics litter contamination at the rate of 16,339 to 520,213 particles per square kilometer (Güven et al., 2017), while surface water of Singapore reportedly has 4 particles of microplastics per liter of water (Ng and Obbard, 2006).

South China Sea has ranked among the top location in the world with a high concentration of plastic debris and microplastics. Qiu et al. (2015) reported that 251-436 pieces of microplastics per 50 grams of dry sediment were found from various places around the South China Sea. On the contrary, Singapore's Sentosa beach has reportedly no microplastics contamination in beach sediments. Other beaches in Singapore have little contamination, which is less than 10 pieces per kilogram of sediment (Ng and Obbard, 2006). In Mangrove Forest, where the tidal effect has an important role in the ecosystem, microplastics were also found. Approximately 12.0 to 62.7 pieces of microplastics per kilogram of dry sediment were reported from mangrove forests around Singapore. The microplastics in mangrove forests consisted of smaller size than the previous study in beaches and sea sediment, most of them were smaller than 40 micrometers (Nor and Obbard, 2014).

The type of plastics polymer also differed between location and type of environment. This might be contributing to plastic usage in certain areas or the nature of the distribution of certain plastic polymers in the environment. Microplastics with low specific gravity, such as polystyrene, were found only on the water surface. Mostly every polymer type can be found in the water column, while in sediments, most plastic polymers found were fiber-type and other hard plastics, which are heavier (Güven et al., 2017).

From the meta-analysis of Erni-Cassola et al. (2019), the distribution of different plastic polymer types in the marine environment was assessed. Polyethylene was the most common polymer type found in the environment, with the predicted prevalence at 23% of all microplastic polymers. The second most common polymer was a fiber-type microplastic polymer of polyester and polyamide group, with predicted prevalence at 20%, followed by polypropylene at 13%, and others polymers at lower ratios.

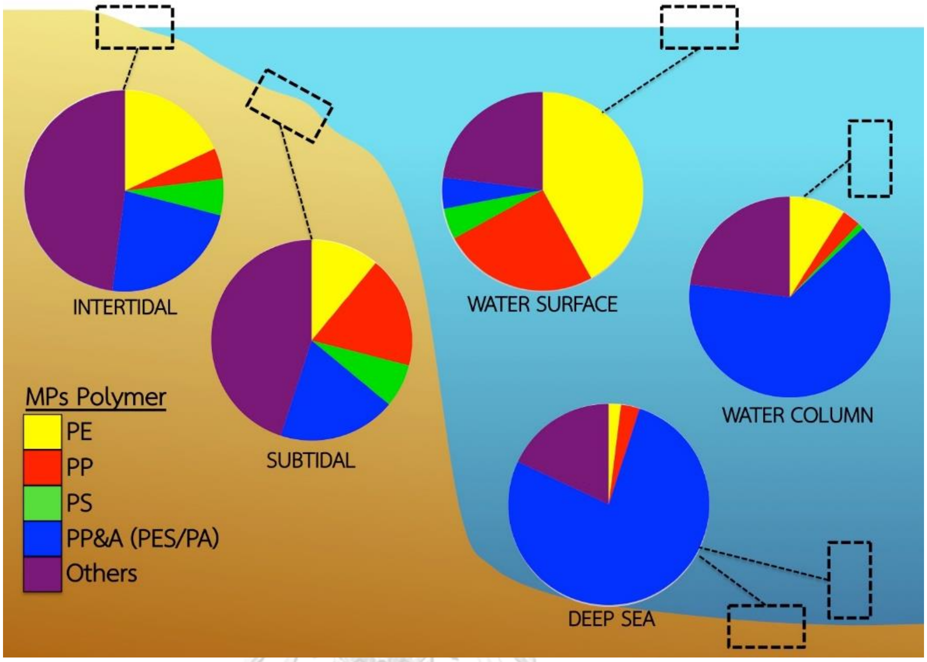


Figure 5 The distribution of microplastics polymer type between different oceanic zone, adapted from Erni-Cassola et al. (2019).

In beaches and sediments of Singapore, many types of polymers were reportedly found, including polyethylene, polypropylene, polystyrene, nylon, polyvinyl alcohol, and acrylonitrile butadiene styrene. On the contrary, a polymer found in the water surface and subsurface layer of Singapore only consisted of 3 major polymer types, including polyethylene, polypropylene, and polystyrene. While in the mangrove forest, the plastics polymers found were mainly polyethylene and polypropylene (Ng and Obbard, 2006; Nor and Obbard, 2014).

In Thailand, few publications have reported the presence of microplastics in the environment. Most of these publications were published after 2017. The study of Matsuguma et al. (2017) has confirmed the presence of microplastics in sediments from the Gulf of Thailand. The sizes of Microplastics were ranging from 315 μm – 1 mm. There was also a difference in the level of microplastics contamination between the depths of the sediment, which can determine the time period that the contamination had occurred.

In the deepest layer of sediment, at 44-46 centimeters or more, no microplastic contamination had been detected. This layer could be dated back to the 1950s. The presence of microplastics has started from the layer that could be dated back to the 1960s. The surface layer (the 2000s) contained markedly higher microplastics than the 6-12 cm layer (1990s), which indicates a marked increase in microplastics contamination rate from the 1990s to 2000s. This can be implied that plastic consumption and microplastic released in Thailand had started in the 1960s and markedly increased after the 1990s. In the same way, Wang et al. (2020) also reported that surface sediments in the Gulf of Thailand have a high level of microplastics contamination. Microplastic particles were detected from all 18 sampling sites around the Gulf of Thailand. The average number of particles count was 150.4 ± 86.2 pieces per kilogram dry weight of sediments, which consider medium level compared to other locations worldwide. More than 70% of microplastics found were in the size of 0.5-1 millimeters. The most abundant types were rayon (37%) and polyester (16%).

The beaches along the eastern coast of Thailand also reportedly had microplastics contamination. All of the samples from 21 beaches had microplastics count ranging from 420 to more than 200,000 pieces per kilograms. Most of the microplastic particles found on the beach were fragment type. Only a few portions were fiber-type microplastics (1.49%). More than 60% of fragment particles were in white color. On the other hand, white color consists of around 40% of fiber microplastics, which followed by blue color at around 35%. Significantly higher microplastics deposition was in Rayong province, where many industrial areas are located, compare to Chonburi, Chantaburi, and Trat (Bissen and Chawchai, 2020).

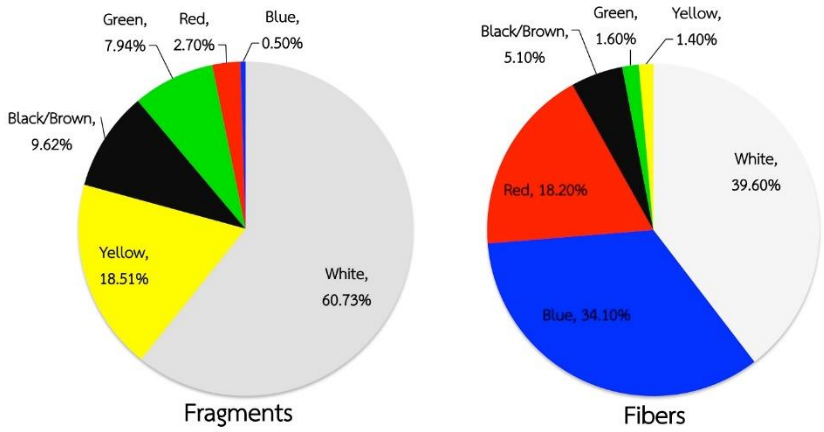


Figure 6 The color distribution of microplastics contaminated on beaches of the eastern coast of Thailand, Adapted from Bissen and Chawchai (2020).

Not only in a marine environment, but microplastics were also reportedly found in the freshwater environment in Thailand. A report revealed that microplastics had contaminated the Chao Phraya River in the Bangkok area. There was 80 ± 65 items/m3 or 53.3 ± 58.4 mg/m3 of microplastics in the water and 91 ± 13 items/kg and 4.9 ± 3.4 mg/kg of microplastics in the sediment. The main morphologies of microplastics were fragments and fibers; most of them were white. Polypropylene (PP) was the dominant polymer type found in both the water and the sediment. A high amount of fiber-type microplastics, for instance, polyester, was found in the sediment of the river. Moreover, heavy metals contamination (Cr, Cu, Ni, and Pb) on microplastics were also determined by ICP-OES in this study (Ta and Babel, 2020).

The color of microplastics can provide some information about the source of microplastics. It can also tell the color preference of ingested organisms or the conditions that the particles had been exposed to. Beaches fragment microplastics from the Eastern coast of Thailand were mostly white while the fiber type collect was mostly white and blue (Bissen and Chawchai, 2020). Currently, there is still no standard scheme for the determination of microplastics color. GESAMP (2019) recommended the use of the color identification scheme proposed by The European Marine Observation and Data Network (EMODnet) in 2017. Eight color groups were proposed for easier and more harmonized work on marine litter identification. The color schemes proposed were Black (including grey), Blue/Green, Brown (including tan), White (including cream), Yellow, Orange/Pink/Red, Transparent, and Opaque (Galgani et al., 2017).

Another important indicator for microplastics contamination in a marine environment is the filter feeders. Microplastic contamination was found in tissues of sessile filter-feeding invertebrates collected from the eastern coast of Thailand. The collecting sites were namely Angsila, Bangsaen, and Samaesarn. Angela had the highest prevalence of microplastics contamination among 3 locations. This can be implied that there is a presence of microplastics in the seawater from the eastern coast of Thailand. The most common plastic polymer types were including polyamide and polyethylene terephthalates, followed by polystyrene (Thushari et al., 2017).

2.5 Contamination of microplastics in aquatic animals

Aquatic animals can be exposed to microplastics because of the tremendous contamination level of microplastics in aquatic environments. Gills exposure and absorption were possible in certain circumstances. Very small particles might be able to absorb through the gills of the animal (GESAMP, 2015).). The most significant exposure route was by ingestion, either intentionally or unintentionally. One of the most frequently used indicators of environmental contamination in the marine ecosystem is a filter-feeding animal. Many publications found that the flesh of filter-feeding animals has microplastic contamination. A study from the eastern coast of Thailand showed that tissues of sessile filter-feeding invertebrates, namely the striped barnacles (*Balanus amphitrite*), the periwinkle (*Littoraria* sp.), and the rock oyster (*Saccostrea forskalii*), have microplastics contamination in the range of 0.2-0.6 particles per gram of animal flesh. Rock oysters have the significantly highest microplastic contamination (0.57 particles per gram). The lowest was periwinkles (0.17 particles/g). Polyamide and polyethylene terephthalates were among the highest microplastics found, followed by polystyrene (Thushari et al., 2017).

Unlike small filter-feeding invertebrates, larger vertebrates can easily ingest microplastics into their gastrointestinal system. Many reports worldwide had confirmed the presence of microplastics in the gastrointestinal tract of aquatic vertebrates especially fish. The prevalence and amount of ingestion usually depend on the location and species of fish. One reason that affects the difference of microplastics exposure among fish species was the feeding habit of certain fish species. For example, Fish species that selectively foraged on small invertebrate prey was less likely to expose microplastics in feed compared to fish species that preferably foraged on a wide range of natural feed, including detritus (Peters et al., 2017). Ingested particles were either accumulate or excrete from the animal (GESAMP, 2015). From the study of Jovanovic et al. (2018), microplastics retention can be found in the gastrointestinal tract of a fish even after 1 month of depuration period. However, the concentration was low compare to the microplastics ingesting period.

A study of fish from the English Channel revealed that 36.5 percent of fish samples had microplastics in their guts. The prevalence was higher than 50% in Blue Whiting (*Micromesistius poutassou*) and Red Gurnard (*Aspitrigla cuculus*), which are pelagic and demersal fish, respectively. The lowest prevalence was found in thick-back sole (*Microchirus variegates*) (22%), which are demersal fish. There was slightly but no statistical difference between pelagic and demersal fish (38% and 35% respectively). The average microplastics count in this study was 1.90±0.10 pieces per fish. The most abundant polymer types are polyamide and polyester (Lusher et al., 2013).

A study from the United States revealed that 42.4% of marine fishes from the Texas Gulf had microplastics in their gastrointestinal tracts. The average particle counts per fish was 0.82 pieces per individual fish. Species with the lowest and highest contamination rate was Grunt (26.8%) and Atlantic Spadefish (46.6%) (Peters et al., 2017).

Higher ratios were reported from the Mediterranean Sea, 28 species of fish from 10 locations were examined for microplastics in their gastrointestinal tract. 58% of fish examined had microplastics in their gastrointestinal tract. A slightly lower percentage of microplastics was detected from the stomach than from the intestine. 34% of fish had microplastics in their stomach while 41% of fish had microplastics in their intestine. The average particles count in the stomach and intestine were 1.80 and 1.81 particles per fish, respectively (Güven et al., 2017).

In Malaysia, many species of fish were reported to have ingested plastic particles. The Highest prevalence was in African catfish (*Clarias gariepinus)* (60%) and Indian mackerel (*Rastrelliger kanagurta)* (50%), which are benthic and pelagic fish, respectively. No microplastics was detected in Oxeye Scad (*Selar boops) and* Kawakawa (*Euthynnus affinis),* which were demersal and pelagic-neritic fish species. Most microplastics found were fragment type (67.4%). Polyethylene was the most abundant microplastics polymer found in this study (88.4%), followed by polypropylene (9.3%) (Karbalaei et al., 2019).

On the contrary to many other location, microplastics contamination in planktivorous fish from the Northern Baltic Sea was relatively low. Only 1.8% of herrings and 0.9% of sprat was found microplastics in their gastrointestinal tract, while Three-spine sticklebacks fish had no microplastic particles at all in their guts (Budimir et al., 2018).

In Thailand, there was a report of microplastics ingestion in marine fish from the lower Gulf of Thailand. From this report, 54.29 percent of fish samples from

Songkhla province contained plastics in their gut. Among these numbers, 27.27% was microplastics (smaller than 5 millimeter). 69.88% was mesoplastics (5-25 mm) and 2.85% macroplastic, (>25 mm). The most abundant plastic types was fibers (Azad et al., 2018). The contamination of microplastics in fish occurred not only in marine fish but also in freshwater fish. 72.9% of freshwater fish from the Chi river in North-eastern Thailand found microplastics in their gastrointestinal tract (Kasamesiri and Thaimuangphol, 2020).

Most of the field studies at present were focus mainly on digestive system of the fish or other animals, very few were studied on distribution or accumulation of microplastics in other tissues or body fluids of filed-collected aquatic animals. However, many experimental studies have shown that microplastics were able to be uptake and distribute to other organ system of the animal apart from digestive tract (GESAMP, 2015).

Apart from fish, fish derived product such as fishmeal was confirmed to have microplastics contamination. This is because of the fish meal making process that use whole fish including gastrointestinal tract (Hanachi et al., 2019). Various fishmeal and Krill meal products from 11 countries were tested for microplastics contamination. The results came out that all samples contain microplastics contamination except only krill meal from Antarctica. The average contamination rate from each country ranged from 33.3 to 337.5 pieces per kilogram of fishmeal. The highest contamination rate was from China (337.6±34.5 pieces per kilograms of fishmeal) (Gündoğdu et al., 2020).

Invertebrates also reported to have microplastics contamination. Sessile invertebrates from the Thai eastern coast were reported to have contamination of microplastics in tissues (Thushari et al., 2017). From many studies, the presence of microplastics does not relate with trophic level of the fish. However, fish from pelagic-neritic zone has slightly higher number of microplastic particles than fish from other habitats (reef, demersal or bentho-pelagic) (Güven et al., 2017; Azad et al., 2018).

2.6 Health effect of microplastics on aquatic animals

2.6.1. Physical effects

These plastics can pose direct physical threat to small-size aquatic organisms, for example, injuries or obstruction of the gastrointestinal tract. This seems to have relations with the structure, shape and size of the particle (Horton et al., 2017). Microplastics have the possibility to cause intestinal blockage in larvae of European sea bass (*Dicentrarchus labrax*) (Mazurais et al., 2015). In another experiment performed on European sea bass (*Dicentrarchus labrax*), Intestinal histopathological lesions were observed in fish fed with PE and PVC microplastics. Fish show no tissue damage lesions in the proximal part of the intestine except the accumulation of leukocytes. In the distal intestine, degrees of intestinal damage were shown on histopathological examination. Intestinal mucosal epithelial damage, decreasing of goblet cells and shortening of villi was observed (Espinosa et al., 2019).

Microplastics are being concerned to interfere the life cycle of small-size organisms in the ecosystem, which can consequently affect the food web. For instance, nanoplastics can affect growth and reproduction of *Daphnia magna*, and the effect were even stronger with the presence fish kairomones (known to cause stress in *Daphnia*) (Besseling et al., 2014).

2.6.2. Cytotoxic effects

Direct effects of microplastics or nanoplastics can be resulting from the translocation of these particles into the circulatory system or tissues of the organism. Currently, there were very few study demonstrate or examined such pathways (GESAMP, 2015). A study had confirmed that small microplastics can be taken up by the gills and digestive glands of the blue mussels (*Mytilus edulis*). Endocytosis and lysosomal system was responsible pathways in up-taking of these microplastic particles. Accumulation of plastic particles in the lysosome can cause the lysosomal membrane breakdown and the releasing of digestive enzyme, which consequently causing cell death (Von Moos et al., 2012). Endocytotic microplastics can also cause cellular stress, which the cell consequently responded by secreting the damage associated molecular patterns (DAMPs). DAMPs can trigger the inflammatory responses or reactive oxygen species (ROS) formation by the induction of innate immunity via toll-like receptors (TLR). Transcytosis or absorption through impair gut/vascular barrier were also among pathways that led microplastics or nanoplastics accessed into the circulatory system (Yong et al., 2020). The conclusive diagram was shown in figure 7.

The study by Von Moos et al. (2012) also demonstrates that microplastic particles in the connective tissue of the digestive tract, which later became phagocytized by attracting eosinophillic granulocytes. This accumulation of granulocytes or so-called the formation of “granulocytoma” was the early signs of host response upon intake of plastic particles that can be seen within 6 hours after the ingestion. These effects can be also histologically observed by the vacuolization and the formation of granulocytomas in digestive tubuli of the mussel (figure 7).

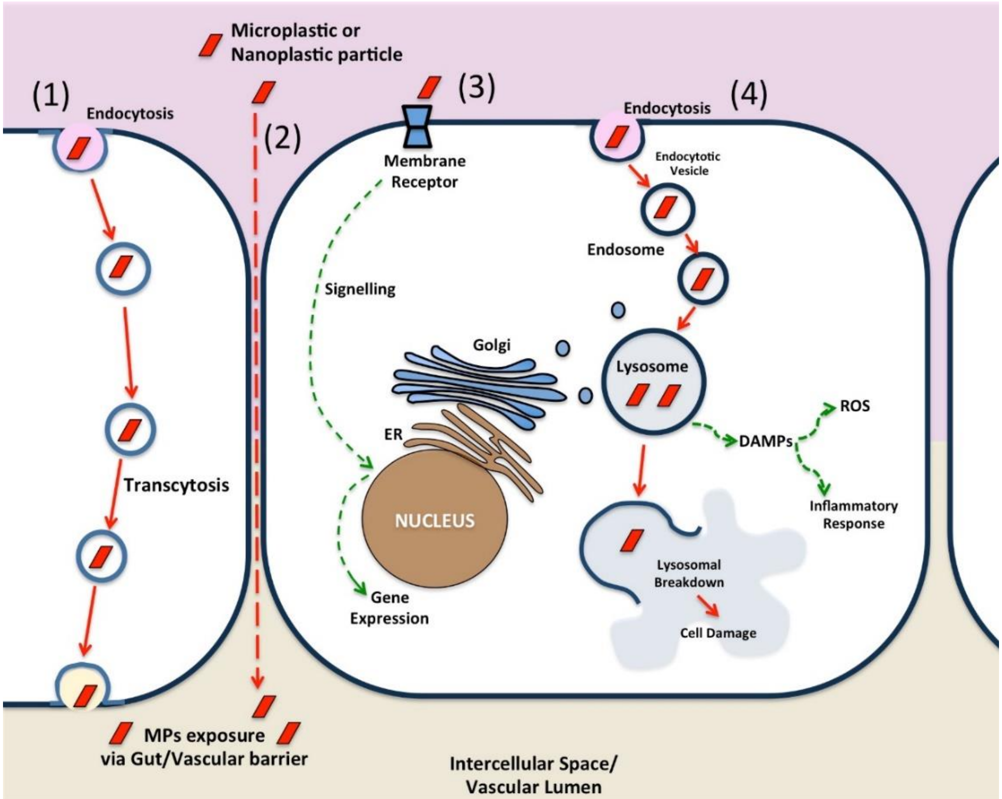


Figure 7 Conclusive diagram of microplastics uptake pathways into Eukaryotic cells and potential pathways of microplastics cytotoxicity. (1) Transcytosis; (2) Gut barrier breach; (3) Altering of gene expression via membrane receptor(s); (4) Endocytosis and inflammatory responses (Modified from Moore, 2006; GESAMP, 2015; Yong et al., 2020)

In Fish, microplastics were also confirmed to be absorbed via intestinal mucosa and get into the circulatory system. These plastics can be consequently accumulated in internal organ (Jovanović, 2017). In experimentally microplastics-fed zebrafish, microplastics were accumulated and caused acute inflammation and lipid accumulation in the liver of the fish. It also caused changes in liver metabolomics profile of the fish (Lu et al., 2016). European sea bass (***Dicentrarchus labrax***) fed with virgin PE and PVC microplastics 0.01 to 0.05% w/w show histopathological lesion in liver and intestine, including hepatocytes morphological change, vacuolation, hypertrophy and signs of blood sinusoid congestion. Fish fed with PE microplastics showed signs of decreasing antioxidant enzymes in liver and intestine of experimental fish. Thus, PE microplastics can cause oxidative stress in European Sea Bass (Espinosa et al., 2019).

These plastics reportedly caused very few histopathological changes in the tissue and did not alter major blood chemistry profiles of the fish. This is because most plastic polymer itself is considered chemically inert and has very little short-term harm to the fish (Jovanovic et al., 2018).

Despite many studies confirmed the presence of microplastics in the gastrointestinal tract of fish, very few demonstrated or tried to examine the presence of those plastics in other organs or tissue. In fact, degree of ingested microplastics translocation from the gastrointestinal tract is low. In an experimental study, only 1 particle of microplastics (1-5 μm in size) can translocate to the fillet of the European Sea Bass (*Dicentrarchus labrax*) for every 1.87×10^7 ingested particles (Zeytin et al., 2020).

2.6.3. Chemical effects

Apart from plastics polymer, microplastics can be contaminated with other chemicals, which is intentionally or unintentionally added to plastics, including unreacted monomers, residue catalyst, solvent or chemical pollutants from the environment. Plastics additives were chemical that added during or after plastics polymerization process to made it desirable function or appearance. Additives can be wide ranges of chemicals from organic to inorganic or less-toxicity to high- toxicity compounds. These additives will have different effects on animal health or organ system (Hahladakis et al., 2018). There was also a report of heavy metals accumulation in microplastics in the environment from Chao Phraya River (Ta and Babel, 2020).

This is because the hydrophobicity of plastic can absorb a wide range of chemical compounds found in aquatic environments, including many toxic pollutants, For example, persistent organic pollutants (POPs). POPs are one of the important groups of organic compounds that have greater affinity in binding to the plastic than staying in the seawater. Examples of common POPs are polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). They can be potentially harmed if they were ingested by marine organisms and can be accumulated up in the food chain (Arthur et al., 2009; Jovanović, 2017; Niaounakis, 2017).

These contaminants are major harm from microplastics. The study of Jovanovic et al. (2018) showed that pure microplastics has no short-term harm to the fish. They also have short retention time and less chance of accumulation in fish body. As well as the study of Rainieri et al.(2018), which revealed that microplastics together with contaminants, including PCBs, BFRs, PFCs and methyl-mercury, posed significantly greater effects on fish more than only contaminants at twice concentration. This study also showed that pure microplastics alone did not alter the expression of certain gene in the Liver, brain and intestine of the affected fish. However, microplastics with those contaminants appeared to be able to alter gene expression in the liver in the same level as chemicals alone with double concentration. It can be implied that microplastics can increase the chance of toxicity and accumulation of these POPs in fish, which could be further potentially harm to fish consumers (Rainieri et al., 2018).

Another body system that is affected from microplastics is reproductive system. Some plastic polymers potentially act as endocrine disruptors. Fish fed with pure polyethylene (PE) microplastics showed some endocrine disrupting signs after 2 months. In addition, fish fed with PE that contaminated with environmental contaminant showed some abnormal histological changes of male germ cells (Rochman et al., 2014). In an experimental study, both virgin LDPE and LDPE loaded with Phenanthrene contaminant cause a significant alteration of reproductive genes transcription in African catfish (*Clarius gariepinus*). Virgin LDPE also caused a disturbance of cholesterol and LDL levels in the the fish (Karami et al., 2016). In Oyster, reproductive disturbances were reportedly caused by the ingestion of polystyrene (PS) microplastics. There were significant decreased in oocyte number and sperm velocity with molecular signs of endocrine disruption (Sussarellu et al., 2016). These phenomena might relate with endocrines disrupting effects of plastic monomers and plastic additives.

Two important and frequently used plastic additives that have endocrine disrupting effects were Bisphenol A (BPA) and Phthalates. BPA can be used as the compound monomer of polycarbonate plastics (PC) or the additive of other plastics, such as polyvinyl chloride (PVC). BPA rank among the top of plastics that known to have endocrine disrupting effects. BPA is also one of the major additives that can migrate from plastic food container into food or drink upon contact exposure. BPA was reported to have many adverse effects in experimental animals. BPA can bind to several steroid receptors on cell membrane causing various effects, ranging from hormonal changes to gonadal neoplasms (Halden, 2010).

Another frequently used plastic additive that also classified as an endocrine disrupting agent was Phthalates. Phthalates are di-ester compounds of phthalic acid, which can be used as plasticizer in various types of plastics, including many food container materials. Phthalates can cause adverse reproductive effects, especially in the male, such as testicular dysgenesis or abnormal sperm production (Halden, 2010).

Apart from aforementioned 2 significant harmful additives, data on effects of additives or pathophysiology in most of other type of plastics were still limit. Fiber-type microplastics or so-called “microfibers” were still need more studies on their effects on animals and human (Henry et al., 2019).

2.7 Microplastics and risk to human health

2.7.1 Microplastics toxicity to humans

Nowadays, few studies have been done in order to characterizing hazard and risk of microplastics to human. Focusing on health effects from contaminants and additives, microplastics and nanoplastics likely pose some hazard to human in the same way as in other animals. However, these micro- and nanoplastics ingestion may have relatively low hazard of transferring additives and contaminants to human compare to other routes of exposure. Such routes were, for instance, dermal uptake, aerosol or inhalation of dust and plastic particles. Therefore, FDA have concluded that microplastics and nanoplastics likely pose negligible threat of transferring additives and contaminants to human via consumption (Lusher et al., 2017). However, the data for direct effect and other effects of microplastics to human health were still not adequate. Moreover, abundance of plastics debris and especially microplastics were growing at very high rate. Therefore, the risk of microplastics to human health in the future may increase (Andrady, 2017; Shahul Hamid et al., 2018).

Few studies were done to evaluate the direct effects of microplastics to humans. The study of *in vivo* toxicity of these plastics to humans was still absent. Some previous studies were performed *in vitro* on cells culture. Noting that most of the studies revealed that toxicity of microplastics or nanoplastics usually occurred at a relatively high degree of exposure (Yong et al., 2020). For instances, a study had confirmed that polypropylene microplastics show some cytotoxic effects to human cells and can induce local immune response. The degree of cytotoxicity was related to the size and dosage of microplastics. In smaller sizes or higher dosages, polypropylene microplastics posed greater cytotoxic effects to human cells than larger sizes or lower dosage (Hwang et al., 2019). From the current understanding, it was suggested that plastics create some negative toxic effects on human. However, the clear degree of toxicity was still not fully illustrated. Further studies should be focused on these potential effects for the clarification of human health threats in the future (Yong et al., 2020).

2.7.2. Microplastics exposure in humans

In actuality, microplastics were proven to be able to be ingested by human. A report was confirmed the presence of microplastic particles in human stools from 8 volunteers in Asia and Europe. All volunteers had lived their daily routine and did not have any dietary restrictions. A median of 20 particles per 10 grams of human stool was reported. The most abundant polymer types found in this study was polypropylene and polyethylene terephthalate. 6 out of 8 participants were reported to had eaten seafood meals during the study (Schwabl et al., 2019).

Risk of microplastics intake for American people was carried out by meta-analysis from 26 publications. Seafood, bottled water and air were among the most likely sources of microplastics to American people, Containing 1.48 particles per grams of seafood, 94.37 particles per liter of bottled water and 9.80 particles per cubic meters of Air. Daily microplastics consumption rate in male children, male adults, female children, and female adults were also different due to physiological differences and other eating habits. The daily consumption rate from food and beverages was estimated to be 113, 142, 106, and 126, respectively. Therefore, microplastics consumption rate for those groups of Americans were around 41000, 52000, 39000, and 46000 particles per person per year, respectively (Cox et al., 2019).

Seafood is one of the major sources of microplastics intake for humans. Average seafood consumption per capita and amount of microplastics contamination in seafood have important roles in determining degree of microplastics intake in different region. For instance, Americans were less likely to received microplastics from seafood compared to Japanese, which has much higher seafood intake rate. However, the amount of microplastics contamination in seafood for American and Japanese might be different. Therefore, the meta-analysis in the study previously mentioned can be used with only American people (Cox et al., 2019). Some intake rate from others publication were ranging from 1 pieces per day to 30 pieces per day per person (Lusher et al., 2017). Therefore, determining amount of microplastics contamination in specific region is important in understanding the risk of people in that region.

Thai people consume relatively high amount of seafood every year. The report of Food and Agricultural Organization revealed that Thai people consume fish and fisheries product 27.2 kilograms per capita in 2016, which is higher than global average at 19.7 per capita (FAO, 2019). Correspondingly, a proposed estimation of average seafood consumption of each country by seafood consumption footprint was published in 2011. From the study, Thailand has an estimation of 1,785,733 tons of total seafood consumption footprint per year. Average seafood consumption per capita in 2011 was 25.7 kilograms, which can be divided to consumption by fisheries, aquaculture and fishmeal product equal 9.8 kg, 8.6 kg and 7.4 kg per capita, respectively. This estimation was also higher than global average at 22.3 kilograms per capita (Guillen et al., 2019).

In public health aspects, the exposure or average daily dose (ADD) of microplastics can be calculated by using exposure dose per weight and time. The exposure dose can be calculated by concentration of microplastics in food multiplies with intake rate of that food. The intake rate of specific food for Thai people can be obtained Food consumption data of Thailand, Office of Standard Development, National Bureau of Agricultural Commodity and Food Standards, Ministry of Agriculture and Cooperatives. The data of 18-35 years old Thai people in 2016 has shown that feed intake rate of group of marine fish, including grouper and seabass, was at 1.70 grams per people per day. While feed intake rate of Short Mackerel was at 9.21 grams per people per day (ACFS, 2016).

In 2016, The European Food Safety Agency (EFSA) made a preliminary assessment of the problem of plastic particles related to food safety. EFSA reported that there were still insufficient data to assess human microplastics exposure by seafood consumption. Therefore, EFSA stated that further investigations on microplastics contamination in various food sources are important to ensure seafood safety. Such monitoring can consisted of both targeted and market sampling (GESAMP, 2019).

2.7.3. Microplastics risks in the food chain

Humans can expose to microplastics in the food web not only by direct intake of microplastics from seafood but also by the intake of seafood-by-products, such as fishmeal. As a high-quality protein source, fishmeal was used in many animal feeds. Therefore the contamination of microplastics in fish meal tend to directly affect the global food web, including humans (Gündoğdu et al., 2020).

Using microplastics-contaminated fishmeal as a feed protein source for livestock animals can lead to microplastics contamination and accumulation in those animals. One of the common examples of animal feed made from fishmeal products was fish feed pellets in aquaculture industries. The Common Carp (*Cyprinus carpio*) is a common aquaculture fish species, which is normally fed with fishmeal-contain feed pellets. In an experimental study carried out by Hanachi et al. (2019), fish fed with pellets made from microplastics-contaminated fishmeal were found to have microplastics in their guts after 4 weeks of exposure, while fish fed with plastic-free soybean meal feed were free of microplastics. The conversion ratio from microplastic counts in fishmeal (counts per gram) to the experimental fish (count per fish) was ranging from 0.205 to 0.584.

In Thailand, fishmeal was used in many aquacultures compound feed. From the FAO data obtained in 2008, fishmeal were used as a protein sources in various proportion in aquaculture feed, for example, 5-25% of Shrimp feed (average FCR 1.5), 5-20% of freshwater prawns feed (average FCR 1.7), 20-50% of Barramundi feed (average FCR 1.8), 0-20% in Tilapia feed (average FCR 1.5), and 5-20% in Catfish feed (average FCR 1.4) (Tacon and Metian, 2008).

2.8 Microplastic detection method

In general, 2 major steps are involved in the detection process of microplastics, which are the visual identification of potential plastic particles and the chemical confirmation by plastic polymer analysis (Shim et al., 2017). However, different sample preparation processes must be used between different sample types to obtain appropriate and accurate microplastic particles for further identification processes. Strict laboratory protocol must be applied to prevent contamination from the examiner or environment.

Because microplastics contamination in the environment may be low and sometimes close to the lower detection limits, a quality control protocol must be carried out to validate and ensure the reliability of the procedure. This is including the adding of procedural blank, replicate sample, spiked blank sample, matrix spike sample, or certified reference materials with every batch of samples analyzed (GESAMP, 2019).

2.8.1. Laboratory protocols for the prevention of contamination

It is generally known that microplastics are ubiquitous in the environment, including the human body, shirt, and laboratory environment. Many studies have developed protocols to limit such contaminants. Dehaut et al. (2018) had published a recommendation for the studies of microplastics in seafood to summarized and harmonized protocols between many laboratories. The details were as follows;

a. Contamination from operators

   Microplastics can be contaminated on the body and especially on the hands. Synthetic fibers cloths were also possible sources of contamination. In the laboratory process, operators must wear clean gloves that had been kept in a clean and closed container. The sterility of the glove had not been mentioned, as it did not affect the contamination of microplastics. Cotton lab clothes or gowns must be worn to minimize the risk of synthetic fibers contamination. A sticky roller can be used to ensure that there were no possible fibers contaminate the cloths.

b. Contamination from work environment

   Dehaut et al. (2018) have described the work environment related to microplastics contamination into 2 groups, including sample processing place and equipment. The processing place can be varying. However, many studies showed that a clean bench, i.e., laminar flow or safety cabinet with HEPA filters, is the best protocol to prevent airborne contamination. Others were fumed hood or cleaned laboratory benches. The equipment used must be clean or rinsed with a clean solution. i.e., deionized water or alcohol. A control experiment must also be done to check for contamination in the process.

Preventing airborne microplastics is essential for accurate results. Dris et al. (2017) reported that there are 5.4 microplastic particles per cubic meter of air in an indoor environment. These numbers can lead to an erroneous result if the preventing protocols were not properly performed.

c. Contamination from used solutions

The solution used in the study must be microplastics-free. Milli-Q, deionized, or other purified water should be used. Filtration before the process is also mandatory. Dehaut et al. (2018) also mentioned that “microplastics-free” grade solutions might be suitable in the future.

d. Controls of contamination

Dehaut et al. (2018) have also described the mandatory of using control blank to monitor for contamination and should be addressed in the publications. Controlling for possible air contamination in the laboratory environment can also be done. Lastly. Positive and negative control of procedures can be added for more reliability of the process.

2.8.2. Detection from environmental samples

Microplastics can sparely distribute in the environment. The most common sample types that usually included in many studies on microplastics were water and sediment samples. Large number of samples usually requires due to the low concentration of microplastics in the environment, especially in seawater. Towed net or other equipment primarily designed for a collection of small biota were commonly applied to collect a large volume of water for microplastics detection. The mesh size will determine the size range of microplastics collected from the water. In the case of sediments sampling, the various methodology also has been applied to collect sediment samples for microplastics detection. Location, type of sediment, and characteristic of sediments can vary the sampling techniques. For example, core samples have been applied to detect microplastics from a different depth of sediment (GESAMP, 2015; Matsuguma et al., 2017).

Microplastic in environmental samples can be easily separated from other materials by density separation method in solutions that have higher specific gravity than plastics, i.e. potassium iodide. In this step, Heavy materials, which have higher specific gravity than the solution, such as sand and other sediments, will sink to the bottom of the container, while microplastics will float on the surface. After that, the supernatant with microplastic particles will be filtered through a small pore mesh to obtained microplastics. Further identification processes might be needed to confirm the existence of microplastics. Lipophilic dye (such as Nile Red) can be used to help to stain microplastics. Most microplastic can be seen by stereomicroscope or optical microscope. However, in the case of nanoplastics, a higher magnification microscope like an electron microscope might be needed (Andrady, 2011).

2.8.3. Detection from animal tissue samples

Apart from detection of microplastics contamination in the environment, monitoring microplastics contamination in animals is also a good indicator for the environmental plastics contamination situation (GESAMP, 2019). In the case of the detection of microplastics contaminant in animal tissues, more processes must be done to effectively separate microplastics from other tissues and contaminants. The processes are described as follows;

a. Criteria for a good indicator species or group of species for monitoring microplastics in marine biota.

Marine organisms can be used as a good indicator of microplastics contamination in the marine environment. In this case, microplastics impacts on the organism will be second to the degree of contamination, which will reflect the overall contamination picture in a certain environment or region. A sessile species can be the indicator for a specific sampling location, while a mobile species can be used as a spatial or regional indicator (GESAMP, 2019).

There were several criteria in selecting a species or a group of species as a bio-indicator. The criteria for good indicator species for monitoring microplastics in marine biota was published in the report and studies no.99 of GESAMP (GESAMP, 2019). These criteria were; as follows.

- A regional representative species living within the geographic range.
- An abundant species in the region (non-threaten or not a protected species).
- An important species in the region (Commercially or Ecologically).
- Comparable to indicator species in other area of the world.
- In case a group of species, there must consist of mixed ecological niches or feeding habits.

b. Sample collection and tissue collection

Animal sampling procedure, particularly marine organism sampling, is important to obtain accurate data for environmental contamination. Target sampling may perform to directly collect target species in the environment. On the other hand, market sampling can also provide cost-effective and reliable data of environmental contamination only if the certain origin of the animals (such as collection method and location) was available (GESAMP, 2019).

The recommendation for the studies of microplastics in seafood (Dehaut et al., 2018) has described the importance of sampling procedure and data record. Recording of essential data related to seafood should be done. These data were including location, method of catching, and biometric data of the animal.

To obtain tissues for microplastics study, whole animal or part of animal tissue will be used. The selection of whole animal or animal tissue will depend on the type of animal. The whole animal sample is usually applied in small animals, such as small crustaceans or mollusks. In larger animals like fish, researchers were recommended to dissect only target tissue or organ.

Samples should be kept either below 0°C or in a preservative agent, i.e. 10% formalin. However keeping below 0°C was recommended by the authors due to the low possibility of chemical reactions between preservatives and plastics or digesting agents in further steps (Dehaut et al., 2018)

c. **Digestion of animal tissues**

Animal tissues must be digested in corrosive reagents to uncover microplastics inside. The reagent could be an acidic or alkaline solution. Various digestive agents were used in many publications. Examples of frequently used chemical are as follow;

- Concentrated Nitric acid (NO_3) (22.5 M); 5 grams of animal soft tissue must be digested in 20 ml of concentrated nitric acid. The digestion temperature is 60°C for 1 hour followed by 100°C for 1 hour. This can result in 99.85% digestibility (Claessens et al., 2013).
- Concentrate potassium hydroxide (KOH) (52.5 M); the same procedure will have resulted in 99.36% digestibility of soft tissue. These methods are validated that more than 97.9 % of 30 μm polystyrene microplastics and 98.3% of 100x400 μm fishing nylon fibers can be detected after digestion. However, these first two methods seem to have digested and obscured the presence of smaller nylon particles with the size of 30x200 μm (Claessens et al., 2013).
- Potassium Hydroxide (KOH) 10% w/v (200ml/sample); incubate at 72°C for 72 hours (Karbalaei et al., 2019).
- 30% H_2O_2 (30 ml/sample); Incubate at 65°C for 24 hours (Kasamesiri and Thaimuangphol, 2020).
- Mixture of 1 M Sodium Hydroxide (NaOH), Sodium Dodecyl Sulphate (SDS), and Fish tissue in the ratio of 10:5:1, respectively. Incubate at 50°C for 48 hours. This method is suggested for better

digestion of organic materials and better preservation of microplastic particles. From the validity test, the microplastics retrieval rate of this method was 90 ± 12% for microplastics in the size range of 300 μm to 1 mm. and 78 ± 16% for microplastics smaller than 300 μm. The physical shape, size, and weight change of spike microplastics were negligible (Budimir et al., 2018).

d. Filtration of microplastics out of digested materials

After digestion, microplastic particles must be collected out from mixtures of digested animal tissue. Claessens et al. (2013), recommended that the digested materials must be diluted in a volume of warm filtered deionized water and then vacuum filtered through a coarse sieve, a mesh, or a porous membrane, which pores are large enough to let smaller unwanted materials but microplastics pass through. The filtration equipment used in publications can be varied, for example, 5 μm cellulose nitrate membrane (Whatman AE 85) (Claessens et al., 2013), 10 μm zooplankton mesh (Jovanovic et al., 2018), 26 μm zooplankton mesh (Güven et al., 2017) or 100 μm mesh filter (Budimir et al., 2018). In some cases, If there were some undigested organic materials or calcified materials left on the filter membrane, Hydrogen peroxide and/or Hydrochloric acid (HCl) could be used to wash the filter membrane for better observation of microplastics (Budimir et al., 2018).

e. Microscopic detection of microplastics.

To manually observe microplastics particles, a stereomicroscope is needed to examine the filtered material thoroughly. Microplastics can be spherical, fiber, or irregular in shape (Claessens et al., 2013). An optical microscope with a High color fidelity camera and image analysis software can be used to aid in distinguishing, measuring, and counting the microplastics (Güven et al., 2017).

Microscopic detection is an easy and fast way to identified microplastics in samples. However, small particles, especially those that are smaller than 100 micrometers, may ambiguously identifiable as microplastics under a microscope. In addition, a remnant of organic particle leftover from digestion may appear like microplastic. Therefore, chemical confirmation might be needed to avoid false-positive identification (Shim et al., 2017). False-positive from only visual identification can be as high as 20% and 70% particularly for transparent color (GESAMP, 2019).

Basic criteria for those particles ambiguously appear as plastics are as follow; (GESAMP, 2019)

- No cellular or organic structures appear on the particle.
- In case of microfibers, the thickness should be equal throughout the length.
- The particle should have homogeneous color.

f. Chemical confirmation and Identification of plastic polymers.

Visual detection of microplastics is an easy and cheap method to identified microplastics. However, there is a high possibility of false positive by only visual detection. Therefore, a further chemical identification method is required to accurately define microplastics in any samples. The study of Karbalaei et al. (2019) found that only 76.8% of visually detected particles were confirmed to be microplastics. These methods also help to identify the chemical properties of plastic, which crucial for plastic polymer type identification (Shim et al., 2017).

Identification of the plastic-type can be done by various methods. Methods that frequently use to identified plastic polymers or study the polymer properties are gas chromatography/mass spectrometry, Hi-pressure liquid chromatography, flow injection polymer analysis, spectroscopic analysis, melt viscosity/Rheology measurement, and some other elemental analysis (Lobo and Bonilla, 2003).

Spectroscopic analysis is an easy, fast, and accurate method to identified plastic polymer types. Spectroscopic analysis methods are namely Fourier transform infrared spectrometry, Raman spectrometry; nuclear magnetic resonance spectrometry, and near-infrared spectrophotometry (Lobo and Bonilla, 2003; Araujo et al., 2018).

The Fourier transform infrared (FTIR) spectrometer is an ideal piece of equipment for such a procedure. Without a prior sample separation process needed, FTIR can directly use in any kind of sample or mixture. This method provides data of specific chemical bonds in samples. By irradiated with infrared (IR) light (wave frequency 400-4000 cm-1), samples will absorb, reflect, and transmits IR light depending on their molecular structure (Käppler et al., 2016). These will be resulting in spectral data of samples. Spectral data of plastics obtained from FTIR will be compared with data library, which is usually easily built or commercially available. Good data libraries are essential for the accuracy and precision of this method (Lobo and Bonilla, 2003; Shim et al., 2017).

FTIR machine that equipped with a microscope is called micro-FTIR or FTIR-microscope. It can switch between the microscopic lens and IR probe without the need to move samples, which makes it perfect for the study of small plastic particles (Shim et al., 2017). FTIR and FTIR-microscope are among the most frequently used equipment to identified microplastics (Qiu et al., 2015; Güven et al., 2017; Matsuguma et al., 2017; Hanachi et al., 2019; Klangnurak and Chunniyom, 2020; Wang et al., 2020). Micro-FTIR with attenuated total reflectance (ATR) is recommended to study microplastics from environmental samples (Shim et al., 2017). In general, lower detection limits of the FTIR-microscope depend on the size of the aperture of the IR probe (e.g., 10 μm). However, microplastics that are smaller than 50 μm usually difficult to obtain precise spectra (Shim et al., 2017).

Raman spectroscopy, which has comparable detection ability, was also being used in various microplastic studies (Shim et al., 2017; Thushari et al., 2017; Araujo et al., 2018). Instead of using IR light, monochromatic (laser) light was used in Raman spectroscopy. Samples interacted with laser light will produce scattered light depending on chemical bond or molecular characteristics, which consequently create a Raman curve for further interpretations (Käppler et al., 2016).

As mentioned earlier, it is still challenging to identify very small microplastics by both FTIR-microscope and Raman spectroscopy. For very small microplastics (smaller than 20 μm) or nanoplastics (smaller than 1 μm), an alternative methodology must be considered to accurately identified plastics polymer. These methods were, for example, Focal plane array FTIR (FTIR-FPA), Nano-IR, Pyro-GC/MS or SEM-EDS. Very few studies were successfully done to detect and confirmed the presence of such small nanoplastics in the environment. Thus, studies on environmental nanoplastics remain challenging for scientists (GESAMP, 2019).

CHAPTER 3

MATERIALS AND METHODS

3.1 Fish samples

Marine fish samples were collected from August 2019 to February 2020 from local fresh seafood markets or fishing piers in 8 coastal provinces of the upper Gulf of Thailand. The methods of sampling, processing, and laboratory protocols were completed following the recommendation for studying microplastics in seafood (Dehaut et al., 2018). Six fish species were obtained. Species were grouped into 2 categories ac cording to their inhabited oceanic zone, including pelagic and benthic species. The pelagic fish species was Short Mackerel (*Rastrelliger brachysoma,* Bleeker, 1851). Benthic fish species were Silver Sillago (*Sillago sihama*, Forsskål, 1775), 2 species of Threadfin Bream (*Nemipterus hexodon*, Quoy & Gaimard, 1824 and *Nemipterus japonicus* Bloch, 1791), Soilder Croakers (*Nibea soldado* Lacepède, 1802), and Striped Eel Catfish (*Plotosus lineatus* Thunberg, 1787). A minimum of 100 fish in each category was collected, which resulted in a total number of at least 200 fish. The sample size was calculated with a 95% confidence interval and 7% margin of error with an estimated population mean of 54%, which was the prevalence of microplastics in fish from Songkhla province, Southern Thailand (Azad et al., 2018). Catching locations and catching methods were interviewed from fishermen. Fish samples were kept cool at 4°C and brought to the Veterinary Medical Aquatic Animal Research Center of Excellence, Chulalongkorn University.

At the Veterinary Medical Aquatic Animal Research Center of Excellence, The total length and weight of all fishes were measured. Each fish was dissected for pathological studies. Any gross pathological lesions observed during the dissection were recorded. Tissue samples that were obtained included the dorsal musculature, liver, kidney, reproductive organ, and gastrointestinal (GI) tract, respectively. Each tissue sample was divided into 2 pieces. The first piece was immediately kept in a pre-clean zip-locked bag and kept frozen at -20°C for further microplastics detection processes. The other piece was preserved in 10% formalin in a clean glass bottle for histopathological study. Clean gloves and cotton lab clothes were always worn to minimize potential contamination (Dehaut et al., 2018).

3.2 Quantitative analysis of microplastics

The quantitative microplastic detection process in fish tissues was conducted according to Claessens et al. (2013) and Budimir et al. (2018). Laboratory processes were carried out under the recommendation for the studies of microplastics in seafood (Dehaut et al., 2018). The process was performed in a clean bench with a pre-cleaned surface and all pieces of equipment used were cleaned and rinsed with Ethanol 95% to prevent from contamination. Tissues were weighed and separately kept in a glass container with a tight lid to perform the tissue digestion process (figure 9). Each Tissue sample was digested with 1 M Sodium hydroxide (NaOH) and 0.5% w/v Sodium Dodecyl Sulfate (SDS) in the ratio of 1:10:5, respectively. The solution was incubated at 50°C in a water bath for 24 hours. At 24 hours, the container was shaken gently to mix contents. The incubation continued for another 24 hours. After 48 hours of total incubation time, the sample solution was filtered through a cellulose nitrate membrane, with pores size of 20 micrometers and 90 millimeters in diameters (Whatman 1004-090, Grade 4). The membrane was placed over the glass funnel with a glass plate cover. To collect any residue contents, which might be left on the container used in the digestion, the container was washed with 95% ethanol 3 times. The washed solution was consequently filtered on the membrane. On the condition that the tissue contains a high amount of calciferous scales or shells of shellfish, 2-3 drops of 2 M hydrochloric acid (HCl) was added to the filter membrane to aid the digestion. The membrane was then transferred to a glass plate to be quantitatively analyzed for microplastic particles under a stereomicroscope (figure 10). The quantities of microplastic particles from each tissue sample were examined.

The ratio of microplastic pieces per fish, fish tissue weight and fish body weight were calculated.

Conclusive diagram of quantitative analysis method was shown in figure 8. The pilot validity test of this method was completed prior to the study. Polypropylene (PP) and High-density polyethylene (HDPE) microplastics were used as positive control samples. As a result, the process was able to detect microplastic pieces that had been added to the fish tissues. Moreover, the qualitative analysis results after this process matched with the positive control samples added. Therefore, this method can digest fish tissue without damaging microplastic pieces and has no effects on microplastics quantitative and qualitative analysis.

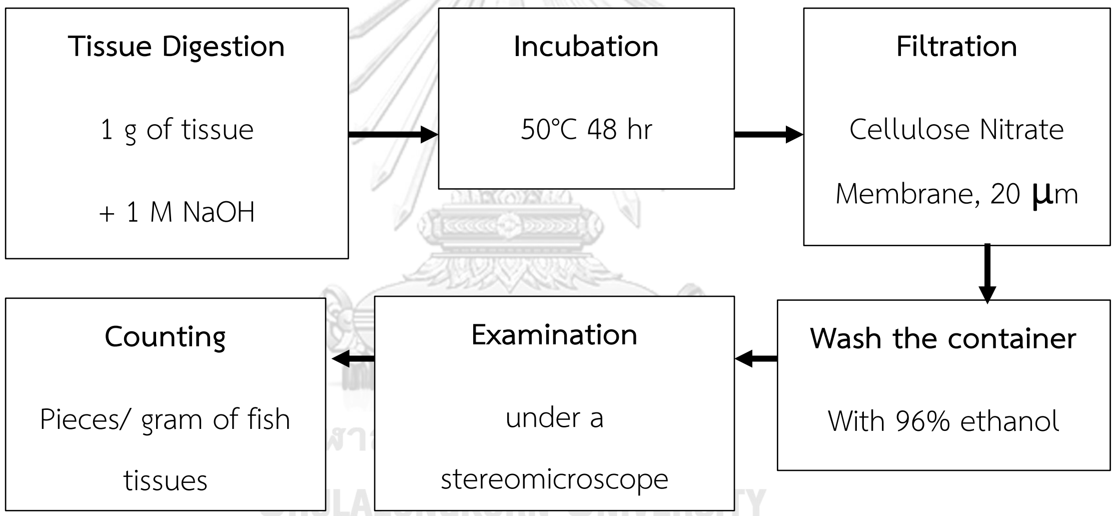


Figure 8 Steps of quantitative analysis of microplastics from fish tissue

Figure 9 Digestion of tissue sample in glass flasks, incubation and filtration.

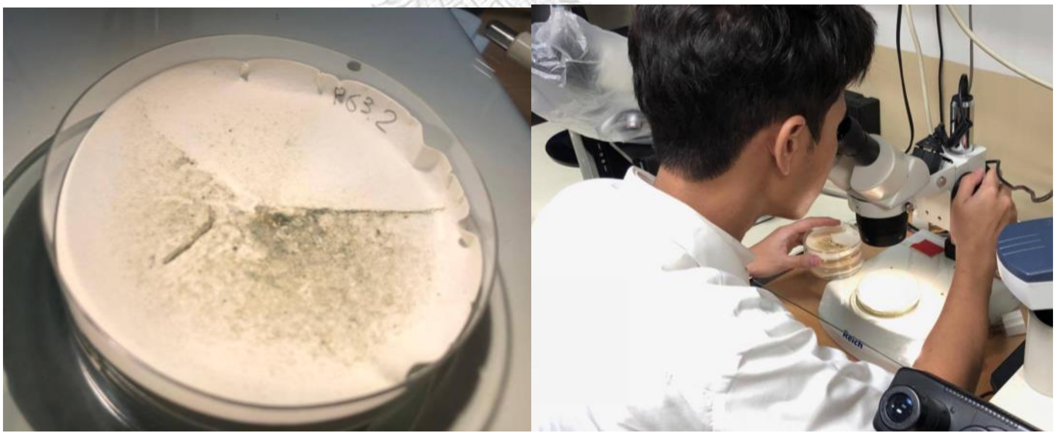


Figure 10 Filtered cellulose nitrate membrane in a closed dish and the screening for microplastics by a stereomicroscope.

3.3 Qualitative analysis of microplastics

Physical characteristics of microplastics were determined in color, shape and size. Colors and shapes of microplastics were classified primarily under a stereomicroscope. Shapes of microplastics in this study were categorized into fiber, spherical and fragment (irregular) type (Matsuguma et al., 2017). An Optical microscope (Olympus BX51RF) with Image Analysis Software (Olympus stream Image Analysis) was used to determine the size of microplastics. Frequencies of each characteristic were recorded.

For chemical properties of microplastics, Fourier transform infrared spectroscopy microscope (FTIR microscope) was used to indicate the diversities of plastic polymers presented (Shim et al., 2017; Thushari et al., 2017). The FTIR that were used is JASCO FT/IR-6600 (JASCO International Co., Ltd.) with an infrared microscope (JASCO IRT-5200 Irtron Infrared microscope). The plate with samples on filter paper was place on the sample stage of FTIR microscope machine. The location of suspected particles was located at the point of IR probe. Attenuated total reflectance (ATR) mode was used (Shim et al., 2017). The specific chemical bonds in plastic particles were obtained as spectral graphs. The spectra of the background, i.e., filter paper and plate, were automatically subtracted. Spectra obtained were compared with reference spectra of major plastic polymer types in the data library to identify types of polymers. The data library was provided by SCG chemicals co., Ltd. The percentage of similarity of samples and data library for each sample was recorded. Percentage that was match to the data library less than 60% was excluded (Hanachi et al., 2019). Frequency of polymer types of each sample was recorded.

3.4 Histopathological analysis of fish tissue

In this process, tissue samples preserved in 10% formalin were embedded into paraffin blocks and cut at 5 micrometers thickness by a microtome blade. Tissue sections were deparaffinized and rehydrated in xylene and ethanol solutions. Sections were stained with Hematoxylin and Eosin staining (H&E) (Gonçalves et al., 2018; Jovanović et al., 2018). The staining process was done by veterinary diagnostic laboratory, faculty of Veterinary Science, Chulalongkorn University. Histopathological changes observed under an optical microscope were described and scored by semi-quantitative scale scoring (Jovanovic et al., 2018) (Table 2-6)

Table 2 *Semi-quantitative histopathological scoring for fish liver tissue*

Score	Histopathological lesions observed from liver tissue samples
0	Normal hepatocytes and hepatic sinusoids. No excess accumulation of fat droplets, pigmentation or inflammatory cells was observed.
1	Very mild changes in hepatocyte and hepatic sinusoids. Very mild accumulation of fat droplets, pigmentation or inflammatory cells was observed.
2	Mild changes in hepatocyte and hepatic sinusoids. Mild accumulation of fat droplets, pigmentation or inflammatory cells was observed.
3	Moderate changes in hepatocyte and hepatic sinusoids. Moderate accumulation of fat droplets, pigmentation or inflammatory cells was observed.
4	Moderate to severe changes in hepatocyte and hepatic sinusoids. Moderate to severe accumulation of fat droplets, pigmentation or inflammatory cells was observed.
5	Severe changes in hepatocyte and hepatic sinusoids. Severe accumulation of fat droplets, pigmentation or inflammatory cells was observed.

Table 3 *Semi-quantitative histopathological scoring for fish Kidney tissue*

Score	Histopathological lesions observed from kidney tissue samples
0	Normal cellular structure in both renal and hematopoietic tissue. No pigmentation or inflammatory cell was observed.
1	Mild changes of kidney tissue. Few pigmentations or inflammatory cells were sparsely seen.
2	Mild changes of kidney tissue. Moderately dense pigmentation,

	inflammatory cells or few melanomacrophage centers were sparsely observed. (1-2 Melanomacrophage centers per field of view at 40x magnification)
3	Moderate changes of kidney tissue. Dense pigmentation, inflammatory cells or multiple melanomacrophage centers were moderately observed. (1-2 Melanomacrophage centers per field of view at 100x magnification)
4	Moderate to severe changes of kidney tissue. Multiple melanomacrophage centers were moderately observed. (≤5 Melanomacrophage centers per field of view at 100x magnification)
5	Severe changes of kidney tissue. Multiple melanomacrophage centers were diffusely observed. (>5 Melanomacrophage centers per field of view at 100x magnification

***Table 4** Semi-quantitative histopathological scoring for fish skin and muscular tissue*

Score	Histopathological lesions observed from skin and muscular tissue samples
0	Normal muscle fiber arrangement. Normal skin structure. No inflammatory cell was observed.
1	Mild changes in muscle fiber arrangement or skin structure. Very few inflammatory cells or melanomacrophages might be seen.
2	Changes in muscle fiber arrangement or the skin structure were seen in more than 25% of the tissue areas. Inflammatory cells or melanomacrophages might be seen.
3	Changes in the muscle fiber arrangement or the skin structure were seen in more than 50% of the tissue areas. Inflammatory cells or melanomacrophages can be seen.
4	Changes in the muscle fiber arrangement or the skin structure were seen in more than 75% of the tissue areas. Moderate Inflammatory cells or melanomacrophages were seen.

5	Changes in the muscle fiber arrangement or skin structure were seen in all of the tissue areas. Numerous inflammatory cells or melanomacrophages were seen.

Table 5 *Semi-quantitative histopathological scoring for fish Gastrointestinal tract tissue*

SCORE	Histopathological lesions observed from Gastrointestinal (GI) tract samples
0	Normal GI tract structure. Normal alignment of GI tract wall. No inflammatory cell was observed.
1	Mild changes of the GI tract structure or tract wall alignment. Inflammatory cells might be seen.
2	Mild changes of the GI tract structure and tract wall alignment. Mild infiltrations of inflammatory cells were observed.
3	Moderate changes of the GI tract structure and tract wall alignment. Moderate infiltrations of inflammatory cells were observed.
4	Moderate to severe changes of the GI tract structure and tract wall alignment. Moderate to severe infiltrations of inflammatory cells were observed.
5	Severe changes of the GI tract structure and tract wall alignment. Severe infiltrations of inflammatory cells were observed in all tissue area.

Table 6 *Semi-quantitative histopathological scoring for fish Gonadal tissue*

Score	Histopathological lesions observed from gonadal tissue samples
0	Normal gonadal structure with normal germ cells proliferation. No inflammatory cell was observed.
1	Normal gonadal structure with mild abnormality in germ cells proliferation process. Inflammatory cells might be seen.
2	Mild changes in gonadal structure with some abnormality in germ cells

	proliferation process. Inflammatory cells might be seen.
3	Moderate changes in gonadal structure with some abnormality in germ cells proliferation process. Inflammatory cells were observed.
4	Moderate to severe changes in gonadal structure with abnormality in germ cells proliferation process. Inflammatory cells were observed.
5	Severe changes in gonadal structure with abnormality or complete lacking in germ cells proliferation process. Inflammatory cells were observed.

3.5 Microplastics Exposure Assessment

Exposure Assessment of microplastics for Thai fish consumers was conducted. The exposure level equals the concentration of microplastics multiplied by the intake rate of the particular food source. The data were formulated according to the Food consumption data of Thailand by the Office of Standard Development, National Bureau of Agricultural Commodity and Food Standards, Ministry of Agriculture and Cooperatives. The data of 18-35 years old Thai people in 2016 has revealed that their intake rate of various marine fishes, i.e. grouper, seabass etc., was at 1.70 grams per people per day. On the other hand, the intake rate of Short Mackerel (*Rastrellinger brachysoma*) for these subjects was at 9.21 grams per people per day (ACFS, 2016). The exposure calculated were in pieces of microplastics per person per day.

3.6 Statistical analysis

Descriptive statistics were used to explain the distribution of microplastics, quantitative data of microplastics, qualitative data of microplastics, and pathological changes observed. The Kolmogorov-Smirnov test was used to examine the distribution pattern of the following; average microplastic counts per fish, average microplastic counts per gram of fish tissue weight, average microplastic counts per gram of fish body weight, average fiber and fragment microplastic counts per fish, semi-quantitative histopathological scores between different category of fish, fish species, and sampling provinces. Normally distributed data were analyzed by independent sample T-Test or One-Way Analysis of Variance with multiple comparisons. The Mann-Whitney U test or the Kruskal-Wallis test was used to analyze the data that were not normally distributed. Associations between the presence of microplastics and fish species, fish category, sampling location or histopathological score were determined using the Chi-square test for association. The confidence level for all tests was set at 95% with a p-value of 0.05. Data were analyzed by SPSS statistics (IBM) version 22.

CHAPTER 4

RESULTS

4.1 Fish Samples

A total of 239 fish was collected from 13 fishing piers or local fish market in 8 coastal provinces of the upper Gulf of Thailand. Sampling provinces were namely Prachuab Kiri Khan, Petchburi, Samut Songkram Samut Sakhon Samut Prakan, Chachoengsao, Chonburi and Rayong. Six species of fish were obtained. One species was categorized as a pelagic fish species, while the other 5 species were categorized as Benthic fish species. Fish scientific name, common name, number of Samples from each source, and fish category were shown on the Table 7. Species list of fish samples were including Short Mackerel (*Rastrelliger brachysoma,* Bleeker, 1851), Silver Sillago (*Sillago sihama*, Forsskål, 1775), Soilder Croaker (*Nibea soldado* Lacepède, 1802), Striped Eel Catfish (*Plotosus lineatus* Thunberg, 1787) and 2 species of Threadfin Bream (*Nemipterus hexodon*, Quoy & Gaimard, 1824 and *Nemipterus japonicus* Bloch, 1791). All samples were locally collected by local fishing boats using small trawling net fishing boat (figure 11), except Short Mackerels from Samut Songkram and Sumut Prakarn, local Stake trap were used.

Samples were brought to Veterinary medical aquatic animal research center, Chulalongkorn University, Bangkok. The weight and total length (TL) were measured and recorded. The average weight and TL of fish samples from each source were shown on the table 7. There was no remarkable external pathological abnormality of fish samples from each province except Threadfin bream from Samut Prakarn province, which showed high prevalence of external parasitic copepod. Upon dissection to collect the organ samples (figure 12), there was also no remarkable internal pathological abnormality shown among fish from different source. The carcass stage of most fish samples was fresh, except few samples that were mild to moderate autolysis. The collection of histopathological samples in autolyzed samples was omitted.

Figure 11 Examples of fish sampling site; (A) Samut Sakhon fish wholesale market, Samut Sakhon province; (B) Suan Son fishing pier market, Rayong province.

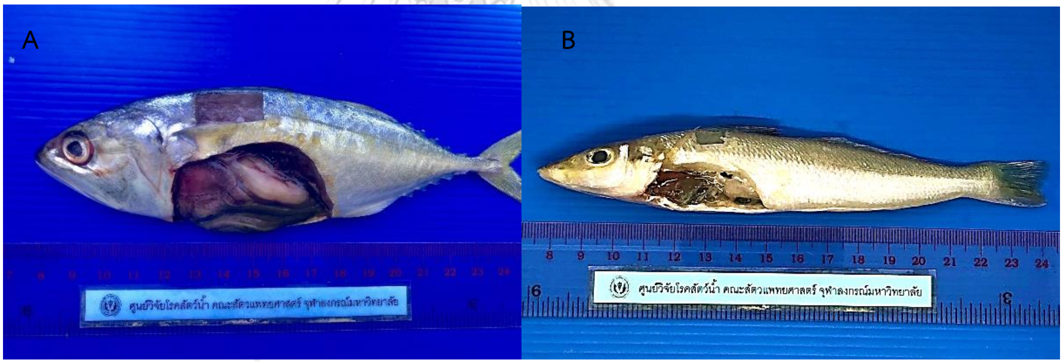


Figure 12 Sample collection process; ***(A)*** *A pelagic fish, Short Mackerel (Rastrelliger brachysoma);* ***(B)*** *A Benthic fish, Silver Sillago (Sillago sihama).*

Table 7 Sampling locations, sources, species, category, numbers, size and weight of fish samples. Size and weight were shown in average and standard deviation.

	Province	Sources/ (Collection time)	Common name (Scientific Name)	Grouping	N (fish)	Average total length (cm.)	Average Weight (grams)
1	Prachuab Kiri Khan	Ban Bang Pu local fishing pier (August 2019)	Short Mackerel (*Rastrelliger brachysoma*)	P	9	21.28±1.37	111.11±16.75
		Thap Sakae fishing pier (August 2019)	Short Mackerel (*R. brachysoma*)	P	9	13.77±0.27	31.33±1.80
			Soilder Croaker (*Nibea soldado*)	B	3	18.00±4.00	81.67±44.02
2	Petchaburi	Ban Laem fisherman community (January 2020)	Short Mackerel (*R. brachysoma*)	P	5	18.57±0.31	73.10±3.12
			Striped Eel Catfish (*Plotosus lineatus*)	B	18	24.14±1.06	98.79±15.72
3	Sumut Songkram	Mae Klong fresh market (November 2019)	Short Mackerel* (*R. brachysoma*)	P	12	19.12±0.85	82.79±14.98
			Silver Sillago (*Sillago sihama*)	B	21	16.89±0.98	39.24±7.85
4	Sumut Sakhon	Samut Sakhon fish wholesale market (November	Short Mackerel (*R. brachysoma*)	P	26	16.95±0.77	57.80±8.31

		2019)					
		Mahachai fresh market (November 2019)	Silver Sillago (*S. sihama*)	B	22	17.24±0.80	35.22±4.92
5	Sumut Prakarn	Pak Nam fresh market (November 2019)	Short Mackerel* (*R. brachysoma*)	P	19	18.86±0.92	75.89±6.87
			Threadfin Bream (*Nemipterus japonicus*)	B	19	19.05±0.99	75.57±12.13
6	Chachoeng sao	Bang Pakong fresh market (September 2019)	Short Mackerel (*R. brachysoma*)	P	13	18.78±1.34	76.62±7.08
			Soilder Croaker (*Nibea soldado*)	B	9	20.60±0.98	91.33±12.14
7	Chonburi	Ang Sila local fresh market (September 2019)	Short Mackerel (*R. brachysoma*)	P	13	17.44±0.53	63.46±4.53
		Won-Napha fishing pier (February 2020)	Silver Sillago (*S. sihama*)	B	17	16.88±0.88	40.39±7.04
8	Rayong	Suan Son fishing pier market (December 2019)	Short Mackerel (*R. brachysoma*)	P	10	19.59±2.76	83.00±37.58
		Ban Pae	Short Mackerel	P	8	21.08±0.61	105.5±10.43

		fresh market (December 2019)	(*R. brachysoma*)				
		Thesaban 4 fresh market (December 2019)	Threadfin Bream (*Nemipterus hexodon*)	B	6	23.03±0.64	173.92±13.21

P= Pelagic fish species; B=Benthic fish species; * = Fish caught by Stake trap method.

4.2 Quantitative analysis of microplastics

Plastic particles were detected in the gastrointestinal tract of 46.86% of Fish samples. The pictures of microplastics observed by stereomicroscope and optical microscope with image analysis software were demonstrated in figure 13. No microplastics were detected from other tissue samples, including muscle, liver, kidney and gonads. The average plastics count in fish sample was 1.556±0.470 pieces per fish or 0.035±0.015 pieces per gram of fish bodyweight. Since there was only microplastics detected from gastrointestinal tract, there were 2.01±0.98 pieces of microplastics per gram of gastrointestinal tract weight.

Categorized by pelagic and benthic fish species, the results came out that benthic fish has higher plastics contamination rate than pelagic fish. Plastics were detected in the gastrointestinal tracts of 54.78 percent of benthic fish samples. In pelagic fish, plastics were detected in the gastrointestinal tract of 39.52 percent of fish samples. The average plastic counts per fish were 2.56±0.878 and 0.629±0.086 particles in benthic and pelagic fish, respectively. From the Chi-square test for association, there was a significant relationship ($p<0.05$) between category of fish and the presence of microplastics in the gastrointestinal tract of fish. The frequencies were shown in table 8 and 9. From the independent sample Mann-Whitney U test, there was a significant difference in average microplastic counts per fish, average counts per gram of fish GI weight, average counts per gram of fish body weight and average fiber counts between pelagic and benthic fish (p<0.05). No difference in average fragment microplastic counts was observed between pelagic and benthic fish (p>0.05).

Table 8 *Frequency table showed the relationship between category of fish and the presence of microplastics in the gastrointestinal tract of fish.*

Grouping	Number of Fish		Total
	Microplastics detected	Microplastics not detected	
Pelagic Fish	49	75	**124**
Benthic Fish	63	52	**115**
Total	**112**	**127**	**239**

Table 9 *Quantitative microplastics analysis results, classified by pelagic and benthic fish.*

	N (Fish)	Fish with MPs (%)	Avg. MPs count per fish (Pieces)*	Avg. Fragment MPs count (Pieces)*	Avg. Fiber MPs count (Pieces)	Avg. MPs count per fish GI weight (Pieces per gram)*	Avg. MPs count per fish body weight (Pieces per gram)*
Pelagic	124	39.52	0.629±0.086*	0.203±0.05	0.431±0.07*	0.38±0.10*	0.010±0.002**
Benthic	115	54.78	2.56±0.878*	0.623±0.31	1.94±0.83*	3.78±1.84*	0.062±0.028**
Overall	239	46.86	1.556±0.470	0.405±0.16	1.16±0.44	2.01±0.98	0.035±0.015

- * = Significant difference (p<0.05) was detected after a Chi-square test using SPSS statistics.
- ** = Significant difference (p<0.001) was detected after a Chi-square test using SPSS statistics.

Six species from 5 genera of fish were selected as samples of this study. The species with the highest plastics contamination rate was Threadfin Bream, which plastics were found in 60.0% of the species samples. The species with the lowest contamination rate was Soilder Croaker, which plastics contaminated in 25% of samples. Species with the highest average plastic counts per fish was Silver Sillago, which the average of 3.5±1.59 pieces of plastic per fish was observed. The lowest average plastic counts per fish were from Soilder Croaker at 0.58±0.36 pieces per fish.

From the Chi-square test for association, there was a significant relationship ($p<0.05$) between species of fish and microplastics contamination rate. An independent-sample Kruskal-Wallis test with Dunn-Bonferroni pairwise multiple comparisons were performed to analyze differences of plastic counts between species. There was a significant difference ($p<0.05$) in average microplastic counts per fish, average microplastic counts per gram of fish GI weight, average microplastic counts per gram of fish body weight and average fragment microplastic counts among species of fish. There was no difference in average fiber microplastics count among species of fish ($p>0.05$). Dunn-Bonferroni pairwise multiple comparisons results were shown on table 10.

The data of microplastic contamination rate in fish samples from different sampling province were shown in figure 14. Provinces with the highest and lowest microplastic contamination rate were Samut Prakarn province and Rayong province, which microplastics were observed in 66.67% and 41.67% of fish samples, respectively. From the Chi-square test for association, there was no significant relationship ($p>0.05$) between microplastics and collection provinces of fish samples.

Table 10 Quantitative microplastics analysis results, grouping by fish species.

	N (Fish)	Fish with MPs (%)	Avg. MPs count per fish (Pieces)*	Avg. Fragment MPs count (Pieces)*	Avg. Fiber MPs count (Pieces)	Avg. MPs count per fish GI weight (Pieces per gram)*	Avg MPs count per fish body weight (Pieces per gram)*
Short Mackerel	124	39.52	0.629±0.09[a]	0.203±0.05	0.431±0.07	0.375±0.097[a]	0.010±0.002[a]
Silver Sillago	60	58.33	3.5±1.59[b]	0.317±0.09	3.183±1.59	6.788±3.518[bc]	0.105±0.053[bc]
Threadfin Bream	25	60.00	2.36±1.38[ab]	1.833±1.42	0.600±0.16	0.729±0.328[ac]	0.024±0.013[ac]
Soilder Croaker	12	25.00	0.58±0.36[ab]	0	0.583±0.36	0.368±0.267[a]	0.006±0.004[a]
Striped Eel Catfish	18	55.56	1.00±0.28[ab]	0.444±0.15	0.556±0.18	0.268±0.077[ac]	0.010±0.003[ac]

- *= Significant difference ($p<0.05$) was detected by an independent-sample Kruskal-Wallis test using SPSS statistics.
- The uppercase letters (a,b,c) demonstrate Dunn-Bonferroni pairwise multiple comparison differences ($p<0.05$) by SPSS statistic

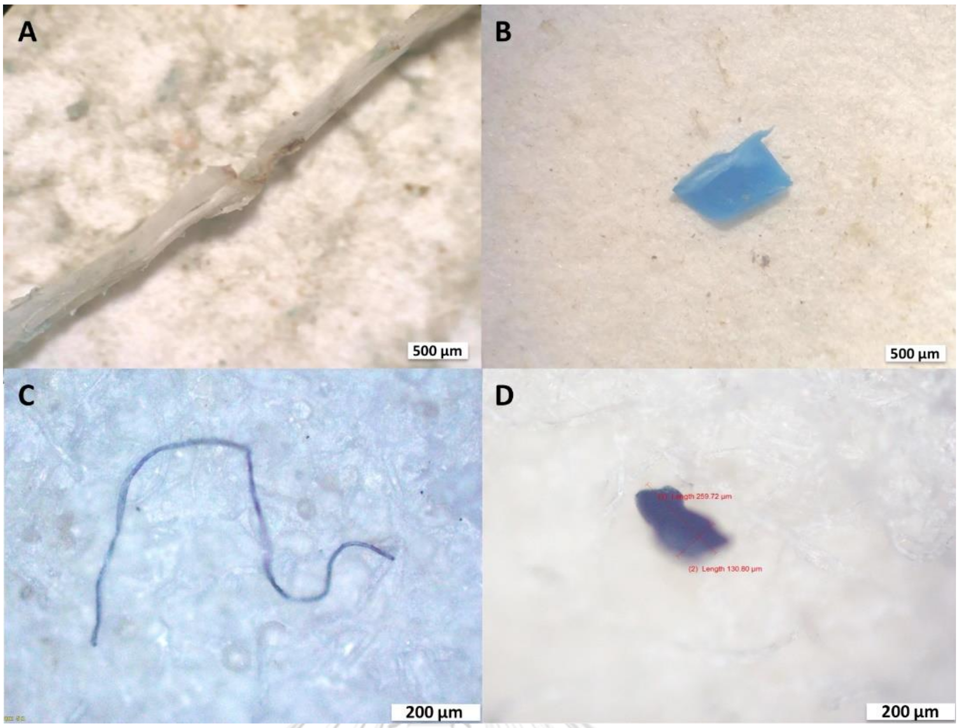


Figure 13 Examples of microplastics observed in this study; (A, B) Particles observed by stereomicroscope; (C, D) Particles observed by optical microscope with Image analysis software.

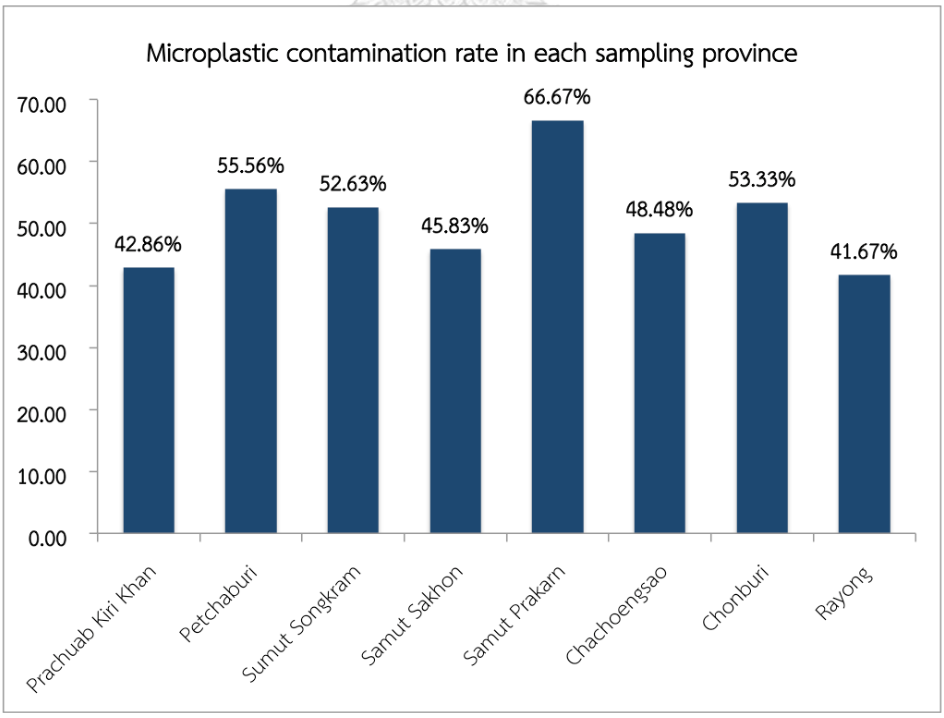


Figure 14 Percentage of microplastics contaminated fish samples classified by area.

4.3 Qualitative analysis of microplastics

Physical characteristics of microplastics were determined in size, shape and color. The average plastics size was 430.08 micrometers. The biggest piece was 8 millimeters in size, which was found in a Short Mackerel. The smallest piece was 13 micrometers in size, which was found in Striped Eel Catfish. “Mesoplastics”, plastic debris that were larger than 5 millimeters, were found in 4.42% of plastic-ingested fish. The plastics’ shapes were defined into fiber, fragment and spherical type. The most abundant shape was fiber type, followed by fragment or irregular type, at 74.3% and 25.7%, respectively. No spherical type was found in this study. The highest abundant plastics colors were blue and white, with frequencies of 55% and 21% of all microplastic pieces, respectively. The results also demonstrates that white plastics dominated in fragment-type microplastics, on the other hand, blue plastics dominated among fiber-type microplastics. The proportion of microplastics color was demonstrated in figure 16 and 17.

Chemical or polymeric characteristics of microplastics were identified by FTIR microscope. Figure 18 demonstrated the laboratory usage of FTIR microscope machine. Figure 19 and 20 depicted polymer identification process from one of microplastics positive control sample and one of unknown sample, which spectral curves obtained from FTIR microscope were compared with spectral curves from the data library.

The proportion of polymer type identified from all fish samples was shown in figure 21. Polyester was the most abundant polymer type at 49.09% of plastic particles detected. Other high-frequency polymers were polyethylene (25.63%), followed by rayon (6.86%), polypropylene (5.05%) and polyamide or Nylon (3.97%). There were differences in polymer types between benthic and pelagic fish. The most abundant polymer type in pelagic fish was polyethylene (22.86%) followed by polyamide (20.00%), polypropylene (14.29), polyurethane (14.29%), and rayon (14.29%). In benthic fish, polyester dominated other polymers with a proportion of 52.63%. The plastic polymers composition in pelagic and benthic fish were shown in figure 22.

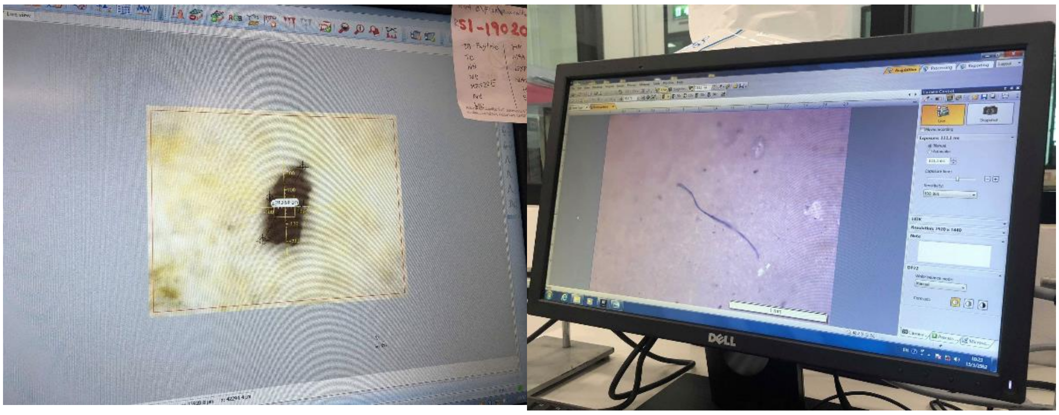


Figure 15 The using of Image analysis software to analyze size and shape of microplastics (Olympus Stream Image).

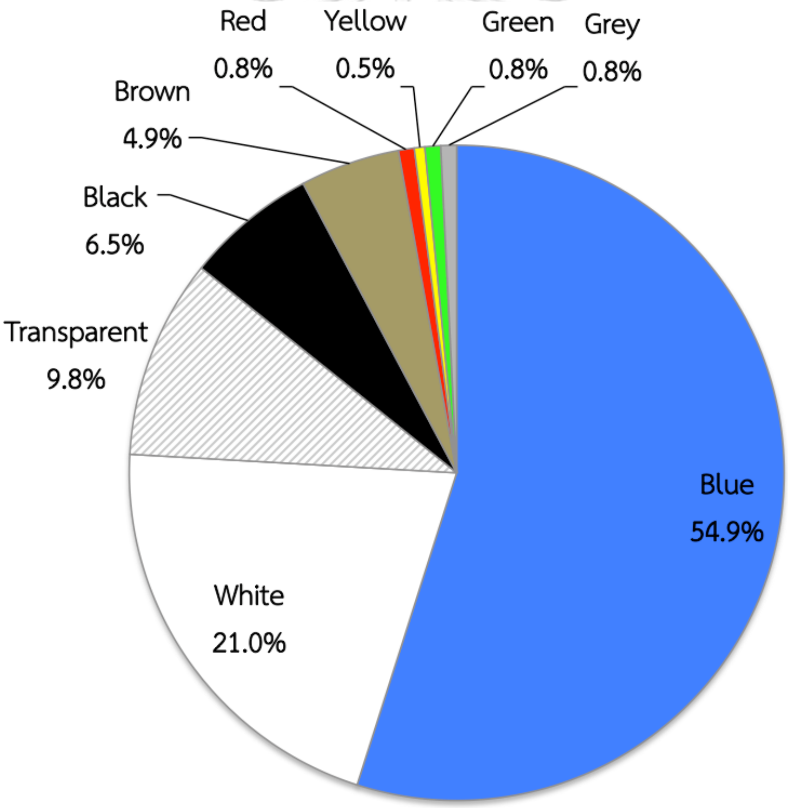


Figure 16 Microplastic color composition.

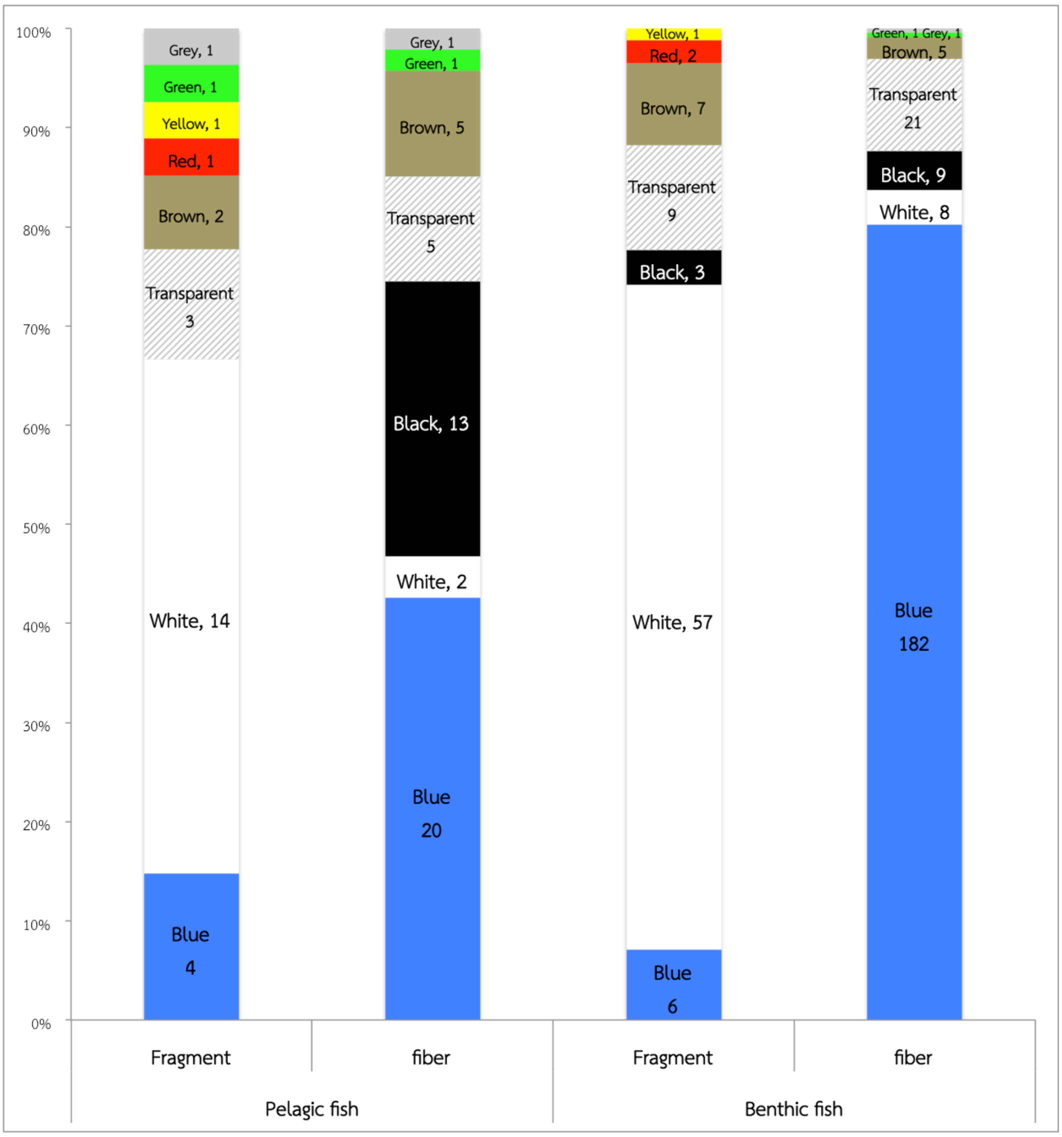


Figure 17 Microplastics color and physical type distribution between 2 grouping.

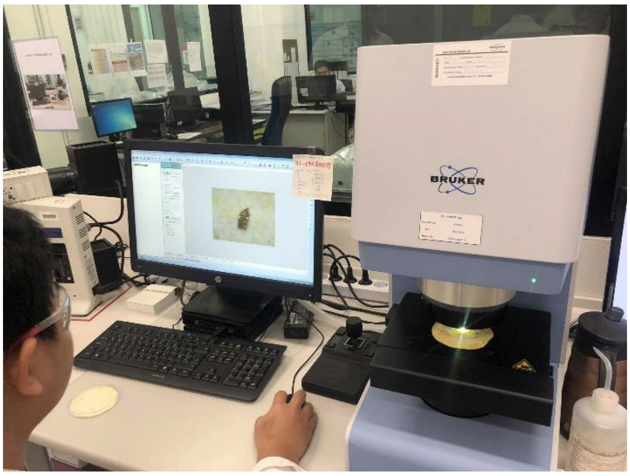
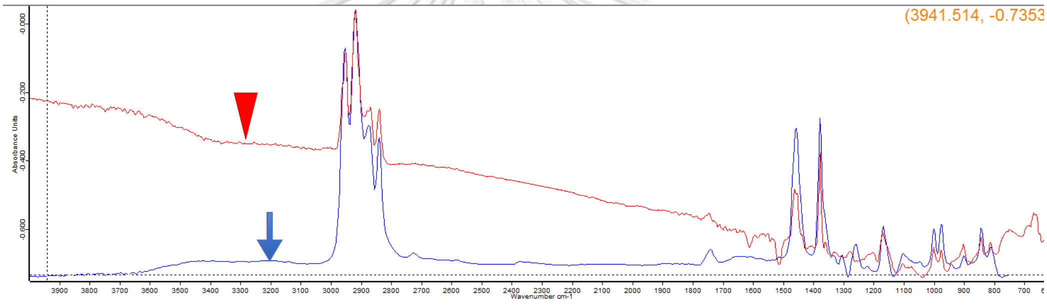


Figure 18 Fourier Transform Infrared Spectroscopy (FTIR) microscope machine. The IR probe, light microscopic probe, and samples stage were on the right of the picture.



Hit No.		Color	Hit Quality	Compound Name	CAS number	Molecular formula	Molecular weight
1	✓		846	100% Permashield BCF Olefin, style- Reliance II			
2			830	Silt fence fill yarn, 550 denier			
3			820	Ground lever fill yarn			
4			814	Spunbonded Polypropylene			
5			810	Dyed Ribbon Fiber, Polypropylene			
6			809	Polypropylene, undyed			

Figure 19 FTIR spectral curve of positive control PP microplastic samples digested with fish tissue. The picture shows sample's spectral curve (Arrowhead) and PP spectral curve from data library (Arrow).

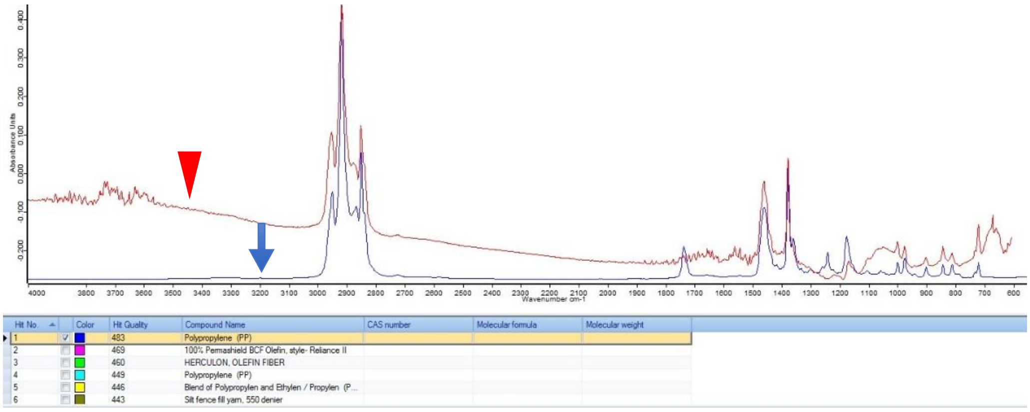


Figure 20 FTIR spectral curve of PP microplastic obtained from a fish sample. Plastic polymer result was shown as PP. The picture shows sample's spectral curve (Arrowhead) and PP spectral curve from data library (Arrow).

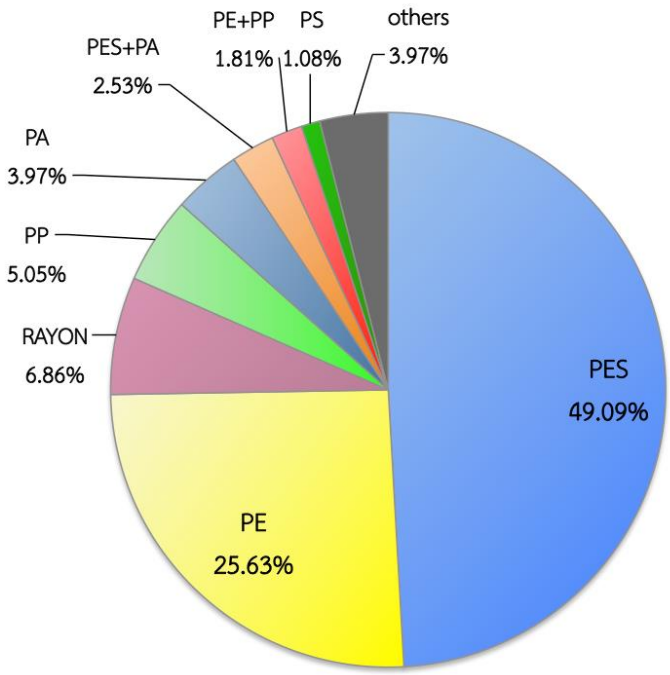


Figure 21 Composition of microplastic polymers.

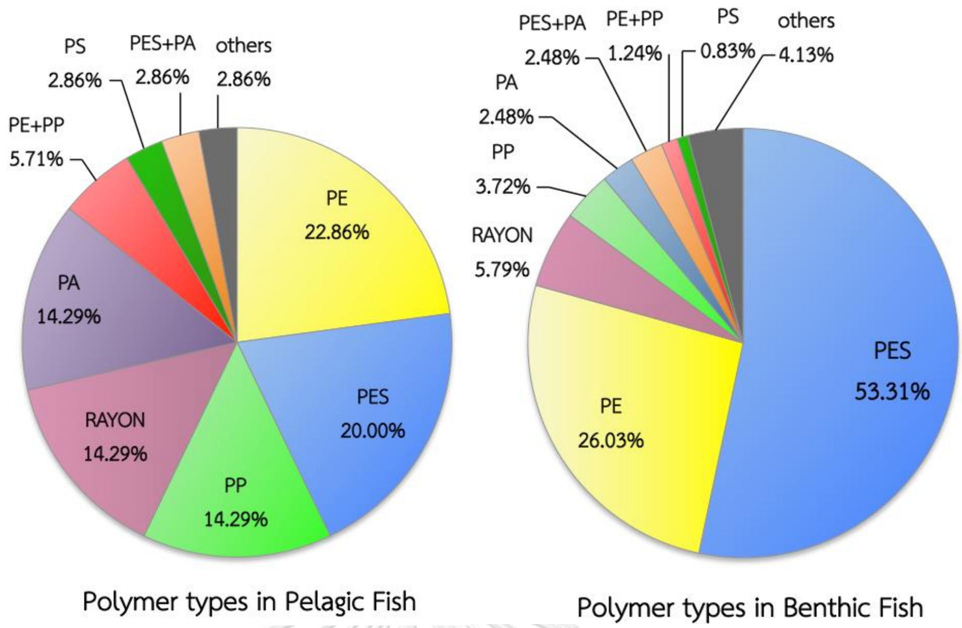


Figure 22 Microplastic polymers composition in benthic and pelagic fish.

4.4 Histopathological analysis of fish tissue

The histopathological study of liver, kidney, muscle, gastrointestinal tract and gonads of fish samples with Hematoxylin and Eosin staining had been carried out. Some fish samples were omitted to perform histopathological study due to putrefaction of tissues, especially fish from small fishing boat without any cooling methods applied before get on the shore. The total number of fish samples with histopathological study was 146 fish, which were 93 pelagic fish and 53 benthic fish samples.

Most of the fish samples were having good histological condition with only a few histopathological lesions were observed. For liver samples, total of 93 and 53 liver samples were obtained from pelagic and benthic fish, respectively. The most common lesion observed was the vacuolation and fatty degeneration of the hepatic parenchyma. Few pigment deposition and melanomacrophage infiltration in Liver was also observed.

The histopathological lesions observed from kidneys of fish samples were mainly the clustering of melanomacrophages into melanomecrophage center (MMC). No remarkable lesion was observed in renal glomerular, tubular or hematopoietic tissue. In 13.43% of Short Mackerel kidney samples, numerous parasitic Microsporidia were observed clustering in renal tubuli or renal parenchyma. No specific distribution pattern of the parasite between provinces was observed. After the chi-square test for association, there was also no relationship ($p>0.05$) between microsporidia infestation and fish with microplastics contamination.

In skin and muscle, total of 93 and 53 samples were obtained from pelagic and benthic fish, respectively. Very few histopathological lesions were seen in skin and muscle samples. The only change was the increase in pigmentation of the dermal tissue. Muscle fibers in all of the fish samples were in a good arrangement.

In the intestine, total of 93 and 53 intestinal samples were obtained from pelagic and benthic fish, respectively. Some of the samples were mild autolysis, which obscured the exact observation of mucosal epithelial lining. In some intestinal samples, the infiltration of granulocytes or other inflammatory cells was observed. Such presences were observed from both fish with plastics and without plastics in the gastrointestinal tracts.

Gonads were putrefied or absent in some fish samples. Total of 63 and 48 gonadal samples were obtained from pelagic and benthic fish, respectively. The major lesion observed from gonads was mainly the decreasing of germ cells production cycle without any evidence of cells proliferation or inflammation.

Histopathological lesions of each organ were interpreted into semi-quantitative score. The scoring results were shown in table 11. Fish with microplastics tend to have a slightly higher average histopathological score than fish with no microplastics in the gastrointestinal tract. Kolmogorov-Smirnov test for distribution showed that the average histopathological scoring results, as well individual organ's scores, were not normally distributed. From the independent sample Mann-Whitney U test, there was no significant difference in histopathological score between fish with microplastics and without microplastics ($p>0.05$) in every individual organ and overall average score. The distribution of histopathological scoring between fish with microplastics and fish without microplastics was shown on figure 23 and table 11

Table 11 Average Semi-quantitative histopathological scoring results between microplastics contaminated fish and non-contaminated fish.

Presence of MPs	Semi-quantitative scoring of histopathological lesion (mean ± S.E.)					
	Liver	Kidney	Muscle	GI	Gonad	All tissue average
YES	1.85±0.143	2.32±0.19	0.17±0.06	0.27±0.08	0.48±0.15	1.10±0.10
NO	1.66±0.117	2.34±0.17	0.14±0.05	0.43±0.10	0.29±0.08	0.97±0.06

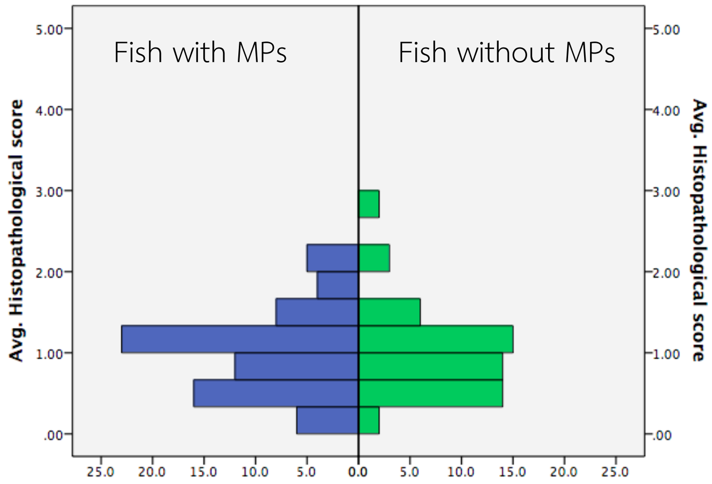


Figure 23 The distribution of average histopathological scoring between fish samples with microplastics in the GI tract (A) and without microplastics contamination in the GI tract (B).

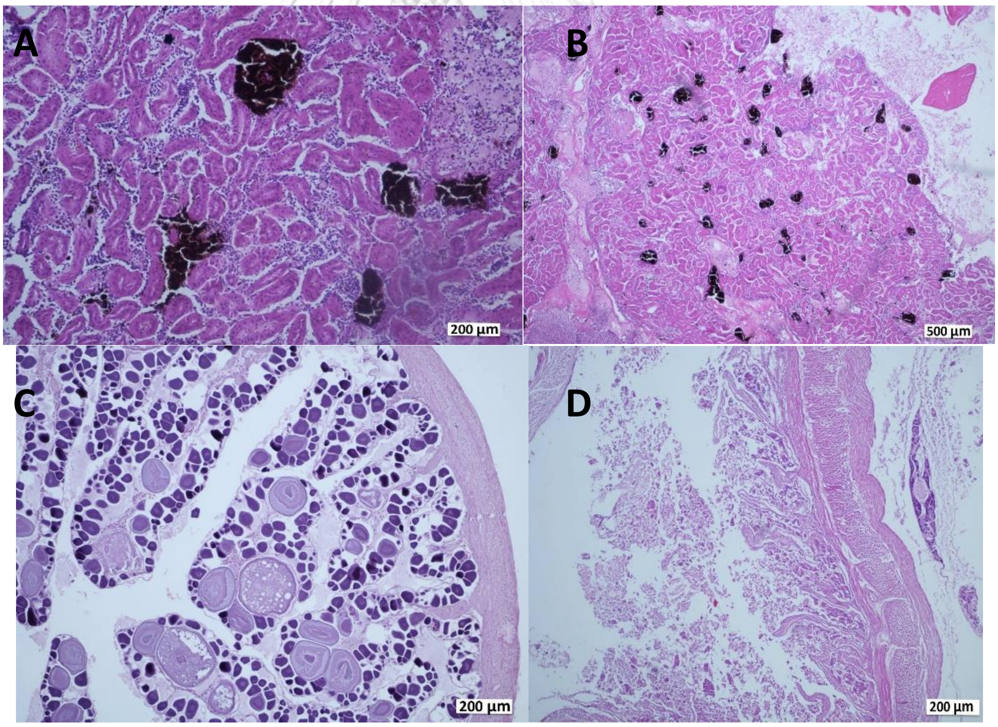


Figure 24 Histopathological lesions observed from fish samples; Melanomacrophage centers observed from kidneys of fish samples (A, B); An ovary with normal germ cell production (C); Intestinal wall with normal arrangement of serosal lining (D).

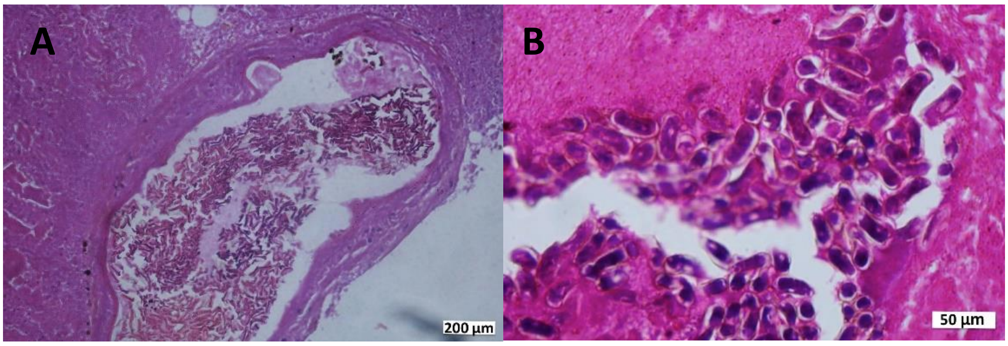


Figure 25 (A, B); Parasites were observed clustering in a tubular-like structure (A); 400x Magnification of the parasites (B).

4.5 Exposure Assessment

Exposure = Dose (Concentration) X Intake Rate

The intake rate of general marine fishes, such as groupers, sea bass, etc. was 1.70 grams per person per day (ACFS, 2016). The concentration of microplastics from overall food fish in this study was 0.035 pieces per gram of fish bodyweight. Therefore, the expected microplastics exposure of Thai people from general marine fishes can be calculated as follows;

Expected exposure from general marine fishes = 0.035 x 1.7
= 0.06 pieces per person per day
= 21.9 pieces per person per year

The intake rate of Short Mackerel was 9.21 grams per person per day (ACFS, 2016). The concentration of microplastics from Short Mackerel was 0.0103 pieces per gram of fish bodyweight. Therefore, the expected microplastics exposure from Short Mackerel for Thai people can be calculated as follows;

Expected exposure from Short Mackerel = 0.0103 x 9.21

= 0.095 pieces per person per day

= 34.68 pieces per person per year

CHAPTER 5

DISCUSSION

Facts from this study have proven the existence of microplastics contamination in a number of marine fishes from the upper Gulf of Thailand. Certain amounts of microplastics and mesoplastics contamination were found in the gastrointestinal (GI) tract of fish samples. These small plastic particles might be directly polluted from industrial products or some microplastics-containing commercial products. Microplastics might also indirectly entered the environment via breaking down processes of large plastic debris (Jovanović, 2017). This result also affirms the presence of microplastics in the marine environment of the Gulf of Thailand, which was previously reported in some published studies (Thushari et al., 2017; Wang et al., 2020).

Tissue digestion was the method used to extract microplastic particles from fish tissue in this study. There were many published methods available, each of them using various chemicals, times, and equipment nowadays. However, each method yields different efficacy and there was still no agreement of a standardized method available in this field of study. In this study, after preliminary studies, tissue digestion protocol according to Budimir et al. (2018) was used due to high validity, high efficacy of organic matter digestion, and negligible physical effects on microplastic particles. This method reportedly yielded 90±12% extractability for microplastics in the size range of 300 μm to 1 mm, and 78±16% extractability for microplastics smaller than 300 μm. It is worth noting that the detection of smaller microplastics can be challenging, not only in this method but also in other published methods. Therefore, the detection method of very small microplastics or nanoplastics from animal tissue samples remains challenging for scientists.

In this study, the smallest particle detected was 13 μm. The average size detected was much larger at 430.08 μm in size. The plastic pieces that were larger than 5 millimeters, or mesoplastics, were found in 4.42% of plastics ingested fish. In some other studies, mesoplastics were found in a higher proportion, as high as 20.8% (Gündoğdu et al., 2020). This proportion depends on the species of fish and plastics contamination characteristics of the area.

During the laboratory process of our study, containers with close lids were used throughout the process. Moreover, tissue digestion procedures were done in a closed fume hood. These processes had performed to prevent airborne microplastics, which is essential for accurate results. Dris et al. (2017) reported that there are as high as 5.4 microplastic particles per cubic meter of air in an indoor environment, which can lead to an erroneous result unless the lid was properly closed. Cotton lab cloth and sanitary gloves were always worn. These protocols were to minimize the risk of microplastics contamination from the examiner and the environment. The protocols in this study have strictly followed the recommendation for the studies of microplastics in seafood by Dehaut et al. (2018).

From this study, 46.86% of marine food fish from the upper Gulf of Thailand contain plastics in the gastrointestinal tract. A higher ratio was previously reported from Songkhla province, the Lower gulf of Thailand, at 54% (Azad et al., 2018). However, no chemical confirmation process had been done in the previous report to confirm the debris as microplastics. Both contamination ratios obtained from this study and from previous study were comparable to previous reports from other locations worldwide. The contamination rates from most previous reports were ranging from less than 1% to 100% of samples collected. For instances, a study from Finland has revealed that 0.9-1.8% of marine fishes from the Baltic Sea contained microplastics (Budimir et al., 2018). On the other hand, marine fishes samples from some other places had a microplastic contamination rate of 100%, for example, the

East China Sea (Jabeen et al., 2017) and Rio de la Plata estuaries, Argentina (Pazos et al., 2017). Examples of previous studies were summarized in table 12.

Table 12 *Summarized examples of previous studies and this study on microplastic contamination rates worldwide.*

Fish with MPs (%)	**Sample type**	**Location**	**References**
100%	Marine fish	East China Sea, China	Jabeen et al. (2017)
100%	Marine fish	Rio de la Plata, Argentina	Pazos et al. (2017)
95%	Freshwater fish	Taihu lake, China	Jabeen et al. (2017)
72.9%	Freshwater fish	Chi River, Thailand	Kasamesiri and Thaimuangphol (2020).
58%	Marine fish	Mediterranean sea, Turkey	Güven et al. (2017)
54.29%	Marine fish	Songkhla, Thailand	Azad et al. (2018)
50%	Marine fish (Indian Mackerel)	Malaysia	Karbalaei et al. (2019)
46.86%	**Marine fish**	**Upper Gulf of Thailand**	**Present study**
42.4%	Marine fish	Texas Gulf, USA	Peters et al. (2017)
36.5%	Marine fish	English Channel, England	Lusher et al. (2017).
0.9-1.8%	Marine fish	Baltic sea, Finland	Budimir et al. (2018).

Many factors, both anthropogenic and natural factors, were important in determining the environmental distribution of microplastics. Most marine plastic debris came from improper-managed inland plastics wastes. These wastes usually ended up in the ocean by the effects of wind, rain, and rivers. Ninety percent of marine plastic litters came from rivers runoff. The monsoon-affected areas were also escalating the flow of these plastics debris into the sea (Lebreton et al., 2017). The coastal area of the Gulf of Thailand, especially the upper Gulf, was a highly populated area with many popular tourist destinations, fishery activities, industrial areas, and drainage from many major rivers. These factors lead to the high risk of plastic debris and microplastics contamination in the environment (Shahul Hamid et al., 2018).

The geographical structure of the area also plays a vital role in determining the degree of microplastics contamination in the area. A closed sea was likely to have a higher microplastics accumulation rate than an open sea. Since microplastics deposition can occur easier in a closed sea, water currents or other hydrodynamics activities are usually lower than in an open sea (Alomar et al., 2016). The Gulf of Thailand, an open sea, which linked to the South China Sea and the Pacific Ocean, were reportedly having lower sediment microplastics deposition compared to the Mediterranean Sea. The sediment of the Gulf of Thailand contained 150.4 ± 86.2 microplastic particles per kilogram (Wang et al., 2020), and the Mediterranean had 270 particles per kilogram of sediment (Alomar et al., 2016). In the same way, microplastics contamination in aquatics organisms was also higher in the Mediterranean compare to the Gulf of Thailand, which was the main area of interest in this study. According to facts, fifty-eight percent of fish from the Mediterranean sea has microplastics in their stomach (Güven et al., 2017), which was higher than the results of our study at 46.86 percent in the Gulf of Thailand.

There were differences in the amount of microplastic particles in the sediments among sampling locations within the Gulf of Thailand. From the study of Wang et al. (2020), the microplastics concentration at the sediment sampling point with the highest microplastics contamination was 17 times higher than the sampling points with the lowest microplastics concentration. The highest contamination point was at the opening of the Bay of Bangkok (A rectangular-shaped bay of the inner Gulf of Thailand). Beached microplastics among Eastern coastal provinces were also different. Rayong province had a higher proportion of beached microplastics compare to other eastern coastal provinces (Bissen and Chawchai, 2020). In contrary to beached microplastics, no significant differences were observed among fish from different provinces in this study. This might be because of the mobility of fish, which were able to move across provinces of their feeding habitat. Therefore, the degree of microplastics contamination in fish may reflect the overall or spatial picture of contamination in the region rather than at a local provincial level. This is according to the suggestion of samples species of GESAMP (2019), which mentioned that a sessile species can be the microplastic contamination indicator for a specific sampling location, while a mobile species, in this case, can be used as a spatial or regional indicator

In this study, fish from Samut Prakarn province, which is the main drainage of the Chao Phraya river system, had higher microplastics contamination compare to other locations. This might be because most marine plastic debris and microplastics came from rivers run-off (Lebreton et al., 2017). As mentioned earlier, the Chao Phraya River, the largest river system in central Thailand, had high microplastics contamination. A report had revealed that the Chao Phraya River in Bangkok had 80 ± 65 microplastic particles per cubic meter of water and 91 ± 13 particles per kilogram of sediment (Ta and Babel, 2020). These microplastics were consequently brought to the Gulf of Thailand at Samut Prakarn province and accumulated within that area. This led to microplastics contamination and accumulation in the Gulf of

Thailand at 150.4 ± 86.2 per kilogram of sediment (Wang et al., 2020), which was higher than the contamination rate in sediments of the Chao Phraya River,

There was a significant difference in microplastics contamination rate between pelagic and benthic fish. Benthic fish has a higher proportion of microplastics contamination than pelagic fish. This might be resulting from the difference in the amount of microplastics contamination between water columns or the difference in the feeding habits of both groups of fishes. Despite the Gulf of Thailand is a relatively shallow sea, with an average depth of 45 meters and a maximum depth of around 80 meters (Wang et al., 2020). The vertical distribution of microplastics should not vary much between depths. Many studies also showed no significant difference in the amount of microplastics contamination among fish living in different habitats (Güven et al., 2017; Azad et al., 2018). The vertical distribution of microplastics in the water column was resulting from the buoyancy changing of plastic particles. Theoretically, each microplastic particle descends to the depth at the same rate. However, with the influence of many physical, chemical, or biological factors, different sinking rates may have occurred, which resulting in the different proportion of microplastics type between the depth (GESAMP, 2015). Therefore, fish from different locations may expose to different scenarios of microplastics exposure.

In this study, 7 species from 6 genera of important marine food fish in the upper Gulf of Thailand were selected. These species lists were compatible with the criteria for good indicator species for monitoring microplastics in marine biota. These criteria were, for example, a regional representative species living within the geographic range, Not a threatened species or abundant in the region, important species in the region, and consist of mixed ecological niches (GESAMP, 2019).

Differences in microplastics contamination rate between fish species were examined in this study. We can conclude that there is a significant relationship between microplastics contamination characteristics and species of marine fish. Many previous studies also revealed different contamination degrees between reported species, ranging from no contamination in some species to more than half of the sample. For instance, only 0.9 to 1.8% of planktivorous fish from the Baltic Sea had microplastics in their stomach. While some species in the same location had no microplastics contamination in fish samples (Budimir et al., 2018). On the other hand, more than 75% of fish species from the Mediterranean have microplastics contamination (Güven et al., 2017). Factors affecting microplastics contamination in different species and locality of fish are, for example, feeding habits of fish, an abundance of microplastics in their natural feed, and the marine environment. Feeding strategies play a very important role in determining how the organism will receive or interrelate with these plastics litter (GESAMP, 2019). Other potentially affecting factors, such as the difference in the gastrointestinal retention time of plastics among species, were still not fully understood.

In this study, there were 1.556±0.470 pieces of plastics per fish. Benthic fish was significantly higher than pelagic fish at 2.56±0.878 and 0.629±0.086 count per fish, respectively. Most of the previously published studies share the same result that the average microplastics count per single fish was usually between 1-2 pieces or lower (Lusher et al., 2017).

Benthic fish, especially Threadfin bream and Silver Sillago, have microplastics count per fish far higher than the other species. Threadfin bream was the species with the highest microplastics contamination rate at 60%. While Silver Sillago was the species with the highest number of microplastic counts in their gastrointestinal tract at 3.50±1.59 pieces per fish. This is because of the feeding habits of these groups of fish that feed on a wide range of prey, including benthic organisms like benthic diatoms, algae, smaller fish, crustaceans, mollusks and other invertebrates (Taghavi et al., 2012; AFSHARI et al., 2013). This wide range of natural prey was resulting in a higher amount of ingested microplastics compare to fish with a narrower range of feeding preference. A study from the Texas Gulf had demonstrated that a fish species that had a wider range of natural food was more likely to expose to microplastics than a fish species that had a narrower feeding preference or more selective foraging preference (Peters et al., 2017).

Short Mackerel (*Rastrelliger brachysoma*) was an example of a narrow feeding preferences species in this study. It fed mainly on planktons, such as diatoms, dinoflagellates and planktonic copepods (Aye, 2020). It was an important food fish species of Thailand, which had microplastics in their guts at 39.52% of samples in this study. This is the very first report of microplastics contamination in this species of fish from the upper Gulf of Thailand. Another related report was from the lower Gulf of Thailand, which found plastic debris in 25% (1 out of 4) of Short Mackerel samples from Songkhla province (Azad et al., 2018). Its close relative, Indian mackerel (*Rastrelliger kanagurta*), from Malaysia, had microplastics in 50% of fish samples (Karbalaei et al., 2019).

Croakers, which are fish in the family Scianidae, are the important food fish for locals. Previous reports had revealed a different ratio of microplastics contamination in this group of fish depending on location and species. *Nibea Soldado*, a species of benthic Croakers were found to have microplastics in 25% of samples in this study. *Nibea soldado*, was a major active carnivore. Their natural preys were mainly small crustaceans, especially shrimp (Hajisamae, 2009; Jeyaraj et al., 2015) *Panna microdon* and *Dendrophysa russelli*, two species of seafloor-dwelling Croakers from Songkhla province had microplastics in GI tract in 44.44% and 56.10% of fish samples, respectively. While 59.46% of *Johnius* spp., a pelagic living Croakers, had microplastics (Azad et al., 2018). Interestingly, *Argyrosomus regius*, benthic Croakers from the Mediterranean Sea had microplastics in 75% of samples (Güven et al., 2017).

In the same way as Croakers, Threadfin Bream, *Nemipterus japonicas*, and *N. hexodon* had a relatively high proportion of microplastics contamination in this study. However, A study reported that only 10% of *N. bipunctatus* from Malaysia had microplastics (Karbalaei et al., 2019). Noting that, even the fish species were from the same genus or same family living in the same region (i.e. samples from Malaysia and Thailand), but different species or different living habitats can also lead to the difference in the degree of microplastics contamination.

The selected species in this study were important food fish species, most of which were caught by trawling boats and bamboo stake traps. In fact, by-catch fish that was not consumable (not a preferential species or not in the market size) were usually brought into the fishmeal industries. Thailand ranked among the world's top fishmeal-producer, comprise 6.6 of global fish meal production in 2019 (Gündoğdu et al., 2020). Apart from small and not consumable fish, fish by-products including guts were also used as fishmeal sources. In 2013, Thailand was the world's highest fish meal producer from fish by-products (Shepherd and Jackson, 2013). Fishmeal consumption per capita of Thai people in 2011 was 7.4 kilograms per capita (Guillen et al., 2019). Because of the use of fish guts as the major raw materials of fishmeal, together with the fact that microplastics were primarily found contaminated in the fish gut, fishmeal was very likely to have microplastics contamination. Although humans did not directly consume fishmeal, many animal feed productions used fishmeal as a major protein source. Hence, contaminations of microplastics in fishmeal affect humans, by its impact on the food web (Gündoğdu et al., 2020).

The level of plastics contamination in fishmeal should be related to the level of contamination in fish species used as raw material and spatial marine environment. Most fishmeal was made of pelagic species fish. Fish that undergo the fishmeal production process were heating up to 95-100°C. Noting that the lowest melting point of a plastic polymer is 110°C, which is the melting point of polyethylene. Therefore, the heating process in fishmeal production should not interfere with microplastics contamination from fish to fishmeal. The only grinding process might affect in breaking plastic particles size into smaller pieces, resulting in a higher number of particles count (Gündoğdu et al., 2020). However, Considering that heating can also cause the releasing of some potentially toxic substances (PoTSs) from plastics polymer. For example, heating can accelerate the migration rate of styrene from polystyrene, or phthalates from PVC, both of which are toxic compounds to humans (Hahladakis et al., 2018). Hence, heating of fishmeal may accelerate the emission of PoTSs from microplastics that may further accumulate in fishmeal deriving products.

In current study, pelagic fish consist of 0.010±0.002 pieces of microplastics per gram of body weight. The selected pelagic species genus in our study (*Rastrelliger* spp.) was among the most common species of fish used as raw material for fishmeal production in Thailand, together with *Sardinella* spp. (Péron et al., 2010). From IFFO database, 1 kilogram of fishmeal came from the average of 4.4-4.6 kilogram of whole fish. In other words, fishmeal production from whole fish has an average of 22.5% yields (Gündoğdu et al., 2020). Theoretically, we can estimate that the average amount of microplastics in fishmeal should be 0.044-0.046 pieces per gram of fishmeal or 44-46 pieces per kilogram of fishmeal. The data from the study of Gündoğdu et al. (2020) showed that fishmeal from 11 countries contains an average of 177.2±19.4 microplastic particles per kilogram of fishmeal. Countries with the lowest microplastics contamination in fishmeal were Norway (33.3±6.7 particles per kg) and South Korea (33.3±6.7 particles per kg), while the highest was from China (337.5±34.5 particles per kg). The calculated estimation of microplastics contamination from our data was in the ranges of this report. Noting that the number of microplastics contamination in fishmeal varies greatly depends on countries and the raw-material fish. Hanachi et al. (2019) stated that there was a significant difference in microplastics count between fishmeal from different fish sources or even the same source but with different protein levels. The actual amount of microplastics contamination in fishmeal may differ depends on many factors. The aforementioned reduction ratio of 4.4-4.6 is theoretically the direct conversion from small pelagic fish into fishmeal. However, with the addition of other types of raw material including trash fish or fish by-products, the actual number of microplastics contamination in fishmeal may lower or higher than this expected value. This depends on the proportion and characteristics of the added raw materials, which vary between seasons and quality requirements of fishmeal products (Péron et al., 2010).

The using microplastics-contaminated fishmeal as a protein source for animal feed can lead to microplastics contamination and accumulation in those animals. An experimental scenario was demonstrated in Common Carp (*Cyprinus carpio*). Carps fed with pellets made from microplastics-contaminated fishmeal have microplastics in their guts after 4 weeks of exposure. On the other hand, fish fed with plastic-free soybean meal feed were free of microplastics. The conversion ratio from microplastic counts in fishmeal (counts per gram) to the experimental fish (counts per fish) was ranging from 0.205 to 0.584 (Hanachi et al., 2019). Given these conversion ratios, if a group of Common Carp were fed with the feed made from fishmeal that made from pelagic from the Upper Gulf of Thailand, which were calculated to have 0.044-0.046 plastic pieces per gram, by the same feed ingredient ratio as the previous experiment. These Carp will contain 0.009 to 0.027 pieces of microplastics in their gastrointestinal tract per individual after 4 weeks of feeding. These numbers may differ depends on fish species, feeding amount, and many factors. In Thailand, one of the commonly used fishmeal in aquaculture feed was in Tilapia feed production. Tilapia feed usually contains 0-20% fishmeal. Given an average FCR of 1.5 (Tacon and Metian, 2008), we can estimate that 1 kilogram of Tilapia should expose to fishmeal around 0-300 grams, which might expose to microplastics up to 13.8 pieces per kilogram of Tilapia throughout the production period. With this low number of estimation, together with the fact that the translocation rate of microplastics from guts to edible muscle tissue was only 1 particle per 1.87×10^7 ingested particles (Zeytin et al., 2020), we can infer that the risk of microplastics translocation from guts to muscles of commercial aquaculture fish is still low. However, the risk from exposing microplastics from aquaculture fishes' gastrointestinal tracts still present. The actual situation of microplastics contamination in commercial fishmeal and aquaculture industries must be further investigated to ensure food safety for fish consumers.

In the present study, plastic polymer types identification was done by FTIR microscope. This process was performed to chemically confirm that each debris observed by the stereomicroscope was an actual microplastic particle. FTIR results revealed that some of the plastic-like debris observed from the stereomicroscope was not plastic pieces. These particles were, for example, sand grain, proteinaceous or calciferous residue from the fish tissue, or gastric content. Hence, this process greatly helps in enhancing the accuracy of the test protocols (Shim et al., 2017).

In Thailand, few studies on microplastic contamination in fish were published. The chemical confirmation process was present in only one previous publication. None of them used FTIR-microscope to identify polymer. A conventional FTIR machine was used by Klangnurak and Chunniyom (2020). This conventional FTIR machine has a remarkable limitation in the identification of very small plastic particles compare to FTIR-microscope (Shim et al., 2017), which was used in the present study. The other published studies about microplastics contamination in fish in Thailand were performed by only visual identification techniques (Azad et al., 2018; Kasamesiri and Thaimuangphol, 2020), which has a significantly lower accuracy compared to studies with chemical confirmation process (Shim et al., 2017).

In this study, a FTIR-microscope machine was used to identify and confirm plastic polymer types. Polyester, a fiber-type plastics polymer, was a dominant type found in fish, followed by polyethylene, rayon, polypropylene, and other mixed polymers in a smaller proportion. Benthic fish had fibers-type polymer, particularly polyester, markedly higher than pelagic fish, which indicates high accumulation of such polymers on the seafloor or sediments. The results were consistent with a report on microplastics in the sediment of the Gulf of Thailand, in which rayon and polyester were the most common polymer types found. Rayon and Polyester had a combined proportion of 53% in the sediment of the Gulf of Thailand (Wang et al., 2020), compared to 59% in Benthic fish in our study. Polyester and other fiber-type plastics were reportedly found on the bottom of the Chao Phraya River as well (Ta and Babel, 2020). Because of the differences in the distribution of microplastics due to their density, Pelagic-feeding organisms may expose to a different set of plastics litters from those bottom-feeding organisms. Those bottom-feeding organisms will expose to a set of denser debris that were accumulating on the sea-floor sediments (GESAMP, 2019).

Because plastic polymer contamination profiles from different parts of the world might be exclusively different depending on human activities or plastics usage in a certain location (Gündoğdu et al., 2020). Therefore, we may suggest that polyester, a fiber-type plastics polymer, was a dominant type of microplastics contamination found in the Gulf of Thailand.

Polyester and other fiber type microplastics, or so-called “microfibers” is a fiber type microplastics that is one of the major consequences of laundering activities of synthetic clothes (Henry et al., 2019; Zambrano et al., 2019). Polyester and other fiber type plastics, such as Nylon, were also primarily used in the fishery industries (GESAMP, 2019), which was heavily presented around the Gulf of Thailand. Marine litter associated with fishing industries and aquaculture were accounting for a large proportion of overall marine litter. In some regions, up to 20% of fishing gears might be lost and become marine litter due to accidents, weather conditions, or intentional abandonment. These reasons could lead to the higher deposition of this group of microplastics in a certain area than in the other area.

Most fiber microplastics have relatively higher specific gravity than other polymers by their nature. The average specific gravity of polyester, rayon, and nylon was 1.35, 1.50, and 1.13-1.15, respectively. For this reason, these groups of polymers are usually found at the bottom of the water body. The polyester group was the most abundant polymer in many studies focusing on sea sediments (GESAMP, 2015; Erni-Cassola et al., 2019). Other lower density polymer, such as Polyethylene, is mostly found near the water surface and water column, While polypropylene exists throughout the water surface to the sediments (Erni-Cassola et al., 2019).

Microfiber plastics enter the food chain through the ingestion of small aquatic fauna. These plastics were likely to persist in the food chain due to their size and shape that make them prone to have longer gut retention or cause entanglement in small fauna. Despite most microfiber may not have a prominent carcinogenic or endocrine-disrupting agent like some plastic polymers; microfibers tend to have a higher surface area than other microplastics. This led to a higher chance of absorbing potentially toxic compounds in the environment and brought them into the food chain (Henry et al., 2019). Therefore, we should be aware that these effects might have occurred in the fish from the Gulf of Thailand.

Despite polyester was the plastic polymer with the highest count in this study; most of them were found in a high amount in some individuals. In the other words, fish with polyester contamination usually found more than few pieces of polyester fiber in their gastrointestinal tracts, while other plastic polymers, such as polyethylene, were found only 1-2 pieces per individual.

Polyethylene was the second most common polymer detected in this study, at 25.63% of the overall polymer identified. It was the highest abundant polymer found in pelagic fish, followed by other polymers in comparable proportion. From a meta-analysis by Erni-Cassola et al. (2019), polyethylene was the polymer with the highest chance of being found in an aquatic environment. It has a predictive prevalence of 23% of total microplastics, followed by polyester and polyamide, at 20%, and polypropylene, at 13%. From this study, we also can assume that polyethylene is the most abundant polymer found in the mid-water column of the Gulf of Thailand.

Smith et al. (2018) reported that 79% of studies base on microplastics polymer type found polyethylene as major microplastic contaminants. On the other hand, some of the frequently used plastics, particularly polyvinylchloride (PVC) and polyethylene terephthalates (PET) had relatively low abundance in the environment. Only 5% and 2% of polymer type studies had found PVC and PET in their studies. In conjunction with the result from the current study that only 1 piece of PVC and no PET had been detected. A major reason that both PVC and PET have been scarcely found in the aquatic environment may due to the fact that both polymers have very long service lives. Thus, very few proportions of these plastics became trash and enter the environment as microplastics (GESAMP, 2015). Both PVC and PET were plastic polymers with many reports of releasing potentially toxic substances (PoTSs), such as phthalates and other additives. These additives can have carcinogenic or endocrine-disrupting effects in animals or humans (Halden, 2010; Hahladakis et al., 2018). Hence, a low level of contamination of both plastic polymers means fewer such risks in aquatic animals and humans around the Gulf of Thailand.

Various factors were contributing to the distribution of polymer type in different geography. Fiber-type microplastics, including polyester, polyamide, and rayon, have a higher tendency to accumulate near the urbanized areas. This is because these polymers came directly from human activities, especially laundry (Zambrano et al., 2019). Besides, with their relatively higher specific gravity than other polymers, these fiber polymers sank faster to the bottom of the sea and accumulated near their sources. On the contrary, fragment type microplastics, which were mostly degradation products of larger plastic debris, usually found in a remote area. This is because large plastic debris was easily carried far away from its origin by the effects of wind or currents (Alomar et al., 2016; Erni-Cassola et al., 2019). A highly urbanized zone together with drainages from many large river basins surrounds the area in this study, the upper Gulf of Thailand. Therefore, fiber-type microplastics might have a higher chance to be detected.

Fiber type microplastics was a dominant type of microplastics observed in various studies. A study from the United States had demonstrated that microfibers consisted of as high as 86.4% of microplastics from marine fishes in the Texas Gulf (Peters et al., 2017). Knowing microplastics polymer type in a certain area can help to predict the general effects of microplastics on living organisms. For example, polyethylene was an endocrine-disrupting agent, which can cause hormonal abnormalities and reproductive disturbances in consumed animals (Rochman et al., 2014). Accordingly, aquatic animals in the upper Gulf of Thailand may have risks of developing such disorders.

Blue and white were the most common microplastics color found in this study. In the same manner, Bissen and Chawchai (2020) revealed that coastal microplastic contamination from Thai eastern coastal provinces was mostly blue and white in color. Interestingly, there was a difference in particle color between fragment-type and fiber-type microplastics. A fragment or irregular-shaped microplastics were mostly white, while fiber microplastics were dominated by blue color. Almost the same manner of color distribution was observed between pelagic and benthic fish.

Color effects on microplastic feeding ratios in aquatic organisms were barely understood. Color similarities between microplastics and animals' natural food may play role in increasing the chance of ingestion. Daphnids (*Daphnia Magna*) reportedly mistake green microplastics for green algae (Chen et al., 2020).

Histopathological studies were carried out from fish tissue samples. The result showed no significant relationship between microplastics detection in the GI tract and histopathological changes. Most lesions found were not specific and not related to the presence of microplastics. These unspecific lesions were possibly found in some fish with normal appearance (Jovanovic et al., 2018; Senarat et al., 2018a).

In Short Mackerel, various histopathological lesions were observed. A noticeable lesion was the presence of the Microsporidian parasites in the kidney of 13.43% of fish. Similar organisms have been reported in the study of Senarat et al. (2018b) in fish from the Samut Songkram Province, which parasites were suspected to be *Myxospora* spp. In the present study, the presences of microsporidian parasites in the kidney were not related with the presence of microplastics in the gastrointestinal tracts. Moreover, no remarkable gross pathological lesions were also observed. Several studies were mentioned some general histopathological features found in captive and natural Short Mackerel, which were in accordance with histopathological lesions in this study. For instance, the presence of melanomacrophage center in liver and kidney, Abnormal renal tubular structure, hepatic lipidosis (Senarat et al., 2018a; Senarat et al., 2018b) or degeneration of ovary (Senarat et al., 2017).

These histopathological lesions presented in this study were not associated with the presence or amount of microplastics in the GI tract. However, microplastics can theoretically affect and cause histopathological lesions in fish and other animals. Such effects were resulted from both plastic polymer and chemical contaminants. Virgin or pure microplastics may not cause prominent histopathological lesions in fish, only alterations such as accumulation of inflammatory cells in the intestine were observed (Jovanovic et al., 2018). Hepatocyte abnormality, hepatic vacuolation, and hepatic sinusoid congestion were only observed in fish fed with high dose PE and PVC microplastic (Espinosa et al., 2019). Hepatic and muscular lesions were also reportedly observed in zebra fish that received microplastics in feed as high as 2,800 pieces daily (Lu et al., 2016).

In addition to pure microplastics, many experimental studies confirmed the histopathological effects of microplastics together with plastics additives and environmental pollutants on histopathological changes in fish. Hepatic vacuolization was observed in fish fed with PE that contains persistent organic pollutants (POPs). The experimental PE dosage was 2% of feed (Rainieri et al., 2018). Another study revealed that PE immersed in seawater with organic pollutants caused abnormal germ cells proliferation in male reproductive organs of fish, which can potentially further become tumor (Rochman et al., 2014).

Despite abnormalities confirmed from experimental studies, the actual concentration of microplastics in the environment was relatively low, compared to those in previously described experimental studies. The natural occurrence of MP in gastrointestinal tract of fish was usually <1-2 pieces/fish (Lusher et al., 2017), or 1.556±0.47 pieces per fish in the present study.

Moreover, little was known about the retention of microplastics in the fish gastrointestinal tract. This is because most experimental fish were received daily dosage of microplastics for a certain period of time in most of previous experimental studies, which lead to continuous exposure to microplastics in the gastrointestinal tract. In experimentally microplastic-fed Gilt-head Seabream (*Sparus aurata*), more than 90% of microplastics were excreted within 24 hours after ingestion. Very little retention of microplastics was observed after 30 days of depuration (Jovanovic et al., 2018). Therefore, more data may be required to confirm the cause of these histopathological lesions whether they were microplastics-related or not. Further studies on the effects of environmental microplastics on histopathological lesions in fish need to be carried out.

From this study, microplastics were only detected from the GI tract. None was detected from other tissues. However, microplastics can theoretically be absorbed via the intestinal wall into the blood circulation and accumulated in internal organs. Using very small microplastics or nanoplastics with the help of pre-stained special colors, some experimentally fed microplastics were detected in other tissues such as liver and muscle in several fish species (Jovanović, 2017). With the limitation of natural microplastics without special staining and low sensitivity for small microplastics and nanoplastics detection method, such accumulations were not detected in natural fish in this study. In addition, these experiments used a very high concentration of microplastics. Zebrafish fed with 2,800 pieces of microplastics daily for 45 days showed accumulation of microplastics in their liver and muscle. There were more than 30 pieces of microplastics in the GI tract of the fish at the end of the study (Jovanovic et al., 2018). This was much higher than the natural occurrence of microplastics count that usually lower than 1-2 pieces per fish (Lusher et al., 2017) or 1.556±0.47 pieces in this study.

Despite many studies confirmed the presence of microplastics in the gastrointestinal tract of fish, very few demonstrated or tried to examine the presence of those plastics in other organs or tissue. In fact, the occurrence of microplastics translocation from the gastrointestinal tract is low. Only 1 particle of microplastics (1-5 μm in size) can translocate to the fillet of the European Sea Bass (*Dicentrarchus labrax*) for every 1.87×10^7 ingested particles in an experimental study (Zeytin et al., 2020).

Therefore, it can be concluded that the current risk of microplastics accumulation outside the GI tract of natural fish is low. However, fish consumers can be exposed to microplastics via ingesting fish guts or the whole fish (GESAMP, 2015; Smith et al., 2018). At the present stage of knowledge, there was still no method to extract or remove microplastic particles from food. Hence, the authors may suggest that avoid consuming fish gastrointestinal tract is currently the best way to minimize microplastics exposure for fish consumers. Further assessment on microplastics contamination in fish tissue as well as methodology development in the future was also crucial for a better understanding of the risk of microplastics to humans.

In 2017, the Food and Agriculture Organization of the United Nation (FAO) reported a negligible risk of microplastics as vehicles of chemical additives and pollutants to humans compared to other modes of exposure, such as direct contact and inhalation. However, understanding of direct effects and other effects of microplastics on humans was still insufficient (Lusher et al., 2017). According to the current knowledge, there was no evidence of remarkable acute toxicity, severe chronic toxicity or any fatal toxicity from microplastics, as well as nanoplastics, to humans. However, others modalities of effects, such as chronic accumulative effect, were still not fully understood. Researches related to these mechanisms were still needed to be done in the future (Yong et al., 2020). In 2016, The European Food Safety Agency (EFSA) reported that there were still insufficient data to assess human food safety from microplastics contamination in seafood. Therefore, EFSA stated that further investigations on microplastics contamination in various food sources are important to ensure seafood safety in the future (GESAMP, 2019). A risk assessment or exposure assessment in other routes apart from seafood ingestion was still not enough. Although those routes were also important exposure routes to humans (GESAMP, 2015). Global plastics production and consumption were still continuously increasing (Andrady, 2017). The trend of environmental microplastics contamination was also increasing at a dramatic rate (Shahul Hamid et al., 2018). From our current understanding, we can predict that environmental plastic debris and microplastics in the environment will be markedly increased in near future. As a consequence, Future microplastics exposure of aquatic animals will be higher than the current days. These high exposures will directly or indirectly affect humans. Therefore, continuing surveillance of microplastics contamination in seafood, as well as other sources together with overall exposure assessment will be necessary for the near future.

Conclusion

This study has revealed the amounts and characteristics of microplastics contamination in marine food fishes from the Gulf of Thailand. Comparing with other previous reports worldwide, a moderate degree of contamination was observed in this study. Significant relationships were observed between the degree of microplastics contamination in marine fishes of the upper Gulf of Thailand and fish species and between the degree of microplastics contamination and ecological niche (pelagic or benthic). Benthic fish has significantly higher microplastics contamination compared to pelagic fish. The most dominant plastic polymers were polyester and polyethylene. Blue and white were the highest abundant color observed. No association was detected between the presence of microplastics and histopathological changes in fish. It was estimated that Thai people ingest microplastics from marine fish 0.06 to 0.095 pieces per person per day. From current knowledge, the risk of transferring microplastics from the gastrointestinal tract to other edible tissue of marine fishes from the upper Gulf of Thailand was low. We can also assume that there was still a risk of microplastics transferring from those fishes to fishmeal, into the food chain, and consequently to humans. The estimated number of microplastics in fishmeal in Thailand according to the results from this study was among the ranges of many countries worldwide. Moreover, the transferring rate of microplastics in fishmeal into animal via animal feed was low. Despite the estimated amount was relatively low, together with the fact that microplastics tend to pose chronic or accumulative risks to animal and human, continuous surveillance or further research for the preventive solution were crucial in the future.

REFERENCES

ACFS 2016. FOOD CONSUMPTION DATA OF THAILAND. National Bureau of Agricultural Commodity and Food Standards Office of Standard Development, ed. [Online]. Available: https://consumption.acfs.go.th

AFSHARI M, VALINASSAB T, SEIFABADI J and KAMALY E 2013. AGE DETERMINATION AND FEEDING HABITS OF NEMIPTERUS JAPONICUS (BLOCH, 1791) IN THE NORTHERN OMAN SEA. IRANIAN JOURNAL OF FISHERIES SCIENCES. 12(2): 248-264.

Alomar C, Estarellas F and Deudero S 2016. Microplastics in the Mediterranean Sea: deposition in coastal shallow sediments, spatial variation and preferential grain size. Marine environmental research. 115: 1-10.

Andrady AL 2011. Microplastics in the marine environment. Marine pollution bulletin. 62(8): 1596-1605.

Andrady AL 2017. The plastic in microplastics: A review. Marine pollution bulletin. 119(1): 12-22.

Araujo CF, Nolasco MM, Ribeiro AMP and Ribeiro-Claro PJA 2018. Identification of microplastics using Raman spectroscopy: latest developments and future prospects. Water research. 142: 426-440.

Arthur C, Baker JE and Bamford HA 2009. Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris, September 9-11, 2008, NOAA Technical Memorandum NOS-OR&R-30.

Aye ZM 2020. Food and feeding habits of short mackerel (Rastrelliger brachysoma, Bleeker, 1851) from Palaw and adjacent coastal waters, Taninthayi region, Myanmar. International Journal of Fisheries and Aquatic Studies. 8(4): 360-364.

Azad SMO, Towatana P, Pradit S, Patricia BG and Hue HT 2018. Ingestion of microplastics by some commercial fishes in the lower Gulf of Thailand: a preliminary approach to ocean conservation. International Journal of

Agricultural Technology. 14(7): 1017-1032.

Besseling E, Wang B, Lürling M and Koelmans AA 2014. Nanoplastic affects growth of S. obliquus and reproduction of D. magna. Environmental science & technology. 48(20): 12336-12343.

Bissen R and Chawchai S 2020. Microplastics on beaches along the eastern Gulf of Thailand–A preliminary study. Marine Pollution Bulletin. 157: 111345.

Budimir S, Setälä O and Lehtiniemi M 2018. Effective and easy to use extraction method shows low numbers of microplastics in offshore planktivorous fish from the northern Baltic Sea. Marine pollution bulletin. 127: 586-592.

Chanda M and Roy SK 2008. Plastics fundamentals, properties, and testing. 1st ed. Boca Raton: CRC press. 280 pp.

Chen Q, Li Y and Li B 2020. Is color a matter of concern during microplastic exposure to Scenedesmus obliquus and Daphnia magna? Journal of Hazardous Materials. 383: 121224.

Claessens M, Van Cauwenberghe L, Vandegehuchte MB and Janssen CR 2013. New techniques for the detection of microplastics in sediments and field collected organisms. Marine pollution bulletin. 70(1-2): 227-233.

Cox KD, Covernton GA, Davies HL, Dower JF, Juanes F and Dudas SE 2019. Human consumption of microplastics. Environmental science & technology. 53(12): 7068-7074.

Critchell K and Lambrechts J 2016. Modelling accumulation of marine plastics in the coastal zone; what are the dominant physical processes? Estuarine, Coastal and Shelf Science. 171: 111-122.

Dehaut A, Hermabessiere L and Duflos G 2018. Current frontiers and recommendations for the study of microplastics in seafood. TrAC Trends in Analytical Chemistry. 116, 346-359.

Dris R, Gasperi J, Mirande C, Mandin C, Guerrouache M, Langlois V and Tassin B 2017. A first overview of textile fibers, including microplastics, in indoor and outdoor environments. Environmental Pollution. 221: 453-458.

Erni-Cassola G, Zadjelovic V, Gibson MI and Christie-Oleza JA 2019. Distribution of plastic polymer types in the marine environment; A meta-analysis. Journal of hazardous materials. 369: 691-698.

Espinosa C, Esteban MÁ and Cuesta A 2019. Dietary administration of PVC and PE microplastics produces histological damage, oxidative stress and immunoregulation in European sea bass (Dicentrarchus labrax L.). Fish & Shellfish Immunology. 95: 574-583.

FAO 2019. FAO yearbook. Fishery and Aquaculture Statistics 2017. Pages 65-70. 2019 ed. FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, Rome. [Online]. Available: http://www.fao.org/fishery/statistics/yearbook/en

Frias J and Nash R 2019. Microplastics: Finding a consensus on the definition. Marine pollution bulletin. 138: 145-147.

Galgani F, Giorgetti A, Vinci M, Le Moigne M, Moncoiffe G, Brosich A, Molina E, Lipizer M, Holdsworth N and Schlitzer R 2017. Proposal for gathering and managing data sets on marine micro-litter on a European scale. 38 pp. [Online] Available: 10.6092/8ce4e8b7-f42c-4683-9ece-c32559606dbd.

GESAMP 2015. Sources, fate and effects of microplastics in the marine environment: a global assessment. IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, Reports and Studies, GESAMP No.90. [Online] Available: http://www.gesamp.org/publications/ publicationdisplaypages/reports-and-studies-no.-90.

GESAMP 2019. Guidelines or the monitoring and assessment of plastic litter and microplastics in the ocean (Kershaw P.J., Turra A. and Galgani F. editors).

IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP/ISA Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, Reports and Studies, GESAMP No.99. 130 pp. [Online] Available: http://dx.doi.org/10.25607/OBP-435

Geyer R, Jambeck JR and Law KL 2017. Production, use, and fate of all plastics ever made. Science advances. 3(7): e1700782.

Gonçalves C, Martins M, Costa MH and Costa PM 2018. Development of a method for the detection of polystyrene microplastics in paraffin-embedded histological sections. Histochemistry and cell biology. 149(2): 187-191.

Guillen J, Natale F, Carvalho N, Casey J, Hofherr J, Druon J-N, Fiore G, Gibin M, Zanzi A and Martinsohn JT 2019. Global seafood consumption footprint. Ambio. 48(2): 111-122.

Gündoğdu S, Eroldoğan O, Evliyaoğlu E, Turchini G and Wu X 2020. Fish out, plastic in: Global pattern of plastics in commercial fishmeal. Aquaculture. 534, 736316.

Güven O, Gökdağ K, Jovanović B and Kıdeyş AE 2017. Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish. Environmental Pollution. 223: 286-294.

Hahladakis JN, Velis CA, Weber R, Iacovidou E and Purnell P 2018. An overview of chemical additives present in plastics: migration, release, fate and environmental impact during their use, disposal and recycling. Journal of hazardous materials. 344: 179-199.

Hajisamae S 2009. Trophic ecology of bottom fishes assemblage along coastal areas of Thailand. Estuarine, Coastal and Shelf Science. 82(3): 503-514.

Halden RU 2010. Plastics and health risks. Annual review of public health. 31: 179-194.

Hanachi P, Karbalaei S, Walker TR, Cole M and Hosseini SV 2019. Abundance and properties of microplastics found in commercial fish meal and cultured common carp (Cyprinus carpio). Environmental Science and Pollution Research.

26(23): 23777-23787.

Hartmann NB, Hüffer T, Thompson RC, Hassellöv M, Verschoor A, Daugaard AE, Rist S, Karlsson T, Brennholt N and Cole M 2019. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. ACS Publications. Environmental Science & Technology. 53: 1039–1047.

Henry B, Laitala K and Klepp IG 2019. Microfibres from apparel and home textiles: Prospects for including microplastics in environmental sustainability assessment. Science of The Total Environment. 652: 483-494.

Horton AA, Walton A, Spurgeon DJ, Lahive E and Svendsen C 2017. Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. Science of the total environment. 586: 127-141.

Hutley TJ and Ouederni M 2016. Polyolefins—the history and economic impact. In: Polyolefin Compounds and materials. Springer. 13-50.

Hwang J, Choi D, Han S, Choi J and Hong J 2019. An assessment of the toxicity of polypropylene microplastics in human derived cells. Science of The Total Environment. 684: 657-669.

Jabeen K, Su L, Li J, Yang D, Tong C, Mu J and Shi H 2017. Microplastics and mesoplastics in fish from coastal and fresh waters of China. Environmental Pollution. 221: 141-149.

Jeyaraj N, Suhaila A, Divya L, Baskaran N, Santhanam P and Ravikumar S 2015. FORAGING BEHAVIOR PATTERNS OF FOUR SELECTED COMMERCIALLY IMPORTANT FISH SPECIES IN KASARAGOD DISTRICT, NORTHWEST PART OF KERALA Indo–Asian Journal of Multidisciplinary Research (IAJMR). 1(1): 38-44.

Jovanović B 2017. Ingestion of microplastics by fish and its potential consequences from a physical perspective. Integrated environmental assessment and management. 13(3): 510-515.

Jovanovic B, Gokdag K, Guven O, Emre Y, Whitley EM and Kideys AE 2018. Virgin microplastics are not causing imminent harm to fish after dietary exposure. Marine Pollution Bulletin. 130: 123-131.

Jovanović B, Gökdağ K, Güven O, Emre Y, Whitley EM and Kideys AE 2018. Virgin microplastics are not causing imminent harm to fish after dietary exposure. Marine pollution bulletin. 130: 123-131.

Käppler A, Fischer D, Oberbeckmann S, Schernewski G, Labrenz M, Eichhorn K-J and Voit B 2016. Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or both? Analytical and bioanalytical chemistry. 408(29): 8377-8391.

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: 58-70.

Karbalaei S, Golieskardi A, Hamzah HB, Abdulwahid S, Hanachi P, Walker TR and Karami A 2019. Abundance and characteristics of microplastics in commercial marine fish from Malaysia. Marine pollution bulletin. 148: 5-15.

Kasamesiri P and Thaimuangphol W 2020. MICROPLASTICS INGESTION BY FRESHWATER FISH IN THE CHI RIVER, THAILAND. International Journal. 18(67): 114-119.

Klangnurak W and Chunniyom S 2020. Screening for microplastics in marine fish of Thailand: the accumulation of microplastics in the gastrointestinal tract of different foraging preferences. Environmental Science and Pollution Research. 27: 27161-27168.

Lebreton L-M, Greer S and Borrero JC 2012. Numerical modelling of floating debris in the world’s oceans. Marine pollution bulletin. 64(3): 653-661.

Lebreton LCM, Van der Zwet J, Damsteeg J-W, Slat B, Andrady A and Reisser J 2017. River plastic emissions to the world’s oceans. Nature communications. 8: 15611.

Lobo H and Bonilla JV 2003. Handbook of plastics analysis. Vol 68. In: Crc Press.

Lu Y, Zhang Y, Deng Y, Jiang W, Zhao Y, Geng J, Ding L and Ren H 2016. Uptake and accumulation of polystyrene microplastics in zebrafish (Danio rerio) and toxic effects in liver. Environmental science & technology. 50(7): 4054-4060.

Lusher A, Hollman P and Mendoza-Hill J 2017. Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety. In: FAO, 2017.

Lusher AL, McHugh M and Thompson RC 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Marine pollution bulletin. 67(1-2): 94-99.

Matsuguma Y, Takada H, Kumata H, Kanke H, Sakurai S, Suzuki T, Itoh M, Okazaki Y, Boonyatumanond R and Zakaria MP 2017. Microplastics in sediment cores from Asia and Africa as indicators of temporal trends in plastic pollution. Archives of environmental contamination and toxicology. 73(2): 230-239.

Mazurais D, Ernande B, Quazuguel P, Severe A, Huelvan C, Madec L, Mouchel O, Soudant P, Robbens J, Huvet A and Zambonino-Infante J 2015. Evaluation of the impact of polyethylene microbeads ingestion in European sea bass (Dicentrarchus labrax) larvae. Marine Environmental Research. 112: 78-85.

Moore M 2006. Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? Environment international. 32(8): 967-976.

Ng KL and Obbard JP 2006. Prevalence of microplastics in Singapore’s coastal marine environment. Marine Pollution Bulletin. 52(7): 761-767.

Niaounakis M 2017. Management of Marine Plastic Debris. In: William Andrew Publishing, 2017).

Nor NHM and Obbard JP 2014. Microplastics in Singapore’s coastal mangrove ecosystems. Marine pollution bulletin. 79(1-2): 278-283.

Pazos RS, Maiztegui T, Colautti DC, Paracampo AH and Gómez N 2017. Microplastics in gut contents of coastal freshwater fish from Río de la Plata estuary. Marine pollution bulletin. 122(1-2): 85-90.

Péron G, François Mittaine J and Le Gallic B 2010. Where do fishmeal and fish oil products come from? An analysis of the conversion ratios in the global fishmeal industry. Marine Policy. 34(4): 815-820.

Peters CA, Thomas PA, Rieper KB and Bratton SP 2017. Foraging preferences influence microplastic ingestion by six marine fish species from the Texas Gulf Coast. Marine Pollution Bulletin. 124(1): 82-88.

Qiu Q, Peng J, Yu X, Chen F, Wang J and Dong F 2015. Occurrence of microplastics in the coastal marine environment: first observation on sediment of China. Marine Pollution Bulletin. 98(1-2): 274-280.

Rainieri S, Conlledo N, Larsen BK, Granby K and Barranco A 2018. Combined effects of microplastics and chemical contaminants on the organ toxicity of zebrafish (Danio rerio). Environmental research. 162: 135-143.

Rochman CM, Kurobe T, Flores I and Teh SJ 2014. Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment. Science of the Total Environment. 493: 656-661.

Schwabl P, Köppel S, Königshofer P, Bucsics T, Trauner M, Reiberger T and Liebmann B 2019. Detection of various microplastics in human stool: a prospective case series. Annals of internal medicine, 171(7): 453-457.

Senarat S, Jiraungkoorskul W and Kettratad J 2017. Ovarian histology and reproductive health of short mackerel, Rastrelliger brachysoma (Bleeker, 1851), as threatened marine fish in Thailand. Songklanakarin Journal of Science & Technology. 39(2).

Senarat S, Kettratad J, Tipdomrongpong S, Pengsakul T, Jiraungkoorskul W, Boonyoung P and Huang S 2018a. Histopathology of kidney and liver in the captive broodstock (Rastrelliger brachysoma) during its juvenile stage. Veterinary Integrative Sciences. 16(2): 87-93.

Senarat S, Kettratad J, Tipdomrongpong S, Plumley FG, Jiraungkoorskul W and Poolprasert P 2018b. Health status in wild and captive short mackerel, Rastrelliger brachysoma from Thailand: Histopathology. Songklanakarin Journal of Science & Technology. 40(5).

Shahul Hamid F, Bhatti MS, Anuar N, Anuar N, Mohan P and Periathamby A 2018. Worldwide distribution and abundance of microplastic: how dire is the situation? Waste Management & Research. 36(10): 873-897.

Shashoua Y 2012. Conservation of plastics, Material science, Degradation, and Preservation. 1st ed. Routledge: Elsevier. 304 pp.

Shepherd CJ and Jackson AJ 2013. Global fishmeal and fish-oil supply: inputs, outputs and marketsa. Journal of Fish Biology. 83(4): 1046-1066.

Shim WJ, Hong SH and Eo SE 2017. Identification methods in microplastic analysis: a review. Analytical Methods. 9(9): 1384-1391.

Smith M, Love DC, Rochman CM and Neff RA 2018. Microplastics in seafood and the implications for human health. Current environmental health reports. 5(3): 375-386.

Sussarellu R, Suquet M, Thomas Y, Lambert C, Fabioux C, Pernet MEJ, Le Goïc N, Quillien V, Mingant C and Epelboin Y 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proceedings of the National Academy of Sciences. 113(9): 2430-2435.

Ta AT and Babel S 2020. Microplastic contamination on the lower Chao Phraya: Abundance, characteristic and interaction with heavy metals. Chemosphere. 257: 127234.

Tacon AGJ and Metian M 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture. 285(1): 146-158.

Taghavi MA, Hakimelahi M, Ghodrati SM, Vahabnezhad A and Taheri MA 2012. Feeding habits and stomach contents of Silver Sillago, Sillago sihama, in the northern Persian Gulf. Iranian Journal of Fisheries Sciences. 11(4): 892-901.

Thushari GGN, Senevirathna JDM, Yakupitiyage A and Chavanich S 2017. Effects of microplastics on sessile invertebrates in the eastern coast of Thailand: An approach to coastal zone conservation. Marine Pollution Bulletin. 124(1): 349-355.

Von Moos N, Burkhardt-Holm P and 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(20): 11327-11335.

Wang Y, Zou X, Peng C, Qiao S, Wang T, Yu W, Khokiattiwong S and Kornkanitnan N 2020. Occurrence and distribution of microplastics in surface sediments from the Gulf of Thailand. Marine Pollution Bulletin. 152: 110916.

Yong CQY, Valiyaveetill S and Tang BL 2020. Toxicity of microplastics and nanoplastics in mammalian systems. International journal of environmental research and public health. 17(5): 1509.

Zambrano MC, Pawlak JJ, Daystar J, Ankeny M, Cheng JJ and Venditti RA 2019. Microfibers generated from the laundering of cotton, rayon and polyester based fabrics and their aquatic biodegradation. Marine pollution bulletin. 142: 394-407.

Zeng EY 2018. Microplastic contamination in aquatic environments: an emerging matter of environmental urgency. In: Elsevier. 424 pp.

Zeytin S, Wagner G, Mackay-Roberts N, Gerdts G, Schuirmann E, Klockmann S and Slater M 2020. Quantifying microplastic translocation from feed to the fillet in European sea bass Dicentrarchus labrax. Marine Pollution Bulletin. 156: 111210.

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