Faculty of Resource Science and Technology

Ingestion and Types of Microplastic in *Meretrix lyrata* from Buntal Bay Sarawak through Nile Red Fluorescent Tagging

Eric Lau Ngie Hao

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Ingestion and Types of Microplastics in *Meretrix lyrata* from Buntal Bay Sarawak through Nile Red Fluorescent Tagging

Eric Lau Ngie Hao

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Signature

Name: Eric Lau Ngie Hao

Matric No.: 18020217

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

Date :

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ABSTRACT

Microplastic had emerged as new form of plastic pollution. Many ingestions in biota were reported worldwide. Filter feeder such as bivalves are prone to microplastic ingestion. Shellfish were usually consumed as whole, without gut removal. This poses a threat towards human consumption. In this study, hard shell clam (*Meretrix lyrata*) was chosen and collected 4 times (2 times in both October 2020 (pre-monsoon) and March 2021 (post-monsoon) from local fish market (Kampung Buntal Wet Market) to investigate the factor of size class and harvesting season towards the ingestion rate of *Meretrix* lyrata. Fluorescent tagging using Nile red dye were used to aid the quantification process. Polymer identification were done on isolated suspected particles using Nicolet IN10 µ-FTIR and compared to an open-source FTIR reference library OpenSpecy. All samples (81 samples) in this study were positive with microplastic ingestion. A total of 15867 microplastic were quantified, the most dominant shape type is particle type (89%). The average microplastic size was 0.043 mm - 0.069 mm, and average of 195.90 ± 43.6 microplastic/individuals. Results shows that smaller sized clam tends to ingest more microplastic compare to larger sized clams. There were no significant differences ($p = 0.05$) in microplastic ingestion according to harvesting season. This might indicate that habitats for *M. lyrata* have pervasive microplastic pollution. PVC, PP with silicate mix, resin dispersion and PDMS were the four polymers identified from the microplastic particles isolated. All four were common polymers that can be found at place with human settlements. Fluorescent tagging using Nile red is a good method in quantification of microplastic however modification and precautionary steps were needed to obtain optimum results are important for microplastic detection improvements.

Keywords: *Meretrix lyrata*, nile red fluorescent tagging, microplastic. µ-FTIR, size-dependent

Dokumentasi Jenis dan Jumlah Mikroplastik dalam* Meretrix lyrata *dari Kampung Buntal, Kuching, Sarawak melalui Penandaan Pendarfluor menggunakan Nile red

ABSTRAK

Mikroplastik telah disahkan sebagai punca utama pencemaran plastik. Sehingga kini, terdapat banyak penerbitan mengenai perjumpaan mikroplastik dalam usus hidupan laut. Organisma penyuap penapis seperti kerang-kerangan merupakan hidupan laut yang kerap dilaporkan perjumpaan mikroplastik dalam usus. Kerang-kerangan seringkali dimakan secara menyeluruh tanpa dibuang sistem usus. Ini merbahaya kepada manusia sebagai kosumer kerang-kerangan kerana mikroplastik memasuki tubuh manusia melalui pemakanan. Dalam kajian ini, Kepah (Meretrix lyrata) dari Kampung Buntal dikaji untuk memastikan pengaruhan saiz kepah dan musim tengkujuh terhadap jumlah mikroplastik dalam usus kepah. Penandaan pendarfluor menggunakan Nile red adalah metodologi utama untuk kajian ini. Pengenalpastian polimer dijalankan dengan penggunaan mesin Nicolet IN10 μ-FTIR dan sumber rujukan terbuka OpenSpecy. Mikroplastik telah dijumpai dalam kesemua sampel kajian ini. Sebanyak 15867 butiran mikroplastik dijumpai dan plastic berbentuk partikal merupakan majoriti (89%). Saiz purata mikroplastik adalah antara 0.043 mm sehingga 0.069 mm, dan jumlah purata mikroplastik adalah 195.90 ± 43.6 mikroplastik/individu. Keputusan menunjukkan bahawa kepah yang bersaiz kecil mempunyai lebih banyak bilangan mikroplasitk dalam usus berbanding kepah bersaiz besar. Musim tengkujuh tidak menpengaruhi jumlah bilangan mikroplastik dalam kepah. Hal ini mungkin disebabkan habitat kepah yang menpunyai pecemaran mikroplastik. Beberapa polimer yang dikenal pasti hadir adalah PVC, PDMS, resin, serta PP. Keempat-empat polimer adalah polimer yang kerap dijumpai di kawasan kediaman atau perpopulasi *manusia. Penandaan pendarfluor menggunakan Nile red adalah metodologi untuk kuantifikasi yang baik serta sesuai untuk kuantifikasi mikroplastik dalam kerrang-kerangan. Pengubahsuaian metodologi seharusnya dilakukan untuk mendapat bacaan tepat serta optimum bagi sampel kerang-kerangan.*

Kata kunci: *Meretrix lyrata, nile red penandaan pendarfluor, mikroplastik, µ-FTIR, bergantung kepada saiz*

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LIST OF ABBREVIATIONS

FTIR	Fourier-transform infrared spectroscopy
HDPE	High density polyethylene
LDPE	Low density polyethylene
PDMS	Polydimethylsiloxanes
PET	Polyethylene terephthalate
PP	Polypropylene
PVC	Polyvinyl chloride

CHAPTER 1

INTRODUCTION

1.1 Introduction

Plastics is currently the most used material worldwide. Plastics Europe (2021) reported global plastic production had increased since 2016 and reached 367 mt in 2020. Characteristics such as low cost, strength, lightweight, corrosion resistance, durability, and good thermal and electrical insulation qualities increase the demand for plastics-based product productions. Asia alone had contributed 52% of the total plastic production in 2020 (Plastics Europe, 2021). Different plastic-based material variants produced have a finite lifespan and are quickly discarded. Most of the waste will either be dumped into landfills or littered into the natural environment, apart from specific recyclable variants. High environmental persistence of plastic causes it to be the top contributor to marine debris (Auta et al., 2017). While authorities and policymakers started working to deal with the marine debris issue, another 'side product' from marine debris received attention: microplastics (NOAA, 2020).

Lebreton et al. (2018) reported plastics as dominant type of marine litter within Great Pacific Garbage Patch. While the amount reported only consist of those positively buoyant plastic materials but the total plastic pollution maybe under-estimated as different types of plastic have different densities in nature. Lower density plastics such as polypropylene, polyethylene and polystyrene have higher transportability than those denser plastic type such as polyvinylchloride (PVC) which settle down but lower transportability due to certain natural events such as runoff from heavy rains or waves. (Woodall et al., 2014; Mani et al., 2015).

Back in 1972, Carpenter and Smith had first reported the presence of small fragment plastics but the microplastic related research only receive attention when large amount of microplastics were reported by Thompson et al. (2004) in sediment and plankton samples. The term microplastics was widely defined as plastic particles with a diameter smaller than 5 mm (Arthur et al., 2009), origin of these micro-sized particles can be defined as primary microplastic and secondary microplastic. Primary microplastic were defined as the plastic particles designed and produced intentionally as micro size. On the other hand, secondary microplastic were product from defragmentation of bigger plastic pieces from marine debris through processes such as mechanical degradation and photo-degradation.

Microplastic pollution has been reported in many marine ecosystems worldwide due to massive input from land source, the persistent of microplastic in environment and are readily to be transported by ocean current. (Lavers & Bond, 2017; Brach et al., 2018; Reed et al., 2018). Lebreton et al. (2019) suggested that buoyant macroplastic accumulate in offshore surface water should be lesser than predicted as a series of repeated activity like stranding, settling and resurfacing in coastal environment tend to trap most of the marine litters. This shows coastal area and estuarine as the second largest sink of microplastic. Usman et al. (2020) stated contribution of plastic marine debris of a country is highly depending on its population size and the efficiency of waste management system.

Seafood provides proteins, vitamins and other essential minerals for human diet (Jayasekara et al., 2020), discovery of microplastic in seafood poses high risk and the side effect of these anthropogenic particles needs high attention. Clams are one of the most popular seafood among Malaysian. It is a perfect vitamin, mineral and lean protein source for consumers. The non-selective feeding strategy of clams increases the abundance of anthropogenic particles such as microplastics.

Small size nature of microplastic increased the tendency of contaminants accumulate from the surroundings due to the hydrophobic nature and the high surface ratio properties (Shahul Hamid et al., 2018; Deng et al., 2021). Small size of microplastics increases the bio-availability for marine organisms. Bivalves are particularly interested in assessment of microplastic ingestion due to their extensive filter-feeding activity, through exposing them directly with microplastic in water column and sediment (Li et al., 2015). Several reports had report direct effect of microplastics on bivalves such as filtration activity and feeding behaviour (Rist et al., 2016; Green et al., 2017). Previous reports from Kolandhasamy et al. (2018) and Li et al. (2019) highlight three pathways of microplastic accumulation in mussels including ingestion, adherence and fusion. The high surface area to volume ratio of microplastic makes them a prefect vector for persistent organic pollutants (POPs) and heavy metals (Zhang et al., 2020). Apart from physical damage caused by microplastic ingestion, bioaccumulation of harmful substances such as persistent organic pollutants and heavy metals along the food chain should also be given attention (Fu et al., 2020).

1.2 Problem statement

Meretrix lyarata or commonly known as ‘Kepah’ or lyrate asiatic hard clams were one of the local favourite shellfish species. The clams can be easily obtained by the locals throughout the year. (either through the imported frozen clams or buy directly with local fishermen at study site Kampung Buntal). The common shell size of the clam (from imported frozen clam) is less than 50 mm (shell length), this draws the attention of current study to investigate the difference in microplastic contamination between different size class. The availability of the clams throughout the year regardless of seasons also draw the interest to investigate the microplastic detection within the clams between different harvesting season.

1.3 Objective

Current study aims to utilize fluorescent tagging method to increase the sensitivity and reduce the bias from the existing quantification method for microplastic quantification in biota samples. This study also aims to investigate different possible factors which could affects the microplastic ingestion rate in white hard clam (*M. lyrata*). Therefore, the objectives of this study are:

1) To combine and adapt different microplastic quantification method and merging rapid screening method using fluorescent tagging.

2) To investigate factors size and seasonality to amount of microplastic ingestion

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy and morphology

Meretrix lyrata clam belongs to family Veneridae which were reported inhibiting in South and Southeast Asia regions (Liu et al., 2006) and also in several locations within Malaysian waters including Sarawak, Malaysia such as current project study site Kampung Buntal and Kabong (Betong District) (Hamli et al., 2016). The key characteristic of *Meretrix lyrate* with other *Meretrix* sp. were the undulate pattern on the shell surface (Appendix 5). The classification of clam according to Nguyen (1996) was shown in Figure 2.1.

Phylum: Mollusca

Class: Bivalvia

Order: Venerida

Family: Veneridae

Genus: Meretrix

Species: Meretrix lyrata (Sowerby, 1851)

Figure 2.1 Classification of clam taxanomy

As one of the commercially important bivalve species, *Meretrix* sp. productions are highly dependent on natural sources. (Duisan et al., 2022). Both Hamli et al. (2016) and Desrita et al. (2019) reported the increase demand of *Meretrix* sp. clam in Southeast Asia and Malaysia.

The *M. lyrata* is described by Helm et al. (2004) as a clam with two equal shells, in which calcium carbonate was the main component of the shells. The connecting side of the two shells which known as umbo were the dorsal part of the clam. Siphons were at the posterior side of the clam and foot were at the anterior side of the clam. Figure 2.2 shows the internal surface view and dorsal view of *M. lyrata*. Duisan et al. (2022) explained *M. meretrix* with size between 50mm-70mm is matured enough to be induced for spawning.

The development stages of *M. lyrata* includes embryonic stage, D-larve stage, mature larvae, early juvenile and spat. (Helm et al., 2004). Sandy tidal flats were the optimum growing environment for *Meretrix* sp. with the sediment composition of 80 - 90% sand and 9 – 14% mud and organic fragments. (Oanh & Lap, 2020). Sediment dwelling nature of the clam poses this species under threat with exposure towards vast amount of microplastics found within coastal sediments. (Urban-Malinga et al., 2021).

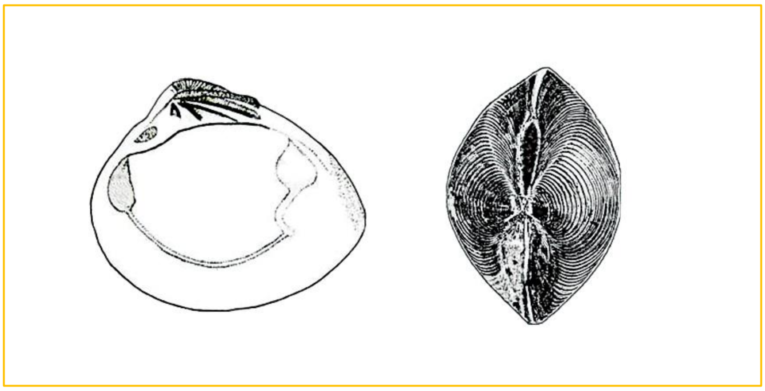

Figure 2.2 *M. lyrata* internal surface of right valve view (left) and dorsal view (right)

2.2 Plastic and its emergences

Plastic had become most of the substitute material due to its durability, versatility and cheap in production. (Klemeš et al., 2020) Packing industry is one of the industries that uses plastic material the most. Plastics are products of monomers connected repeatedly through polymerization and forming large molecular chain. The combination of different monomers is possible and gives the end product both of the characteristics of the monomers. This widely increase the uses of plastics material in our daily life. Ranging from car parts, building materials, packing, and down to microbeads in cosmetic products.

Plastic can be classified into two categories, thermosetting plastics and thermoplastics (Kazemi et al., 2021). Majority of thermosetting plastics such as bakelite are permanently mold, cannot be melted and reformed. This irreversible chemical change characteristic made thermosetting plastics non-recyclable. On the other hand, thermoplastics such as polyethylene terephthalate (PET) and polypropylene which are far more abundant are recyclable by heated until melted and remolded to form into new shapes.

In 2021 ASTM International revised the symbols from the code with ASTM D7611 standard, replacing the familiar three chasing arrows with solid equilateral triangles (ASTM International, 2021). The updated version of the ASTM International codes was shown in Table 2.1. This is to prevent the confusion of public because the old symbol looks similar to universal recycling symbols.

Table 2.1 Type of plastic according to ASTM designator code and its application

American Society for Testing and Materials designator code	Plastic	Abbreviation	Typical application
01 PET	Polyethylene terephthalate	PET	Beverage bottles Food containers Film and sheeting Strapping Fibres Fleece Filling material
02 PE-HD	High-density polyethylene	HDPE	Chemical containers Beverage bottles Food containers Crates Buckets Tubing Pipes
03 PVC	Polyvinyl chloride (rigid)	PVC	Containers Electrical conduit Pipes Gutters Cladding Window frames
03 PVC	Polyvinyl chloride (plasticised)	PVC	Cable insulation Garden hose Footwear Sheeting Flooring Mats
04 PE-LD	Low-density polyethylene	LDPE	Bags Squeezable bottles Food packaging Carton linings Shrink wrap Pallet sheets Mulch film Waste bins Outdoor furniture

Table 2.1 continued

American Society for Testing and Materials designator code	Plastic	Abbreviation	Typical application
05 PP	Polypropylene	PP	Bottle caps and container lids Packaging tape Pipes Rope Automotive Applications Outdoor furniture
06 PS	Polystyrene (rigid)	PS	Disposable cutlery and tableware Rigid disposable food containers Other low-cost applications requiring rigidity
06 PS	Polystyrene foams (expanded or extruded)	EPS, XPS	Disposable food containers and cups Packaging foam Foam boxes Thermal insulation
07 O	Polycarbonate	PC	Information storage discs Traffic light lenses Riot shields Security windows Eye protection
07 O	Poly(methyl methacrylate)	PMA	Glazing Aircraft canopies and windows Fluorescent light diffusers Vehicle lighting covers and lenses Contact lenses
07 O	Polytetrafluoroethylene	PTFE	Non-stick coatings for cookware and bakeware Breathable fabrics Plumbing tape Waterslides Low-friction coatings Automotive applications
07 O	Acrylonitrile butadiene styrene	ABS	Electrical and electronic equipment Automotive applications Pipes
07 O	Polyamide (nylon)	PA	Fibres Bristles Monofilament Bearings Automotive applications
07 O	Polychloroprene (neoprene)	CR	Gloves Footwear Diving garments Medical bandages and supports Automotive gaskets Electrical insulation Weather balloons

2.3 Plastic after use and fate

Generally recycling of plastic is possible for certain polymer type which manufactured through injection molding at low temperatures, thermoplastics such as PET, PP, and HDPE. Recycling other waste material such as metal (80%), paper (60%), glass (50%) but recycling of plastic waste is only at 14-18%. (Haward, 2018; Hossain et al., 2021). One reason for the recycling industry does not receive much support and attention might be the confusion and misunderstanding of certain plastic are recyclable. (Worrell & Reuter, 2014). Acceptance of general public towards recycled plastic products is still low, reported by van der Vegt et al. (2022), general public's image towards both used plastics and perception of recycled material are associated with being dirty, unhygienic, unsafe or in lower quality. Another factor voiced out by van der Vegt et al. (2022) which hinder or disturb sorting of used plastic materials in recycling process is manufacture of the plastic products involves combinations of different materials, coloration, additives and adhesives. Xanthos (2012) reported addition of additive in polypropylene can increase the resistance of PP to oxidation.

A survey done by Dilkes-Hoffman et al. (2019) shows that most of the respondents are aware of high load of general waste will be landfilled and concerns about the amount of plastic waste that will be disposed in landfill. Taking the easiest example PET which is the plastic type for mineral water bottles, although recycling of PET is possible but people tend to dispose it as general waste. Food and drinking manufacturers refuse to use recycled products as there are possible contamination such as chemical additives, food residue and degraded product sorb on the plastic prior to recycling process.

The fate of these plastic waste will either end up in landfills or being burn to reduce the waste produced. (Boucher & Friot, 2017). However, burning of plastics could produce