University of Nottingham

UK | CHINA | MALAYSIA

MICROPLASTIC LOADS IN FISH AND INVERTEBRATES IN MALAYSIAN RIVERS

Ng Yong Sin, BSc

Thesis submitted to The University of Nottingham

for the degree of Master of Research

December 2022

Abstract

Microplastic pollution is a global problem, but little is known about the degree of microplastic contamination in tropical rivers, and even less is known of contamination levels in the animals in these systems. Additionally, there is a gap in knowledge regarding the extent of microplastic pollution in rapidly developing regions, where environmental infrastructure (e.g. sewage systems) and waste management are often inadequate. This study assessed the microplastic loads in invertebrates and fish in the Semenyih River, Malaysia. The Semenyih is a rapidly urbanising catchment on edge of Kuala Lumpur, Malaysia's capital city. Microplastic contamination levels in river sediment and water were assessed at 8 sites along the river to develop a better understanding of the relations between environmental contamination levels and body loads of fish and invertebrates. Relations between body sizes and feeding preferences of animals were also examined. Microplastic loads in animals were assessed using two methods, one capable of detecting material in the 0.1-5 mm size range (low-resolution, manual microscopy), and the other down to 0.004 mm (semi-automated high-resolution, counterstaining dye method). Thus, microplastics including nanoparticles were assessed as part of this research.

Using the widely used low-resolution method, 94.7% of fish, 44.2% of insect larvae, and 58.3% of mussels were found to contain microplastic. For the four insect families studied, this low-resolution method detected an average of 1.1 pieces of microplastic per individual. When expressed per body weight, individuals of these families contained an average of 11.06 pieces of microplastic per mg of dry tissue. Numbers

increased markedly when the high-resolution method was used, with an average of 128.8. pieces per individual and 704.3 per mg dry weight for the four insect families. The overall ratio between microplastic detected using high- and low-resolution methods was around 120:1.

Microplastic loads in aquatic animals varied between major taxonomic groups (i.e. between fish, mussels, and insects) but not between all four of the insect families studied; Hydropsychidae contained more pieces per individual than Simuliidae, but Baetidae and Chironomidae did not differ significantly from each other or the other insect taxa. Microplastic contamination of water and sediment differed significantly between sites. However, there was no simple direct relationship between bed contamination levels and body loads in invertebrates at the site. Analysing all the taxonomic groups together, there was a significant relationship between body size and contamination levels; the same positive relation was found for insects and mussels but not fish.

Fibre was the most abundant microplastic in water and sediments in the Semenyih River, though fragments were present in greater abundance on the bed than in the water column. There was a much greater prevalence of fragments in the bodies of animals than either in the water column or in the sediment. Microplastic loads differed significantly between functional feeding groups when expressed as microplastic concentration per mg of dry tissue, with filterers containing less microplastics in their

tissue per unit weight than gatherers. Invertebrates and fish ingested microplastic of all four different shapes (beads, fibres, fragments, and films).

The work shows widespread contamination by microplastic in the Semenyih River and its aquatic biota. Organisms appear to ingest microplastics in ways that do not simply reflect their gross abundance in the environment but reflect their mode of feeding. Contamination loads also reflect body size, although differences between taxa depend on whether the load is expressed simply as pieces of microplastic per individual or unit body weight. The work also indicates that body loads may be much higher than suggested using conventional low-resolution enumeration methods, averaging 120 times greater across the four insect families when the high-resolution method was used. The high-resolution method applied in this study is more time-consuming than low-resolution ones, so may not always be practical. However, the 120:1 ratio could potentially be applied to data generated using the low-resolution method, to provide an estimate of the likely true load in the body of aquatic organisms.

Acknowledgement

First and foremost, I would like to thank The Habitat Foundation and The University of Nottingham Malaysia, for sponsoring this research. Besides, I am extremely grateful to my supervisors, Prof. Christopher Gibbins, Dr Sivathass Bannir Selvam, and Prof. Ting Kang Nee for providing me with the opportunity to continue my studies, and their invaluable advice, continuous support, and patience throughout my MRes journey. I would also like to thank Dr Kasturi Muthoosamy and Bee Hui for their support and suggestion on developing the counterstaining dyes method.

I am particularly grateful to work with the members of the Hydro Ecology Lab, especially my research partner, Kitty. It is their kind help and support that have made my master's degree journey a wonderful time. Finally, I would like to express my gratitude to my family, Simon, and friends, for their understanding and encouragement throughout this journey.

Table of Contents

List of Appendices

List of Tables

List of Figures

INTRODUCTION

1. Introduction

1.1 Background

Plastic, a ubiquitous material with a thousand uses, has taken over many aspects of daily life. The first synthetic plastic, Bakelite, was introduced at the beginning of the twentieth century (Klun et al., 2022). Its beneficial properties, such as its lightweight, durability, corrosion resistance, and low cost, have made plastic one of the world's most important and widely used man-made materials. Since its commercial introduction in the mid-twentieth century, plastic production and consumption have grown at exponential rates, particularly for single-use purposes (Andrady and Neal, 2009). Plastic production now takes up 6% of the world's oil consumption (World Economic Forum, 2016). Against this background trend of increased use, the COVID-19 pandemic has enhanced consumer reliance on single-use plastic, especially for hygiene purposes. An estimated 3.4 billion tonnes of single-use plastic waste related to personal protective equipment has been generated daily since the start of the outbreak (Benson et al., 2021). Not all plastics are recycled, and as of 2015 79% of global plastic waste was either disposed of in landfills or released into the environment in an uncontrolled manner (Geyer et al., 2017).

Due to the long-lasting nature of this synthetic material, plastic waste does not decompose but breaks down into smaller fragments when released into the environment. According to their size, these fragmented plastic materials can be classified as macroplastics (>25 mm), mesoplastics (5mm to 25mm), microplastics (< 5mm), and nanoplastics (< 0.1um) (Boyle and Örmeci, 2020). Of these groups, only

microplastics have been classified into several types based on their source and shape. According to their source, microplastic can be divided into two categories: primary microplastic and secondary microplastic. Primary microplastics are microplastics that were initially manufactured in the micron size range. This includes plastic resin pellets used as industrial raw materials for the plastic industry (Mato et al., 2001), micron-sized polyester film that is used as glitters (Yurtsever, 2019), and plastic microbeads used as mechanical exfoliants in pharmaceutical and personal care products (Chang, 2015). On the other hand, secondary microplastics are derived from the fragmentation of larger plastic waste. Secondary microplastics account for the vast majority of microplastics found in the environment (Xia et al., 2022). This includes microfibres released during the washing of synthetic textiles (Napper and Thompson, 2016), synthetic rubber fragments derived from car tyre shedding (Sommer et al., 2018), and fragmentation of larger plastic waste via a variety of mechanisms such as photodegradation (Andrady et al., 2003), biodegradation (Shah et al., 2008), and environmental erosion (Andrady, 2017).

Microplastics can be grouped into four shapes: (1) films, (2) fragments, (3) fibres, and (4) beads, see figure 1.1. The shape of microplastics can be used as a general indicator of their origin (Ugwu et al., 2021). For example, films are thin plastic sheets that are usually formed from degraded plastic bags. In contrast, fragments are thicker and result from the degradation of a variety of plastic products. Fibres, on the other hand, are long cylindrical plastics with equal diameters along their long axis and are frequently released during the washing of synthetic textiles. Finally, beads are

distinguished by their perfectly spherical shape and are often found in personal care products (Kooi and Koelmans, 2019).

Much attention has been paid to microplastics, especially as they can pass through wastewater treatment plants and be released into rivers and oceans more easily than larger materials (Murphy et al., 2016). The problem posed by microplastic is discussed in the following section.

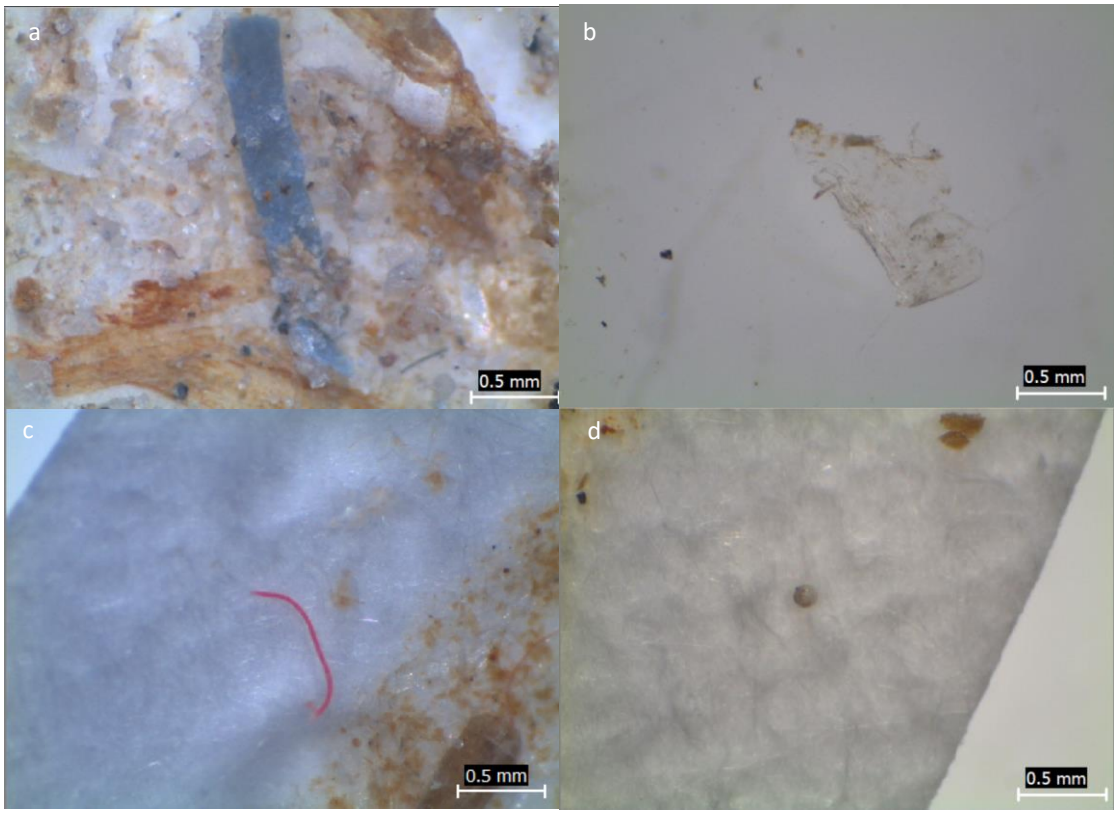


Figure 1.1. *Microplastics with different shapes extracted from freshwater organisms sampled from the Semenyih River, Malaysia (a, fragment; b, film; c, fibre; d, bead). Photos by author.*

1.2 Microplastic pollution

Microplastics are not a new pollutant. Plastic debris was first reported in the environment in the 1970s when researchers found that microplastics were ingested by more than half of the fish species sampled from coastal waters of southern New England (Carpenter et al., 1972). Nonetheless, microplastics were only considered as a contaminant of emerging concern in the early 2000s, after mounting evidence that marine animals ingested microplastics (Browne et al., 2007; Thompson et al., 2004).

Now, because of its pervasiveness in our daily lives, plastic has emerged as a potential marker for the Anthropocene strata (Zalasiewicz et al., 2016). Despite more easily degradable polymers such as oxo-biodegradable plastics made from polyethene and additives having been produced to tackle plastic pollution, the issue remains – not least because of the ongoing degradation of larger plastic waste already in the environment into microplastics (Thompson et al., 2009). Microplastics will continue to accumulate in the environment due to their long-lasting characteristics, with the level of plastic pollution now exceeding the planetary boundary's safe operating space (Persson et al., 2022).

Microplastic contamination is ubiquitous. At least 5 trillion plastic particles are currently floating in the ocean, with 92 per cent of them being microplastics (Eriksen et al., 2014). These plastic particles have a wide range of effects on ecosystems. Microplastic can entangle or be ingested by smaller organisms, and this may further lead to a change in ecosystem services such as carbon cycling (Rillig et al., 2021) and ocean carbon sequestration (Shen et al., 2020). Ingestion of microplastic, for example,

results in a higher chance of mortality, skin lesions, and a lower reproduction rate of earthworms, which has influenced the soil carbon cycle by decreasing organic matter transportation into deeper soil layers (Büks et al., 2020).

A critical issue is that a wide range of additives is used in plastic production to improve durability and performance (Hahladakis et al., 2018). Some of these additives, such as Bisphenol A (BPA), are endocrine disruptors that are harmful to human and animal health even in low doses. Although many of these toxic additives have been banned, previously manufactured plastics are still present in the environment, and the ingestion of these materials poses a problem to aquatic organisms and, via trophic transfer, potentially to humans (Barboza et al., 2020). Moreover, the fragmentation and ageing of microplastics will enhance the biofouling and pollutant sorption rate (Binda et al., 2021; Hüffer et al., 2018). Thus, microplastics can act as a vector and increase the bioavailability of environmental pollutants and pathogens in ecosystems (Atugoda et al., 2021).

1.3 Problem statement

Despite the adverse environmental impact of microplastics being widely studied, relatively few studies have been conducted to investigate microplastic pollution in freshwater ecosystems compared to marine systems (Akdogan and Guven, 2019). Furthermore, microplastic research is primarily conducted in the laboratory, with few studies evaluating its impact on the natural environment (O'Connor et al., 2022). Published field data on freshwater systems has primarily focused on the abundance of

microplastics in water and sediment; although numbers are growing, there have been few field studies assessing levels of microplastic contamination in freshwater organisms (Kukkola et al., 2021). As a result, more field research is needed to gain a better understanding of the occurrence and abundance of microplastics in freshwater organisms, the characteristics of microplastics, and the factors that influence freshwater organisms' intake of microplastics. In addition, a pervasive problem is that most studies only focus on larger-sized microplastics (see Literature Review). It is possible therefore that by 'missing' finer materials, much current work underestimates microplastic body loads in animals.

More broadly, information on microplastic pollution in freshwater environments is geographically fragmented, with most publications coming from Europe, North America, and China (Blettler et al., 2018). Thus, information on the extent of microplastic pollution in rapidly developing regions, where environmental infrastructure such as sewage systems and waste management are commonly inadequate, is lacking (Chen et al., 2021b).

Malaysia has been identified as a hotspot of microplastic pollution, largely because its rivers are the world's third-largest source of plastic pollution in the ocean (Meijer et al., 2021). However, few microplastic studies have been conducted in the country. To date, 19 studies in Malaysia have been conducted to investigate microplastic pollution in the environment (see table 1 in Literature Review), but only five were conducted in freshwater environments. Only one out of the five studies examined body loads of

microplastic in fish. Thus, there are no adequate field data on the occurrence and abundance of microplastics in freshwater organisms in Malaysia.

1.4 Aim and objectives of the study

The overall aim of this study is to assess the microplastic loads in freshwater invertebrates and fish in the Semenyih catchment, in Malaysia.

The research has the following objectives:

i. **To assess the nature and extent of microplastic contamination in freshwater organisms.** *This objective focuses on determining the number of individual pieces of microplastic found in aquatic animals and their frequency (the proportion of individual organisms of the same family that ingested microplastic), using both lower- and higher-resolution microplastic enumeration methods.*

ii. **To examine the spatial differences in microplastic loads in aquatic animals in relation to site contamination levels.** *This objective is to understand whether animals at highly contaminated sites have more microplastic in their bodies than those at less contaminated ones, as might logically be expected.*

iii. **To assess the factors influencing microplastic ingestion of freshwater organisms.** *The objective is to understand the extent to which site contamination level, feeding preference and body size influence the amount of microplastic ingested by freshwater fish and invertebrates.*

iv. **To evaluate the composition and characteristics of microplastic consumed by fish and invertebrates**. *This objective is to determine the shape and colour of microplastic ingested by freshwater fish and invertebrates and compare these characteristics to microplastic found in the river to see if availability and ingestion are related.*

1.5 Significance of the study

This is the first study in Malaysia to provide information on the extent of microplastic pollution in freshwater organisms. Furthermore, this study looked at microplastic load across a diverse taxonomic range, providing data on microplastic pollution in Malaysia's freshwater ecosystem across various taxonomic groups and trophic levels. Notably, it uses both lower and higher resolution techniques (the later capable of detecting materials in the nano size-range), in order to provide a more complete picture of body loads. The work represents a critical starting point for informing management and policy in Malaysia, to combat microplastic pollution in freshwater environments in the country.

LITERATURE REVIEW

2. Literature review

Small plastic debris has been reported from every continent on the planet (Shahul Hamid et al., 2018); for instance, deep-sea sediment in the remote Antarctic region has been described as a microplastic sink due to its high microplastic load (Woodall et al., 2014). Moreover, microplastics were also found in the snow and water samples from the Mount Everest (Napper et al., 2020). However, many of the studies conducted to date have focused on marine microplastic pollution (Akdogan and Guven, 2019; Chen *et al.*, 2021). In contrast, less attention has been given to microplastic pollution in the freshwater environment, although as the threats to freshwater ecosystems become clearer, more research is being directed to lakes and rivers.

The freshwater ecosystem acts as a biodiversity reservoir. Despite covering only 0.8% of the Earth's surface area, it contains up to 6% of the world's biodiversity (Dudgeon et al., 2006). Though, the ecosystem is challenged by a variety of anthropogenic stressors such as climate change, land cover change, eutrophication and the introduction of alien species (Angeler et al., 2014). As a result, 82 % of the global population relies on upstream freshwater sources that are severely threatened (Green et al., 2015).

Due to their small size, microplastics can be easily ingested by organisms and so are highly bioavailable in the environment (Botterell et al., 2018). Furthermore, microplastics are a vector that promotes pollutant bioaccumulation and pathogen transport in the environment (Amelia et al., 2021). They have created an ideal

environment for attached pathogens to develop higher antimicrobial resistance genes because of their ability to absorb pollutants (Bowley et al., 2021). This is alarming as recent research suggests that airborne microplastic from clinical waste contaminated with SARS-CoV-2 may aid the transmission of the virus in the environment (Liu and Schauer, 2021). Moreover, some plastics contain additives and potentially harmful substances that have adverse impacts on environmental and human health when released into the environment (Hahladakis et al., 2018).

Understanding the impact of microplastic on the freshwater ecosystem is critical for better management and the development of policies designed to reduce the impact of this material. Accordingly, the purpose of this review is to summarise knowledge of (1) the sources and factors influencing microplastic abundances in freshwater ecosystems, (2) the occurrence and ecological impacts of microplastics, with a particular focus on what is known about Southeast Asian freshwater ecosystems, and (3) the factors determining microplastic abundance in freshwater organisms. The review concludes with a summary and a section which identifies key knowledge gaps and makes recommendations to address these gaps.

2.1 Microplastics in the freshwater environment

When compared to microplastic-related studies conducted in marine ecosystems, freshwater microplastic pollution is still a relatively new research area. The presence of microplastics in freshwater systems was first reported in 2012 in Lake Geneva, where researchers discovered the presence of macro- and microplastics in the water column and fish collected from the lake (Faure et al., 2012). The first microplastic

occurrence in the riverine environment was reported in 2014, with evidence of microplastic ingestion in wild Gudgeon (*Gobio gobio*) from the French rivers (Sanchez et al., 2014). Chen *et al* (2021) provided a detailed synthesis of the number of papers on marine and freshwater systems, as well as the geographic spread of published work. Since the first paper in 2014, an increasing number of studies have found microplastics in both lentic (standing waters like lakes, ponds, and swamps) and lotic (flowing waters like rivers and streams) freshwater ecosystems (Kukkola et al., 2021).

Microplastics enter the freshwater ecosystem via a diverse set of pathways (Wang et al., 2021). Like many other contaminants, microplastics in freshwater systems originate from point and non-point sources. The former are more easily identified, which include wastewater treatment plants (WWTPs), domestic drainage, and industrial discharges (Kataoka et al., 2019). Non-point sources include microplastics delivered to lakes and rivers as a result of runoff from urban and agricultural areas and atmospheric deposition (Kataoka et al., 2019). Primary microplastic, such as manufactured resin pellets, is often found in close proximity to plastic processing plants, while secondary microplastic found in water samples may be more difficult to pin to a source.

When looking at the domestic sources, microplastics are released from laundry activities and entered wastewater channels and pipes. Before entering natural waters, the effluent is typically, but not always, treated by WWTPs (Gaylarde et al., 2021). However, most WWTPs were not designed to remove microplastics, and microplastics

are still able to pass through the WWTP's bioreactor and effluent polishing systems (Leslie et al., 2017). As a result, many studies found that WWTPs did not lead to a reduction in microplastic concentrations in domestic wastewater before water was released to rivers (Leslie et al., 2017; Stanton et al., 2020). Nevertheless, evidence of effective microplastic removal in domestic wastewater via WWTPs has also been reported. A field study in the Clyde River in Scotland reported a 98 per cent removal of microplastics after wastewater treatment, but the authors also acknowledged the large volume of treated effluent released into the river from the WWTP as a potential source of microplastics (Murphy et al., 2016).

Another source of microplastic contamination is domestic wastewater released directly to drainage systems. This type of contamination can therefore be considered an impact of urbanisation (Dikareva and Simon, 2019; Kataoka et al., 2019), with urban rivers sometimes suffering extreme microplastic pollution (Blettler et al., 2018). Domestic wastewater often includes a large number of microscopic textile fibres, resulting from the domestic washing of textiles, primarily synthetic clothing (Murphy et al., 2016). Microplastics are also present in personal care products such as skin cleansers and toothpaste, which may also be discharged directly into watercourses (Duis and Coors, 2016).

The lack of proper waste management can result in a large number of microplastics being released directly into the environment. For example, due to the lack of a WWTP in Lahore City, Pakistan, the Ravi River received a large amount of microplastic directly from sullage carriers (Irfan et al., 2020). Similarly, African rivers have been reported to

have high microplastic concentrations as a result of the region's limited plastic waste management strategy (Alimi et al., 2021).

Atmospheric transport is another route for microplastic. Atmospheric microplastics can directly enter inland waters via dry atmospheric deposition. This was found in both rural and urbanised areas by Bianco and Passananti (2020) and Stanton et al (2020). Microplastic in such fallout is dominated by fibres (Cai et al., 2017). Furthermore, microplastics in the atmosphere can be washed into freshwater ecosystems along with microplastics on land via rainfall and surface runoff (Stanton et al., 2020; Xia et al., 2020).

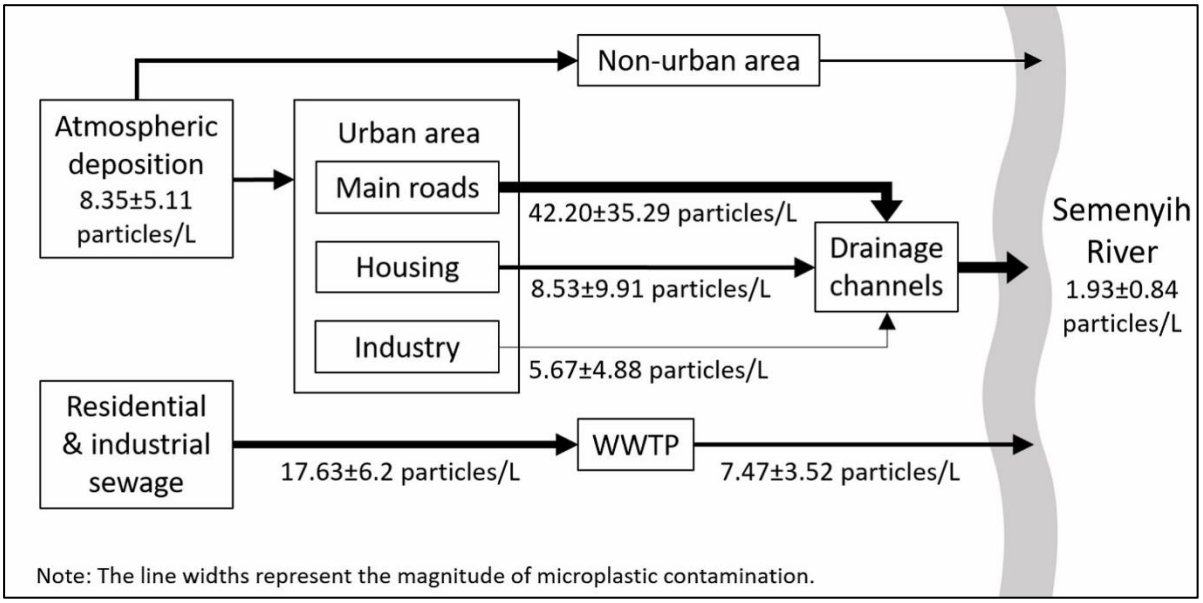


Figure 2.1 *Microplastic budget of the Semenyih River. Figure adapted from Chen et al (2022).*

Runoff from urban surfaces is an obvious source of microplastics (Figure 2.1). Chen *et al* (2022) found roads had the highest microplastic concentration compared to other sources such as industrial, residential, WWTP, and atmospheric deposition sampled in Semenyih River, Malaysia, with 36 per cent of the sampled microplastics being styrene-butadiene rubber fragments, a synthetic rubber commonly used for car tires. A study in Norway estimated daily tire tread emissions of approximately 8.1 kg per day draining a road junction that extended for approximately 0.75 km; this equates to around 3,000 kg of tread per year (Vogelsang et al., 2018). Rainfall and surface runoff aided in the deposition of microplastics in the atmosphere (Bianco and Passananti, 2020) and the remobilisation of microplastic settled on the riverbed (Chen et al., 2021a). In many developed countries, processed sewage sludge is applied as fertiliser in agricultural areas (Nizzetto et al., 2016b). However, The high concentrations of microplastic trapped in sewage sludge may be a source of microplastic in rivers after it is repurposed as biosolids for agricultural use (Leslie et al., 2017; Stanton et al., 2020).

It is clear that there are multiple sources of MP in freshwater, with these often-contributing different types of microplastics (e.g., airborne material dominated by fibres). This finding suggested that microplastic load is highly dependent on the surrounding sources and varies temporally and spatially in the freshwater ecosystem.

2.1.1 Microplastics in the lotic ecosystem

A high concentration of microplastic was reported from rivers. In a highly urbanized river in Chicago, USA, the concentrations of microplastic equalled or exceeded those

in the oceans and the Great Lakes (McCormick et al., 2014, 2016). In Asia, the average concentration of MPs has been found to be as high as 0.71 particles per litre in the urban Pearl River, China (Fan et al., 2019). Nevertheless, the fate of microplastic in running waters is complex. Spatial and temporal variation in microplastic in lotic systems is influenced by hydrological (Klein et al., 2015; Rodrigues et al., 2018) and anthropogenic factors (Chinfak et al., 2021; Dikareva and Simon, 2019).

Nizzetto *et al.*, (2016) used a modelling approach to help understand the factors influencing the transport and retention of microplastic in catchments, including terrestrial soils and riverbed sediments. They reported strong hydrological controls on microplastic dynamics, with deposition, storage, and retention of microplastic predominant in streams with low stream power. While this was a modelling study that lacked field-based validation, their general conclusions have been supported by the small number of studies that have been conducted on this topic. Besseling *et al* (2017), for example, found that sedimentation and resuspension of microplastics are governed by the river width, depth, and discharge. These channel scale variables interact to produce the hydraulic conditions (e.g., shear stress) that directly influence the entrainment and transport of bed material. For instance, the river bed will act as the temporary sink of microplastics during low flow (Stanton et al., 2020).

Due to differences in the density and shape of microplastics compared to fine sediment, they settled and are entrained under different hydraulic conditions. Pore-scale microplastics (20–50 µm) are more likely to be accumulated in the hyporheic

zone of the river (Frei et al., 2019). Larger and denser microplastics, on the other hand, are more likely to be transported back into the water column and fragmented as a result of the hyporheic exchange, even at low flow rates (Drummond et al., 2020). Nevertheless, just as fine sediment, the behaviour of microplastics (e.g. whether they are entrained or not) can be explained using hydraulic parameters such as shear stress and represented using Shield's diagrams (Waldschläger and Schüttrumpf, 2019).

River flow affects the microplastic concentrations (due to dilution of the total load) and transport versus settlement rates (Su et al., 2018). For example, a WWTP on Africa's Mvudi River increased the volume of water in the river and diluted the microplastic concentration in the river by increasing the flow of treated wastewater into the system (Dalu et al., 2021). Samples collected before and after catchment-wide flooding in the upper Mersey and Irwell catchment, United Kingdom, also showed a significant reduction in microplastics in the riverbed as the microplastics in the river were entrained and transported downstream towards the ocean (Hurley et al., 2018). This finding corresponded to the global model that shows rivers as the expressways that transport microplastics into the world's oceans (Lebreton et al., 2017). In turn, the model indicates how understanding ocean contamination requires us to understand freshwater source areas.

Now, most of the publications available on freshwater microplastic pollution were conducted in Europe, North America and China (Blettler et al., 2018), and information on freshwater microplastic pollution in other regions is still scare. In Asia, the majority of studies were conducted in China, leaving information from other Asian countries

lacking (Chen et al., 2021c; Kukkola et al., 2021). This geographical gap is concerning as environmental pollution issues are present in many Asian countries due to rapid urbanisation (Zafar et al., 2020). Moreover, Southeast Asian countries such as Malaysia, Vietnam and Thailand were now known as the main importer of global plastic wastes (Liang et al., 2021), but information related to microplastic pollution in the region remains limited (Chen *et al.*, 2021).

2.2 Microplastic pollution in Malaysia

2.2.1 Scale of the problem

Malaysia's plastic industry contributes significantly to the country's economy. In 2020, the plastic manufacturing sector in Malaysia reported an annual 2.3% rise in sales turnover to RM48.46 billion, due to the high demand for plastics goods from the essential sectors during the COVID-19 pandemic (Malaysia Plastic Manufacturer Association, 2021). However, plastic wastes in Malaysia were mainly unrecycled and landfilled (Chen, *et al.*, 2021). The rapid urbanisation, lack of environmental infrastructure and limited proper waste management in the country has resulted in notable adverse environmental and economic impacts the country (World Bank, 2021). Consequently, Malaysia is a potential hotspot of freshwater microplastic. This is borne out by a modelling study which showed 9% of the total plastic waste generated in the country was released into the global ocean annually, making the country the world's third largest emitter of plastics to the marine environment (Meijer et al., 2021).

2.2.2 Microplastics contamination in Malaysia

To date, microplastic occurrences were reported in all nineteen field studies conducted in Malaysia (manual search on Scopus with keywords: “microplastic” and “Malaysia”; Table 1). The majority of the studies were conducted on Peninsular Malaysia's East Coast, with Terengganu being the most studied state in the country (Table 1). Only five of the nineteen studies concerned freshwater environments, and only one of them looked at the abundance of microplastics in freshwater organisms.

The majority of studies conducted in the freshwater environment assessed microplastic loads in the surface water of Malaysian rivers (Table 1). The average microplastic concentration of surface water ranged from 0.0042 pieces per cubic metre (Cherating River, Terengganu; Pariatamby *et al.*, 2020) to 102.8 pieces per cubic metre (Dungun River, Terengganu; Yang Hwi, Shuaib Ibrahim and Wan Mohd Khalik, 2020). Sarijan *et al* (2018) provided the only data for MP in sediments in freshwater systems in Malaysia; it consisted of data from only a single site on each river, collected on a single sampling date (December). Average concentrations of MP in sediments (from 3 replicate samples per site) were 200 (River Skudai) and 680 (River Tebrau) particles per kg. In addition, fish were also collected from the River Skudai, where the researcher identified an average of 1.07 ± 1.76 pieces of microplastics per individual of fish sampled (Sarijan et al., 2019).

This knowledge gap is consistent with findings from other countries where freshwater microplastic pollution research is limited (Bellasi et al., 2020; Kumar et al., 2021).

However, understanding the abundance, source, and fate of microplastic in the freshwater ecosystem is critical, as microplastic is listed as one of the emerging threats to freshwater biodiversity (Reid et al., 2019). As a result, scientists and policymakers in Malaysia need a better understanding of the risks posed by microplastic in the country, and the starting point for this is to assess the loads in animals (Krause et al., 2021; Kukkola et al., 2021; Meng et al., 2020). The following sections review what is known about body loads in and the impacts of microplastic on organisms. As far as possible literature is drawn from tropical Southeast Asia, but because of the insufficient research from Malaysia, the section it is not possible to cite examples of impacts in the country.

Table 1. *Publications of microplastic occurrences in marine and freshwater environments in Malaysia (Scopus; December 2022), with mean microplastic concentration ± SD, if available.*

Ecosystem	Location	Sample	Microplastic extraction method	Mean microplastic concentration	References
Marine	Santubung Beach	Sediment	NaCl (1.2 g cm^{-3}) 30 minutes; HCl (37%) + HNO_3 (65%) 10 hours	0.0358 ± 0.062 pieces g^{-1}	Noik and Tuah (2015)
	Trombol Beach	Sediment	NaCl (1.2 g cm^{-3}) 30 minutes; HCl (37%) + HNO_3 (65%) 10 hours	1.7343 ± 2.173 pieces g^{-1}	Noik and Tuah (2015)
	Straits of Johor	Sediment	H_2O_2 (30%) 1 week; Nal (1.6 g cm^{-3}) 3 hours	4 pieces kg^{-1}	Matsuguma et al (2017)
	Kuala Nerus	Surface water	Direct filtration	0.41 ± 0.28 pieces L^{-1}	Khalik et al (2018)
	Kuantan Port	Surface water	Direct filtration	0.145 ± 0.005 pieces L^{-1}	Khalik et al (2018)
	Setiu Wetlands	Asian Sea Bass (*Lates calcarifer*)	NaOH (10M) 60°C, 21 days	1124.5 pieces $individual^{-1}$	Khalik et al (2017)
		Polychaete (*Namalycastis sp*)	NaOH (10M) 60°C	34.5 pieces $individual^{-1}$	Hamzah et al (2021)

	Surface water	NaCl	0.36 ± 0.250 pieces L^{-1}	Ibrahim et al (2021)
	Sediment	NaCl	5.97 pieces g^{-1}	Ibrahim et al (2021)
Terengganu Coasts	Surface water	Direct filtration	Offshores: 211.2 ± 104 pieces m^{-3} Estuaries: 421.8 ± 110 pieces m^{-3}	Taha et al (2021)
	Zooplankton	HNO_3 (65%) 80 °C, 30 minutes	0.104 pieces $individual^{-1}$	Taha et al (2021)
	Sediment	NaCl (1.2 g cm^{-3})	2.07 pieces m^{-3}	Hamza et al (2020)
Klang River estuary	Gastropod (*Nerita sp* and *Chicoreus sp)*	HNO_3 (69%) + H_2O_2 (30%) 50°C, 30 minutes; NaCl (1.2 g cm^{-3})	0.92 pieces g^{-1}	Zaki et al (2021a)
	Surface water	Wet peroxide oxidation 75 ℃; NaCl	2.47 ± 1.19 pieces L^{-1}	Zaki et al (2021b)

	Pangkor Island	Sea cucumber (*Stichopus horrens*)	NaOH 40 °C, 72 hours	72.3 pieces individual^{-1}	Muhammad Husin et al (2021)
	Remis Beach	Commercial marine fish	KOH (10%) 40 °C, 72 hours	9.88 pieces individual^{-1}	Jaafar et al (2021)
	Cape Penyabung	Commercial marine fish	KOH (10%) 40 °C, 72 hours	5.17 pieces individual^{-1}	Jaafar et al (2021)
	Miri coast	Sediment	H_2O_2 (30%), 24 hours; $ZnCl_2$, 24 hours	4.5 pieces g^{-1}	Alexander Tampang and Viswanathan (2022)
	Kebagu Beach	Sediment	NaCl (1.2 g cm^{-3})	131 pieces m^{-2}	Zahari et al (2022)
	ODEC Beach	Sediment	NaCl (1.2 g cm^{-3})	66 pieces m^{-2}	Zahari et al (2022)
Freshwater	Skudai River	Sediment	H_2O_2 (30%), 1 week; NaCl (1.2 g cm^{-3}), 3 minutes	200 ± 80 pieces kg^{-1}	Sarijan et al (2018)

	Fish	KOH (10%, 1:10 v/w), 2 days; NaCl (1.2 g cm^{-3}), 2 minutes	1.07 ± 1.76 pieces $individual^{-1}$	Sarijan et al (2019)
Tebrau River	Sediment	H_2O_2 (30%), 1 week; NaCl (1.2 g cm^{-3}), 3 minutes	680 ± 140 pieces kg^{-1}	Sarijan et al (2018)
Cherating River	Surface water	Alcohol (20%), overnight	0.0042 pieces m^{-3}	Pariatamby et al (2020)
Dungun River	Surface water	H_2O_2 (30%) room temperature, 24 hours	102.8 pieces m^{-3}	Yang Hwi et al (2020)
Langat River	Surface water	Wet peroxide oxidation, 75 ℃	4.39 ± 5.11 pieces L^{-1}	Chen et al (2021a)

2.3 Microplastics in freshwater organisms

2.3.1 Microplastic occurrence in freshwater organisms

Microplastic is distributed across all trophic levels in freshwater ecosystems. A high abundance of microplastic was found entangled with and absorbed by macroalgae (*Cladophora sp*) in the Laurentian Great Lakes, North America (Peller et al., 2021). Microplastic was found to reduce the photosynthetic ability of microalgae (*Chlorella sp*) in a laboratory setting (Wu et al., 2019). This implies that microplastics entered the food web via primary producers, causing adverse effects on the freshwater ecosystem from the lowest trophic level.

Microplastics have also been found in freshwater consumers such as invertebrates (Table 2) and fishes (Table 3). Due to their commercial importance, many studies have been conducted on freshwater fish species and identified microplastic in commonly consumed families such as Cyprinidae (Jabeen et al., 2017; Kasamesiri and Thaimuangpho, 2020; Sarijan et al., 2019), Cichlidae (Biginagwa et al., 2016; Sarijan et al., 2019), Bagridae (Kasamesiri et al., 2021; Kasamesiri and Thaimuangpho, 2020; Zhang et al., 2017), and Chanidae (Agustian Fareza and Sembiring, 2020).

In comparison to freshwater fish species, the microplastic loads and their effects on freshwater invertebrates were less well understood (Azevedo-Santos et al., 2021). Table 2 compiled the studies that assessed microplastic load in wild freshwater invertebrates (manual search on Scopus with keywords: “microplastic”, “freshwater”, “river”, “lake”, and “invertebrate”). Freshwater invertebrates have a higher chance of interacting with microplastic because they are typically found in the benthos or bed

sediment, which serves as the microplastic sink in a freshwater environment (Nel et al., 2018; Stanton et al., 2020). For instance, 100% microplastic occurrence was reported in wild freshwater mussels (Unionidae) from Höje river, Sweden (Berglund et al., 2019). Freshwater benthic invertebrates not only provide energy to aquatic and terrestrial vertebrates, but they also play an important role in redistributing nutrients to lower trophic level organisms such as bacteria and algae (Covich et al., 1999). As a result, it is critical to understand the abundance of microplastics in freshwater invertebrates because of how, by impacting invertebrates, microplastic may influence nutrient and energy transfer within freshwater systems.

However, it was worth noting that there is no standardised method for microplastic extraction, identification, and quantification (Caldwell et al., 2022; Lusher et al., 2020; O'Connor et al., 2020). As a result, publications included in this review have used a different method for microplastic extraction (Table 1) and identification and quantification for invertebrate (Table 2) and fish (Table 3) samples. Inconsistency in the extraction method will alter the microplastic load findings. Different extraction methods, such as the types and concentrations of solvent used, will have different extraction efficiency depending on the sample type. For instance, Hydrogen peroxide (H_2O_2) and Iron (II) Sulphate ($FeSO_4$) were found to be most efficient in digesting organic matter derived from plants, whereas Potassium Hydroxide (KOH) was most suitable for animal tissue digestion (Kühn et al., 2017; Lusher et al., 2020; Prata et al., 2019a).

Furthermore, the methods for identifying and quantifying microplastics were not standardised. Some studies only used a stereomicroscope to inspect microplastics, whereas some conducted an additional confirmation analysis on suspected microplastics using spectroscopy such as Raman and Fourier-transform infrared spectroscopy (FTIR) to identify the polymer type (Bertoli et al., 2022; McNeish et al., 2018; Parker et al., 2022a). The magnification of the stereomicroscope used affects the size range of microplastic that the studies can detect. For example, higher microplastic abundance was reported in caddisfly larvae (Trichoptera) when a higher magnification stereomicroscope (120x; Parker et al., 2022a) was used compared to 35x magnification (Windsor et al., 2019), as the higher magnification stereomicroscope has detected microplastic from a size range of 100 µm to 5000 µm, whereas the other study only detected size range from 500 µm to 5000 µm, see Table 2. Despite the fact that both studies were conducted in the United Kingdom (Table 2), the spatial and temporal variation of microplastics must be considered when comparing the microplastic load of animals across different studies. As a result, future studies must use a method that is commonly used and best suits its sample type, as well as a higher magnification for data comparison with other studies and to avoid underestimation of microplastic load.

Table 2. *Publications of microplastic load (occurrence, abundance, and concentration) in freshwater invertebrates (Scopus; December 2022), with the microplastic identification and quantification method used and the size range of microplastic, if available. Microplastic abundance and concentration were presented either as range or mean value.*

Class/Order	Suborder/ Family	MP occurrence (%)	Mean MP abundance (items/ individual)	MP concentration	MP identification and quantification	Size range (µm)	Location	Country	Reference
Amphipoda		25	0.070	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected MPs (≥ 100 µm)	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)
		33	0.110	-	Stereomicroscope (120x) and FTIR with micro-ATR on 200 suspected MPs (≥ 100 µm)	100-5000	River Bourne	United Kingdom	Parker et al (2022b)

	Gammaridae	-	0.092	-	FTIR microscope (µFT-IR)	20−5000	Dommel River	Netherlands	Pan et al (2021)
Annelida		75	0.560	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected MPs (≥ 100 µm)	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)
		67	0.560	-	Stereomicroscope (120x) and FTIR with micro-ATR on 200 suspected MPs (≥ 100 µm)	100-5000	River Bourne	United Kingdom	Parker et al (2022b)
	Naididae	-		129 ± 65.4 (SD) items/g	Stereomicroscope (20-50x) and FTIR with ATR	50-5000	River Irwell	United Kingdom	Hurley et al (2017)
Arachnida	Hydrachnidiae	-	0.057	-	Stereomicroscope (10-80x) and FTIR	10-5000	Vipacco River	Italy	Bertoli et al (2022)

					microscope (µFT-IR)				
Arhynchobdellida	Erpobdellidae	-	0.570	-	FTIR microscope (µFT-IR)	20–5000	Dommel River	Netherlands	Pan et al (2021)
Bivalvia	Cyrenidae	96	0.4-5.0	0.3/4.9 items/g	Stereomicroscope (25-80x) and FTIR microscope (µFT-IR) on 150 MPs	21-4020	Yangtze River Basin	China	Su et al (2018)
	Unionidae	71.5	1.900	21 items/g	Stereomicroscope (7x - 45X) and Raman Microscope on 60% of suspected MPs (up to 500x)	21–298	Grand River Watershed	Canada	Wardlaw and Prosser (2020)
		100	25.310	-	Stereomicroscope	-	Höje river	Sweden	Berglund et al (2019)
Coleoptera		33	0.290	-	Stereomicroscope (120x) and FTIR with micro-ATR on	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)

				98 suspected MPs (≥ 100 µm)				
Elmidae	-	0.094	-	Stereomicroscope (10-80x) and FTIR microscope (µFT-IR)	10-5000	Vipacco River	Italy	Bertoli et al (2022)
	-	0.333	-	Stereomicroscope (10-80x) and FTIR microscope (µFT-IR)	10-5000	Vipacco River	Italy	Bertoli et al (2022)
	-	0.011	-	Stereomicroscope (10-80x) and FTIR microscope (µFT-IR)	10-5000	Vipacco River	Italy	Bertoli et al (2022)
	-	0.167	-	Stereomicroscope (10-80x) and FTIR microscope (µFT-IR)	10-5000	Vipacco River	Italy	Bertoli et al (2022)

Decapoda	Astacidea	-	17.20	-	FTIR microscope (µFT-IR)	20−5000	Dommel River	Netherlands	Pan et al (2021)
Diptera		25	0.060	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected MPs (≥ 100 µm)	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)
		24	0.130	-	Stereomicroscope (120x) and FTIR with micro-ATR on 200 suspected MPs (≥ 100 µm)	100-5000	River Bourne	United Kingdom	Parker et al (2022b)
	Chironomidae	75 (Summer)/ 98 (Winter)	-	0.37 ± 0.44 (SD) items/mg (summer);1.12 ± 1.19 (SD) items/mg (winter)	Stereomicroscope (100x)	-	Bloukrans River	South Africa	Nel et al (2018)

	-	0.083	-	Stereomicroscope (10-80x) and FTIR microscope (μFT-IR)	10-5000	Vipacco River	Italy	Bertoli et al (2022)
	-	-	0 - 2.87 items/mg	Stereomicroscope (7.8-160x) and scanning electron microscope (SEM)	< 500	Wu River Basin	Taiwan	Lin et al (2021)
	-	9.60	-	FTIR microscope (μFT-IR)	20−5000	Dommel River	Netherlands	Pan et al (2021)
	100	-	56.2 particles g^{-1}	Stereomicroscope	500-5000	Braamfontein Spruit	South Africa	Dahms et al (2020)
Ephemeroptera	-	2.50	-	FTIR microscope (μFT-IR)	20−5000	Dommel River	Netherlands	Pan et al (2021)
	50	0.74	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)

				MPs (≥ 100 µm)				
	33	0.08	-	Stereomicroscope (120x) and FTIR with micro-ATR on 200 suspected MPs (≥ 100 µm)	100-5000	River Bourne	United Kingdom	Parker et al (2022b)
Baetidae	-	0-0.14	0-6 items/mg	Stereomicroscope (8-35x) and confirmation using light microscopy, bright- and dark-field spectroscopy (Olympus BX40)	500-5000	Taff catchment	United Kingdom	Windsor et al (2019)
Caenis	-	0.067	-	Stereomicroscope (10-80x) and FTIR	10-5000	Vipacco River	Italy	Bertoli et al (2022)

				microscope (µFT-IR)				
Ephemera	-	0.667	-	Stereomicroscope (10-80x) and FTIR microscope (µFT-IR)	10-5000	Vipacco River	Italy	Bertoli et al (2022)
Heptageniidae	-	0-0.14	0-6 items/mg	Stereomicroscope (8-35x) and confirmation using light microscopy, bright- and dark-field spectroscopy (Olympus BX40)	500-5000	Taff catchment	United Kingdom	Windsor et al (2019)
Potamanthus	-	0.200	-	Stereomicroscope (10-80x) and FTIR microscope (µFT-IR)	10-5000	Vipacco River	Italy	Bertoli et al (2022)

Gastropoda		67	0.290	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected MPs (≥ 100 µm)	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)
		50	0.330	-	Stereomicroscope (120x) and FTIR with micro-ATR on 200 suspected MPs (≥ 100 µm)	100-5000	River Bourne	United Kingdom	Parker et al (2022b)
	Lymnaeidae	43.3	0.200	-	Stereomicroscope (10-80x) and FTIR microscope (µFT-IR)	10-5000	Vipacco River	Italy	Bertoli et al (2022)
	Neritidae	90	0.23±0.05 (SEM)	6.10±1.05 (SEM; items/g)	Stereomicroscope and FTIR microscope (µFT-IR)	-	Rhine river	Europe	Akindele et al (2019)
	Ampullariidae	100	3.80±0.83 (SEM)	1.71±0.46 (SEM; items/g)			Osun River	West Africa	
	Thiaridae	80	1.70±0.42 (SEM)	4.57±1.07 (SEM; items/g)					

Hemiptera	Corixidae	-	1.363	-	FTIR microscope (µFT-IR)	20−5000	Dommel River	Netherlands	Pan et al (2021)
Hemiptera (Herbivorous)		20	0.120	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected MPs (≥ 100 µm)	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)
		33	0.090	-	Stereomicroscope (120x) and FTIR with micro-ATR on 200 suspected MPs (≥ 100 µm)	100-5000	River Bourne	United Kingdom	Parker et al (2022b)
Hemiptera (Predatory)		14	0.120	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected MPs (≥ 100 µm)	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)

		33	0.300	-	Stereomicroscope (120x) and FTIR with micro-ATR on 200 suspected MPs (≥ 100 µm)	100-5000	River Bourne	United Kingdom	Parker et al (2022b)
Isopoda		44	0.170	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected MPs (≥ 100 µm)	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)
		24	0.060	-	Stereomicroscope (120x) and FTIR with micro-ATR on 200 suspected MPs (≥ 100 µm)	100-5000	River Bourne	United Kingdom	Parker et al (2022b)
Malacostraca	Asellidae	-	8.700	-	FTIR microscope (µFT-IR)	20−5000	Dommel River	Netherlands	Pan et al (2021)

		-	0.016	-	Stereomicroscope (10-80x) and FTIR microscope (µFT-IR)	10-5000	Vipacco River	Italy	(Bertoli et al., 2022)
Megaloptera		25	0.250	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected MPs (≥ 100 µm)	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)
	Sialidae	-	3.667	-	FTIR microscope (µFT-IR)	20−5000	Dommel River	Netherlands	Pan et al (2021)
Odonata		36	0.240	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected MPs (≥ 100 µm)	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)
		48	0.290	-	Stereomicroscope (120x) and	100-5000	River Bourne	United Kingdom	Parker et al (2022b)

				FTIR with micro-ATR on 200 suspected MPs (≥ 100 µm)				
Calopterygidae	-	0.40	-	FTIR microscope (µFT-IR)	20−5000	Dommel River	Netherlands	Pan et al (2021)
	-	0.043	-	Stereomicroscope (10-80x) and FTIR microscope (µFT-IR)	10-5000	Vipacco River	Italy	Bertoli et al (2022)
Coenagrionidae	-	0.100	-	Stereomicroscope (10-80x) and FTIR microscope (µFT-IR)	10-5000	Vipacco River	Italy	Bertoli et al (2022)
	-	0.455	-	FTIR microscope (µFT-IR)	20−5000	Dommel River	Netherlands	Pan et al (2021)
Gomphidae	-	0.081	-	Stereomicroscope (10-80x) and FTIR	10-5000	Vipacco River	Italy	Bertoli et al (2022)

					microscope (µFT-IR)				
Plecoptera	Leuctra	12.4	0.003	-	Stereomicroscope (10-80x) and FTIR microscope (µFT-IR)	10-5000	Vipacco River	Italy	Bertoli et al (2022)
Trichoptera		57	0.620	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected MPs (≥ 100 µm)	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)
		46	0.240	-	Stereomicroscope (120x) and FTIR with micro-ATR on 200 suspected MPs (≥ 100 µm)	100-5000	River Bourne	United Kingdom	Parker et al (2022b)
	Hydropsychidae	-	0-0.14	0-6 items/mg	Stereomicroscope (8-35x) and		Taff catchment	United Kingdom	Windsor et al (2019)

					confirmation using light microscopy, bright- and dark-field spectroscopy				
		19.6	0.003	-	Stereomicroscope (10-80x) and FTIR microscope (µFT-IR)	10-5000	Vipacco River	Italy	Bertoli et al (2022)
Tubificida	Tubificidae	-	0.867	-	FTIR microscope (µFT-IR)	20–5000	Dommel River	Netherlands	Pan et al (2021)
Multiple taxa	36 taxa	2	0.025 (50 microplastics, 2010 individuals)	-	Stereomicroscope and FTIR with ATR	700 - 5000	Garonne river	France	Garcia et al (2021)

High microplastic abundances have been reported in the freshwater surface water and sediment from Thailand (Ta and Babel, 2020), Indonesia (Buwono et al., 2021; Wicaksono et al., 2021), Viet Nam (Strady et al., 2021), Philippines (Osorio et al., 2021) and Malaysia (Chen et al., 2021a; Sarijan et al., 2018; Yang Hwi et al., 2020). To date, only four journal articles from the region have reported microplastic occurrence in freshwater fishes (Agustian Fareza and Sembiring, 2020; Kasamesiri et al., 2021; Kasamesiri and Thaimuangpho, 2020; Sarijan et al., 2019) but microplastic contamination in other freshwater organisms remained unknown.

In Thailand, microplastic occurrences were reported in all fifteen common freshwater fish species studied (Kasamesiri et al., 2021; Kasamesiri and Thaimuangpho, 2020). Interestingly, the highest microplastic occurrence was reported in omnivorous fish from Chi River (Kasamesiri and Thaimuangpho, 2020), but microplastics were more commonly ingested by carnivorous fish in Ubolratana Reservoir (Kasamesiri et al., 2021).

In Malaysia, six ornamental and economically important freshwater fish species from the Skudai River, Johor, were assessed for the microplastic load (Sarijan et al., 2019). 40% of the fish's gastrointestinal tract (GIT) contained microplastic, with an average of 1.07 ± 1.76 (mean ± SD) items per fish. A significant difference was found between the abundance of microplastic ingested by different species. For instance, the highest amount of microplastic (9 items/individual) was found in the Striped Catfish

(*Pangasianodon hypophthalmus*). However, no significant difference was observed when compared across different feeding habits of the fish.

In contrast, milkfish (*Chanos chanos*) was the only freshwater species investigated in Indonesia (Agustian Fareza and Sembiring, 2020). Microplastics were found in fish sampled from all sampling locations, but the microplastic concentration in fish was not correlated with the microplastic concentration in water and sediment (Agustian Fareza and Sembiring, 2020). Nevertheless, none of these studies investigated the ecological impact of microplastic.

Table 3. *Publications of microplastic load (occurrence, abundance, and concentration) in freshwater fish (Scopus; December 2022), with the microplastic identification and quantification method used and the size range of microplastic, if available. Microplastic abundance and concentration were presented either as range or mean value.*

Family/ Genus	*Species/ Genus*	Microplastic occurrence (%)	Microplastic abundance (items/ individual)	Microplastic concentration	Microplastic Identification and Quantification	Size range	Location	Country	Reference
Ailiidae	*Laides longibarbis*	83.3	1 - 2	-	Stereomicroscope (0.67x - 5x)	30-3840	Chi River	Thailand	Kasamesiri and Thaimuangpho , (2020)
Ambassidae	*Parambassis siamensis*	100	4.11 ± 1.08	-	Stereomicroscope (0.67x - 5x)	30-4770	Ubolratana reservoir	Thailand	Kasamesiri et al (2021)
Anabantidae	*Anabas testudineus*	23.08	0.38 ± 0.87	-	Stereomicroscope (40x-45x)	100 to 5000	Skudai River	Malaysia	(Sarijan et al., 2019)
Bagridae	*Mystus mysticetus*	100	2.92 ± 1.30 (all taxa)	-	Stereomicroscope (0.67x - 5x)	30-4770	Ubolratana reservoir	Thailand	Kasamesiri et al (2021)
	Pelteobaggrus vachelli	25.7 (all taxa)	1 ± 1.41	-	Stereomicroscope (40x) and Raman microscope	300-1800	Xiangxi River	China	Zhang et al (2017)
	Pelteobagrus fulvidraco		0.33 ± 0.58	-					
	Pseudobagrus ussuriensis		1	-					

	Hemibagrus spilopterus	100	2.92 ± 1.30 (all taxa)	-	Stereomicroscope (0.67x - 5x)	30-4770	Ubolratana reservoir	Thailand	Kasamesiri et al (2021)
		70	1 to 2	-	Stereomicroscope (0.67x - 5x)	30-3840	Chi River	Thailand	Kasamesiri and Thaimuangpho (2020)
	Mystus bocourti	73.3	1 to 2	-					
Butidae	*Oxyeleotris marmorata*	100	2	-	Stereomicroscope (40x-45x)	100 to 5000	Skudai River	Malaysia	Sarijan et al (2019)
Callichthyidae	*Hoplosternum littorale*	83	3.6	-	Stereomicroscope (45x)	1000-5000	Pajeú river	Brazil	Silva-Cavalcanti et al (2017)
Catostomidae	*Carpoides cyprinus*	60	13	-	Stereomicroscope (25-50x) and FTIR on 160 fibres	1500-5000	Lake Michigan and its tributaries	USA	McNeish et al (2018)
	Catostomus commersonii	94.11764706	0.4	-					
Centrarchidae	*Lepomis macrochirus*	100	26	-	FTIR microscope (µFT-IR)	100-5000	Han river	South Korea	Park et al (2020a)
		45.3	-	-	Stereomicroscope (2.5-180x)	53-5000	Brazos River Basin	USA	Peters and Bratton (2016)
	Lepomis megalotis	44.1	-	-					
	Micropterus sp.	100	14.67	-	Stereomicroscope (25-50x) and FTIR on 160 fibres	1500-5000	Lake Michigan and its tributaries	USA	McNeish et al (2018)
		100	2	-	FTIR microscope (µFT-IR)	100-5000	Tanchon stream	South Korea	Park et al (2020b)

		100	20	-	FTIR microscope (µFT-IR)	100-5004	Han river	South Korea	Park et al (2020a)
Chanidae	*Chanos*	-	2.22 ± 3.768 (gut and gills) / 1.111 ± 1.167 (tissue)	-	Stereomicroscope and ATR-FTIR	300-500	Citarum river	Indonesia	Agustian Fareza and Sembiring (2020)
Channidae	*Channa argus*	100	38	-	FTIR microscope (µFT-IR)	100-5006	Han river	South Korea	Park et al (2020a)
Cichlidae	*Oreochromis mossambicus*	55.56	1.61 ± 1.79	-	Stereomicroscope (40x-45x)	100 to 5000	Skudai River	Malaysia	Sarijan et al (2019)
	Oreochromis niloticus	20	-	-	Stereomicroscope and ATR-FTIR	250-5000	Lake Victoria	Tanzania	Biginagwa et al (2016b)
Clariidae	*Clarias gariepinus*	19.05	0.33 ± 0.80	-	Stereomicroscope (40x-45x)	100 to 5000	Skudai River	Malaysia	Sarijan et al (2019)
Clupeidae	*Clupeichtys aesarnensis*	100	2.92 ± 1.30 (all taxa)	-	Stereomicroscope (0.67x - 5x)	30-4770	Ubolratana reservoir	Thailand	Kasamesiri et al (2021)
	Dorosoma cepedianum	-	-	-	Stereomicroscope (25-50x) and FTIR on 160 fibres	1500-5000	Lake Michigan and its tributaries	USA	McNeish et al (2018)
Cottidae	*Cottus gobio*	43	0.71	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected MPs (≥ 100 µm)	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)

Cyprinidae	*Puntioplites proctozysron*	86.7	1 - 2	-	Stereomicroscope (0.67x - 5x)	30-3840	Chi River	Thailand	Kasamesiri and Thaimuangpho (2020)
		~95	2.92 ± 1.30 (all taxa)	-	Stereomicroscope (0.67x - 5x)	30-4770	Ubolratana reservoir	Thailand	Kasamesiri et al (2021)
	Alburnus	32	0.45	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected MPs (≥ 100 µm)	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)
	Barbobymus goniontus	100	2.92 ± 1.30 (all taxa)	-	Stereomicroscope (0.67x - 5x)	30-4770	Ubolratana reservoir	Thailand	Kasamesiri et al (2021)
	Carassius auratus	95.7 (all fishes)	1.9 ± 1.0	1.7 ± 1.0 items/g	Stereomicroscope (25-80x) and FTIR microscope (µFT-IR) on some of the MPs	<200-5000	Taihu Lake	China	Jabeen et al (2017)
		100	14	-	FTIR microscope (µFT-IR)	100-5000	Tanchon stream	South Korea	Park et al (2020b)
	Culter alburnus	25.7 (All fishes)	1.5 ± 1.38	-	Stereomicroscope (40x) and Raman microscope	300-1800	Xiangxi River	China	Zhang et al (2017)
	Culter dabryi	25.7 (All fishes)	0.5 ± 0.71	-					
	Cyclocheilichthys apogon	50	0.50 ± 0.71	-	Stereomicroscope (40x-45x)	100 to 5000	Skudai River	Malaysia	Sarijan et al (2019)

Cyclocheilichthys repasson	70.4	1 to 2	-	Stereomicroscope (0.67x - 5x)	30-3840	Chi River	Thailand	Kasamesiri and Thaimuangpho (2020)
	~90	2.92 ± 1.30 (all taxa)	-	Stereomicroscope (0.67x - 5x)	30-4770	Ubolratana reservoir	Thailand	Kasamesiri et al (2021)
Cyprinus carpio	95.7 (all taxa)	2.5 ± 1.3	0.5 ± 0.3 items/g	Stereomicroscope (25-80x) and FTIR microscope (µFT-IR) on some of the MPs	<200-5000	Taihu Lake	China	Jabeen et al (2017)
	-	32.0 ± 12.8	-	FTIR microscope (µFT-IR)	100-5000	Tanchon stream	Sout h Korea	Park et al (2020b)
	100	56	-	FTIR microscope (µFT-IR)	100-5001	Han river	South Korea	Park et al (2020a)
Carassius cuvieri	100	5	-	FTIR microscope (µFT-IR)	100-5002	Han river	South Korea	Park et al (2020a)
Gobio	12	-	-	Stereomicroscope	-	French rivers	France	Sanchez et al (2014)
Gymnocypris przewalskii	N/A	5.4 ± 3.6	-	Stereomicroscope (40x)	333-5000	Qinghai Lake	China	Xiong et al (2018)
Hemiculter bleekeri	95.7 (all fishes)	2.1 ± 1.1	1.1 ± 0.5items/g	Stereomicroscope (25-80x) and FTIR microscope (µFT-IR) on some of the MPs	<200-5000	Taihu Lake	China	Jabeen et al (2017)
Henicorhynchus siamensis	71.4	1 to 2	-	Stereomicroscope (0.67x - 5x)	30-3840	Chi River	Thailand	Kasamesiri and Thaimuangpho (2020)

	~95	2.92 ± 1.30 (all taxa)	-	Stereomicroscope (0.67x - 5x)	30-4770	Ubolratana reservoir	Thailand	Kasamesiri et al (2021)
Hypophthalmichthys molitrix	95.7 (all fishes)	3.8 ± 2.0	2.1 ± 1.1 items/g	Stereomicroscope (25-80x) and FTIR microscope (µFT-IR) on some of the MPs	<200-5000	Taihu Lake	China	Jabeen et al (2017)
Labeo chrysophekadion	75	1 to 2	-	Stereomicroscope (0.67x - 5x)	30-3840	Chi River	Thailand	Kasamesiri and Thaimuangpho (2020)
Labiobarbus leptocheilus	~80%	2.92 ± 1.30 (all taxa)	-	Stereomicroscope (0.67x - 5x)	30-4770	Ubolratana reservoir	Thailand	Kasamesiri et al (2021)
Labiobarbus siamensis	50	1 to 2	-	Stereomicroscope (0.67x - 5x)	30-3840	Chi River	Thailand	Kasamesiri and Thaimuangpho (2020)
Leuciscus	38	0.57	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected MPs (≥ 100 µm)	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)
Megalobrama amblycephala	95.7 (all fishes)	1.8 ± 1.7	0.2 ± 0.1items/g	Stereomicroscope (25-80x) and FTIR microscope (µFT-IR) on some of the MPs	<200-5000	Taihu Lake	China	Jabeen et al (2017)
Mystacoleucus marginatus	100	2.92 ± 1.30 (all taxa)	-	Stereomicroscope (0.67x - 5x)	30-4770	Ubolratana reservoir	Thailand	Kasamesiri et al (2021)

Notropis stramineus	100	1.588	-	Stereomicroscope (25-50x) and FTIR on 160 fibres	1500-5000	Lake Michigan and its tributaries	USA	McNeish et al (2018)
Osteochilus vittatus	100	2.92 ± 1.30 (all taxa)	-	Stereomicroscope (0.67x - 5x)	30-4770	Ubolratana reservoir	Thailand	Kasamesiri et al (2021)
Paralaubuca harmandi	100	2.92 ± 1.30 (all taxa)	-	Stereomicroscope (0.67x - 5x)	30-4770	Ubolratana reservoir	Thailand	Kasamesiri et al (2021)
Phoxinus	47	0.76	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected MPs (≥ 100 µm)	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)
	48	0.86	-	Stereomicroscope (120x) and FTIR with micro-ATR on 200 suspected MPs (≥ 100 µm)	100-5000	River Bourne	United Kingdom	Parker et al (2022b)
Pimephales promelas	-	0.46	-	Stereomicroscope (25-50x) and FTIR on 160 fibres	1500-5000	Lake Michigan and its tributaries	USA	McNeish et al (2018)
Pseudorasbora parva	95.7 (all fishes)	2.5 ± 1.8	5.6 ± 3.9 items/g	Stereomicroscope (25-80x) and FTIR microscope (µFT-IR) on some of the MPs	<200-5000	Taihu Lake	China	Jabeen et al (2017)

Pseudogobio esocinus	100	3	-	FTIR microscope (µFT-IR)	100-5000	Tanchon stream	South Korea	Park et al (2020b)
Rasbora aurotaenia	100	2.92 ± 1.30 (all taxa)	-	Stereomicroscope (0.67x - 5x)	30-4770	Ubolratana reservoir	Thailand	Kasamesiri et al (2021)
Rutilus R	35	0.57	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected MPs (≥ 100 µm)	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)
	51	0.89	-	Stereomicroscope (120x) and FTIR with micro-ATR on 200 suspected MPs (≥ 100 µm)	100-5000	River Bourne	United Kingdom	Parker et al (2022b)
	32.8	0.69±1.25	-	binocular microscope and Raman Spectroscopy on 50% of MPs	-	River Thames	United Kingdom	Horton et al (2018)
Squalius cephalus	38	0.69	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected MPs (≥ 100 µm)	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)
	42	0.63	-	Stereomicroscope (120x) and FTIR with micro-ATR on 200	100-5000	River Bourne	United Kingdom	Parker et al (2022b)

					suspected MPs (≥ 100 µm)				
	Zacco platypus	-	2.9 ± 2.2	-	FTIR microscope (µFT-IR)	100-5000	Tanchon stream	South Korea	Park et al (2020b)
Freshwater fishes	*Multiple taxa (44 species and 12 families)*	8.2	-	-	Stereomicroscope and ATR-FTIR on 32 MPs	-	Various rivers in Texas	USA	Phillips and Bonner (2015)
Fundulidae	*Fundulus diaphanus*	100	2.5	-	Stereomicroscope (25-50x) and FTIR on 160 fibers	1500-5000	Lake Michigan and its tributaries	USA	McNeish et al (2018)
Gasterosteidae	*Gasterosteus aculeatus*	41	0.56	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected MPs (≥ 100 µm)	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)
		63	1.46	-	Stereomicroscope (120x) and FTIR with micro-ATR on 200 suspected MPs (≥ 100 µm)	100-5000	River Bourne	United Kingdom	Parker et al (2022b)
Gobiidae	*Neogobius melanostomus*	100	3.81	-	Stereomicroscope (25-50x) and FTIR on 160 fibers	1500-5000	Lake Michigan and its tributaries	USA	McNeish et al (2018)

Latidae	*Lates niloticus*	20	-	-	Stereomicroscope (25-50x) and FTIR on 160 fibers	250-5000	Lake Victoria	Tanzania	Biginagwa et al (2016b)
Leuciscidae	*Cyprinella spiloptera*	100	5	-	Stereomicroscope (25-50x) and FTIR on 160 fibers	1500-5000	Lake Michigan and its tributaries	USA	McNeish et al (2018)
	Notropis atherinoides	100	6.5	-	Stereomicroscope (25-50x) and FTIR on 160 fibers	1500-5000	Lake Michigan and its tributaries	USA	McNeish et al (2018)
	Notropis hudsonius	100	7.75	-	Stereomicroscope (25-50x) and FTIR on 160 fibers	1500-5000	Lake Michigan and its tributaries	USA	McNeish et al (2018)
Nemacheilidae	*Barbatula*	47	0.89	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected MPs (≥ 100 µm)	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)
		69	1.19	-	Stereomicroscope (120x) and FTIR with micro-ATR on 200 suspected MPs (≥ 100 µm)	100-5000	River Bourne	United Kingdom	Parker et al (2022b)

Osmeridae	*Osmerus eperlanus*	20	0.2 ± 0.42	-	Stereomicroscope and ATR-FTIR	-	Thames Estuary:	United Kingdom	McGoran et al (2017)
Pangasiidae	*Pangasius hypophthalmus*	100	4.00 ± 3.16	-	Stereomicroscope (40x-45x)	100 - 5000	Skudai River	Malaysia	Sarijan et al (2019)
Percidae	*Perca fluviatilis*	29	0.35	-	Stereomicroscope (120x) and FTIR with micro-ATR on 98 suspected MPs (≥ 100 µm)	100-5000	Dorset Stour	United Kingdom	Parker et al (2022a)
Pleuronectidae	*Platichthys flesus*	75	0.33 ± 0.49	-	Stereomicroscope and ATR-FTIR	-	Thames Estuary:	United Kingdom	McGoran et al (2017)
Pristolepididae	*Pristolepis fasciatus*	100	2.92 ± 1.30 (all taxa)	-	Stereomicroscope (0.67x - 5x)	30-4770	Ubolratana reservoir	Thailand	Kasamesiri et al (2021)
Siluridae	*Silurus asotus*	100	37	-	FTIR microscope (µFT-IR)	100-5005	Han river	South Korea	Park et al (2020a)

2.3.2 Ecological impacts

Individual-level impacts

Many studies have documented the uptake and ecological effects of microplastic ingestion. At the lower trophic levels, grazers may accidentally ingest microplastic when feeding on biofilm (Rummel et al., 2017). For instance, the freshwater grazer, Common bladder snail (*Physa fontinalis*), has shown a significant decrease in growth and reproduction after being exposed to, and grazed on biofilm formed on microplastic (Michler-Kozma et al., 2021). Similarly, microplastic has hindered the growth and reproduction of another grazer crustacean, *Hyalella azteca*, even at the lowest microplastic concentration exposure (5000 microplastics mL^{-1}) condition set by the authors (Au et al., 2015). Nevertheless, the microplastic concentration set is still high and unrealistic for most environment. Microplastic not only slowed the growth rate of freshwater invertebrates, but it also delayed imago emergence of chironomid larvae (*Chironomus riparius*) (Silva et al., 2019). However, this alteration in growth speed does not apply to all freshwater organisms. Redondo-Hasselerharm et al (2018) only observed a significant reduction in the growth rate of freshwater shrimps, *Gammarus pulex,* but no difference was found for the other five freshwater benthic invertebrate species after being exposed to high microplastic concentration (40% plastic weight in the total sediment mixture).

Other than influencing the life cycle of freshwater organisms, microplastic was also found to reduce locomotor activity, making individuals more vulnerable to predators. A meta-analysis found that when freshwater organisms were exposed to environmentally relevant concentrations (≤ 1 mg L^{-1}), their average speed and moved distance decreased by 5% and 8%, respectively when compared to controls (Sun et al., 2021). This corresponded to the earlier finding that showed microplastic significantly reduced activity of the acetylcholinesterase that was responsible for voluntary muscle movement in Red tilapia (*Oreochromis niloticus*) (Ding et al., 2018). Furthermore, low microplastic concentrations can affect the morphology of freshwater invertebrates, as deformities in the mandibles and mentums of chironomid (*Chironomus sp*) larvae were observed after they were exposed to low microplastic concentrations (Stanković et al., 2020).

It is important to note that microplastic has a negative impact on freshwater organisms not only through ingestion but also through interactions with the organisms. Many freshwater organisms were found to interact with microplastic in the natural waters. For example, wild caddisfly larvae (*Lepidostoma basale*) were reported to build their cases using microplastics and natural substrates from the riverbed (Ehlers et al., 2019). Furthermore, a laboratory experiment confirmed the finding by showing that all caddisfly larvae (*Odontocerum albicorne*) in the experiment used microplastic to rebuild their case rather than only natural building materials (Gallitelli et al., 2021). Similarly, burrowing mayflies (*Ephemera danica)* preferred to burrow in plastic substrates more than natural substrates due to the lightweight of plastic (Gallitelli et

al., 2021). These interactions between the synthetic material and macroinvertebrates were discovered to have a negative impact on the animal. For example, the mortality of caddisfly larvae (*Hydropsyche pellucidula*) after exposure to microplastic was a result of chemical risk and abrasion effect, but not microplastic ingestion (Piccardo et al., 2021).

Community-level impacts

Over the long term, microplastic can impose adverse ecological impacts on the community level. For example, microplastic sampled from an urbanised river in Jiaxing, China has changed the microbial community of its biofilm by selectively increasing the antibiotic-resistant genes of bacterial colonised on it (Wang et al., 2020). Significant colonisation of oligochaete worms (*Naididae sp)* in the presence of environmental-related microplastic concentration (5% plastics per sediment dry weight), has been found to cause a decrease in the Shannon diversity index of benthic communities after 15 months of exposure (Redondo-Hasselerharm et al., 2020). In contrast, 8 days of exposure of deposit feeders (Chironomidae) and grazers (Baetidae and Ephemerellidae) to baseline microplastic concentration only reduced the community abundance, but not the community diversity (Silva et al., 2022). This disparity in findings suggested that the community-level impacts of microplastics vary over time, and the time frame set for mesocosms must be chosen carefully.

Evidence of microplastic's ecological impact on the community level suggests that it may impact ecosystem functioning. A lower nitrogen removal rate was found after

chironomid larvae (*Chironomus sp*) were exposed to microplastic for 28 days (Huang et al., 2021). Furthermore, nanoplastics were also reported to inhibit the decomposition of leaf litter in the freshwater environment by altering microbial metabolic activity and the community structure of fungi (Du et al., 2022). In contrast, due to a decrease in the abundance of grazers in the system, microplastic exposure did not reduce primary production or leaf litter decomposition in an 8-day mesocosms experiment (Silva et al., 2022).

As with the work of Silva et al (2022) most studies of the ecotoxicity of microplastics on freshwater invertebrates have been conducted in laboratory settings, whereas studies on fish have mostly been conducted in the field (Azevedo-Santos et al., 2021). The results of laboratory experiments can be misleading if the microplastic concentrations and characteristics used do not accurately reflect microplastic pollution in the field (Burns and Boxall, 2018; Karami, 2017). Field studies that reported the microplastic abundance and patterns in a range of freshwater organisms from the field were still lacking (Meng et al., 2020) and very few have looked into the ecological impact of microplastic in the field (Stanković et al., 2021).

2.4 Factors influencing the microplastic loads in the freshwater organism

2.4.1 Biotic factors

Feeding mode is one of the biotic factors influencing the number of microplastic ingested. Filter feeders ingested significantly more microplastic because their position in the benthic zone and non-selective feeding habits caused them to ingest

microplastic along with suspended sediment (Setälä et al., 2016; Su et al., 2018). Similarly, a higher abundance of microplastics was observed in the faeces of filter-feeding freshwater birds, Cape Shovelers *(Spatula smithii),* compared to the grazer, Egyptian goose (*Alopochen aegyptiaca*) (Reynolds and Ryan, 2018).

Bertoli et al (2022) found that collector-gatherers contained a significantly higher number of microplastics when compared to freshwater macroinvertebrates from different functional feeding guilds. According to the authors, this is because collector-gatherers ingest microplastics that are attached to algae or embedded in the sediment at random. A similar finding was reported in freshwater fish, where a large amount of sediment and microplastic was found in benthic feeder fish (*Platichthys flesus*) when compared to a pelagic predator (*Osmerus eperlanus*) sampled from the River Thames in the United Kingdom (McGoran et al., 2017). However, it is notable that no clear relationship between microplastic abundance and functional feeding groups of macroinvertebrates was identified by Windsor *et al* (2019) so questions remain about this issue.

Many field studies on microplastic ingestion in freshwater organisms have included data on the morphology of the microplastics found. The type of microplastic ingested by freshwater organisms was discovered to be taxon-specific, with different taxa ingesting different types of microplastic (Pan et al., 2021). For instance, microfibres were the most commonly ingested microplastic shape in freshwater fish (Galafassi et al., 2021; Yan et al., 2021). Some authors have suggested the size of microplastic was

more important than shape as a factor influencing ingestion (Lehtiniemi et al., 2018; Li et al., 2019). Nevertheless, the type of microplastic ingested by freshwater organisms might simply reflect its prevalence in the surrounding environment; e.g. the relative uptake of microplastic types has been found to be similar to the microplastic found in sediment (Su et al., 2018) and surface water (Parker et al., 2022a).

Several authors have looked at body loads of microplastic as a function of body size. For example, the amount of microplastic ingested was positively correlated with the biomass of freshwater fish (McNeish et al., 2018) and invertebrates (Berglund et al., 2019; Windsor et al., 2019). According to a review of published data, the size ratio of an animal's body length to the largest microplastic it may ingest is around 20:1 (Jâms et al., 2020). However, some studies found no significant relationship between body loads and size (Parker et al., 2022a).

2.4.2 *Abiotic factors*

Microplastic abundance in freshwater organisms varies spatially in ways connected to the distribution of sources. The microplastic concentration in Zebra mussels (*Dreissena polymorpha*) was found to increase in proximity to the WWTP in Lake Iseo, Italy (Pastorino et al., 2021). Similar findings were also observed in freshwater fish sampled from Tanchon Stream, South Korea (Park et al., 2020b). Other than that, the degree of industrialisation and urbanisation also positively correlated with the microplastic concentration found in freshwater organisms such as Armored catfish (*Hoplosternum littorale*) in River Pajeú, Brazil (Silva-Cavalcanti et al., 2017), Nile tilapia (*Oreochromis*

niloticus) and Mud carp (*Cirrhinus molitorella*) from Guangdong, China (Sun et al., 2021).

Many studies have documented the effect of hydrological conditions on microplastic abundances in freshwater sediment and surface water (Chen et al., 2021a; Rodrigues et al., 2018). Similarly, microplastic abundance in freshwater organisms was influenced by the microplastic concentration in the surrounding (Yan et al., 2021). In Braamfontein Spruit, Africa, higher microplastic abundances were observed in the sediment and larvae of *Chrironomus sp* at upstream of a weir compared to downstream (Dahms et al., 2020). Likewise, Windsor et al. (2019) also reported a negative correlation between the microplastic abundance in freshwater macroinvertebrates and river discharge.

Due to observed relationships, several workers have suggested that freshwater species that are widely distributed and have non-selective feeding habits could be used as bioindicators of microplastic. The Asian clam (*Corbicula fluminea*) has been proposed as a potential bioindicator because this bivalve is widely distributed in the region, and the abundance, size distribution, and colour of microplastic ingested by the species are similar to that found in sediment (Su et al., 2018). Similarly, because of their dominance in polluted environments, chironomid larvae (*Thienemannimyia spp*., *Chironomus spp*., and *Orthocladius spp.*) have been proposed as bioindicators for microplastic pollution in sediment (Lin et al., 2021). Others have proposed using the cases of caddisfly larvae as a bioindicator for freshwater microplastic pollution (Ehlers

et al., 2019). However, some challenges remain, as for example no correlation was discovered between microplastic loadings in sediment, water, and freshwater organisms (macroinvertebrates and fish) collected from the Bourne Stream in Southwest England (Parker et al., 2022a). More research is needed to compare the correlation between microplastic abundance found in aquatic organisms and the level of microplastic pollution in the environment, both to better understand the risk to organisms of environmental loads and assess the utility of using organisms as indicators.

2.5 Summary

When compared to the marine environment, microplastic pollution in freshwater still remains relatively under-researched. More studies have recently begun to report on the occurrence, abundance, and fate of microplastics in freshwater environments. However, there is a geographical bias in freshwater microplastic pollution studies; few studies have been conducted to assess microplastic pollution in rapidly urbanising Southeast Asia and Africa, regions that have been identified as potential hotspots for microplastic due to poor waste management and legislation governing water pollution. These factors suggest that organism microplastic loads in Southeast Asian countries such as Malaysia might be high, but the evidence is so far completely lacking.

Microplastic abundance in freshwater organisms, like microplastic abundance in sediment and the water column, is influenced by a variety of biotic and abiotic factors, but more research is needed to fully understand the mechanisms underlying spatial

and temporal variation. Moreover, higher magnification methods are recommended to prevent the underestimation of microplastic loads. Questions remain about the relations between organism microplastic loads and (i) environmental contamination levels, (ii) trophic guild or feeding mode, and (iii) body size. Future studies are recommended to:

- **Investigate and report on the microplastic concentration of freshwater organisms in the field, particularly in rapidly urbanising regions such as Southeast Asia.** Furthermore, it is suggested to adopt a commonly used method for sampling and lab processing for effective comparison of findings across published data. These findings will help scientists and policymakers understand the impact of microplastics on freshwater ecosystems.
- **Investigate the ecological factors influencing microplastic load in freshwater organisms**. This includes understanding how factors such as feeding preference, habitats, and body size alter the microplastic ingestion of aquatic animals.
- **Examine the relationship between microplastic in animals and the river (water and sediment).** The primary goal is to gain a better understanding of the mechanisms underlying the correlation (if any) and the hotspot of microplastic in the river.
- **Investigate the impact of microplastics on freshwater organisms at the individual and community levels.** Future study is recommended to use more environmentally relevant microplastic concentrations, sizes and types when conducting ecological risk assessments in a laboratory setting.

MATERIALS AND METHODS

3. Materials and methods

3.1 Study area and sampling sites

Semenyih River is one of the main tributaries of the Langat Basin in Selangor, Malaysia. With a total catchment area of 266.60 km^2, the river catchment receives approximately 3000 mm of rainfall annually (Al-Badaii et al., 2013). The Semenyih River has its source approximately 4 km upstream from Semenyih Dam, then flows southwest across Semenyih town and Bangi town before reaching its confluence with the Langat River. The Langat Basin overall, because of Semenyih and Langat Dams, is an important water catchment area for Selangor and Kuala Lumpur. These dams provide 30% of portable water supplies to Selangor and Kuala Lumpur and, via agricultural and industrial use, support the livelihood of the river basin's population of 1,499,079 people (Selangor Water Management Authority, 2015).

The Semenyih River was chosen due to its strong transition from rural to urban areas (Figure 3.1). Furthermore, existing studies in the river have looked at the microplastic concentration in river water (Chen et al., 2021a), its source (Chen et al., 2022), and how hydrological controls the fate of microplastic in the Semenyih River (Chen et al., 2021a). Furthermore, Chen et al (2021a) also discovered a significant spatial variation in microplastic concentration in the river from upstream to downstream. As a result, spatial gradients may be visible in animals as well. Hence, the Semenyih River is an ideal location for assessing microplastic contamination and loads in organisms as the concentration in the river water and sediment was already known.

A total of eight sampling sites were established along the Semenyih River (see Appendix 1 for coordinates). Figure 3.1 show the land use and land cover map of Semenyih, surrounding the sampling sites. As the town is undergoing rapid urbanisation (Jesmin Haque and Roslan, 2017), the sampling sites spread along the river represented a transition of the river from forested upstream sites to urbanised downstream areas. Site 1 is located upstream of the Semenyih Dam, whereas other sites are located downstream of the dam with increased prevalence in built-up areas (Figure 3.1).

The goal was to study a wide range of taxonomic and functional feeding groups collected from the river. However, pilot studies failed to detect any mussels. As an important filter feeder, we therefore included mussels by collecting animals (Unionidae: *Sinanodonta woodiana*) from Semenyih lake. The Semenyih Lake is a eutrophic man-made lake with a surface area of 0.06 km^2 and with a maximum water depth of 2m. The lake is commonly used for fishing and recreational activities. It is a suitable location for mussels sampling as an existing study reported a high density of *Sinanodonta woodiana* from the lake (Zieritz et al., 2019).

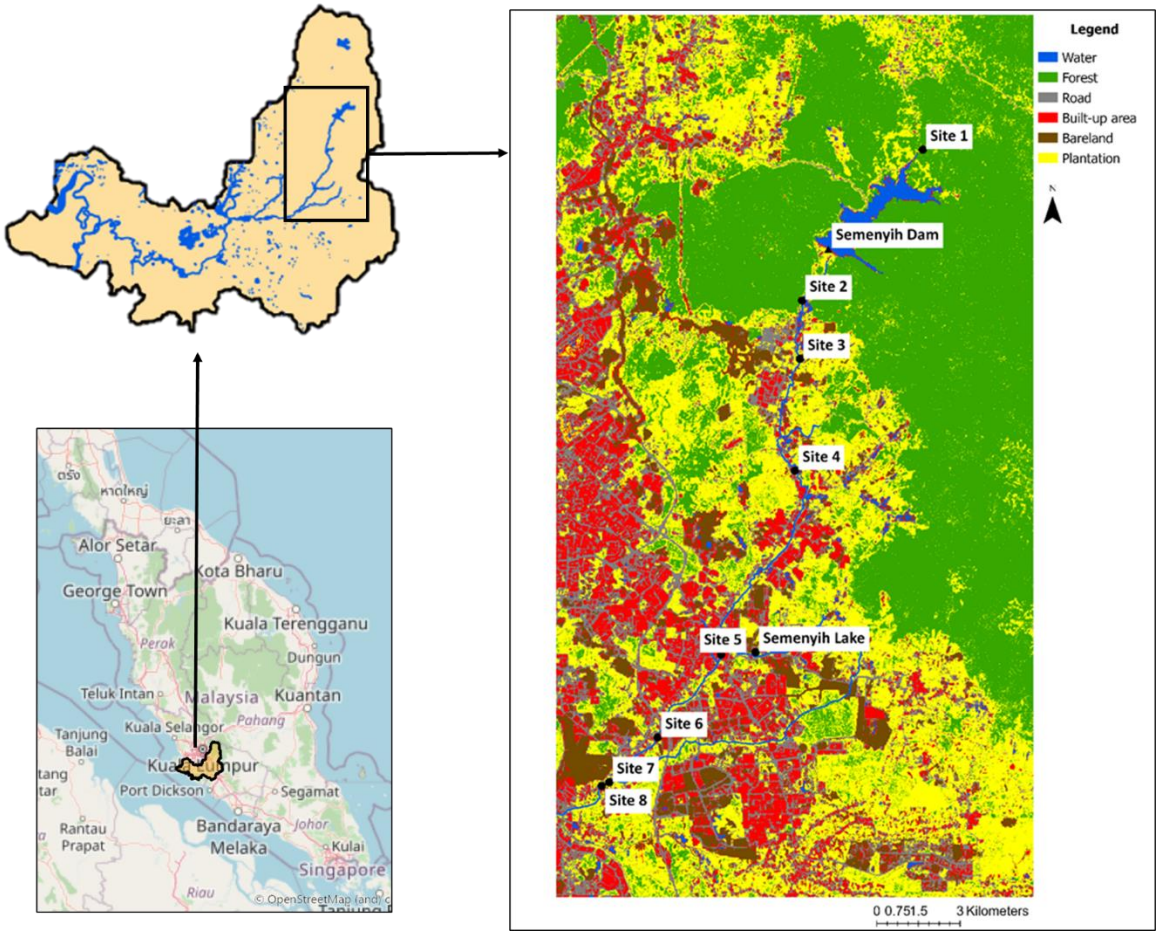


Figure 3. 1. *Land use map of the Semenyih River study area. Data from Sentinel-2, from January 2021 to January 2022. The labels show the locations of the sampling sites. Orange polygon showed the location of the Langat Basin.*

3.2 Sample collection

3.2.1 Overview

To address the objectives, simultaneous water and bed samples were collected from each site, along with samples of benthic invertebrates. Five bed and water samples were collected from each site. These were paired (i.e., bed and water samples from

each of the five locations), whereas invertebrates were collected simply by kick sampling across the site. The objective of the invertebrate sampling was simply to collect sufficient animals of each taxon to allow meaningful assessment of respective body loads and compare loads between sites.

3.2.2 *Water and sediment samples*

Forty water and forty sediment samples were collected from the river, five from each of the eight sites. For each site, five sampling points were selected to cover the range of morphological habitats present (riffle and pools); the five were spread proportionally between the habitats to reflect their relative areas at respective sites. At each sampling point, 2L of water samples were collected from the middle of the water column using a glass bottle and sieved through a pre-rinsed 53-micron stainless steel sieve. For each site, five sets of water samples (10L) were collected. Then, residues retained on the sieve were rinsed into a glass bottle using a squeeze bottle filled with ultrapure water (PURELAB Chorus 1, ELGA). 1L of the water sample was used as the blank for the relevant sediment samples.

For each of the same points as the water sample, a sample of microplastics deposited on the bed surface was collected using the sediment resuspension technique. This sample was collected immediately after the water sample was collected. The resuspension technique was designed and is commonly used to quantify the amount of fine sediment deposited on river beds (Lambert and Walling, 1988), and recently used to assess the amount of microplastic on the riverbed (Hurley et al., 2018). Note

that this is not a volumetric method (I.e., it does not quantify the amount per volume of bed sediment) but rather the amount deposited per unit bed area. The resuspension technique was chosen due to its ability to sample microplastic deposited on the surface of the riverbed, rather than including the subsurface zone. Grab samplers (such as hand spades and spoons) are good for quantifying coarser sediment but have the problem that, when used in wetted areas, very fine material, including microplastic, may be released immediately and lost. Sediment corers (such as corers and Van Veen grab samplers) are commonly used for sediment sampling for microplastic studies, but these are more useful for evaluating subsurface storage of fine sediment or microplastic (Razeghi et al., 2021). As the animals that were collected were from only the surface zone, it was deemed more appropriate to use techniques that quantified microplastic in this zone. focused on sediment sampling littoral zone or deeper in the riverbed.

The resuspension technique involves using a large open cylinder to isolate a target patch of bed. In the present case, an open-ended slightly graded cylinder (upper radius 21.5 cm, lower (base) radius 19 cm, and height 65 cm, see Figure 3.2) was used. The cone was modified by attaching a layer of foam to the bottom to ensure the formation of a tight seal when the base was pressed onto the riverbed. The foam was brightly coloured so that any fragments in the sample could be easily identified and discarded. Once the cylinder was pressed to the bed, the depth of the water column inside was measured. Then, a wooden stick was used to agitate the water and the bed surface inside. Note that the bed was not 'dug up', but rather simply disturbed so as to resuspend material. Once fully agitated, a 1L sample of the turbid water was collected

using a rinsed glass bottle. This water sample was then sieved through a pre-rinsed 53-micron stainless steel sieve, and the residue remaining on the sieve was transferred into a glass bottle using ultrapure water. To avoid microplastic contamination, microplastic identified in the blank sample (1L of water sample) was deducted from the microplastic load of sediment samples. The microplastic load in a replicate was identified as absent if a higher amount of microplastics were identified in its blank replicate compared to its suspended sediment replicate. For quality assurance, an experiment was conducted (see section 3.5) on the sampling cone, to ensure the sampling apparatus is sealed and did not introduce additional microplastic to the sample as contamination.

Figure 3.2. *Open-ended truncated cone used for sediment sampling.*

3.2.3 Biological samples

No recent study has reported the species composition in the Semenyih River; the only work is by Yap et al (2003). To develop a better understanding of the available taxa in the river, as well as likely concentrations in animals, a pilot study was conducted in September 2021. Samples were collected from the Semenyih River and Semenyih Lake. 1567 invertebrates belonging to numerous taxonomic groups were identified (Appendix 2). In the river, the five commonly found invertebrate families representing different functional feeding groups and trophic levels were selected for the main study, namely Hydropsychidae, Chironomidae, Odonata, Baetidae and Simuliidae. No

mussels were found, so to include a filter-feeding Unionidae in the study, mussels (*S. woodiana*) were collected from the lake.

Macroinvertebrates were collected in the river via kick sampling using a bottom kick net and stored in 70% ethanol (R&M Chemical, Selangor, Malaysia). In the lab, macroinvertebrates were identified and categorised according to their sampling location and family grouping. Twenty individuals of each taxon were randomly selected for further processing (i.e., to determine microplastic loads). If a taxon contained less than 20 individuals, all organisms were processed (see Table 5 for details of final sample sizes).

A total of 24 mussels were hand-picked from Semenyih Lake on one sampling occasion. Note: the mussels are not included in any analysis of relations between body loads and microplastic in the water or bed of the river, but simply as an example of a large, filter-feeding invertebrate.

Fish samples were collected opportunistically from local anglers who fish along the Semenyih river. In total, 19 fish were obtained in this way. They include 8 families that are commonly consumed by the locals and sold locally (Appendix 6). Upon collection, both mussel and fish samples were frozen at -50^{o}C until further laboratory processing. All methods were carried out in compliance with ethical guidelines and regulations

and approved by the Animal Welfare and Ethical Review Body of the University of Nottingham.

3.3 **Laboratory analysis**

3.3.1 *Overview*

The following text details the microplastic extraction and enumeration methods. Two enumeration methods were used, one that followed the approach commonly used to assess loads in the bodies of aquatic organisms (i.e., larger microplastics) and the other which allowed smaller materials down into the nano size range to be counted. The overarching goal here was to understand the ratio between the larger and smaller fragments, to assess the extent to which studies that only include the former may be underestimating true (total) body loads. Macroinvertebrates were also measured to assess the relations between body mass and microplastic loads.

3.3.2 *Microplastic extraction*

In the lab, organic material in the water and sediment samples were digested by wet peroxide oxidation (WPO) using Fenton's reagent (20mL 30% H_2O_2 and 20mL $FeSO_4$) following the protocol from Masura et al (2015). WPO was chosen due to its relatively short reaction time and high digestion efficiency compared to other digestion methods (Prata et al., 2019a). After this step, microplastic in digestates with residues remaining (such as sediments or undigested organic matter) was extracted by density separation.

Samples were density separated twice using NaCl (ρ = 1.2g/cm^3) followed by $ZnCl_2$ (ρ = 1.7-1.8g/cm^3) in a centrifuge (9500 RPM, 15^{o}C, 10 minutes). The combination of the lower-density salt solution with a higher-density salt solution helps to isolate microplastics of different densities from the digestate. Next, the supernatant from the previous step was collected and vacuumed through a glass microfiber filter (Whatman GF/C 47 mm diameter, GE Healthcare Whatman). The filter was then transferred into the oven and dried at 70^{o}C overnight.

For macroinvertebrates, the body length (mm) of each animal was measured using a camera-attached stereomicroscope (Leica EZ4D, Leica, Germany) along with the LAS EZ V.3.4.0 software. Body length was measured from the pronotum to the tip of the abdomen. The dry weight (mg) of each insect larva was estimated according to the relevant body length-dry weight regression equation summarised by Cummins et al (2022), see Table 4. Then, each larvae was transferred to a test tube separately and processed individually. Each larva was homogenised using a glass rod to better digest the soft tissue under the chitin layer. Following, 20mL of 10% KOH (R&M Chemical, Selangor, Malaysia) was added to each homogenised sample. KOH is commonly used to digest biota samples for microplastic-related studies with high efficiency (Dehaut et al., 2016; Prata et al., 2019a). In addition, KOH does not cause foaming during tissue digestion, unlike H_2O_2 and Fenton reagent (Avio et al., 2015). The test tubes were then covered with aluminium foil and placed in an incubator shaker for 48 hours for effective digestion of tissues (50 ^{o}C, 120 RPM, WiseCube WIS-20).

Table 4. *The equation used to estimate dry body weight (mg) of macroinvertebrates, modified from Cummins et al (2022). $Y = aX^b$ where Y = dry biomass in mg; X = total body length in mm; a = intercept of Y on X; and b = slope of Y on X.*

Family	**a**	**b**	**Equation ($Y = aX^b$)**
Baetidae	0.0057	2.966	0.0057*(X^2.966)
Hydropsychidae	0.0038	3.61	0.0038*(X^3.61)
Odonata (dragonfly)	0.0086	2.821	0.0086*(X^2.821)
Simuliidae	0.0027	3.084	0.0027*(X^3.084)
Chironomidae	0.0019	2.614	0.0019*(X^2.614)
Odonata (damselfly)	0.0048	3.256	0.0048*(X^3.256)

The total body length and width (mm) of mussels and fish were measured directly using a ruler, with the soft tissue such as the whole body of mussels, the gills, muscle, and GIT of fish then dissected and transferred into a pre-weighed aluminium tray, separately. After this, the aluminium trays were covered using aluminium foil and frozen at -60 ^{o}C for at least 24 hours. Next, the samples were transferred to a freeze dryer and lyophilized for 48 hours at -40^{o}C and 0.12 mbar (ALPHA 1-2 LD plus, Martin Christ, Germany).

The dry weight of lyophilized soft tissue was then measured. The soft tissues were transferred into a conical flask and digested individually using 10% KOH for 48 hours at 50^{o}C for 120RPM in an incubator shaker. Five times the volume of KOH to the weight of the tissue was used for each sample. Similar to the water and sediment samples, digestates with remaining residues after the digestion were further processed using density separation. Samples were centrifuged twice using NaCl (ρ = 1.2g/cm^3) followed by $ZnCl_2$ (ρ = 1.7-1.8g/cm^3) in 50mL falcon tubes. Then, the supernatant was collected and vacuumed through a glass microfiber filter (Whatman GF/C, 47 mm diameter, GE Healthcare Whatman). The filter was then transferred into the oven and dried at 70^{o}C overnight.

3.3.3 Microplastic identification and enumeration

Lower- and higher- resolution methods were used. The lower resolution method was performed via visual inspection and manual enumeration of microplastic using a stereomicroscope. This is the standard visualization method commonly used by many studies for freshwater(Lu et al., 2021; Windsor et al., 2019) and marine (Marrone et al., 2021; Muhammad Husin et al., 2021) ecosystems.

For this, microplastic retained on the filter was identified and enumerated 180x magnification under the stereomicroscope (Nikon SMZ1500, Nikon, Japan). Microplastics were detected based on their morphological characteristics and colour. For example, synthetic fibre should have characteristics such as a uniform surface and equal diameter along its length, whereas fibre without such features was likely to be natural fibre (Stanton et al., 2019). In addition, a hot needle test was carried out on

potential microplastic on a random basis. The particle that remains intact when prodded with a needle but melted when approached by a hot needle was counted as microplastic (De Witte et al., 2014). Once confirmed as microplastic, the material was classified according to its shape (fibre, fragment, film, and bead) and colour.

A further confirmation analysis was conducted on the microplastic to establish the accuracy of this lower-resolution method. For this, a total of 52 pieces of larger-sized microplastic larger than 100µm were extracted from biota samples and picked out randomly. The polymer composition of these particles was verified using an ATR-FTIR spectrometer (Frontier^{TM}, PerkinElmer, United States). For this, each particle was scanned with 4 scans under transmission mode and a spectra range between 4000 and 400 cm $^{-1}$. This range falls under the mid-infrared region of the electromagnetic spectrum and was commonly used in the field of microplastic (Veerasingam et al., 2021). Spectra were then compared with spectrums reference on Open Specy (Cowger et al., 2021). In total, 24 particles with spectra that was similar (Pearson's $r > 0.7$) to the data base were chosen. 8 types of synthetic plastics, 1 type of synthetic rubber, and other materials such as cellulose and plants were identified (Figure 3.3). This check demonstrated that the accuracy of the lower resolution method, with 92% of the particles determined by analysed using FTIR were indeed plastic.

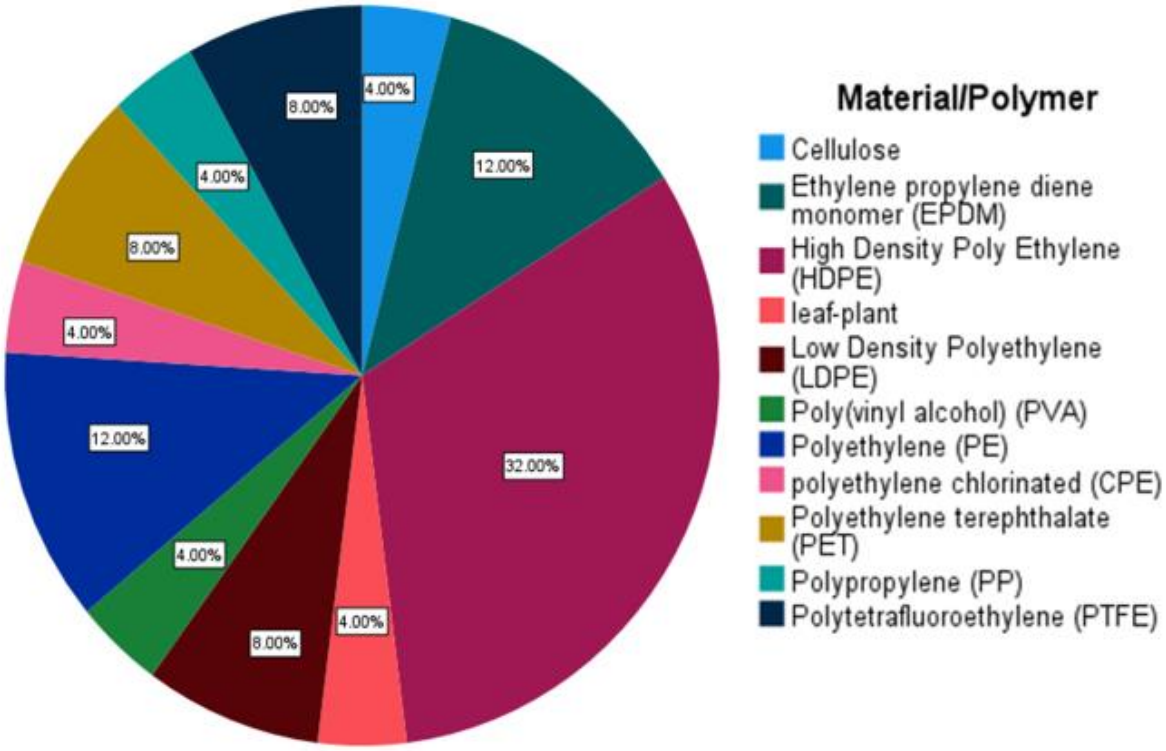


Figure 3.3. P*olymer type of microplastics ingested by freshwater animals.*

This lower-resolution method relied on the manual enumeration of microplastic through a visual examination and was only able to detect microplastic with a diameter larger than 100 µm. One of the observations from the pilot study was that microplastic appeared to be absent from the smaller animals, raising concerns that using the conventional methods we may be completely missing the material in the smaller ranges that smaller animals consume. This, together with the fact that smaller sizes are also consumed by larger animals may lead to an underestimation of their total body loads.

In order to quantify the smaller-sized microplastics, a higher-resolution method was applied. For this, 25 individuals of each of the four abundant insect families (Baetidae, Chironomidae, Simuliidae, and Hydropsychidae) were randomly selected from the field samples. Odonata was not included due to its small sample size. Moreover, a comparison between different insect families processed using the lower resolution approach indicated there was no significant difference in microplastic concentration and abundance between Odonata and the other insect taxa (Appendix 5). Thus, excluding Odonata in the higher resolution method would not affect the result. For each family, 5 individuals were pooled as one replicate sample, with each family having 5 sets of samples.

The higher resolution method adopted was an automated microplastic identification and enumeration technique that uses multiple dyes to identify microplastic from samples (Maxwell et al., 2020; Tarafdar et al., 2022). The method has three stages. First, Calcofluor White/Evans blue solution (1.0 g L^{-1} Calcofluor white, 0.5 g L^{-1} Evans blue, Sigma-Aldrich) and Nile red (0.05g L^{-1} in acetone, diluted 10 times in n-hexane, Sigma-Aldrich) are used to stain the organic matter (ex: chitin and cellulose) and microplastic extracted on the glass microfibre filter, respectively. Second, a confocal laser scanning microscope (TSC SP5 II, Leica, Germany) with Z volume (30 stacks with a total of 40 microns, captured with a 10x objective lens) was used to distinguish plastic from non-plastic. Each replicate (with 5 individuals from the same taxa) was scanned 10 times. For each scan, Calcofluor white/Evans blue solution was excited with 405nm laser with emission wavelength ranges from 425-465nm, followed by Nile red excited with 488nm laser (with emission wavelength ranges from 495-535nm) and

543nm laser (with emission wavelength ranges from 630-700nm), and 10 sets of images were produced for each scan.

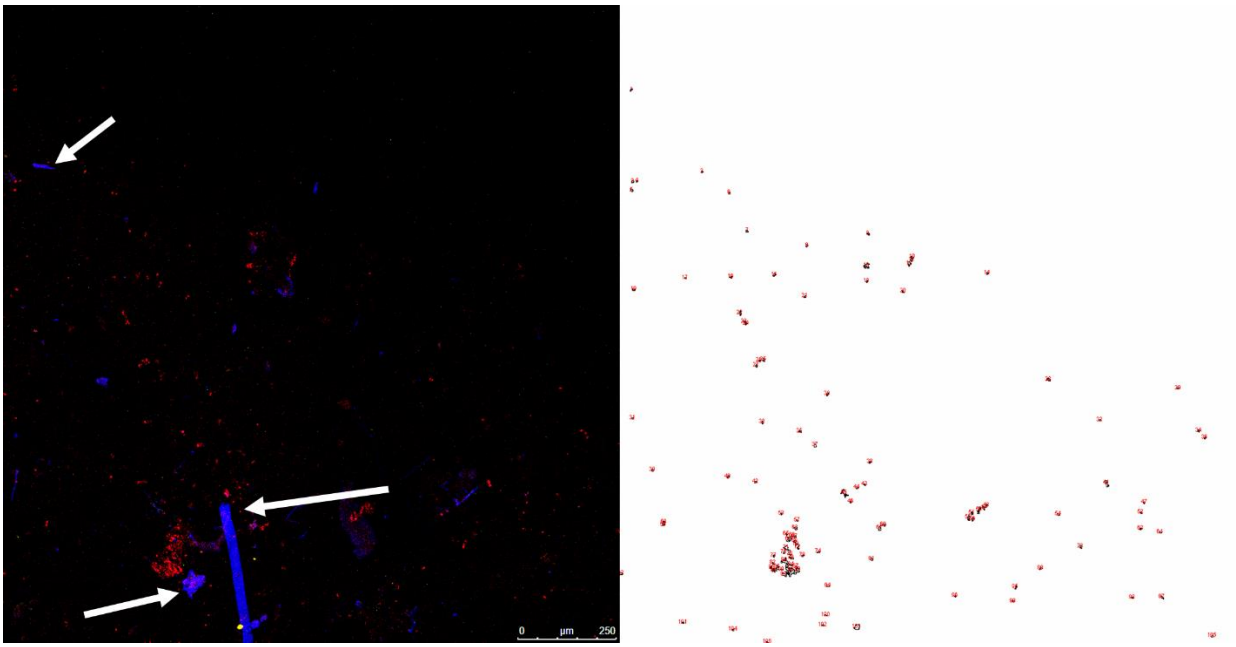


Figure 3.4. *Fluorescent photo (a) of a sub-sample of net-spinning caddisfly (Hydropsychidae) and the automated counting result (b). Blue colour (white arrow) represents particles derived from animal origin whereas red, yellow, and green were microplastic.*

Each set contained four fluorescent images (blue, green, yellow, and red colour) excited when different lasers were used, see Figure 3.4 and Appendix 3. Finally, stage 3 involves using the Fiji software (Schindelin et al., 2012) for automated counting. To ensure a consistent analysis for all samples, the code from (Prata et al., 2019b) was modified to identify microplastic from the images (see Appendix 4). This method helps to shorten the time taken for microplastic enumeration and reduces the risk of

overestimation when compared to a sample solely stained by Nile red (Tarafdar et al., 2022).

3.4 Statistical treatment and analysis of data

Microplastic concentration in water was expressed as the number of microplastic identified per litre of water sample (items L^{-1}). For the amount of material deposited on the bed, microplastic was expressed in the unit of number of pieces per square meter of bed (items m^{-2}). For this, the following equation was used to convert the data from the water sample collected inside the cylinder to pieces per unit area:

$$MP_i = \frac{C_i \times V_i}{A} \quad (1)$$

C (items/L) was the amount of microplastic identified per litre of suspended sediment sample replicate. The volume of water in the cylinder (*V; L*) was calculated from the depth of the water column in the sampling container and *A* (0.0057m^2) was the area of the river bed isolated using the sampling cone.

Microplastic load in animals was expressed as microplastic occurrence (percentage of animals of given taxa that have ingested microplastic), abundance (number of pieces per individual animal), and concentration (number per dry body weight).

All statistical analysis were conducted on IBM SPSS Statistics 28. A non-parametric Kruskal-Wallis test (K-W) with Dunn-Bonferroni post hoc test was used to assess differences between sites, taxa, and trophic groups. A Mann-Whitney U test was used to assess differences in microplastic abundance and concentration between visualisation methods (counterstaining dyes methos and visual inspection) used. The data were not normally distributed, and hence analysis of variance (ANOVA) could not be used. Pearson's correlation coefficient was adopted to assess the correlation and relationship between body size and microplastic abundance. Spearman rank-order correlation coefficient (Spearman's rho) was used to assess the correlation between microplastic load in animals and on the river (sediment and surface water). The Chi-square test was used to assess the differences in the frequencies of different shapes and colours of microplastics identified in animals, water, and sediment samples. Note that due to small sample sizes, differences in body loads between the fish species recorded were not assessed. Also, neither fish nor mussels were used in any inter-site comparison as these groups were not collected systematically from the river sites.

3.5 Quality assurance and control

To minimise the microplastic contamination, several precautionary steps were taken throughout the sampling and lab processing process. Non-plastic attire such as a pure cotton lab coat and nitrile glove was used at all times in the lab. Moreover, all chemicals and ultrapure water were filtered twice using a glass microfibre filter (Whatman GF/C, 47 mm diameter, GE Healthcare Whatman). In addition, all glass wares were rinsed thoroughly with ultrapure water before usage, and a laboratory

procedure was conducted under a fume chamber to prevent atmospheric deposition of microplastic.

As a quality assurance step, an experiment was conducted on the open-ended truncated cone to assess whether the sampling apparatus will introduce microplastic contamination during the sampling process. Briefly, the cone was pressed onto a larger container filled with tap water. The water was then agitated, and 5 litres of the water were sieved through a pre-rinsed 53-micron stainless steel sieve, and the residue remaining on the sieve was transferred into a glass bottle using ultrapure water. On the other hand, 5 litres of blanks (tap water) were collected and underwent the same processing as the agitated sample. After, the samples were filtered through a glass microfibre filter (Whatman GF/C, 47 mm diameter, GE Healthcare Whatman), and analysed under a stereomicroscope (Nikon SMZ1500). No black fragment (potential microplastic colour and shape from the cone) or green foam (potential microplastic colour and shape from the seal) was identified in the blank and agitated sample when inspected under a stereomicroscope. This indicated that the sampling cone did not introduce addition microplastic to the sample.

At least two procedural blanks were conducted for each batch of samples. The characteristic of microplastic identified in the blank samples was noted and excluded from the count if a similar microplastic was identified in the sample. However, only a minimal amount of microplastic (less than 5 microplastics per blank) was found on the blank sample and was excluded from the sample.

RESULTS

4. Results

4.1 Overall microplastic load in freshwater animals from the Semenyih catchment

For the four insect families, the standard lower-resolution method detected an average of 1.1 pieces in microplastic per individual (Figure 4.1). When expressed per body weight, individuals of these families contained an average of 11.06 pieces of microplastic per mg of dry tissue. Numbers increased markedly when the high-resolution method was used, with an average of 128.8. pieces per individual and 704.3 per mg dry weight. The overall ratio between high- and low-resolution methods was 120:1. A difference is to be expected since the high-resolution counterstaining method detects microplastic with a diameter down to 4 µm.

The ratios differed somewhat between families: for every 1 microplastic identified using the lower resolution method, the higher resolution method was able to detect additional 107, 81, 180, and 116 microplastic particles for Baetidae, Chironomidae, Hydropsychidae, and Simuliidae, respectively. Thus, freshwater invertebrates mainly ingested microplastics that were smaller than the minimum microplastic size reported in most studies (around 10 µm – 100 µm, depending on the microplastic detection method used; Table 2), with a potentially important underestimation of body loads of two orders of magnitude.

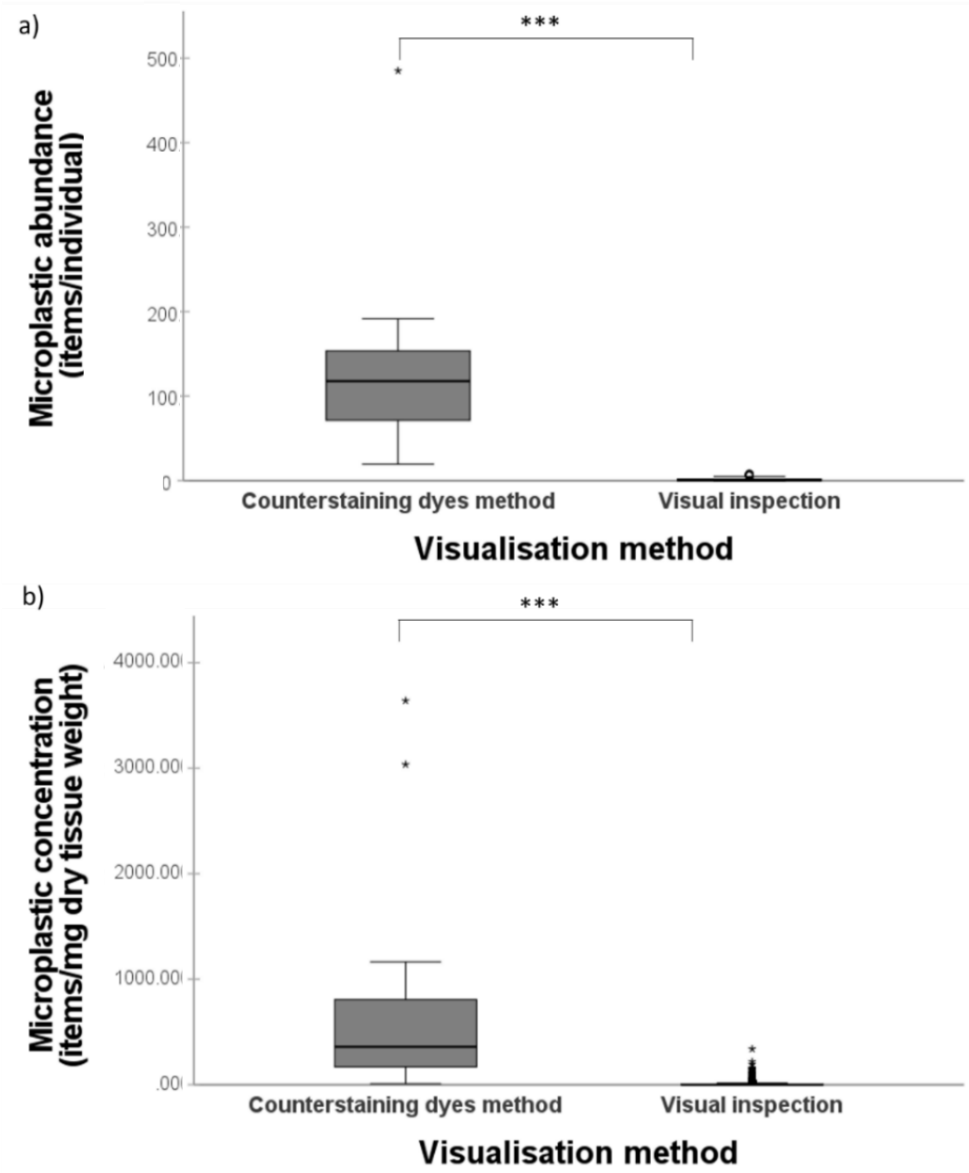


Figure 4. 1. *Box and whisker plot showing median (a) microplastic abundance (items individual^{-1}) and (b) microplastic concentration (items mg dry tissue weight^{-1}) of freshwater invertebrates using two different visualisation methods. Horizontal lines indicate significant differences at p<0.05 (Mann-Whitney U test). p <0.05 represented by a single asterisk (*), p<0.01 represented by a double asterisk (**), and p <0.001 represented by a triple asterisk (***).*

Table 5. *The freshwater fish and invertebrate taxa sampled from the Semenyih River, and its sample size (n), mean and standard deviation of its microplastic occurrence (%), abundance (items individual^{-1}), and concentration (items mg dry tissue weight^{-1}).*

Taxa	n	Microplastic occurrence (%)	Microplastic abundance (items individual^{-1})		Microplastic concentration (items mg dry tissue weight^{-1})	
			Mean	Standard Deviation	Mean	Standard Deviation
Actinopterygii	19.00	94.7	19.421	23.310	0.004	0.006
Insecta	588	44.2	1.199	2.554	10.609	29.400
Baetidae	182.00	57.7	1.137	1.394	6.805	17.901
Chironomidae	160.00	53.1	1.006	1.256	18.286	31.630
Hydropsychidae	132.00	59.8	1.205	1.402	1.562	9.241
Odonata	13.00	69.2	2.000	2.309	0.646	1.822
Simuliidae	77.00	46.8	0.818	1.060	24.145	55.976
Bivalvia						
Unionidae	24.00	58.3	3.708	10.720	0.0003	0.001

Based on the low-resolution, visual inspection method, microplastic was identified in 56.3% of all animals (I.e. insects, fish and mussels, *n*=607) sampled from the Semenyih River and Lake (Table 5). The mussels contained up to 53 pieces of microplastic per individual, while the fish had up to 97 microplastic per individual. Expressed as the number of pieces of microplastic per dry body weight, mussels contained a maximum of 338.65 pieces mg^{-1} and fish 0.024 pieces mg^{-1}. Thus, patterns across different groups

varied according to whether data were represented as abundance or per individual dry weight.

4.1.1 Differences in microplastic contamination between taxa

Microplastic load (abundance and concentration) across different classes obtained using the visual inspection method was compared. There was a significant difference in the microplastic abundance between Actinopterygii, Bivalvia, and Insecta *(K-W:* $H(2)=9.94$, $p=0.007$). Of the three classes, Insecta had significantly lower microplastic abundance than Actinopterygii (Dunn-Bonferroni post-hoc test*,* $p=0.006$), see Figure 4.2a. Microplastic concentration also differed significantly between classes (*K-W*, $H(2)=9.67$, $p=0.008$), with Insecta having a significantly higher microplastic load per milligram of dry tissue than Bivalvia (Dunn-Bonferroni post-hoc test, $p=0.020$); see Figure 4.2b.

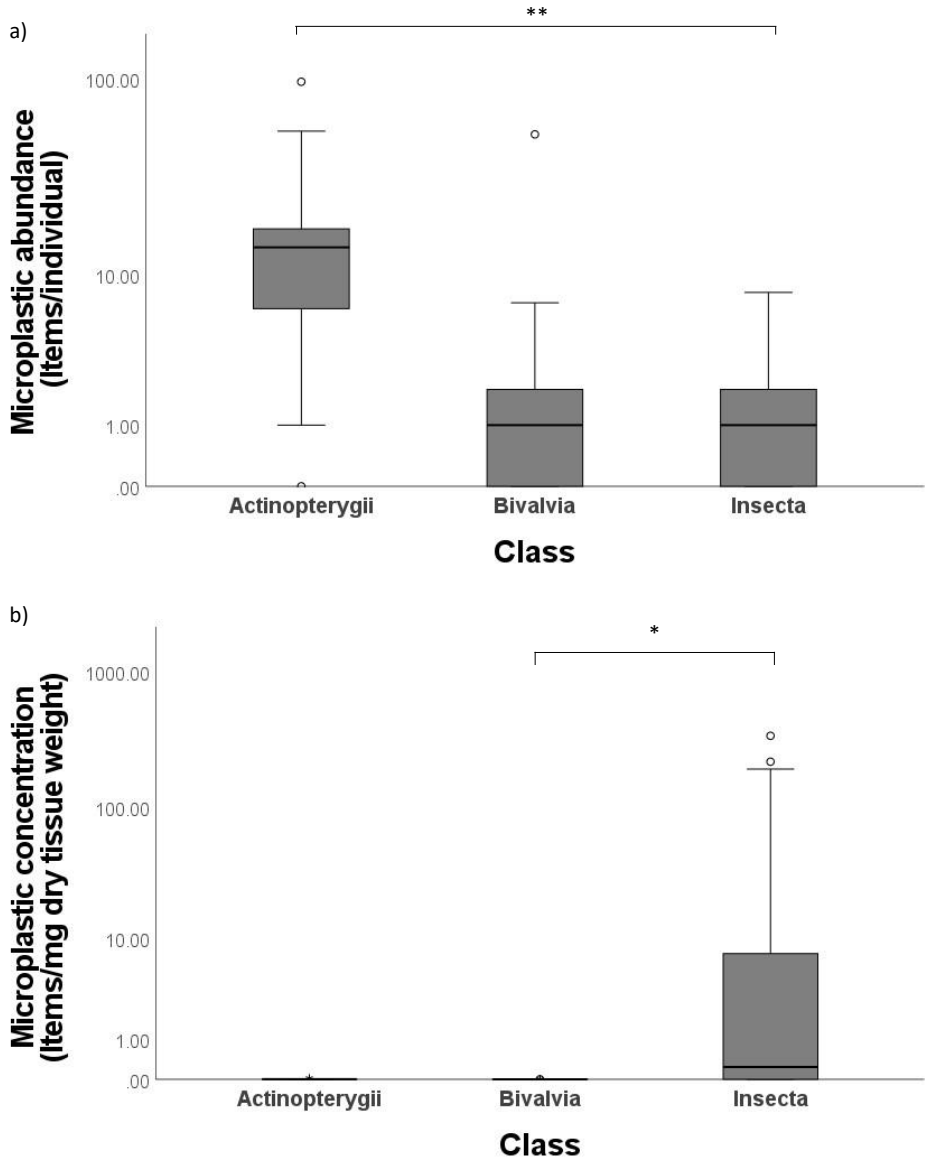


Figure 4. 2. *Box and whisker plot showing median (a) microplastic abundance (items individual^{-1}) and (b) microplastic concentration (items mg dry tissue weight^{-1}) of the major taxonomic groups (Class: Actinopterygii, Bivalvia, and Insecta), under lower resolution method and in logarithmic scale. Horizontal lines indicate significant differences at p<0.05 (Dunn-Bonferroni post-hoc test), p<0.05 represented by a single asterisk (*), p<0.01 represented by a double asterisk (**), and p <0.001 represented by a triple asterisk (***).*

To investigate inter-family differences in the Insecta, data from the high-resolution method were used because this method yielded a more complete picture of total body loads. Tests indicated significant differences in concentration between the families (*K-W*: $H(3)=9.629$, $p=0.022$, Figure 4.3b). Hydropsychidae has significantly lower microplastic concentration than Simuliidae (*K-W:* $H(3) = -10.200$, $p=0.038$, Figure 4.3b). However, there was no significant difference between the four insect families when the load was expressed using microplastic abundance (*K-W:* $H(3) = 7.343$, $p=0.062$, Figure 4.3a). Differences between the fish families (Appendix 6) were not assessed due to the small numbers within some families that prevented meaningful statistical analysis. Overall, it is clear that body loads differ between different taxonomic groups, although this depends partly on whether microplastic is expressed as abundance in the body or concentration. The higher-resolution counterstaining dye method was able to detect differences between some benthic invertebrate families.

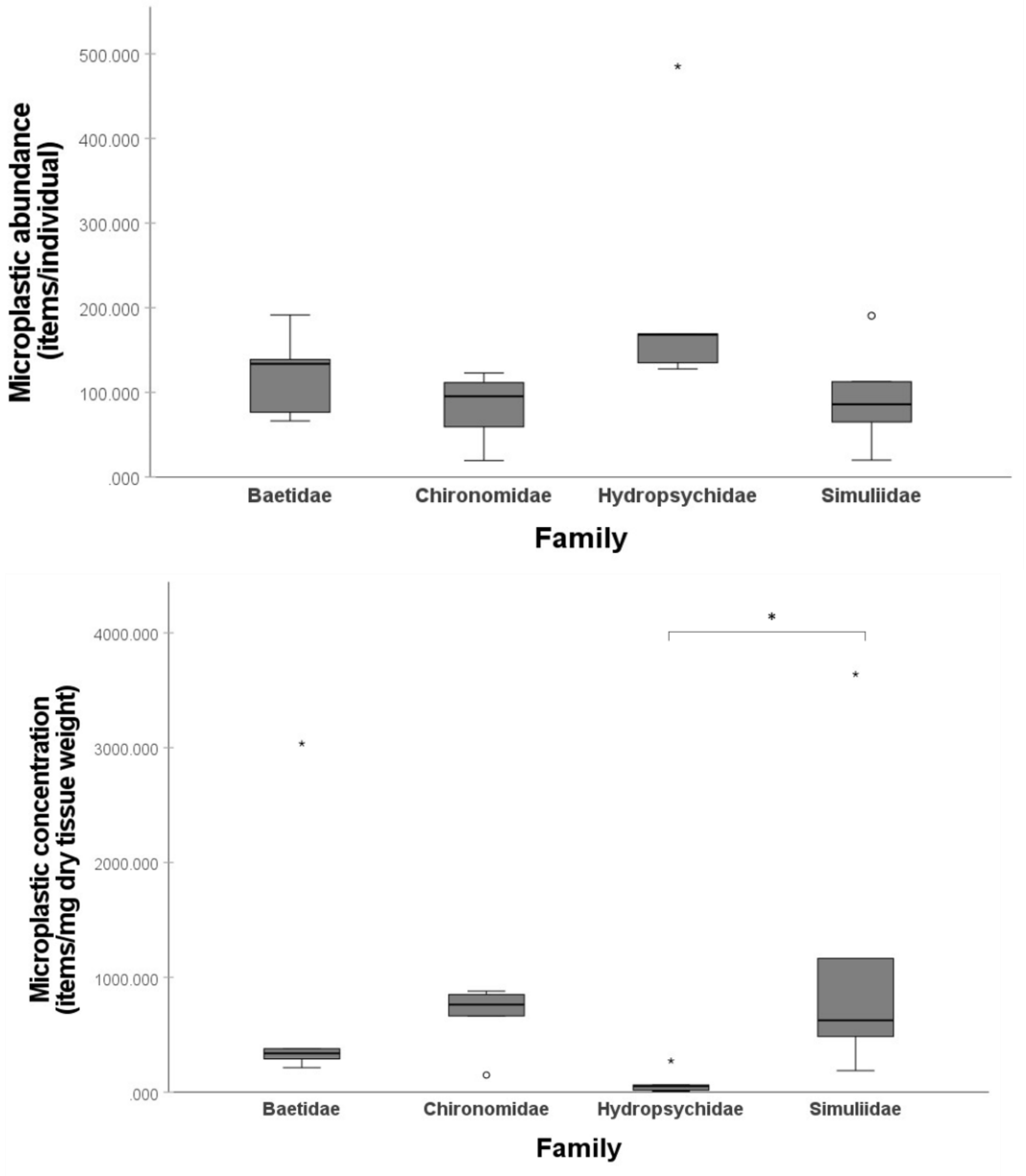


Figure 4.3. *Box and whisker plot showing median (a) microplastic abundance (items individual^{-1}) and (b) microplastic concentration (items mg dry tissue weight^{-1}) of four freshwater invertebrate families processed using the counterstaining dyes approach. Horizontal lines indicate significant differences at p<0.05 (Dunn-Bonferroni post-hoc test), p<0.05 represented by a single asterisk (*), p<0.01 represented by a double asterisk (**), and p <0.001 represented by a triple asterisk (***).*

4.1.2 *Distribution of microplastic between different body parts of fish*

For each fish, gills, GIT, and muscles were dissected and the organ part was processed separately using the visual inspection method. There was a significant difference in microplastic concentration between these three organ parts (K-W: $H(2)=11.05$, $p<0.004$, see Figure 4.4). The load of plastic contaminant for each organ is ranked as GIT > gills > muscle, showing most plastic particles are accumulating in the GIT.

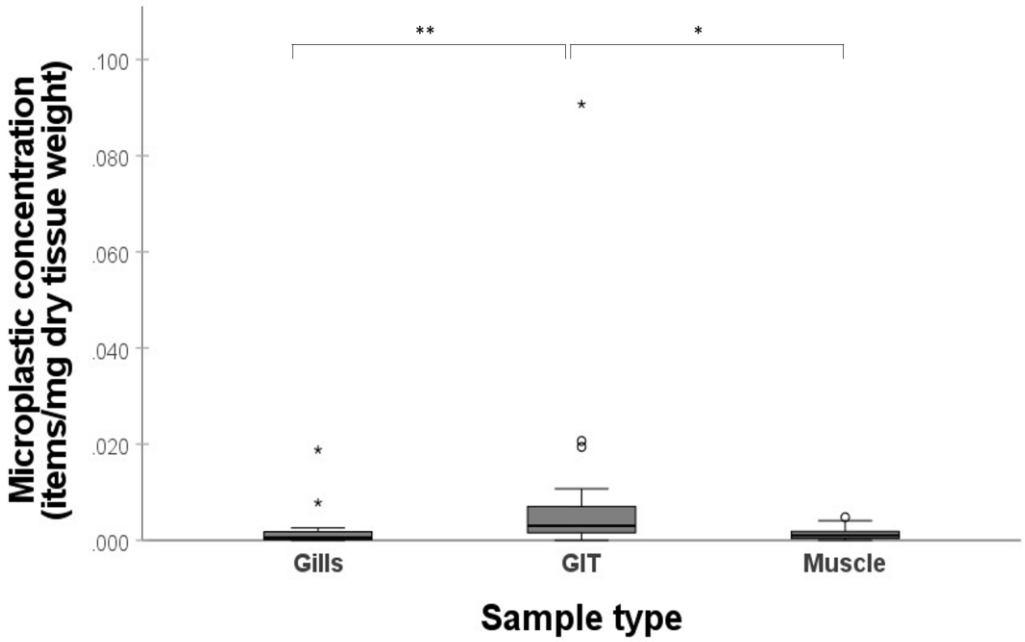


Figure 4.4. *Box and whisker plot showing median microplastic concentration (items mg dry tissue weight^{-1}) of different body parts of Actinopterygii. Horizontal lines indicate significant differences at p<0.05 (Dunn-Bonferroni post-hoc test), p<0.05 represented by a single asterisk (*), p<0.01 represented by a double asterisk (**), and p <0.001 represented by a triple asterisk (***).*

4.2 Spatial differences in microplastic loads

Insects were collected from 8 sites along the Semenyih River, with samples of microplastic in the water and bed obtained at the same time from each site (Figure 4.5). The microplastic concentration in water and on the bed, both differed between sampling sites (K-W: $H(6) = 19.425$, $p = 0.004$, and $H(6) = 26.203$, $p < 0.001$, respectively). The downstream sites were generally more highly contaminated with microplastic, although patterns were complex and did not follow a continuous downstream pattern. For instance, site 1 (the uppermost site) was as highly contaminated as the downstream sites, despite being a rural site located in a forest upstream from the dam. The bed data showed a more progressive downstream increment than the water column data, though again some sites (notably site 2) appeared as anomalies.

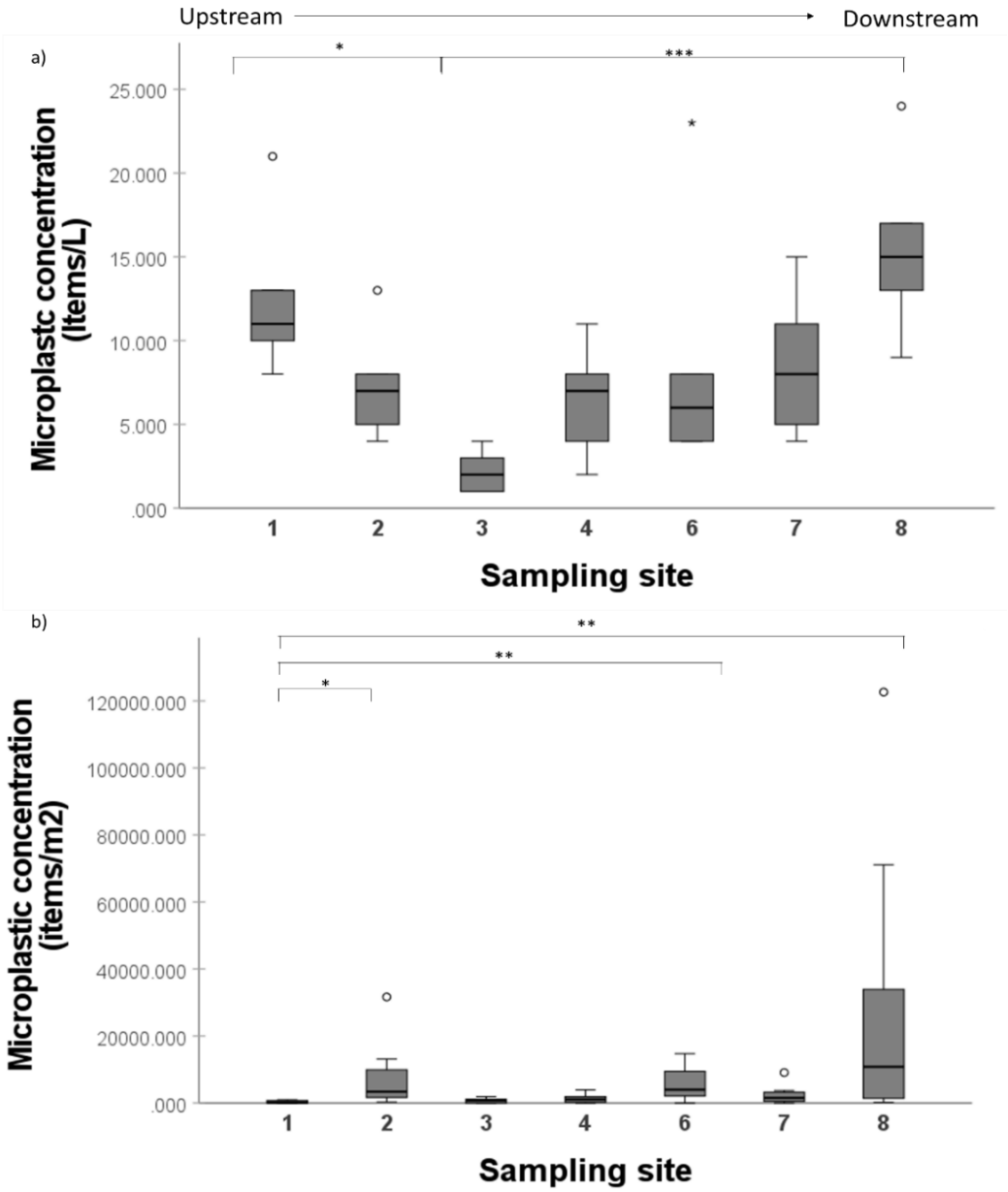


Figure 4.5. *Box and whisker plot showing median microplastic concentration in (a) water (items L^{-1}) and (b) bed sediment (items per m^2) samples collected from the Semenyih River. Sampling sites were named according to their location along the river; Site 1 was the most upstream site and Site 8 was the most downstream site. Horizontal lines indicate significant differences at p<0.05 (Dunn-Bonferroni posthoc test), p<0.05 represented by a single asterisk (*), p<0.01 represented by a double asterisk (**), and p <0.001 represented by a triple asterisk (***).*

There was a significant difference in microplastic abundance in invertebrates collected from different sites on the river (K-W: $H(6)=23.184$, $p<0.001$, see Figure 4.6a), with those collected from site 2 containing significantly more pieces of microplastic than those collected in site 1 (Dunn-Bonferroni post-hoc test $p=0.009$), 3 ($p=0.003$), and 4 ($p=0.005$). The data for microplastic concentration showed some similar patterns with animals at site 2 having high concentrations: those at site 2 had higher loads per body mass than site 4 ($p=0.028$) and were no different from those collected in further downstream sites. Thus, overall, animals from upstream sampling site 2 were equally contaminated with microplastics as the ones collected from further downstream sites (site 6, site 7, and site 8). Note that this and all the subsequent analyses used only invertebrates processed using the low-resolution method (as the sample sizes of animals were higher than the high-resolution method).

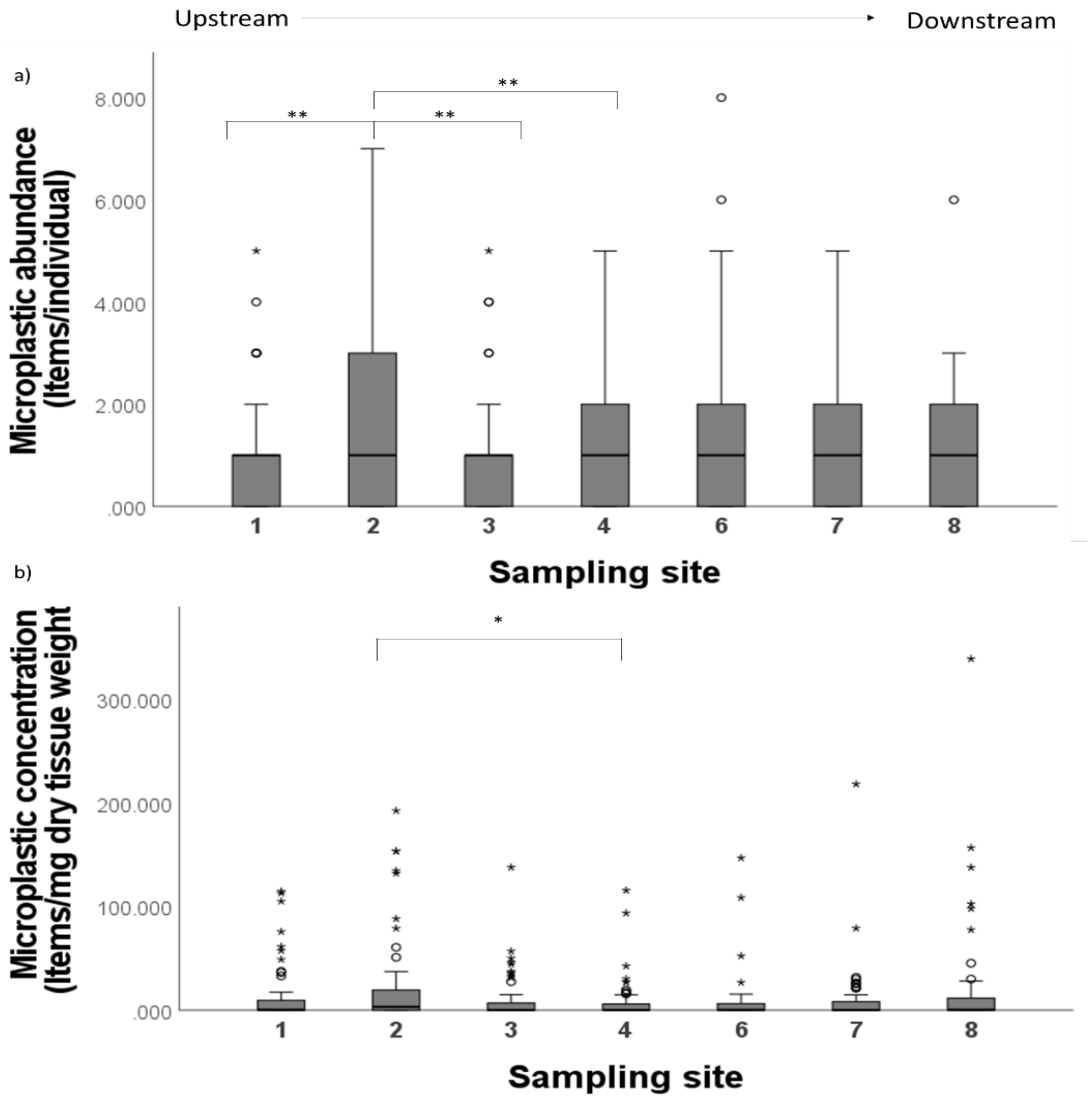


Figure 4.6. *Box and whisker plot showing median (a) microplastic abundance (items individual^{-1}) and (b) microplastic concentration (items mg dry tissue weight^{-1}) of macroinvertebrates (class: Insecta) from the Semenyih River in this study, processed using the visual inspection method. Sampling sites were named according to their location along the river; Site 1 was the most upstream site and Site 8 was the most downstream site. Horizontal lines indicate significant differences at p < 0.05 (Dunn-Bonferroni posthoc test). P <0.05 is represented by a single asterisk (*), P <0.01 is represented by a double asterisk (**), and P <0.001 is represented by a triple asterisk (***).*

4.3 Factors influencing microplastic body loads in aquatic insect larvae

4.3.1 Site contamination

There was no significant correlation between ranked microplastic abundance in invertebrates and the ranked microplastic concentration in water (Spearman's rho: $r_s(6)=0.429$, $p=0.289$) and sediment (Spearman's rho: $r_s(6)=0.667$, $p=0.071$). When expressed in microplastic concentration, the loads in invertebrates also did not correlate with the water (Spearman's rho: $r_s(6)=0.643$, $p=0.086$) and sediment (Spearman's rho: $r_s(6)=0.429$, $p=0.289$).

4.3.2 Body size

Animals included in the study ranged from a body length of 1.237 mm (invertebrate) to 335.00mm (fish). As most animals collected were invertebrates, most were in the smaller size classes (Figure 4.7). Linear regression was used to examine the relationship between the body size of animals and the amount of microplastic ingested. The microplastic abundance of animals was significantly correlated to their body length ($R^2 = 0.113$, $F(1,605) =76.81$, $p<0.001$).

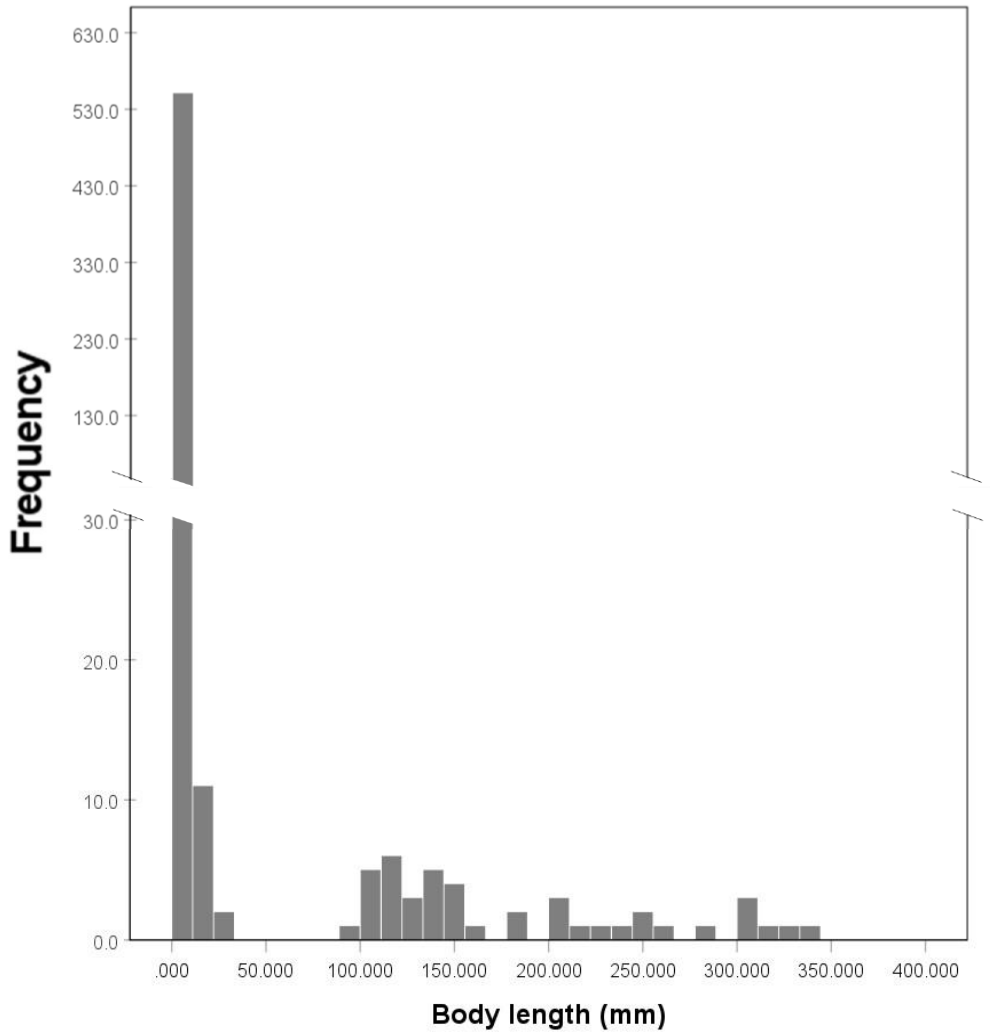


Figure 4.7. *Histogram of the body length (mm) of freshwater animals (N=607) sampled.*

When analysed separately for different classes (Figure 4.8.), only the insects had a body load that was significantly related to their length. However, while formally significant (p=0.041), little of the variability in microplastic could be accounted for by their length (model R^2 = 0.086, F(1,562) = 0.007, p=.041). There was no significant correlation between the body size and microplastic abundance for Actinopterygii (p=0.788) and Bivalvia (p=0.368).

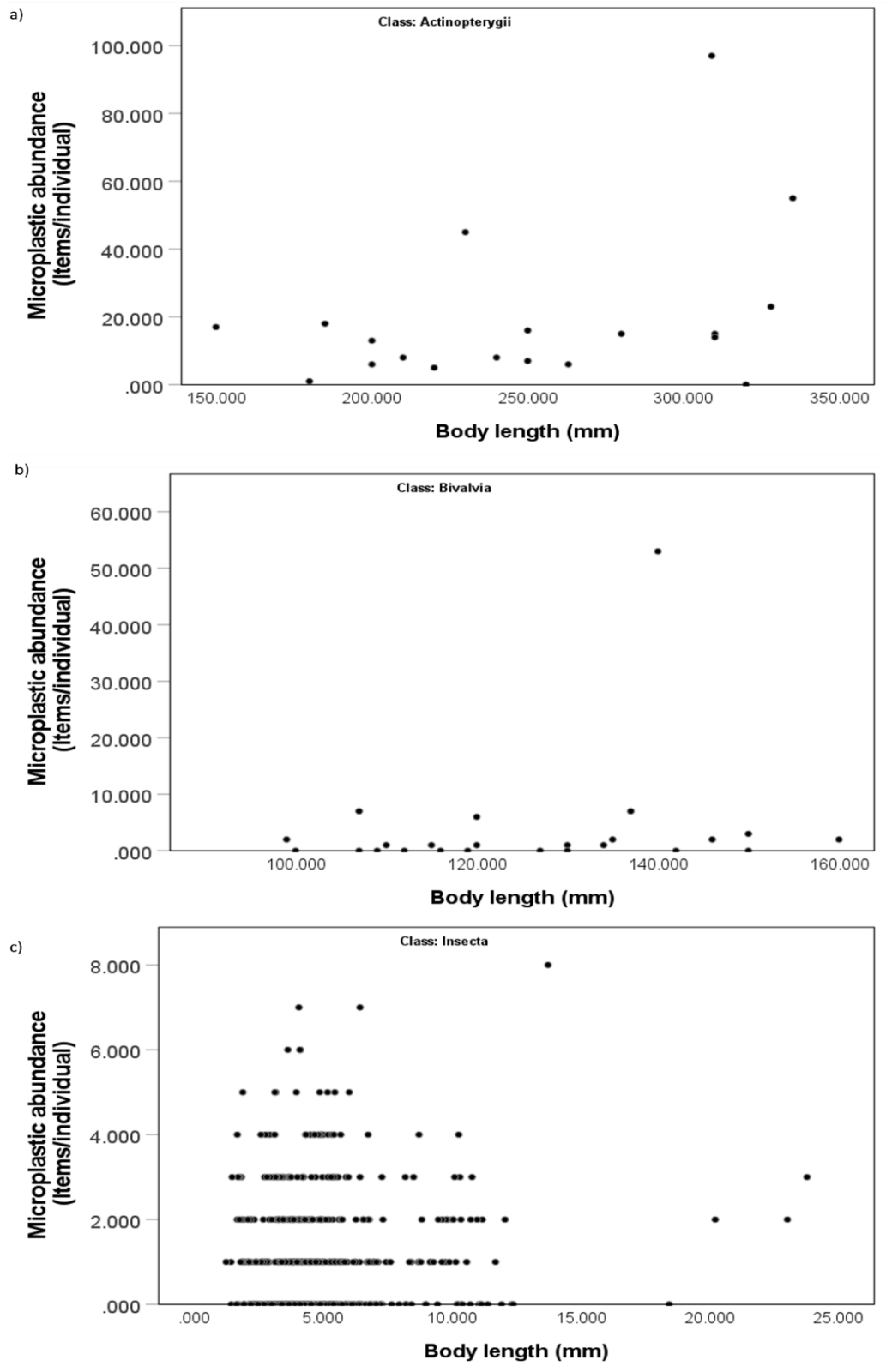


Figure 4.8. *Scatter plots of microplastic abundance (items per individual) of three main freshwater classes included in this study (a; Actinopterygii, b; Bivalvia, and c; Insecta) with their body length (mm).*

4.3.3 Feeding preferences

Invertebrates were grouped according to their functional feeding groups and consisted of a gatherer (Baetidae), a filterer (Simuliidae, Hydropsychidae, and Unionidae), and a predator (Odonata). Chironomidae was not included due to the wide range of feeding preferences of the many species making up this group. Kruskal-Wallis test indicated that microplastic abundance did not differ significantly between functional feeding groups, $H(2)=2.493$, $p=0.288$ (Figure 4.9a). However, the microplastic load between different functional feeding groups differed significantly when expressed in microplastic concentration per mg of dry tissue, $H(2)=7.073$, $p=0.029$, with the filterers containing less microplastics in their tissue per unit weight than gatherers ($p=0.037$; Figure 4.9b).

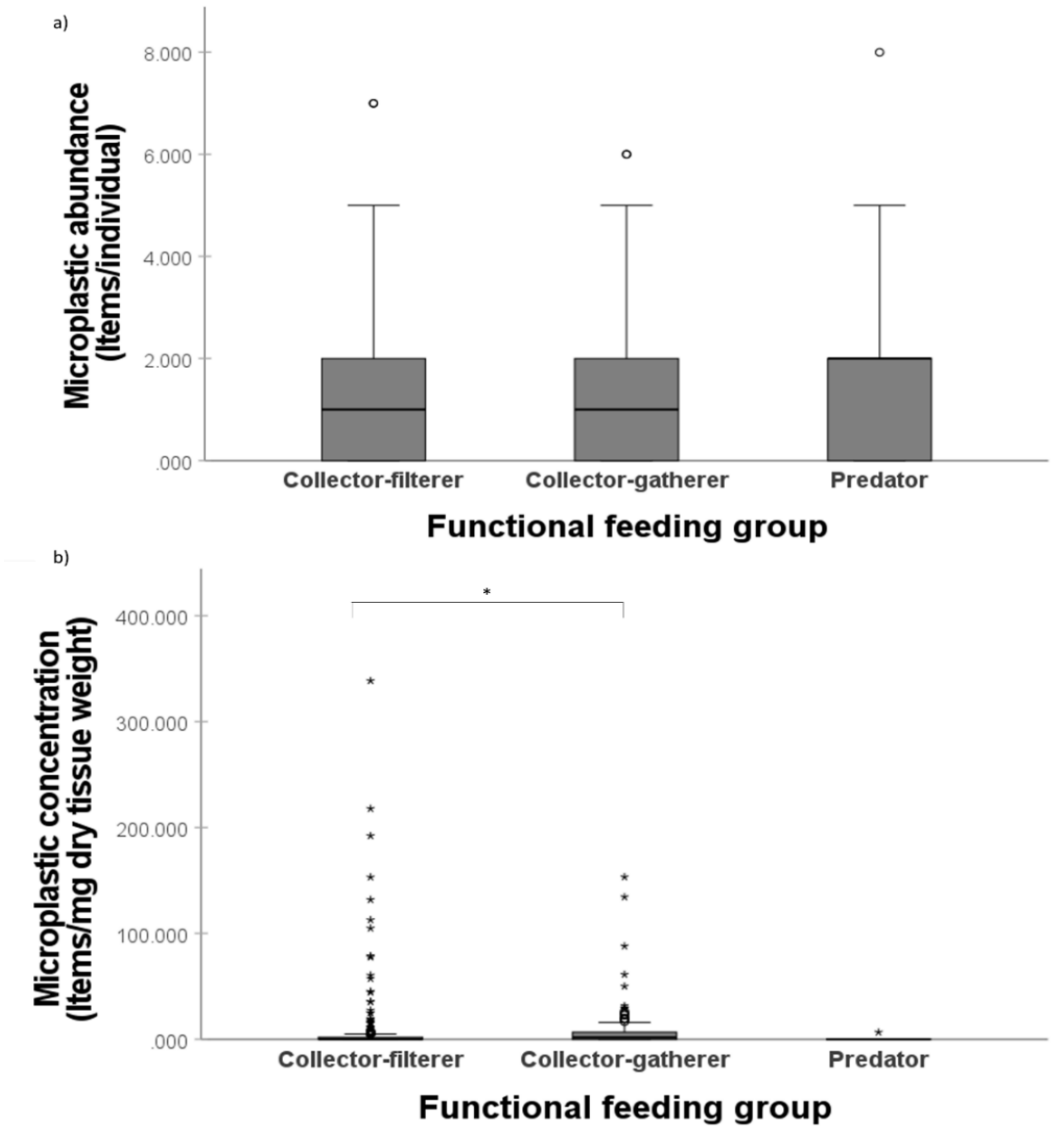


Figure 4.9. *Box and whisker plot showing median (a) microplastic abundance (items individual^{-1}) and (b) microplastic concentration (items mg dry tissue weight^{-1}) of macroinvertebrates from different functional feeding groups. Horizontal lines indicate significant differences at p<0.05 (Dunn-Bonferroni post-hoc test), p <0.05 represented by a single asterisk (*), p<0.01 represented by a double asterisk (**), and p <0.001 represented by a triple asterisk (***).*

Fish were categorised into carnivorous and omnivorous species (see Appendix 6). There was no difference in body concentration between the two groups, Mann-Whitney U test, $U(N_{carnivorous} = 10, N_{omnivorous} = 9,) = 43.00, z = -.163, p = 0.870$ (Figure 4.10). Hence, feeding preference did not appear to influence the ingestion of microplastic by these fish.

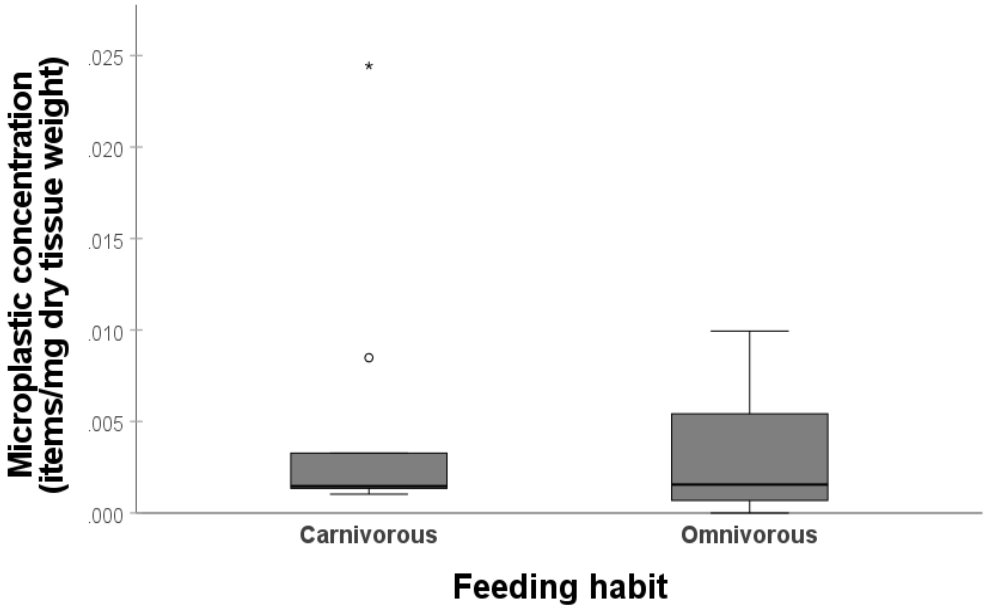


Figure 4.10. *Box and whisker plot showing median microplastic concentration (items mg^{-1} dry tissue weight) of freshwater fish, grouped by their feeding habits.*

4.4 Microplastic characteristics

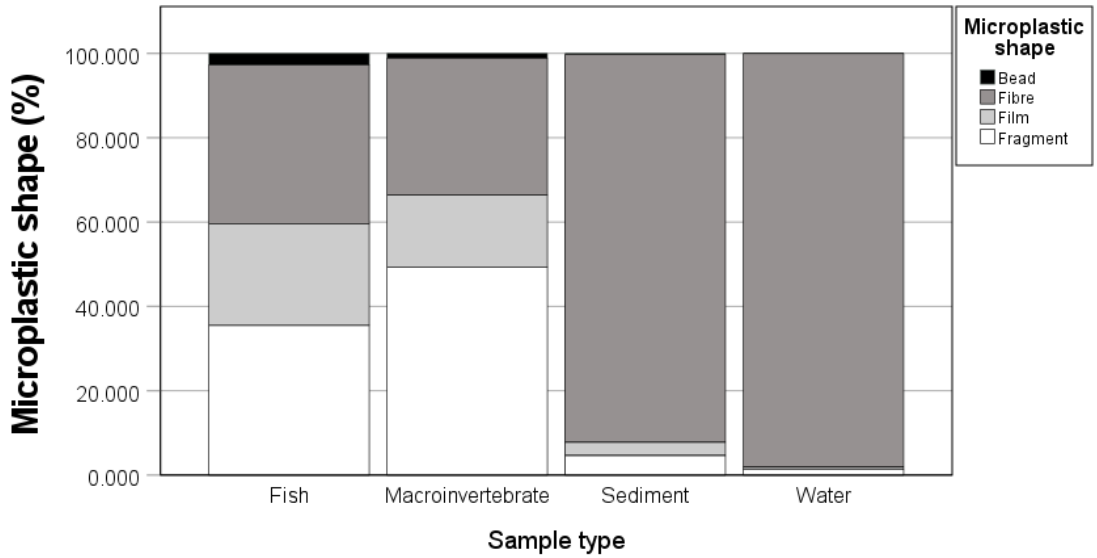


Figure 4.11. *Composition of microplastic shape (bead, fibre, film, and fragment) identified in fish, invertebrates, sediment, and water samples.*

The relative abundance of different shapes/types of microplastic differed between the animals and their environment (*Chi-square test*, X^2 (9, N = 2513) = 1041.23, p <0.001 (Figure 4.11). Bed and water samples were dominated by fibres (91.97% and 97.97% respectively), whereas fragments and films were more prevalent in animals.

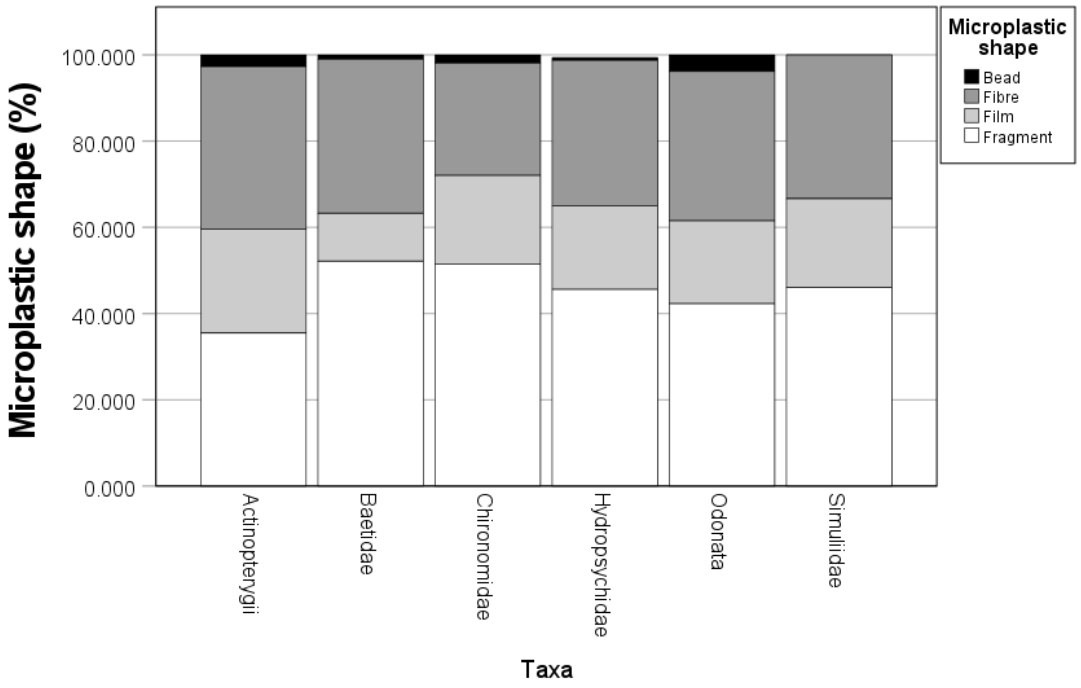


Figure 4.12. *Composition of microplastic shape (bead, fibre, film, and fragment) identified in different freshwater taxa included in this study.*

Invertebrates and fish ingested microplastic of all four different shapes (beads, fibres, fragments, and films). The prevalence of these shapes differed significantly between the taxa, X^2 (15, N = 985) = 33.118, p=0.005 (Figure 4.12). Fibre was the most abundant type ingested by Actinopterygii (37.7%) whereas fragments were most frequently identified in Insecta (Baetidae; 52.2%, Chironomidae; 51.6%, Hydropsychidae, 45.9%; Simuliidae; 46.0%, and Odonata; 42.3%). Most taxa contained all 4 types of microplastic, although Simuliidae lacked beads. Thus, the relative composition of shapes ingested differed between taxa, and microplastic was 'selectively' ingested by the animals rather than randomly ingested from the environment.

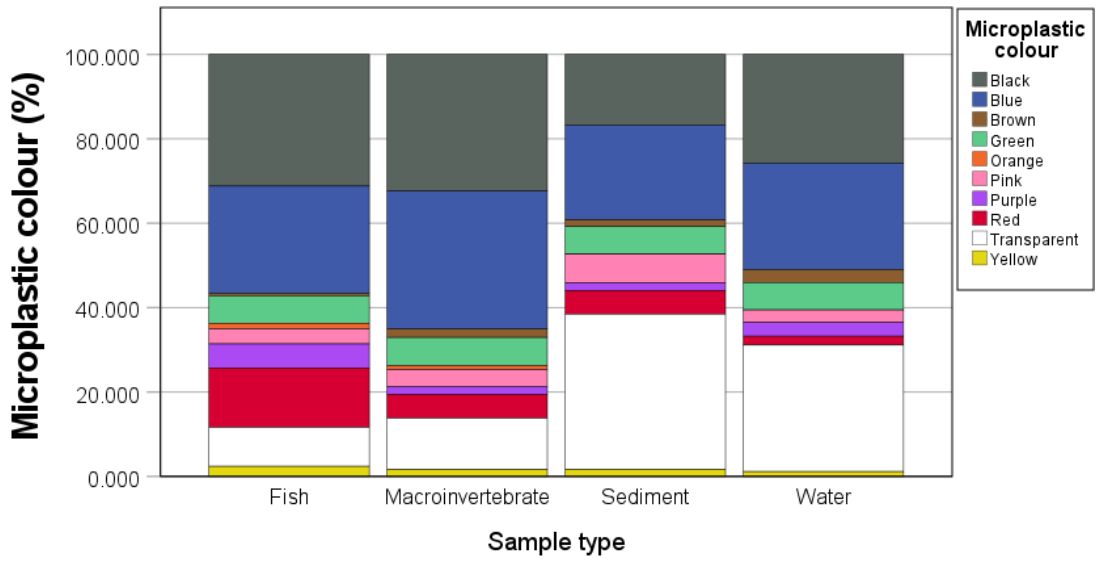


Figure 4.13. *Composition of microplastic colours identified in fish, macroinvertebrates, sediment, and water samples collected from the Semenyih River.*

Microplastics of many different colours were identified (Figure 4.13). The composition of microplastic colours identified in different sample types differed (X^2 (27, N = 2513) = 307.60, p <0.001 (Figure 4.14.). In general, black-coloured microplastic was most frequently found in biota samples (fish 31.17%, macroinvertebrates 33.87%) whereas white/transparent coloured microplastic occurred the most in the sediment (34.07%) and water (29.65%) samples from the Semenyih River.

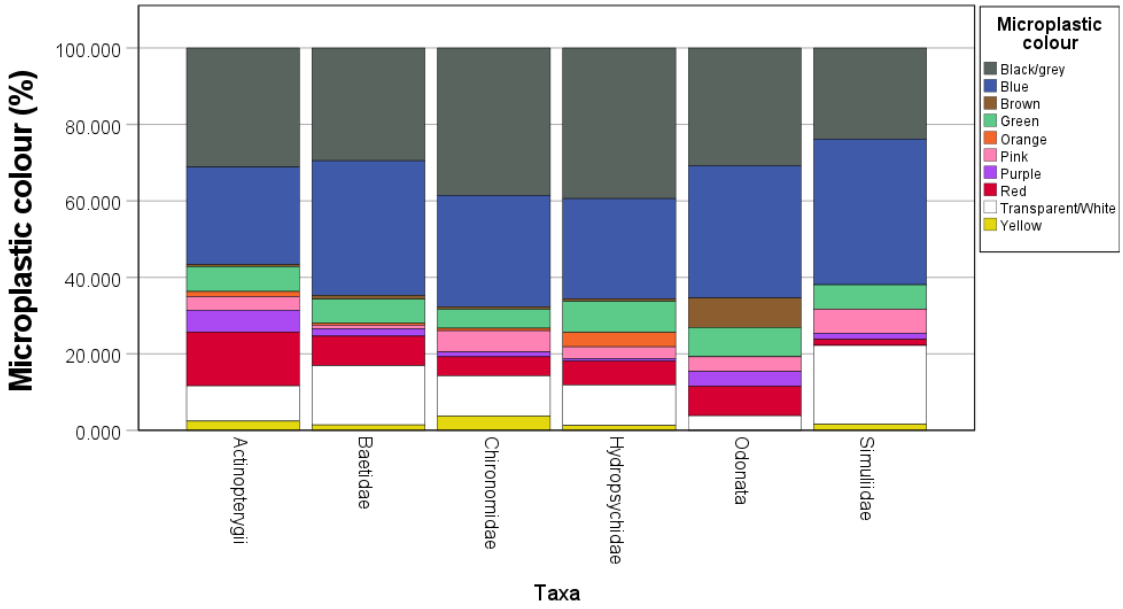


Figure 4.14. *Composition of microplastic colours identified in fish and invertebrate taxa collected from the Semenyih River.*

In general, blue- and black-coloured microplastics were most ingested, followed by transparent microplastic. The Chi-square test showed the colour of microplastics ingested differed significantly between the taxa, X^2 (45, N = 985) = 96.118, p <0.001 (Figure 4.14). Odonata contained a higher percentage of brown-coloured microplastic (7.69%) that was less frequently ingested by the other taxa, while Actinopterygii ingested a higher percentage of red-coloured microplastic (14.09%) than the other taxa. Thus, aquatic animals might selectively ingest microplastic of a certain colour or shape, instead of randomly intaking microplastic available from the surrounding environment.

DISCUSSION

5. Discussion

5.1 Overall microplastic load in freshwater animals from the Semenyih catchment

This is the first study from Malaysia to establish the presence and abundance of microplastics in a broad range of freshwater invertebrates and fish taxa. Using the low-resolution method (which as the common method means that comparison with other work is possible), 44.2% of the macroinvertebrates in the Semenyih River contained microplastic (Table 5). This finding is consistent with 48.5% of the Vipacco River in Italy (Bertoli et al., 2022) and 50% of the Taff catchment in the United Kingdom (Windsor et al., 2019). However, the microplastic occurrence in macroinvertebrates is lower than 88.6% from the Dommel River in the Netherlands (Pan et al., 2021).

Chironomidae have been reported to have high microplastic prevalence, with 75%-100% of individuals from South African rivers containing microplastic (Dahms et al., 2020; Nel et al., 2018). Again, using the low-resolution data to allow comparison, Chironomidae from the Semenyih River had a higher mean microplastic concentration (18.286 items mg dry tissue weight^{-1}) and abundance (1.006 items individual^{-1}) than the maximum loads so far reported in other countries like South Africa (2.31 items mg^{-1}, Nel et al., 2018) and Taiwan (2.87 items mg^{-1}, Lin et al., 2021). Similarly, Baetidae from this study contained higher microplastic load than other taxa from the same order reported in other countries such as Caenis (0.067 items individual^{-1}, Bertoli et al., 2022), Potamanthus (0.2 items individual^{-1}Bertoli et al., 2022), and Ephemeroptera (0.74 items individual^{-1}, Parker et al., 2022a; 0.08 items individual^{-1}, Parker et al., 2022b). Also, the microplastic occurrence of Hydropsychidae in the Semenyih River is

higher than Trichoptera assessed in other countries (19.6%, Bertoli et al., 2022; 57%, Parker et al., 2022a; 46%, Parker et al., 2022b). Finally mean microplastic abundance of Odonata (2 items individual^{-1}) is higher than in other regions, where values ranged from 0.043 to 0.45 items individual^{-1} (Bertoli et al., 2022; Pan et al., 2021; Parker et al., 2022b, 2022a).

This is only the second study conducted in Malaysia that assessed microplastic in freshwater fish. The mean microplastic abundance of some species sampled in Semenyih River including *Oreochromis mossambicus* (12 items individual^{-1}), *Oxyeleotris marmorata* (6 items individual^{-1}), and *Anabas testudineus* (1 item individual^{-1}) is greater than 1.61 items individual^{-1}, 2 items individual^{-1}, and 0.38 items individual^{-1} from the Skudai River (Sarijan et al., 2019), respectively. Also suggesting high rates of contamination is the fact that 94.7% of the fish sampled in this study contained microplastic. This finding is higher than the 40% reported in the Skudai River (Sarijan et al., 2019), 25.7% in XiangXi River, China (Zhang et al., 2017), 45% in Texas (Peters and Bratton, 2016), and 54% in River Bourne, United Kingdom (Parker et al., 2022b). However, the occurrence in Semenyih River is lower than in some Asian countries such as 95.7% in Taihu Lake in China (Jabeen et al., 2017) and 100% in Han River, South Korea (Park et al., 2020a). Microplastic identified in GIT of some species (appendix 7) such as *Notopterus notopterus* (39 items individual^{-1}), *Clarias batrachus* (23 items individual^{-1}) and *Hypostomus plecostomus* (50 items individual^{-1}) are higher than the maximum observation reported in most studies ever conducted (~6-30 items per fish, reviewed by Galafassi et al., 2021).

In contrast, the microplastic load of Unionidae (58.3%, 3.708 items individual^{-1}, and 0.0003 items mg^{-1}) from the Semenyih Lake is lower than *Lasmigona costata* in the Grand River watershed, Canada (71.5%, 1.90 items individual^{-1}, 21 items g^{-1}; Wardlaw and Prosser, 2020) and *Anodonta anatine* (100%, 25.31 individual^{-1}; Berglund et al., 2019).

5.1.1 Differences in microplastic contamination between taxa

The higher-resolution counterstaining dye method detected differences between some benthic invertebrate families. Thus, Simuliidae contained more microplastic per mg dry tissue weight compared to Hydropsychidae, but there was no significant difference between other invertebrate families (Figure 4.3b). This did not align with the pattern established by Windsor et al (2019), who found that Baetidae had a larger microplastic load than Hydropsychidae and Heptageniidae. Other studies also reported differences in microplastic load between taxa. For instance, higher body loads of microplastic were found in Tubificidae, Chironomidae, and Asellidae compared to other taxa assessed in the Dommel River, Netherlands (Pan et al., 2021).

Many studies have failed to detect differences in body loads between taxa. The taxa selected covered a wide range of feeding guilds and trophic positions (Parker et al., 2022a) and the apparent lack of difference might be a result of individual specialisation that caused diet to be highly variable in space and time, especially for the fish taxa (Araújo et al., 2011). However, this study did not assess the difference between fish

species due to the small sample size. As reported here for the Semenyih, the variation in microplastic load between taxonomic grouping also partly depends on whether microplastic is expressed as abundance in the body or concentration. This mirrored the finding from Akindele et al (2019) where the microplastic abundance in *L.varicus* (1.71 ± 0.46 g^{-1}) was the highest compared to *T.fluviatilis* (6.1 ± 1.05 g^{-1}), but the pattern reversed when expressed in concentration.

Along with the visual inspection (lower resolution) method for microplastic identification, this study also adopted the counterstaining dye method (higher resolution) to identify smaller-sized microplastic. It was possible to enumerate material down to 4µm, which is within the nanoparticle size range. This counterstaining method prevents the overestimation of microplastic that can result from using Nile Red dye alone (Maxwell et al., 2020). All biological samples that were analysed using the higher-resolution method contained microplastic, while overall around 50% of those processed using the low-resolution method contained microplastic. Although the ratio of higher to lower resolution approach varied among the taxa, treated as a global value the microplastic abundance of invertebrates processed using the higher resolution method was 120 times higher than the lower resolution method. Future assessments of contamination can use this ratio to avoid underestimating the amount of microplastic in freshwater invertebrates when processing and identifying microplastic down to the nanoscale is not possible.

5.1.2 *Distribution of microplastic between different body parts of fish*

There was evidence that microplastics can translocate from GIT to other body parts of fish (Collard et al., 2018). In the Semenyih data, GIT contained more microplastic than muscle and gills tissue (Figure 4.2; Appendix 7). This is consistent with the findings from Colombia (Garcia et al., 2021) and Indonesia (Agustian Fareza and Sembiring, 2020), where higher abundance was identified in GIT compared to gills and muscle. Although the size of microplastic identified in different body parts was not measured in this study, evidence has shown that microplastic in gills are generally smaller than GIT (Su et al., 2019), and probably was uptake by the fish passively rather than active ingestion (Parker et al., 2021).

This is the first study in Malaysia that reported the presence of microplastic in the flesh of commercially important freshwater fish (Appendix 7). Although, in previous studies no microplastic was found in the flesh of fish sampled from the Han River in South Korea (Park et al., 2020a) and Marne and Seine Rivers (Collard et al., 2018) in France. This finding is concerning as the presence of microplastic in flesh indicated there was a translocation of microplastic from GIT to muscle tissue.

5.2 Spatial differences

Microplastic concentration in the water and deposited on the bed differed between sampling sites with a general increase in concentration downstream. This aligned generally with the pattern reported by Chen et al (2021a). The pattern, however, is complex. The upstream site (site 2) was just as contaminated with microplastic as the

downstream sites, and the same pattern was shown in the microplastic load of invertebrates. Site 2 is a recreation area with significant levels of trash input. Sampling was done following a major flood in the area, although it was unclear whether this increased microplastic input into the river through runoff and precipitation as mentioned in several studies (Matjašič et al., 2023; Werbowski et al., 2021). Thus, spatial patterns in the water and bed reported here may not be consistent over time, under different flow conditions.

The microplastic abundance and concentration in invertebrates significantly differed between sampling sites. This mirrored the pattern observed in invertebrates (Dahms et al., 2020; Garcia et al., 2021; Nel et al., 2018) and fish (Horton et al., 2018; Park et al., 2020b) from other regions. Nevertheless, this pattern is not typical to all rivers; e.g. Parker et al (2022a) failed to detect differences in, the microplastic load of fish captured from different parts of River Stour, Dorset. However, it was worth noting that the authors did not collect accompanying samples from the water and/or sediment, so it remains unclear whether the lack of pattern in the biota reflects a lack of pattern in environmental contamination, or that the two are unrelated.

5.3 Factors influencing microplastic body loads in aquatic insect larvae

5.3.1 Site contamination

Numerous studies have hypothesised that the degree of site pollution would alter the microplastic load in freshwater animals. Water velocity and depth would affect the dispersion and settlement of microplastics and their bioavailability to benthic

macroinvertebrates, and correspondingly the microplastic load in Chironomidae was found to be more connected with the microplastic concentrations in sediment than water (Dahms et al., 2020). In contrast, body loads in fish may be more correlated with the microplastic concentration in the water column (Park et al., 2020b). The amount of microplastic in mosquitofish (*Gambusia affinis*) was more closely related to the microplastic concentration in the water because the fish were more likely to ingest microplastic after a flood when the amount of microplastic in the water rose (Eppehimer et al., 2021).

Notably, no correlation was found between the abundance of microplastic in invertebrates in the Semenyih River and the amount of microplastic in the water or deposited on sediment. Both a simple rank correlation (rank of body loads vs. rank of bed loads for the 8 sites) and a more complex Generalised Additive Mixed Model (applied to raw site values) failed to detect a relationship between bed and body loads in the insects. A lack of pattern was also noted by Garcia et al (2021), although the most urbanised sites of the study contained the highest microplastic loads in the water, sediment, and invertebrates. Similarly, the microplastic load of fish sampled from Lake Michigan in the USA was not correlated with the patterns observed in the water column (McNeish et al., 2018). High sediment microplastic concentration was shown in the site with a lower velocity (Parker et al., 2022b), but microplastic load in invertebrates between sites was not significantly different, suggesting that aquatic animals at sites with high concentrations are not necessarily at greater risk of microplastic ingestion (Parker et al., 2022b).

Chen et al (in press) found that water and sediment collected from the same sampling sites of this study did not correlate and that the amount of microplastic deposited on the bed was highly variable within sites (within-site variation was as great as between site variation). This suggests that bed levels are highly spatially heterogeneous over scales of metres and that site-averaged analysed may overlook true patterns of exposure to benthic invertebrates. This may partly explain the lack of relations between site and biological contamination reported in this thesis. The sampling was designed to assess body loads in a range of taxa and generated data to evaluate different enumeration methods, and a different approach would be needed to assess whether body loads reflect bed contamination levels. Microplastic abundance and size in sediment are known to influence the size and microplastic in organisms within small areas (Pan et al., 2021), and other factors such as hydraulic heterogeneity would influence the bioavailability of microplastic (Dahms et al., 2020; Eppehimer et al., 2021; Matjašič et al., 2023; Windsor et al., 2019). Thus, paired bed and biological samples would need to be collected for the same patches of bed to properly assess links between environmental and biological contamination.

5.3.2 Body size

Body lengths of aquatic animals were weakly correlated to the microplastic abundance, with the pattern only present when all classes were pooled, or within insect larvae, but not for fish and mussels. Macroinvertebrates with higher biomass were found to ingest more microplastic than those with smaller biomass (Windsor et

al., 2019). Some other studies have failed to find such relationships; e.g. the body size of macroinvertebrates from the Vipacco River in Italy did not correlate with the microplastic abundance in the invertebrates (Bertoli et al., 2022).

Our finding aligned with Garcia et al (2021), although the body size of fish also influenced the abundance of microplastic ingested in their study. Similarly, the body length of fish captured from the Dorset Stour also did not influence the microplastic abundance of the fish, but the authors have narrowed the size range of fish in the study (Parker et al., 2022a). In contrast, larger fish in the River Thames in the UK had a higher likelihood of ingesting the most microplastic as they require a larger volume of food to meet energy demands; body loads, however, were not related to other ecological factors such as gender (Horton et al., 2018). Also, larger-sized round goby (*Neogobius melanostomus)* ingested more microplastic than the smaller individuals, although this trend was absent in other species or when taxa were pooled (McNeish et al., 2018). Similar patterns were observed in another Malaysian river, where the authors found body weights of freshwater fish correlated with microplastic abundance (Sarijan et al., 2019). Nevertheless, only 19 individual fishes were included in this study of the Semenyih, and so as small sample size might have hindered the relationship between body size and microplastic abundance in fish.

5.3.3 Feeding preference

Fish and invertebrates with various feeding preferences were compared for the microplastic load. In terms of invertebrates, collector-gatherers had a larger

concentration of microplastic than filterers. Collector-gatherer included in this study was the mayfly larvae (Ephemeroptera: Baetidae). Several studies have reported significantly higher microplastic accumulation in this taxon when compared to taxa from the other functional feeding groups (Akindele et al., 2019; Bertoli et al., 2022; Parker et al., 2022a). For instance, Parker et al (2022a) identified significantly more microplastic in the Ephemeroptera than in other taxa, but microplastic abundance between the other groups was the same. Gatherers were found to have ingested a more diverse range of microplastic types than predatory taxa (Akindele et al., 2019). However, results from stable isotope analyses suggested that feeding preference did not influence the microplastic load in aquatic animals, but taxa in higher trophic positions tend to contain a higher abundance of microplastic (Garcia et al., 2021).

There was no significant difference between the carnivorous and omnivorous fish from the Semenyih River. In another Malaysian river, herbivorous fish contained more microplastic than omnivorous fish, although there was no significant difference between bottom or water column feeders (Sarijan et al., 2019). However, in the Garonne River in France, bottom feeders had higher microplastic concentrations than column feeders; this trend was argued to be more influenced by the origins of the microplastic (sediment vs. water column) than by the trophic position of the fish (Garcia et al., 2021).

5.4 Microplastic characteristics

Microfibre was most commonly identified in the fish, sediment, and water sample in this study. This is in line with the findings of a recent study carried out in the same river, which also observed fibre as the river's most common type of microplastic (Chen et al., 2021a). However, compared to other places where more than half of the microplastic type retrieved from fish was fibre, fibre was less frequently consumed by fish in the Semenyih River (37.2%) (Galafassi et al., 2021; Horton et al., 2018; McGoran et al., 2017; Sarijan et al., 2019). In contrast, invertebrates included in this study mainly ingested fragmented microplastic. This is consistent with the discovery from the River Stour, Dorset, where macroinvertebrates mainly consumed blue-green fragments.

Microplastics of many different colours were identified in this study. The composition of microplastic colours identified in different sample types and taxa differed significantly. Despite white/transparent microplastics being most found in the environment (sediment/water), the fish and invertebrates from the same river mainly ingested blue and black microplastics. Similarly, Parker et al (2022a) also identified mainly blue/green and grey/black microplastic in their macroinvertebrate and fish samples. This is in line with a laboratory study in which wild omnivorous fish (*P. eigenmanniorum*) primarily consumed yellow and blue microplastics while avoiding white microplastic, despite the quantity of different coloured microplastics added to the experimental group was the same (Ríos et al., 2022). However, the mechanisms leading to the differential in microplastic colour identified in different taxa remain to be identified, as the authors did not provide an explanation for why the fish only ingested microplastics of a certain colour (Ríos et al., 2022). Nevertheless, some

suggest blue/green microplastic was most commonly ingested by macroinvertebrates as it was comparable to the food resources (algae) of the animals (Bertoli et al., 2022; Parker et al., 2022a).

The microplastic shapes and colours were significantly different between animals (fish and invertebrates), water and sediment. This suggests the aquatic animals might selectively/actively ingest certain types or colours of microplastic, instead of passively ingesting materials in proportions reflecting their availability in the surrounding environment (Garcia et al., 2021; Nan et al., 2020; Ríos et al., 2022). Benthic organisms were found to selectively ingest microplastic based on factors such as microplastic characteristics and environmental conditions (Pan et al., 2021). Parker et al. (2022a), on the other hand, recognised dominant microplastic types (size, colour, and form) that were consistent in macroinvertebrate and fish samples, indicating that the majority of the microplastic found at the sampling sites is of the same kind. A clear pattern from the Semenyih is that animals ingested more fragments than their availability in water or sediments might suggest, but it remains unclear whether patterns in ingestion of different colours reflect selection for colour per se or autocorrelation between colour and type.

5.5 Future implications

Microplastics are being reported in increasing frequency in different parts of the human body, such as the lungs (Amato-Lourenço et al., 2021), placenta (Ragusa et al., 2021), blood (Leslie et al., 2022), and even colon tissue (Ibrahim et al., 2021b). This is

concerning since exposure to microplastic might impair physiological processes by raising the risk of neurotoxicity, cancer, and GIT disturbance (Pang et al., 2021). However, very few studies used environmentally realistic exposure concentrations when assessing the ecological risk of microplastic (Cunningham and Sigwart, 2019). As more studies provide baseline microplastic abundance in different environments (Akdogan and Guven, 2019; Boyle and Örmeci, 2020; Cheung and Fok, 2016; Reid et al., 2019), it is important to conduct a risk assessment using these concentrations to improve assessment of biological implications of microplastic (Kotta et al., 2022).

The baseline concentration for a variety of freshwater invertebrate and fish taxa from the Semenyih River in Malaysia was presented in this study, along with information on the factors that would influence the microplastic load in wild aquatic animals, and the characteristics of microplastics that were most commonly consumed by these organisms. This is especially important in countries such as Malaysia, where many local communities rely on fish consumed directly from local rivers. This is certainly the case across the Semenyih, where fishermen are ever-present along the river and either consume fish themselves or sell it to local markets. Future research should use this baseline data to evaluate the ecological risk of microplastics to better understand how exposure to such microplastic concentrations might affect both ecological and human health. Moreover, it is advised to replicate the study in the other rivers to determine whether the outcomes provided here are typical of Malaysian rivers. To avoid underestimating the amount of microplastic in freshwater invertebrates, future assessments could use the ratio of higher resolution to lower resolution microplastic

identification methods provided in this study for a few commonly found invertebrate families in Malaysia, when processing and identifying microplastic down to the nanoscale is not feasible. It would be useful to test this ratio in other areas, to evaluate its consistency and hence utility for assessment.

CONCLUSION

6. Conclusion

This study provided the first evidence of microplastic occurrence in a wide range of freshwater fish and invertebrates in Malaysia, and the microplastic loads in the animals were higher than so far reported in the other studies. In addition, this study is also the first to report the presence of microplastic in the gills and flesh of fish, with GIT being the most contaminated part. The microplastic load in aquatic animals were found to varied between invertebrate species and sampling sites, despite the site contamination level would not alter the abundance of microplastic ingested by the invertebrates. Ecological factors such as body size and functional feeding groups only influenced the microplastic load in insect larvae, but not for mussels and fish. The most commonly found microplastic shape in the Semenyih River were fibre (in fish, water, and sediment) and fragment (in invertebrates), with the colour of white, blue, and black being the most dominant colour. However, there were notable differences in the microplastic shapes and colours retrieved between fish and invertebrate samples, as well as between water and sediment. This showed that the aquatic creatures may have intentionally or deliberately ingested microplastic. Finally, future research could use the ratio of higher resolution to lower resolution microplastic identification methods provided in this study for a few commonly found invertebrate families in Malaysia, especially when processing and identifying microplastic down to the nanoscale is not practical. This would prevent underestimating the amount of microplastic in freshwater invertebrates.

REFERENCES

7. References

Agustian Fareza, A., Sembiring, E., 2020. Occurence of Microplastics in Water, Sediment and Milkfish (Chanos chanos) in Citarum River Downstream (Case Study: Muara Gembong). E3S Web Conf. 148, 07005. https://doi.org/10.1051/e3sconf/202014807005

Akdogan, Z., Guven, B., 2019. Microplastics in the environment: A critical review of current understanding and identification of future research needs. Environ. Pollut. 254, 113011. https://doi.org/10.1016/j.envpol.2019.113011

Akindele, E.O., Ehlers, S.M., Koop, J.H.E., 2019. First empirical study of freshwater microplastics in West Africa using gastropods from Nigeria as bioindicators. Limnologica 78, 125708. https://doi.org/10.1016/j.limno.2019.125708

Al-Badaii, F., Shuhaimi-Othman, M., Gasim, M.B., 2013. Water Quality Assessment of the Semenyih River, Selangor, Malaysia. J. Chem. 2013, e871056. https://doi.org/10.1155/2013/871056

Alexander Tampang, A.M., Viswanathan, P.M., 2022. Occurrence, distribution and sources of microplastics in beach sediments of Miri coast, NW Borneo. Chemosphere 305, 135368. https://doi.org/10.1016/j.chemosphere.2022.135368

Alimi, O.S., Fadare, O.O., Okoffo, E.D., 2021. Microplastics in African ecosystems: Current knowledge, abundance, associated contaminants, techniques, and research needs. Sci. Total Environ. 755, 142422. https://doi.org/10.1016/j.scitotenv.2020.142422

Amato-Lourenço, L.F., Carvalho-Oliveira, R., Júnior, G.R., dos Santos Galvão, L., Ando, R.A., Mauad, T., 2021. Presence of airborne microplastics in human lung tissue. J. Hazard. Mater. 416, 126124. https://doi.org/10.1016/j.jhazmat.2021.126124

Amelia, T.S.M., Khalik, W.M.A.W.M., Ong, M.C., Shao, Y.T., Pan, H.-J., Bhubalan, K., 2021. Marine microplastics as vectors of major ocean pollutants and its hazards to the marine ecosystem and humans. Prog. Earth Planet. Sci. 8, 12. https://doi.org/10.1186/s40645-020-00405-4

Andrady, A.L., 2017. The plastic in microplastics: A review. Mar. Pollut. Bull. 119, 12–22. https://doi.org/10.1016/j.marpolbul.2017.01.082

Andrady, A.L., Hamid, H.S., Torikai, A., 2003. Effects of climate change and UV-B on materials. Photochem. Photobiol. Sci. 2, 68–72. https://doi.org/10.1039/B211085G

Andrady, A.L., Neal, M.A., 2009. Applications and societal benefits of plastics. Philos. Trans. R. Soc. B Biol. Sci. 364, 1977–1984. https://doi.org/10.1098/rstb.2008.0304

Angeler, D.G., Allen, C.R., Birgé, H.E., Drakare, S., McKie, B.G., Johnson, R.K., 2014. Assessing and managing freshwater ecosystems vulnerable to environmental change. Ambio 43, 113–125. https://doi.org/10.1007/s13280-014-0566-z

Araújo, M.S., Bolnick, D.I., Layman, C.A., 2011. The ecological causes of individual specialisation. Ecol. Lett. 14, 948–958. https://doi.org/10.1111/j.1461-0248.2011.01662.x

Atugoda, T., Vithanage, M., Wijesekara, H., Bolan, N., Sarmah, A.K., Bank, M.S., You, S., Ok, Y.S., 2021. Interactions between microplastics, pharmaceuticals and personal care products: Implications for vector transport. Environ. Int. 149, 106367. https://doi.org/10.1016/j.envint.2020.106367

Au, S.Y., Bruce, T.F., Bridges, W.C., Klaine, S.J., 2015. Responses of Hyalella azteca to acute and chronic microplastic exposures. Environ. Toxicol. Chem. 34, 2564–2572. https://doi.org/10.1002/etc.3093

Avio, C.G., Gorbi, S., Regoli, F., 2015. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from Adriatic Sea. Mar. Environ. Res., Particles in the Oceans: Implication for a safe marine environment 111, 18–26. https://doi.org/10.1016/j.marenvres.2015.06.014

Azevedo-Santos, V., Brito, M., Manoel, P., Perroca, J., Rodrigues-Filho, J.L., Paschoal, L., Gonçalves, G., Wolf, M., Blettler, M., Andrade, M., Nobile, A., Pontieri de Lima, F., Ruocco, A., da Silva, C., Perbiche-Neves, G., Portinho, J., Giarrizzo, T., Arcifa, M., Pelicice, F., 2021. Plastic pollution: A focus on freshwater biodiversity. AMBIO J. Hum. Environ. https://doi.org/10.1007/s13280-020-01496-5

Barboza, L.G.A., Cunha, S.C., Monteiro, C., Fernandes, J.O., Guilhermino, L., 2020. Bisphenol A and its analogs in muscle and liver of fish from the North East Atlantic Ocean in relation to microplastic contamination. Exposure and risk to human consumers. J. Hazard. Mater. 393, 122419. https://doi.org/10.1016/j.jhazmat.2020.122419

Bellasi, A., Binda, G., Pozzi, A., Galafassi, S., Volta, P., Bettinetti, R., 2020. Microplastic Contamination in Freshwater Environments: A Review, Focusing on Interactions with Sediments and Benthic Organisms. Environments 7, 30. https://doi.org/10.3390/environments7040030

Benson, N.U., Bassey, D.E., Palanisami, T., 2021. COVID pollution: impact of COVID-19 pandemic on global plastic waste footprint. Heliyon 7, e06343. https://doi.org/10.1016/j.heliyon.2021.e06343

Berglund, E., Fogelberg, V., Nilsson, P.A., Hollander, J., 2019. Microplastics in a freshwater mussel (Anodonta anatina) in Northern Europe. Sci. Total Environ. 697, 134192. https://doi.org/10.1016/j.scitotenv.2019.134192

Bertoli, M., Pastorino, P., Lesa, D., Renzi, M., Anselmi, S., Prearo, M., Pizzul, E., 2022. Microplastics accumulation in functional feeding guilds and functional habit groups of freshwater macrobenthic invertebrates: Novel insights in a riverine ecosystem. Sci. Total Environ. 804, 150207. https://doi.org/10.1016/j.scitotenv.2021.150207

Besseling, E., Quik, J.T.K., Sun, M., Koelmans, A.A., 2017. Fate of nano- and microplastic in freshwater systems: A modeling study. Environ. Pollut. 220, 540–548. https://doi.org/10.1016/j.envpol.2016.10.001

Bianco, A., Passananti, M., 2020. Atmospheric Micro and Nanoplastics: An Enormous Microscopic Problem. Sustainability 12, 7327. https://doi.org/10.3390/su12187327

Biginagwa, F.J., Mayoma, B.S., Shashoua, Y., Syberg, K., Khan, F.R., 2016. First evidence of microplastics in the African Great Lakes: Recovery from Lake Victoria Nile perch and Nile tilapia. J. Gt. Lakes Res. 42, 146–149. https://doi.org/10.1016/j.jglr.2015.10.012

Binda, G., Spanu, D., Monticelli, D., Pozzi, A., Bellasi, A., Bettinetti, R., Carnati, S., Nizzetto, L., 2021. Unfolding the interaction between microplastics and (trace) elements in water: A critical review. Water Res. 204, 117637. https://doi.org/10.1016/j.watres.2021.117637

Blettler, M.C.M., Abrial, E., Khan, F.R., Sivri, N., Espinola, L.A., 2018. Freshwater plastic pollution: Recognizing research biases and identifying knowledge gaps. Water Res. 143, 416–424. https://doi.org/10.1016/j.watres.2018.06.015

Botterell, Z., Beaumont, N., Dorrington, T., Steinke, M., Thompson, R., Lindeque, P., 2018. Bioavailability and effects of microplastics on marine zooplankton: A review. Environ. Pollut. 245. https://doi.org/10.1016/j.envpol.2018.10.065

Bowley, J., Baker-Austin, C., Porter, A., Hartnell, R., Lewis, C., 2021. Oceanic Hitchhikers – Assessing Pathogen Risks from Marine Microplastic. Trends Microbiol. 29, 107–116. https://doi.org/10.1016/j.tim.2020.06.011

Boyle, K., Örmeci, B., 2020. Microplastics and Nanoplastics in the Freshwater and Terrestrial Environment: A Review. Water 12, 2633. https://doi.org/10.3390/w12092633

Browne, M.A., Galloway, T., Thompson, R., 2007. Microplastic—an emerging contaminant of potential concern? Integr. Environ. Assess. Manag. 3, 559–561. https://doi.org/10.1002/ieam.5630030412

Büks, F., Loes van Schaik, N., Kaupenjohann, M., 2020. What do we know about how the terrestrial multicellular soil fauna reacts to microplastic? SOIL 6, 245–267. https://doi.org/10.5194/soil-6-245-2020

Burns, E.E., Boxall, A.B.A., 2018. Microplastics in the aquatic environment: Evidence for or against adverse impacts and major knowledge gaps. Environ. Toxicol. Chem. 37, 2776–2796. https://doi.org/10.1002/etc.4268

Buwono, N.R., Risjani, Y., Soegianto, A., 2021. Distribution of microplastic in relation to water quality parameters in the Brantas River, East Java, Indonesia. Environ. Technol. Innov. 24. https://doi.org/10.1016/j.eti.2021.101915

Cai, L., Wang, J., Tan, Z., Zhan, Z., Tan, X., Chen, Q., 2017. Characteristic of microplastics in the atmospheric fallout from Dongguan city, China: preliminary research and first evidence. Environ. Sci. Pollut. Res. 24. https://doi.org/10.1007/s11356-017-0116-x

Caldwell, J., Taladriz-Blanco, P., Lehner, R., Lubskyy, A., Ortuso, R.D., Rothen-Rutishauser, B., Petri-Fink, A., 2022. The micro-, submicron-, and nanoplastic hunt: A review of detection methods for plastic particles. Chemosphere 293, 133514. https://doi.org/10.1016/j.chemosphere.2022.133514

Carpenter, E., Anderson, S., Harvey, G., Miklas, H., Peck, B., 1972. Polystyrene Spherules in Coastal Waters. Science 178, 749–50. https://doi.org/10.1126/science.178.4062.749

Chang, M., 2015. Reducing microplastics from facial exfoliating cleansers in wastewater through treatment versus consumer product decisions. Mar. Pollut. Bull. 101, 330–333. https://doi.org/10.1016/j.marpolbul.2015.10.074

Chen, H.L., Gibbins, C.N., Selvam, S.B., Ting, K.N., 2021a. Spatio-temporal variation of microplastic along a rural to urban transition in a tropical river. Environ. Pollut. 289, 117895. https://doi.org/10.1016/j.envpol.2021.117895

Chen, H.L., Nath, T.K., Chong, S., Foo, V., Gibbins, C., Lechner, A.M., 2021b. The plastic waste problem in Malaysia: management, recycling and disposal of local and global plastic waste. SN Appl. Sci. 3, 437. https://doi.org/10.1007/s42452-021-04234-y

Chen, H.L., Selvam, S.B., Ting, K.N., Gibbins, C.N., 2021c. Microplastic pollution in freshwater systems in Southeast Asia: contamination levels, sources, and ecological impacts. Environ. Sci. Pollut. Res. 28, 54222–54237. https://doi.org/10.1007/s11356-021-15826-x

Chen, H.L., Selvam, S.B., Ting, K.N., Gibbins, C.N., In Press. Microplastic concentrations in river water and bed sediments in a tropical river: implications for water quality monitoring. Environ. Monit. Assess.

Chen, H.L., Selvam, S.B., Ting, K.N., Tshai, K.Y., Gibbins, C.N., 2022. Relative contributions of different local sources to riverborne microplastic in a mixed landuse area within a tropical catchment. Environ. Res. 210, 112972. https://doi.org/10.1016/j.envres.2022.112972

Cheung, P.K., Fok, L., 2016. Evidence of microbeads from personal care product contaminating the sea. Mar. Pollut. Bull. 109, 582–585. https://doi.org/10.1016/j.marpolbul.2016.05.046

Chinfak, N., Sompongchaiyakul, P., Charoenpong, C., Shi, H., Yeemin, T., Zhang, J., 2021. Abundance, composition, and fate of microplastics in water, sediment, and shellfish in the Tapi-Phumduang River system and Bandon Bay, Thailand. Sci. Total Environ. 781, 146700. https://doi.org/10.1016/j.scitotenv.2021.146700

Collard, F., Gasperi, J., Gilbert, B., Eppe, G., Azimi, S., Rocher, V., Tassin, B., 2018. Anthropogenic particles in the stomach contents and liver of the freshwater fish

Squalius cephalus. Sci. Total Environ. 643, 1257–1264. https://doi.org/10.1016/j.scitotenv.2018.06.313
Covich, A., Palmer, M., Crowl, T., 1999. The Role of Benthic Invertebrate Species in Freshwater Ecosystems. Bioscience 49. https://doi.org/10.2307/1313537
Cowger, W., Steinmetz, Z., Gray, A., Munno, K., Lynch, J., Hapich, H., Primpke, S., De Frond, H., Rochman, C., Herodotou, O., 2021. Microplastic Spectral Classification Needs an Open Source Community: Open Specy to the Rescue! Anal. Chem. 93, 7543–7548. https://doi.org/10.1021/acs.analchem.1c00123
Cummins, K.W., Wilzbach, M., Kolouch, B., Merritt, R., 2022. Estimating Macroinvertebrate Biomass for Stream Ecosystem Assessments. Int. J. Environ. Res. Public. Health 19, 3240. https://doi.org/10.3390/ijerph19063240
Cunningham, E.M., Sigwart, J.D., 2019. Environmentally Accurate Microplastic Levels and Their Absence from Exposure Studies. Integr. Comp. Biol. 59, 1485–1496. https://doi.org/10.1093/icb/icz068
Dahms, H.T.J., van Rensburg, G.J., Greenfield, R., 2020. The microplastic profile of an urban African stream. Sci. Total Environ. 731, 138893. https://doi.org/10.1016/j.scitotenv.2020.138893
Dalu, T., Banda, T., Mutshekwa, T., Munyai, L.F., Cuthbert, R.N., 2021. Effects of urbanisation and a wastewater treatment plant on microplastic densities along a subtropical river system. Environ. Sci. Pollut. Res. 28, 36102–36111. https://doi.org/10.1007/s11356-021-13185-1
De Witte, B., Devriese, L., Bekaert, K., Hoffman, S., Vandermeersch, G., Cooreman, K., Robbens, J., 2014. Quality assessment of the blue mussel (Mytilus edulis): Comparison between commercial and wild types. Mar. Pollut. Bull. 85, 146–155. https://doi.org/10.1016/j.marpolbul.2014.06.006
Dehaut, A., Cassone, A.-L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A., Duflos, G., Paul-Pont, I., 2016. Microplastics in seafood: Benchmark protocol for their extraction and characterization. Environ. Pollut. Barking Essex 1987 215, 223–233. https://doi.org/10.1016/j.envpol.2016.05.018
Dikareva, N., Simon, K.S., 2019. Microplastic pollution in streams spanning an urbanisation gradient. Environ. Pollut. 250, 292–299. https://doi.org/10.1016/j.envpol.2019.03.105
Ding, J., Zhang, S., Razanajatovo, R.M., Zou, H., Zhu, W., 2018. Accumulation, tissue distribution, and biochemical effects of polystyrene microplastics in the freshwater fish red tilapia (Oreochromis niloticus). Environ. Pollut. 238, 1–9. https://doi.org/10.1016/j.envpol.2018.03.001
Drummond, J.D., Nel, H.A., Packman, A.I., Krause, S., 2020. Significance of Hyporheic Exchange for Predicting Microplastic Fate in Rivers. Environ. Sci. Technol. Lett. 7, 727–732. https://doi.org/10.1021/acs.estlett.0c00595
Du, J., Qv, W., Niu, Y., Qv, M., Jin, K., Xie, J., Li, Z., 2022. Nanoplastic pollution inhibits stream leaf decomposition through modulating microbial metabolic activity and fungal community structure. J. Hazard. Mater. 424, 127392. https://doi.org/10.1016/j.jhazmat.2021.127392
Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.-I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.-H., Soto, D., Stiassny, M.L.J., Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. 81, 163–182. https://doi.org/10.1017/S1464793105006950
Duis, K., Coors, A., 2016. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environ. Sci. Eur. 28, 2. https://doi.org/10.1186/s12302-015-0069-y

Ehlers, S.M., Manz, W., Koop, J., 2019. Microplastics of different characteristics are incorporated into the larval cases of the freshwater caddisfly Lepidostoma basale. Aquat. Biol. https://doi.org/10.3354/AB00711

Eppehimer, D.E., Hamdhani, H., Hollien, K.D., Nemec, Z.C., Lee, L.N., Quanrud, D.M., Bogan, M.T., 2021. Impacts of baseflow and flooding on microplastic pollution in an effluent-dependent arid land river in the USA. Environ. Sci. Pollut. Res. 28, 45375–45389. https://doi.org/10.1007/s11356-021-13724-w

Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. PLOS ONE 9, e111913. https://doi.org/10.1371/journal.pone.0111913

Fan, Y., Zheng, K., Zhu, Z., Chen, G., Peng, X., 2019. Distribution, sedimentary record, and persistence of microplastics in the Pearl River catchment, China. Environ. Pollut. 251, 862–870. https://doi.org/10.1016/j.envpol.2019.05.056

Faure, F., Corbaz, M., Baecher, H., Felippe, L., 2012. Pollution due to plastics and microplastics in Lake Geneva and in the Mediterranean Sea. Arch. Sci. 7.

Frei, S., Piehl, S., Gilfedder, B.S., Löder, M.G.J., Krutzke, J., Wilhelm, L., Laforsch, C., 2019. Occurence of microplastics in the hyporheic zone of rivers. Sci. Rep. 9, 15256. https://doi.org/10.1038/s41598-019-51741-5

Galafassi, S., Campanale, C., Massarelli, C., Uricchio, V.F., Volta, P., 2021. Do Freshwater Fish Eat Microplastics? A Review with A Focus on Effects on Fish Health and Predictive Traits of MPs Ingestion. Water 13, 2214. https://doi.org/10.3390/w13162214

Gallitelli, L., Cera, A., Cesarini, G., Pietrelli, L., Scalici, M., 2021. Preliminary indoor evidences of microplastic effects on freshwater benthic macroinvertebrates. Sci. Rep. 11, 720. https://doi.org/10.1038/s41598-020-80606-5

Garcia, A.G., Suárez, D.C., Li, J., Rotchell, J.M., 2021. A comparison of microplastic contamination in freshwater fish from natural and farmed sources. Environ. Sci. Pollut. Res. Int. 28, 14488–14497. https://doi.org/10.1007/s11356-020-11605-2

Garcia, F., de Carvalho, A.R., Riem-Galliano, L., Tudesque, L., Albignac, M., ter Halle, A., Cucherousset, J., 2021. Stable Isotope Insights into Microplastic Contamination within Freshwater Food Webs. Environ. Sci. Technol. 55, 1024–1035. https://doi.org/10.1021/acs.est.0c06221

Gaylarde, C., Baptista-Neto, J.A., da Fonseca, E.M., 2021. Plastic microfibre pollution: how important is clothes' laundering? Heliyon 7, e07105. https://doi.org/10.1016/j.heliyon.2021.e07105

Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3, e1700782. https://doi.org/10.1126/sciadv.1700782

Green, P.A., Vörösmarty, C.J., Harrison, I., Farrell, T., Sáenz, L., Fekete, B.M., 2015. Freshwater ecosystem services supporting humans: Pivoting from water crisis to water solutions. Glob. Environ. Change 34, 108–118. https://doi.org/10.1016/j.gloenvcha.2015.06.007

Hahladakis, J.N., Velis, C.A., Weber, R., Iacovidou, E., Purnell, P., 2018. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. J. Hazard. Mater. 344, 179–199. https://doi.org/10.1016/j.jhazmat.2017.10.014

Hamza, A., Aidil, M., Rusli, U., Ibrahim, Y., 2020. Microplastic Occurrence in Seaturtle Nesting Beach Sediments from Terengganu, Malaysia. J. Green Eng. 10, 5712–5729.

Hamzah, S.R., Altrawneh, R.S., Anuar, S.T., Khalik, W.M.A.W.M., Kolandhasamy, P., Ibrahim, Y.S., 2021. Ingestion of microplastics by the estuarine polychaete, Namalycastis sp. in the Setiu Wetlands, Malaysia. Mar. Pollut. Bull. 170, 112617. https://doi.org/10.1016/j.marpolbul.2021.112617

Horton, A.A., Jürgens, M.D., Lahive, E., van Bodegom, P.M., Vijver, M.G., 2018. The influence of exposure and physiology on microplastic ingestion by the freshwater fish Rutilus rutilus (roach) in the River Thames, UK. Environ. Pollut. Barking Essex 1987 236, 188–194. https://doi.org/10.1016/j.envpol.2018.01.044

Huang, Y., Li, W., Gao, J., Wang, F., Yang, W., Han, L., Lin, D., Min, B., Zhi, Y., Grieger, K., Yao, J., 2021. Effect of microplastics on ecosystem functioning: Microbial nitrogen removal mediated by benthic invertebrates. Sci. Total Environ. 754, 142133. https://doi.org/10.1016/j.scitotenv.2020.142133

Hüffer, T., Weniger, A.-K., Hofmann, T., 2018. Sorption of organic compounds by aged polystyrene microplastic particles. Environ. Pollut. 236, 218–225. https://doi.org/10.1016/j.envpol.2018.01.022

Hurley, R., Woodward, J., Rothwell, J., 2017. Ingestion of Microplastics by Freshwater Tubifex Worms. Environ. Sci. Technol. 51, 12844–12851. https://doi.org/10.1021/acs.est.7b03567

Hurley, R., Woodward, J., Rothwell, J.J., 2018. Microplastic contamination of river beds significantly reduced by catchment-wide flooding. Nat. Geosci. 11, 251–257. https://doi.org/10.1038/s41561-018-0080-1

Ibrahim, Y.S., Hamzah, S.R., Khalik, W.M.A.W.M., Ku Yusof, K.M.K., Anuar, S.T., 2021a. Spatiotemporal microplastic occurrence study of Setiu Wetland, South China Sea. Sci. Total Environ. 788, 147809. https://doi.org/10.1016/j.scitotenv.2021.147809

Ibrahim, Y.S., Tuan Anuar, S., Azmi, A.A., Wan Mohd Khalik, W.M.A., Lehata, S., Hamzah, S.R., Ismail, D., Ma, Z.F., Dzulkarnaen, A., Zakaria, Z., Mustaffa, N., Tuan Sharif, S.E., Lee, Y.Y., 2021b. Detection of microplastics in human colectomy specimens. JGH Open 5, 116–121. https://doi.org/10.1002/jgh3.12457

Irfan, M., Qadir, A., Mumtaz, M., Ahmad, S.R., 2020. An unintended challenge of microplastic pollution in the urban surface water system of Lahore, Pakistan. Environ. Sci. Pollut. Res. 27, 16718–16730. https://doi.org/10.1007/s11356-020-08114-7

Jaafar, N., Azfaralariff, A., Musa, S.M., Mohamed, M., Yusoff, A.H., Lazim, A.M., 2021. Occurrence, distribution and characteristics of microplastics in gastrointestinal tract and gills of commercial marine fish from Malaysia. Sci. Total Environ. 799, 149457. https://doi.org/10.1016/j.scitotenv.2021.149457

Jabeen, K., Su, L., Li, J., Yang, D., Tong, C., Mu, J., Shi, H., 2017. Microplastics and mesoplastics in fish from coastal and fresh waters of China. Environ. Pollut. 221, 141–149. https://doi.org/10.1016/j.envpol.2016.11.055

Jâms, I.B., Windsor, F.M., Poudevigne-Durance, T., Ormerod, S.J., Durance, I., 2020. Estimating the size distribution of plastics ingested by animals. Nat. Commun. 11, 1594. https://doi.org/10.1038/s41467-020-15406-6

Jesmin Haque, S., Roslan, F., 2017. Groundwater Quality Aspect Owing to Urbanization in Langat Basin (Kajang, Semenyih) Malaysia. Asian J. Environ. Ecol. 4, 1–9. https://doi.org/10.9734/AJEE/2017/36049

Karami, A., 2017. Gaps in aquatic toxicological studies of microplastics. Chemosphere 184, 841–848. https://doi.org/10.1016/j.chemosphere.2017.06.048

Kasamesiri, P., Meksumpun, C., Meksumpun, S., Ruengsorn, C., 2021. Assessment on Microplastics Contamination in Freshwater Fish: A Case Study of the Ubolratana Reservoir, Thailand. Int. J. Geomate 20, 62–68. https://doi.org/10.21660/2020.77.6108

Kasamesiri, P., Thaimuangpho, W., 2020. Microplastics ingestion by freshwater fish in the Chi River, Thailand. Int. J. GEOMATE 18, 114–119. https://doi.org/10.21660/2020.67.9110

Kataoka, T., Nihei, Y., Kudou, K., Hinata, H., 2019. Assessment of the sources and inflow processes of microplastics in the river environments of Japan. Environ. Pollut. 244, 958–965. https://doi.org/10.1016/j.envpol.2018.10.111

Khalik, W., Ibrahim, Y., Tamilselvam, R.R., Anuar, S., 2017. Isolation and Characterisation of Microplastic Abundance in Lates calcarifer from Setiu Wetlands, Malaysia. Malays. J. Anal. Sci. 21, 1054–1064.

Khalik, W.M.A.W.M., Ibrahim, Y.S., Tuan Anuar, S., Govindasamy, S., Baharuddin, N.F., 2018. Microplastics analysis in Malaysian marine waters: A field study of Kuala Nerus and Kuantan. Mar. Pollut. Bull. 135, 451–457. https://doi.org/10.1016/j.marpolbul.2018.07.052

Klein, S., Worch, E., Knepper, T.P., 2015. Occurrence and Spatial Distribution of Microplastics in River Shore Sediments of the Rhine-Main Area in Germany. Environ. Sci. Technol. 49, 6070–6076. https://doi.org/10.1021/acs.est.5b00492

Klun, B., Rozman, U., Ogrizek, M., Kalčíková, G., 2022. The first plastic produced, but the latest studied in microplastics research: The assessment of leaching, ecotoxicity and bioadhesion of Bakelite microplastics. Environ. Pollut. 307, 119454. https://doi.org/10.1016/j.envpol.2022.119454

Kooi, M., Koelmans, A.A., 2019. Simplifying Microplastic via Continuous Probability Distributions for Size, Shape, and Density. Environ. Sci. Technol. Lett. 6, 551–557. https://doi.org/10.1021/acs.estlett.9b00379

Kotta, J., Lenz, M., Barboza, F.R., Jänes, H., Grande, P.A.D., Beck, A., Van Colen, C., Hamm, T., Javidpour, J., Kaasik, A., Pantó, G., Szava-Kovats, R., Orav-Kotta, H., Lees, L., Loite, S., Canning-Clode, J., Gueroun, S.K.M., Kõivupuu, A., 2022. Blueprint for the ideal microplastic effect study: Critical issues of current experimental approaches and envisioning a path forward. Sci. Total Environ. 838, 156610. https://doi.org/10.1016/j.scitotenv.2022.156610

Krause, S., Baranov, V., Nel, H.A., Drummond, J.D., Kukkola, A., Hoellein, T., Sambrook Smith, G.H., Lewandowski, J., Bonet, B., Packman, A.I., Sadler, J., Inshyna, V., Allen, S., Allen, D., Simon, L., Mermillod-Blondin, F., Lynch, I., 2021. Gathering at the top? Environmental controls of microplastic uptake and biomagnification in freshwater food webs. Environ. Pollut. 268, 115750. https://doi.org/10.1016/j.envpol.2020.115750

Kühn, S., van Werven, B., van Oyen, A., Meijboom, A., Bravo Rebolledo, E.L., van Franeker, J.A., 2017. The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. Mar. Pollut. Bull. 115, 86–90. https://doi.org/10.1016/j.marpolbul.2016.11.034

Kukkola, A., Krause, S., Lynch, I., Sambrook Smith, G.H., Nel, H., 2021. Nano and microplastic interactions with freshwater biota – Current knowledge, challenges and future solutions. Environ. Int. 152, 106504. https://doi.org/10.1016/j.envint.2021.106504

Kumar, R., Sharma, P., Manna, C., Jain, M., 2021. Abundance, interaction, ingestion, ecological concerns, and mitigation policies of microplastic pollution in riverine ecosystem: A review. Sci. Total Environ. 782, 146695. https://doi.org/10.1016/j.scitotenv.2021.146695

Lambert, C.P., Walling, D.E., 1988. Measurement of channel storage of suspended sediment in a gravel-bed river. CATENA 15, 65–80. https://doi.org/10.1016/0341-8162(88)90017-3

Lebreton, L.C.M., van der Zwet, J., Damsteeg, J.-W., Slat, B., Andrady, A., Reisser, J., 2017. River plastic emissions to the world's oceans. Nat. Commun. 8, 15611. https://doi.org/10.1038/ncomms15611

Lehtiniemi, M., Hartikainen, S., Näkki, P., Engström-Öst, J., Koistinen, A., Setälä, O., 2018. Size matters more than shape: Ingestion of primary and secondary microplastics by small predators. Food Webs 17, e00097. https://doi.org/10.1016/j.fooweb.2018.e00097

Leslie, H.A., Brandsma, S.H., van Velzen, M.J.M., Vethaak, A.D., 2017. Microplastics en route: Field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota. Environ. Int. 101, 133–142. https://doi.org/10.1016/j.envint.2017.01.018

Leslie, H.A., J. M. van Velzen, M., Brandsma, S.H., Vethaak, D., Garcia-Vallejo, J.J., Lamoree, M.H., 2022. Discovery and quantification of plastic particle pollution in human blood. Environ. Int. 107199. https://doi.org/10.1016/j.envint.2022.107199

Li, L., Su, L., Cai, H., Rochman, C.M., Li, Q., Kolandhasamy, P., Peng, J., Shi, H., 2019. The uptake of microfibers by freshwater Asian clams (Corbicula fluminea) varies based upon physicochemical properties. Chemosphere 221, 107–114. https://doi.org/10.1016/j.chemosphere.2019.01.024

Liang, Y., Tan, Q., Song, Q., Li, J., 2021. An analysis of the plastic waste trade and management in Asia. Waste Manag. 119, 242–253. https://doi.org/10.1016/j.wasman.2020.09.049

Lin, C.-T., Chiu, M.-C., Kuo, M.-H., 2021. Effects of anthropogenic activities on microplastics in deposit-feeders (Diptera: Chironomidae) in an urban river of Taiwan. Sci. Rep. 11, 400. https://doi.org/10.1038/s41598-020-79881-z

Liu, Q., Schauer, J., 2021. Airborne Microplastics from Waste as a Transmission Vector for COVID-19. Aerosol Air Qual. Res. 21, 200439. https://doi.org/10.4209/aaqr.2020.07.0439

Lu, H.-C., Ziajahromi, S., Neale, P.A., Leusch, F.D.L., 2021. A systematic review of freshwater microplastics in water and sediments: Recommendations for harmonisation to enhance future study comparisons. Sci. Total Environ. 781, 146693. https://doi.org/10.1016/j.scitotenv.2021.146693

Lusher, A.L., Munno, K., Hermabessiere, L., Carr, S., 2020. Isolation and Extraction of Microplastics from Environmental Samples: An Evaluation of Practical Approaches and Recommendations for Further Harmonization. Appl. Spectrosc. 74, 1049–1065. https://doi.org/10.1177/0003702820938993

Malaysia Plastic Manufacturer Association, 2021. Annual Report 2021.

Marrone, A., La Russa, M., Randazzo, L., La Russa, D., Cellini, E., Pellegrino, D., 2021. Microplastics in the Center of Mediterranean: Comparison of the Two Calabrian Coasts and Distribution from Coastal Areas to the Open Sea. Int. J. Environ. Res. Public. Health 18, 10712. https://doi.org/10.3390/ijerph182010712

Masura, J., Baker, J., Foster, G., Arthur, C., Herring, C.E., 2015. Laboratory Methods for the Analysis of Microplastics in the Marine Environment : Recommendations for Quantifying Synthetic Particles in Waters and Sediments. undefined.

Matjašič, T., Mori, N., Hostnik, I., Bajt, O., Kovač Viršek, M., 2023. Microplastic pollution in small rivers along rural–urban gradients: Variations across catchments and between water column and sediments. Sci. Total Environ. 858, 160043. https://doi.org/10.1016/j.scitotenv.2022.160043

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., Kaminuma, T., 2001. Plastic Resin Pellets as a Transport Medium for Toxic Chemicals in the Marine Environment. Environ. Sci. Technol. 35, 318–324. https://doi.org/10.1021/es0010498

Matsuguma, Y., Takada, H., Kumata, H., Kanke, H., Sakurai, S., Suzuki, T., Itoh, M., Okazaki, Y., Boonyatumanond, R., Zakaria, M.P., Weerts, S., Newman, B., 2017. Microplastics in Sediment Cores from Asia and Africa as Indicators of Temporal Trends in Plastic Pollution. Arch. Environ. Contam. Toxicol. 73, 230–239. https://doi.org/10.1007/s00244-017-0414-9

Maxwell, S.H., Melinda K, F., Matthew, G., 2020. Counterstaining to Separate Nile Red-Stained Microplastic Particles from Terrestrial Invertebrate Biomass. Environ. Sci. Technol. 54, 5580–5588. https://doi.org/10.1021/acs.est.0c00711

McCormick, A., Hoellein, T.J., Mason, S.A., Schluep, J., Kelly, J.J., 2014. Microplastic is an Abundant and Distinct Microbial Habitat in an Urban River. Environ. Sci. Technol. 48, 11863–11871. https://doi.org/10.1021/es503610r

McCormick, A.R., Hoellein, T.J., London, M.G., Hittie, J., Scott, J.W., Kelly, J.J., 2016. Microplastic in surface waters of urban rivers: concentration, sources, and associated bacterial assemblages. Ecosphere 7, e01556. https://doi.org/10.1002/ecs2.1556

McGoran, A.R., Clark, P.F., Morritt, D., 2017. Presence of microplastic in the digestive tracts of European flounder, Platichthys flesus, and European smelt, Osmerus eperlanus, from the River Thames. Environ. Pollut. 220, 744–751. https://doi.org/10.1016/j.envpol.2016.09.078

McNeish, R.E., Kim, L.H., Barrett, H.A., Mason, S.A., Kelly, J.J., Hoellein, T.J., 2018. Microplastic in riverine fish is connected to species traits. Sci. Rep. 8, 11639. https://doi.org/10.1038/s41598-018-29980-9

Meijer, L.J.J., van Emmerik, T., van der Ent, R., Schmidt, C., Lebreton, L., 2021. More than 1000 rivers account for 80% of global riverine plastic emissions into the ocean. Sci. Adv. 7, eaaz5803. https://doi.org/10.1126/sciadv.aaz5803

Meng, Y., Kelly, F.J., Wright, S.L., 2020. Advances and challenges of microplastic pollution in freshwater ecosystems: A UK perspective. Environ. Pollut. 256, 113445. https://doi.org/10.1016/j.envpol.2019.113445

Michler-Kozma, D.N., Neu, T.R., Gabel, F., 2021. Environmental conditions affect the food quality of plastic associated biofilms for the benthic grazer Physa fontinalis. Sci. Total Environ. 151663. https://doi.org/10.1016/j.scitotenv.2021.151663

Muhammad Husin, M.J., Mazlan, N., Shalom, J., Saud, S.N., Abdullah Sani, M.S., 2021. Evaluation of microplastics ingested by sea cucumber Stichopus horrens in Pulau Pangkor, Perak, Malaysia. Environ. Sci. Pollut. Res. 28, 61592–61600. https://doi.org/10.1007/s11356-021-15099-4

Murphy, F., Ewins, C., Carbonnier, F., Quinn, B., 2016. Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment. Environ. Sci. Technol. 50, 5800–5808. https://doi.org/10.1021/acs.est.5b05416

Nan, B., Su, L., Kellar, C., Craig, N.J., Keough, M.J., Pettigrove, V., 2020. Identification of microplastics in surface water and Australian freshwater shrimp Paratya australiensis in Victoria, Australia. Environ. Pollut. 259, 113865. https://doi.org/10.1016/j.envpol.2019.113865

Napper, I.E., Davies, B.F.R., Clifford, H., Elvin, S., Koldewey, H.J., Mayewski, P.A., Miner, K.R., Potocki, M., Elmore, A.C., Gajurel, A.P., Thompson, R.C., 2020. Reaching New Heights in Plastic Pollution—Preliminary Findings of Microplastics on Mount Everest. One Earth 3, 621–630. https://doi.org/10.1016/j.oneear.2020.10.020

Napper, I.E., Thompson, R.C., 2016. Release of synthetic microplastic plastic fibres from domestic washing machines: Effects of fabric type and washing conditions. Mar. Pollut. Bull. 112, 39–45. https://doi.org/10.1016/j.marpolbul.2016.09.025

Nel, H.A., Dalu, T., Wasserman, R.J., 2018. Sinks and sources: Assessing microplastic abundance in river sediment and deposit feeders in an Austral temperate urban river system. Sci. Total Environ. 612, 950–956. https://doi.org/10.1016/j.scitotenv.2017.08.298

Nizzetto, L., Bussi, G., Futter, M.N., Butterfield, D., Whitehead, P.G., 2016a. A theoretical assessment of microplastic transport in river catchments and their retention by soils

and river sediments. Environ. Sci. Process. Impacts 18, 1050–1059. https://doi.org/10.1039/c6em00206d
Nizzetto, L., Futter, M., Langaas, S., 2016b. Are Agricultural Soils Dumps for Microplastics of Urban Origin? Environ. Sci. Technol. 50, 10777–10779. https://doi.org/10.1021/acs.est.6b04140
Noik, V.J., Tuah, P.M., 2015. A First Survey on the Abundance of Plastics Fragments and Particles on Two Sandy Beaches in Kuching, Sarawak, Malaysia. IOP Conf. Ser. Mater. Sci. Eng. 78, 012035. https://doi.org/10.1088/1757-899X/78/1/012035
O'Connor, J.D., Lally, H.T., Koelmans, A.A., Mahon, A.M., O'Connor, I., Nash, R., O'Sullivan, J.J., Bruen, M., Heerey, L., Murphy, S., 2022. Modelling the transfer and accumulation of microplastics in a riverine freshwater food web. Environ. Adv. 8, 100192. https://doi.org/10.1016/j.envadv.2022.100192
O'Connor, J.D., Mahon, A.M., Ramsperger, A.F.R.M., Trotter, B., Redondo-Hasselerharm, P.E., Koelmans, A.A., Lally, H.T., Murphy, S., 2020. Microplastics in Freshwater Biota: A Critical Review of Isolation, Characterization, and Assessment Methods. Glob. Chall. 4, 1800118. https://doi.org/10.1002/gch2.201800118
Osorio, E.D., Tanchuling, M.A.N., Diola, Ma.B.L.D., 2021. Microplastics Occurrence in Surface Waters and Sediments in Five River Mouths of Manila Bay. Front. Environ. Sci. 9, 364. https://doi.org/10.3389/fenvs.2021.719274
Pan, C.-G., Mintenig, S.M., Redondo-Hasselerharm, P.E., Neijenhuis, P.H.M.W., Yu, K.-F., Wang, Y.-H., Koelmans, A.A., 2021. Automated µFTIR Imaging Demonstrates Taxon-Specific and Selective Uptake of Microplastic by Freshwater Invertebrates. Environ. Sci. Technol. 55, 9916–9925. https://doi.org/10.1021/acs.est.1c03119
Pang, L.-Y., Sonagara, S., Oduwole, O., Gibbins, C., Nee, T.K., 2021. Microplastics - an emerging silent menace to public health. Life Sci. Med. Biomed. 5. https://doi.org/10.28916/lsmb.5.1.2021.72
Pariatamby, A., Hamid, F.S., Bhatti, M.S., Anuar, Norkhairah, Anuar, Norkhairiyah, 2020. Status of Microplastic Pollution in Aquatic Ecosystem with a Case Study on Cherating River, Malaysia. J. Eng. Technol. Sci. 52, 222–241. https://doi.org/10.5614/j.eng.technol.sci.2020.52.2.7
Park, T.-J., Lee, Seung-Hyun, Lee, M.-S., Lee, J.-K., Lee, Soo-Hyung, Zoh, K.-D., 2020a. Occurrence of microplastics in the Han River and riverine fish in South Korea. Sci. Total Environ. 708, 134535. https://doi.org/10.1016/j.scitotenv.2019.134535
Park, T.-J., Lee, S.-H., Lee, M.-S., Lee, J.-K., Park, J.-H., Zoh, K.-D., 2020b. Distributions of Microplastics in Surface Water, Fish, and Sediment in the Vicinity of a Sewage Treatment Plant. Water 12, 3333. https://doi.org/10.3390/w12123333
Parker, B., Andreou, D., Green, I.D., Britton, J.R., 2021. Microplastics in freshwater fishes: Occurrence, impacts and future perspectives. Fish Fish. 22, 467–488. https://doi.org/10.1111/faf.12528
Parker, B., Andreou, D., Pabortsava, K., Barrow, M., Green, I., Britton, R., 2022a. Microplastic Loads within Riverine Fishes and Macroinvertebrates are Not Predictable from Ecological or Morphological Characteristics. SSRN Electron. J. https://doi.org/10.2139/ssrn.4045481
Parker, B., Britton, J.R., Pabortsava, K., Barrow, M., Green, I.D., Dominguez Almela, V., Andreou, D., 2022b. Distinct microplastic patterns in the sediment and biota of an urban stream. Sci. Total Environ. 838, 156477. https://doi.org/10.1016/j.scitotenv.2022.156477
Pastorino, P., Prearo, M., Anselmi, S., Menconi, V., Bertoli, M., Dondo, A., Pizzul, E., Renzi, M., 2021. Use of the Zebra Mussel Dreissena polymorpha (Mollusca, Bivalvia) as a Bioindicator of Microplastics Pollution in Freshwater Ecosystems: A Case Study from Lake Iseo (North Italy). Water 13, 434. https://doi.org/10.3390/w13040434

Peller, J., Nevers, M.B., Byappanahalli, M., Nelson, C., Ganesh Babu, B., Evans, M.A., Kostelnik, E., Keller, M., Johnston, J., Shidler, S., 2021. Sequestration of microfibers and other microplastics by green algae, Cladophora, in the US Great Lakes. Environ. Pollut. 276, 116695. https://doi.org/10.1016/j.envpol.2021.116695

Persson, L., Carney Almroth, B.M., Collins, C.D., Cornell, S., de Wit, C.A., Diamond, M.L., Fantke, P., Hassellöv, M., MacLeod, M., Ryberg, M.W., Søgaard Jørgensen, P., Villarrubia-Gómez, P., Wang, Z., Hauschild, M.Z., 2022. Outside the Safe Operating Space of the Planetary Boundary for Novel Entities. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.1c04158

Peters, C.A., Bratton, S.P., 2016. Urbanization is a major influence on microplastic ingestion by sunfish in the Brazos River Basin, Central Texas, USA. Environ. Pollut. 210, 380–387. https://doi.org/10.1016/j.envpol.2016.01.018

Phillips, M.B., Bonner, T.H., 2015. Occurrence and amount of microplastic ingested by fishes in watersheds of the Gulf of Mexico. Mar. Pollut. Bull. 100, 264–269. https://doi.org/10.1016/j.marpolbul.2015.08.041

Piccardo, M., Bertoli, M., Pastorino, P., Barceló, D., Provenza, F., Lesa, D., Anselmi, S., Elia, A.C., Prearo, M., Pizzul, E., Renzi, M., 2021. Lethal and Sublethal Responses of Hydropsyche pellucidula (Insecta, Trichoptera) to Commercial Polypropylene Microplastics after Different Preconditioning Treatments. Toxics 9, 256. https://doi.org/10.3390/toxics9100256

Prata, J.C., da Costa, J.P., Girão, A.V., Lopes, I., Duarte, A.C., Rocha-Santos, T., 2019a. Identifying a quick and efficient method of removing organic matter without damaging microplastic samples. Sci. Total Environ. 686, 131–139. https://doi.org/10.1016/j.scitotenv.2019.05.456

Prata, J.C., Reis, V., Matos, J.T.V., da Costa, J.P., Duarte, A.C., Rocha-Santos, T., 2019b. A new approach for routine quantification of microplastics using Nile Red and automated software (MP-VAT). Sci. Total Environ. 690, 1277–1283. https://doi.org/10.1016/j.scitotenv.2019.07.060

Ragusa, A., Svelato, A., Santacroce, C., Catalano, P., Notarstefano, V., Carnevali, O., Papa, F., Rongioletti, M.C.A., Baiocco, F., Draghi, S., D'Amore, E., Rinaldo, D., Matta, M., Giorgini, E., 2021. Plasticenta: First evidence of microplastics in human placenta. Environ. Int. 146, 106274. https://doi.org/10.1016/j.envint.2020.106274

Razeghi, N., Hamidian, A.H., Wu, C., Zhang, Y., Yang, M., 2021. Microplastic sampling techniques in freshwaters and sediments: a review. Environ. Chem. Lett. 19, 4225–4252. https://doi.org/10.1007/s10311-021-01227-6

Redondo-Hasselerharm, P.E., Falahudin, D., Peeters, E.T.H.M., Koelmans, A.A., 2018. Microplastic Effect Thresholds for Freshwater Benthic Macroinvertebrates. Environ. Sci. Technol. 52, 2278–2286. https://doi.org/10.1021/acs.est.7b05367

Redondo-Hasselerharm, P.E., Gort, G., Peeters, E.T.H.M., Koelmans, A.A., 2020. Nano- and microplastics affect the composition of freshwater benthic communities in the long term. Sci. Adv. 6, eaay4054. https://doi.org/10.1126/sciadv.aay4054

Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T.J., Kidd, K.A., MacCormack, T.J., Olden, J.D., Ormerod, S.J., Smol, J.P., Taylor, W.W., Tockner, K., Vermaire, J.C., Dudgeon, D., Cooke, S.J., 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. Biol. Rev. 94, 849–873. https://doi.org/10.1111/brv.12480

Reynolds, C., Ryan, P.G., 2018. Micro-plastic ingestion by waterbirds from contaminated wetlands in South Africa. Mar. Pollut. Bull. 126, 330–333. https://doi.org/10.1016/j.marpolbul.2017.11.021

Rillig, M.C., Leifheit, E., Lehmann, J., 2021. Microplastic effects on carbon cycling processes in soils. PLOS Biol. 19, e3001130. https://doi.org/10.1371/journal.pbio.3001130

Ríos, J.M., Tesitore, G., de Mello, F.T., 2022. Does color play a predominant role in the intake of microplastics fragments by freshwater fish: an experimental approach with Psalidodon eigenmanniorum. Environ. Sci. Pollut. Res. 29, 49457–49464. https://doi.org/10.1007/s11356-022-20913-8

Rodrigues, M.O., Abrantes, N., Gonçalves, F.J.M., Nogueira, H., Marques, J.C., Gonçalves, A.M.M., 2018. Spatial and temporal distribution of microplastics in water and sediments of a freshwater system (Antuã River, Portugal). Sci. Total Environ. 633, 1549–1559. https://doi.org/10.1016/j.scitotenv.2018.03.233

Rummel, C.D., Jahnke, A., Gorokhova, E., Kühnel, D., Schmitt-Jansen, M., 2017. Impacts of Biofilm Formation on the Fate and Potential Effects of Microplastic in the Aquatic Environment. Environ. Sci. Technol. Lett. 4, 258–267. https://doi.org/10.1021/acs.estlett.7b00164

Sanchez, W., Bender, C., Porcher, J.-M., 2014. Wild gudgeons (Gobio gobio) from French rivers are contaminated by microplastics: Preliminary study and first evidence. Environ. Res. 128, 98–100. https://doi.org/10.1016/j.envres.2013.11.004

Sarijan, S., Azman, S., Said, M., Lee, M.H., 2019. Ingestion of Microplastics by Commercial Fish in Skudai River, Malaysia. EnvironmentAsia 12, 75–84. https://doi.org/10.14456/ea.2019.47

Sarijan, S., Azman, S., Said, M.I.M., Andu, Y., Zon, N.F., 2018. Microplastics in sediment from Skudai and Tebrau river, Malaysia: a preliminary study. MATEC Web Conf. 250, 06012. https://doi.org/10.1051/matecconf/201825006012

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682. https://doi.org/10.1038/nmeth.2019

Selangor Water Management Authority, 2015. Selangor State of River Report- Sungai Langat.

Setälä, O., Norkko, J., Lehtiniemi, M., 2016. Feeding type affects microplastic ingestion in a coastal invertebrate community. Mar. Pollut. Bull. 102, 95–101. https://doi.org/10.1016/j.marpolbul.2015.11.053

Shah, A.A., Hasan, F., Hameed, A., Ahmed, S., 2008. Biological degradation of plastics: A comprehensive review. Biotechnol. Adv. 26, 246–265. https://doi.org/10.1016/j.biotechadv.2007.12.005

Shahul Hamid, F., Bhatti, M.S., Anuar, Norkhairiyah, Anuar, Norkhairah, Mohan, P., Periathamby, A., 2018. Worldwide distribution and abundance of microplastic: How dire is the situation? Waste Manag. Res. 36, 873–897. https://doi.org/10.1177/0734242X18785730

Shen, M., Ye, S., Zeng, G., Zhang, Y., Xing, L., Tang, W., Wen, X., Liu, S., 2020. Can microplastics pose a threat to ocean carbon sequestration? Mar. Pollut. Bull. 150, 110712. https://doi.org/10.1016/j.marpolbul.2019.110712

Silva, C.J.M., Machado, A.L., Campos, D., Rodrigues, A.C.M., Patrício Silva, A.L., Soares, A.M.V.M., Pestana, J.L.T., 2022. Microplastics in freshwater sediments: Effects on benthic invertebrate communities and ecosystem functioning assessed in artificial streams. Sci. Total Environ. 804, 150118. https://doi.org/10.1016/j.scitotenv.2021.150118

Silva, C.J.M., Silva, A.L.P., Gravato, C., Pestana, J.L.T., 2019. Ingestion of small-sized and irregularly shaped polyethylene microplastics affect Chironomus riparius life-history traits. Sci. Total Environ. 672, 862–868. https://doi.org/10.1016/j.scitotenv.2019.04.017

Silva-Cavalcanti, J.S., Silva, J.D.B., França, E.J. de, Araújo, M.C.B. de, Gusmão, F., 2017. Microplastics ingestion by a common tropical freshwater fishing resource. Environ. Pollut. 221, 218–226. https://doi.org/10.1016/j.envpol.2016.11.068

Sommer, F., Dietze, V., Baum, A., Sauer, J., Gilge, S., Maschowski, C., Gieré, R., 2018. Tire Abrasion as a Major Source of Microplastics in the Environment. Aerosol Air Qual. Res. 18. https://doi.org/10.4209/aaqr.2018.03.0099

Stanković, J., Milošević, D., Jovanović, B., Savić-Zdravković, D., Petrović, A., Raković, M., Stanković, N., Stojković Piperac, M., 2021. In Situ Effects of a Microplastic Mixture on the Community Structure of Benthic Macroinvertebrates in a Freshwater Pond. Environ. Toxicol. Chem. etc.5119. https://doi.org/10.1002/etc.5119

Stanković, J., Milošević, D., Savić-Zdraković, D., Yalçın, G., Yildiz, D., Beklioğlu, M., Jovanović, B., 2020. Exposure to a microplastic mixture is altering the life traits and is causing deformities in the non-biting midge Chironomus riparius Meigen (1804). Environ. Pollut. Barking Essex 1987 262, 114248. https://doi.org/10.1016/j.envpol.2020.114248

Stanton, T., Johnson, M., Nathanail, P., MacNaughtan, W., Gomes, R.L., 2020. Freshwater microplastic concentrations vary through both space and time. Environ. Pollut. 263, 114481. https://doi.org/10.1016/j.envpol.2020.114481

Stanton, T., Johnson, M., Nathanail, P., MacNaughtan, W., Gomes, R.L., 2019. Freshwater and airborne textile fibre populations are dominated by 'natural', not microplastic, fibres. Sci. Total Environ. 666, 377–389. https://doi.org/10.1016/j.scitotenv.2019.02.278

Strady, E., Dang, T.H., Dao, T.D., Dinh, H.N., Do, T.T.D., Duong, T.N., Duong, T.T., Hoang, D.A., Kieu-Le, T.C., Le, T.P.Q., Mai, H., Trinh, D.M., Nguyen, Q.H., Tran-Nguyen, Q.A., Tran, Q.V., Truong, T.N.S., Chu, V.H., Vo, V.C., 2021. Baseline assessment of microplastic concentrations in marine and freshwater environments of a developing Southeast Asian country, Viet Nam. Mar. Pollut. Bull. 162, 111870. https://doi.org/10.1016/j.marpolbul.2020.111870

Su, L., Cai, H., Kolandhasamy, P., Wu, C., Rochman, C.M., Shi, H., 2018. Using the Asian clam as an indicator of microplastic pollution in freshwater ecosystems. Environ. Pollut. 234, 347–355. https://doi.org/10.1016/j.envpol.2017.11.075

Su, L., Deng, H., Li, B., Chen, Q., Pettigrove, V., Wu, C., Shi, H., 2019. The occurrence of microplastic in specific organs in commercially caught fishes from coast and estuary area of east China. J. Hazard. Mater. 365. https://doi.org/10.1016/j.jhazmat.2018.11.024

Sun, D., Wang, J., Xie, S., Tang, H., Zhang, C., Xu, G., Zou, J., Zhou, A., 2021. Characterization and spatial distribution of microplastics in two wild captured economic freshwater fish from north and west rivers of Guangdong province. Ecotoxicol. Environ. Saf. 207, 111555. https://doi.org/10.1016/j.ecoenv.2020.111555

Sun, T., Zhan, J., Li, F., Ji, C., Wu, H., 2021. Environmentally relevant concentrations of microplastics influence the locomotor activity of aquatic biota. J. Hazard. Mater. 414, 125581. https://doi.org/10.1016/j.jhazmat.2021.125581

Ta, A.T., Babel, S., 2020. Microplastic contamination on the lower Chao Phraya: Abundance, characteristic and interaction with heavy metals. Chemosphere 257. https://doi.org/10.1016/j.chemosphere.2020.127234

Taha, Z.D., Md Amin, R., Anuar, S.T., Nasser, A.A.A., Sohaimi, E.S., 2021. Microplastics in seawater and zooplankton: A case study from Terengganu estuary and offshore waters, Malaysia. Sci. Total Environ. 786, 147466. https://doi.org/10.1016/j.scitotenv.2021.147466

Tarafdar, A., Choi, S.-H., Kwon, J.-H., 2022. Differential staining lowers the false positive detection in a novel volumetric measurement technique of microplastics. J. Hazard. Mater. 432, 128755. https://doi.org/10.1016/j.jhazmat.2022.128755

Thompson, R.C., Moore, C.J., vom Saal, F.S., Swan, S.H., 2009. Plastics, the environment and human health: current consensus and future trends. Philos. Trans. R. Soc. B Biol. Sci. 364, 2153–2166. https://doi.org/10.1098/rstb.2009.0053

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at Sea: Where Is All the Plastic? Science 304, 838–838. https://doi.org/10.1126/science.1094559

Ugwu, K., Herrera, A., Gómez, M., 2021. Microplastics in marine biota: A review. Mar. Pollut. Bull. 169, 112540. https://doi.org/10.1016/j.marpolbul.2021.112540

Veerasingam, S., Ranjani, M., Venkatachalapathy, R., Bagaev, A., Mukhanov, V., Litvinyuk, D., Mugilarasan, M., Gurumoorthi, K., Guganathan, L., Aboobacker, V.M., Vethamony, P., 2021. Contributions of Fourier transform infrared spectroscopy in microplastic pollution research: A review. Crit. Rev. Environ. Sci. Technol. 51, 2681–2743. https://doi.org/10.1080/10643389.2020.1807450

Vogelsang, C., Lusher, A., Dadkhah, M., Sundvor, I., Umar, M., Ranneklev, S., Pettersen Eidsvoll, D., Meland, S., 2018. Microplastics in road dust - characteristics, pathways and measures.

Waldschläger, K., Schüttrumpf, H., 2019. Effects of Particle Properties on the Settling and Rise Velocities of Microplastics in Freshwater under Laboratory Conditions. Environ. Sci. Technol. 53, 1958–1966. https://doi.org/10.1021/acs.est.8b06794

Wang, J., Qin, X., Guo, J., Jia, W., Wang, Q., Zhang, M., Huang, Y., 2020. Evidence of selective enrichment of bacterial assemblages and antibiotic resistant genes by microplastics in urban rivers. Water Res. 183, 116113. https://doi.org/10.1016/j.watres.2020.116113

Wang, Z., Zhang, Y., Kang, S., Yang, L., Shi, H., Tripathee, L., Gao, T., 2021. Research progresses of microplastic pollution in freshwater systems. Sci. Total Environ. 795, 148888. https://doi.org/10.1016/j.scitotenv.2021.148888

Wardlaw, C., Prosser, R.S., 2020. Investigation of Microplastics in Freshwater Mussels (Lasmigona costata) From the Grand River Watershed in Ontario, Canada. Water. Air. Soil Pollut. 231, 405. https://doi.org/10.1007/s11270-020-04741-5

Werbowski, L.M., Gilbreath, A.N., Munno, K., Zhu, X., Grbic, J., Wu, T., Sutton, R., Sedlak, M.D., Deshpande, A.D., Rochman, C.M., 2021. Urban Stormwater Runoff: A Major Pathway for Anthropogenic Particles, Black Rubbery Fragments, and Other Types of Microplastics to Urban Receiving Waters. ACS EST Water 1, 1420–1428. https://doi.org/10.1021/acsestwater.1c00017

Wicaksono, E.A., Werorilangi, S., Galloway, T.S., Tahir, A., 2021. Distribution and seasonal variation of microplastics in tallo river, makassar, eastern indonesia. Toxics 9. https://doi.org/10.3390/toxics9060129

Windsor, F.M., Tilley, R.M., Tyler, C.R., Ormerod, S.J., 2019. Microplastic ingestion by riverine macroinvertebrates. Sci. Total Environ. 646, 68–74. https://doi.org/10.1016/j.scitotenv.2018.07.271

Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for microplastic debris. R. Soc. Open Sci. 1, 140317. https://doi.org/10.1098/rsos.140317

World Bank, 2021. Plastics Market Study for Malaysia [WWW Document]. World Bank. URL https://www.worldbank.org/en/country/malaysia/publication/market-study-for-malaysia-plastics-circularity-opportunities-and-barriers (accessed 12.19.21).

World Economic Forum, 2016. The New Plastics Economy: Rethinking the future of plastics (Industry Agenda). World Economic Forum, Geneva.

Wu, Y., Guo, P., Zhang, X., Zhang, Y., Xie, S., Deng, J., 2019. Effect of microplastics exposure on the photosynthesis system of freshwater algae. J. Hazard. Mater. 374, 219–227. https://doi.org/10.1016/j.jhazmat.2019.04.039

Xia, B., Sui, Q., Du, Y., Wang, L., Jing, J., Zhu, L., Zhao, X., Sun, X., Booth, A.M., Chen, B., Qu, K., Xing, B., 2022. Secondary PVC microplastics are more toxic than primary PVC microplastics to Oryzias melastigma embryos. J. Hazard. Mater. 424, 127421. https://doi.org/10.1016/j.jhazmat.2021.127421

Xia, W., Rao, Q., Deng, X., Chen, J., Xie, P., 2020. Rainfall is a significant environmental factor of microplastic pollution in inland waters. Sci. Total Environ. 732, 139065. https://doi.org/10.1016/j.scitotenv.2020.139065

Yan, Z., Chen, Y., Bao, X., Zhang, X., Ling, X., Lu, G., Liu, J., Nie, Y., 2021. Microplastic pollution in an urbanized river affected by water diversion: Combining with active biomonitoring. J. Hazard. Mater. 417, 126058. https://doi.org/10.1016/j.jhazmat.2021.126058

Yang Hwi, T., Shuaib Ibrahim, Y., Wan Mohd Khalik, W.M.A., 2020. Microplastic Abundance, Distribution, and Composition in Sungai Dungun, Terengganu, Malaysia. Sains Malays. 49, 1479–1490. https://doi.org/10.17576/jsm-2020-4907-01

Yap, C., Ismail, Ahmad, Ismail, A, Tan, S., 2003. Species Diversity of Macrobenthic Invertebrates in the Semenyih River, Selangor, Peninsular Malaysia. Pertanika J. Trop. Agric. Sci. 26.

Yurtsever, M., 2019. Glitters as a Source of Primary Microplastics: An Approach to Environmental Responsibility and Ethics. J. Agric. Environ. Ethics 32. https://doi.org/10.1007/s10806-019-09785-0

Zafar, A., Ullah, S., Majeed, M.T., Yasmeen, R., 2020. Environmental pollution in Asian economies: Does the industrialisation matter? OPEC Energy Rev. 44, 227–248. https://doi.org/10.1111/opec.12181

Zahari, N.Z., Tuah, P.M., Junaidi, M.R., Mohd Ali, S.A., 2022. Identification, Abundance, and Chemical Characterization of Macro-, Meso-, and Microplastics in the Intertidal Zone Sediments of Two Selected Beaches in Sabah, Malaysia. Water 14, 1600. https://doi.org/10.3390/w14101600

Zaki, M.R.M., Ying, P.X., Zainuddin, A.H., Razak, M.R., Aris, A.Z., 2021a. Occurrence, abundance, and distribution of microplastics pollution: an evidence in surface tropical water of Klang River estuary, Malaysia. Environ. Geochem. Health 43, 3733–3748. https://doi.org/10.1007/s10653-021-00872-8

Zaki, M.R.M., Zaid, S.H.M., Zainuddin, A.H., Aris, A.Z., 2021b. Microplastic pollution in tropical estuary gastropods: Abundance, distribution and potential sources of Klang River estuary, Malaysia. Mar. Pollut. Bull. 162, 111866. https://doi.org/10.1016/j.marpolbul.2020.111866

Zalasiewicz, J., Waters, C.N., Ivar do Sul, J.A., Corcoran, P.L., Barnosky, A.D., Cearreta, A., Edgeworth, M., Gałuszka, A., Jeandel, C., Leinfelder, R., McNeill, J.R., Steffen, W., Summerhayes, C., Wagreich, M., Williams, M., Wolfe, A.P., Yonan, Y., 2016. The geological cycle of plastics and their use as a stratigraphic indicator of the Anthropocene. Anthropocene 13, 4–17. https://doi.org/10.1016/j.ancene.2016.01.002

Zhang, K., Xiong, X., Hu, H., Wu, C., Bi, Y., Wu, Y., Zhou, B., Lam, P.K.S., Liu, J., 2017. Occurrence and Characteristics of Microplastic Pollution in Xiangxi Bay of Three Gorges Reservoir, China. Environ. Sci. Technol. 51, 3794–3801. https://doi.org/10.1021/acs.est.7b00369

Zieritz, A., Mahadzir, F.N., Chan, W.N., McGowan, S., 2019. Effects of mussels on nutrient cycling and bioseston in two contrasting tropical freshwater habitats. Hydrobiologia 835, 179–191. https://doi.org/10.1007/s10750-019-3937-4

APPENDICES

7. Appendices

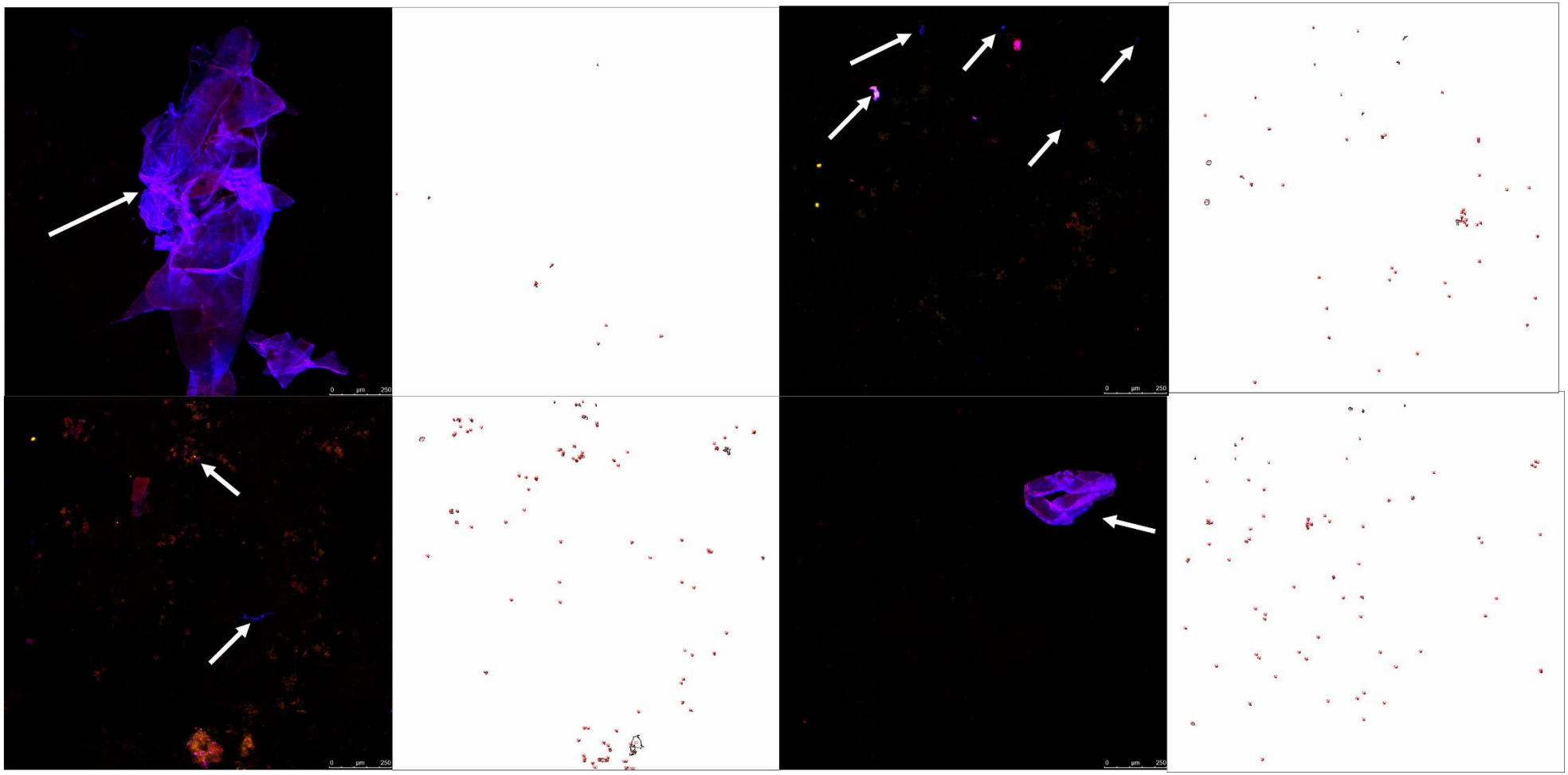
Appendix 1. *Geographical coordinates of the sampling sites.*

Sampling site	Coordinates
1	101.9128281, 3.1093625
2	101.8737889, 3.060739
3	101.8731406, 3.0418375
4	101.8714844, 3.0058125
5	101.8476177, 2.94643381
6	101.827078, 2.919683
7	101.811433, 2.9052202
8	101.8089981, 2.9040708
Semenyih Lake	2.9470 N, 101.8598 E

Appendix 2. *The number of species (N) found in the Semenyih river and lake from the preliminary study conducted in September 2021.*

Taxa	N
Bivalvia	3
Annelida-Hirudinea	7
Annelida-Oligochaetes	5
Branchiopoda-Conchostraca	28
Chironomidae-Chironominae	57
Chironomidae-Orthocladiinae	15
Chironomidae-Tanypodinae	125
Coleoptera-Elmidae	1
Diptera-Simuliidae	4
Ephemeroptera-Baetidae	1141
Ephemeroptera-Caenidae	56
Gastrapoda	2
Lepidotera-Acentropinae	1
Bivalvia-Unionidae	22
Nematoda	12
Odonata-Lestidae	3
Odonata-Libellulidae	1
Trichoptera-Hydropsychidae	55
Trichoptera-Leptoceridae	27
Trichoptera-Odontoceridae	2

Appendix 3. *Flurorescent photos of macroinvertebrate samples processed using the higher resolution (counterstaining dyes) method. Blue colour (white arrow) represents particles derived from animal origin whereas red, yellow, and green were microplastics.*



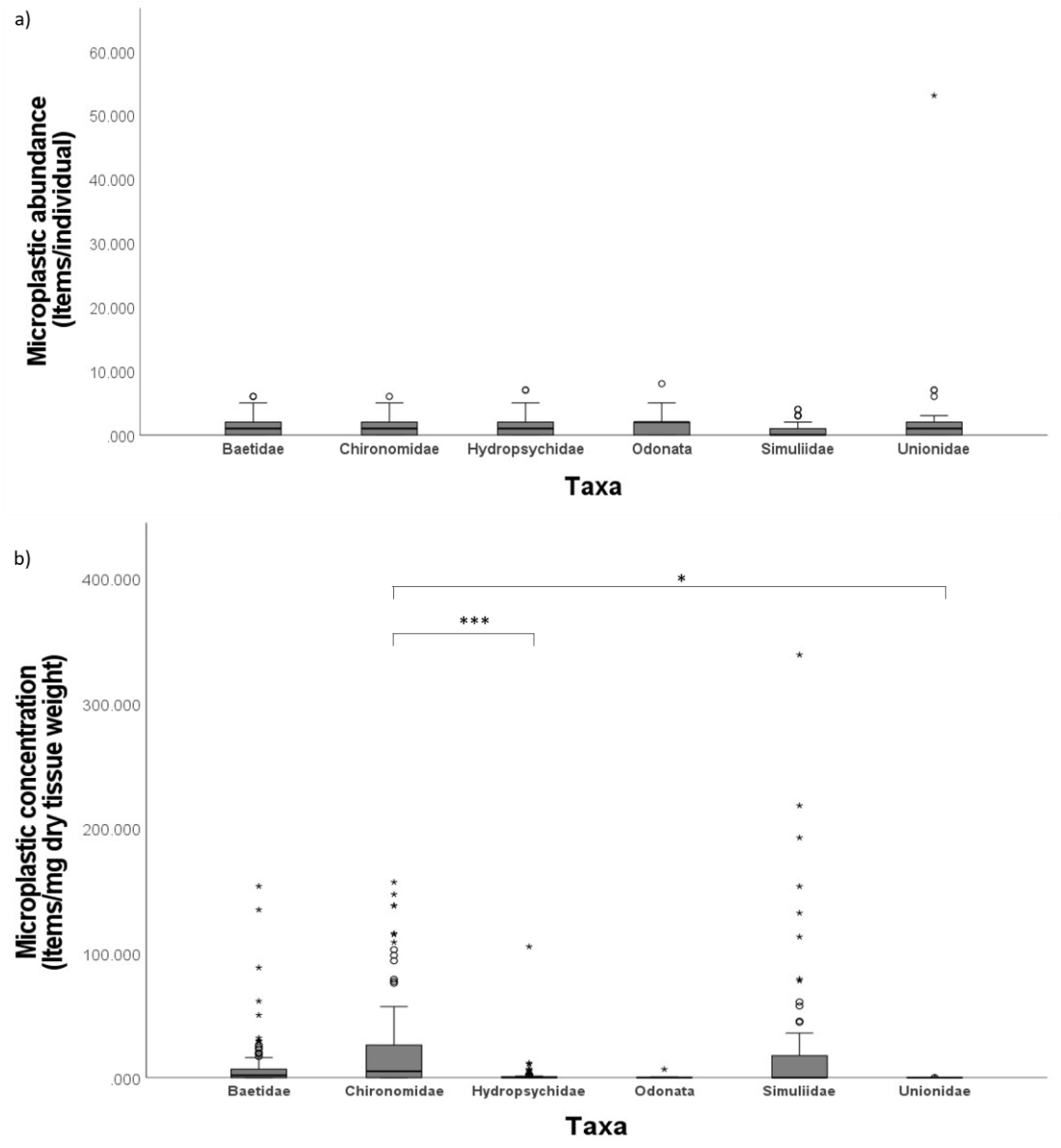
Appendix 4. *Fiji Macros code was used for automated counting of microplastic processed by counterstaining dye method.*

```
///stack 4 images (ch00,ch02,ch03,ch04), ch00 (blue only), ch002 (green only),
ch003 (yellow only), ch004 (red only).
run("Images to Stack", "use");
//substract scale bar from stack using a blank image with scale bar after
calibrating.
open("C:/Users/user/OneDrive - University of Nottingham
Malaysia/Desktop/Blank with scale bar.tif");
imageCalculator("Subtract create stack", "Stack","Blank with scale bar.tif");
//set threshold and fill holes
selectWindow("Result of Stack");
run("8-bit");
run("Set Scale...", "distance=0.6604 known=1 unit=µm global");
setAutoThreshold("MaxEntropy");
//run("Threshold...");
setThreshold(14, 255, "raw");
setOption("BlackBackground", false);
run("Convert to Mask", "method=MaxEntropy background=Dark calculate");
run("Fill Holes", "stack");
run("Stack to Images");


title = getTitle();
```

```
if
ch00 = endsWith(title, "ch00");
ch02 = endsWith(title, "ch02");
ch03 = endsWith(title, "ch03");
ch04 = endsWith(title, "ch04");
//ch02 add ch03
imageCalculator("Add create",ch02,ch03);
selectWindow("Result of"+ch02);
//ch0302 add ch04
imageCalculator("Add create", "Result of"+ ch02,ch04);
selectWindow("Result of Result of"+ ch02);
imageCalculator("Subtract create", "Result of Result of" + ch02,
currentTitle+ch00);
selectWindow("Result of Result of Result of" + ch02);
run("Analyze Particles...", "size=10-5000 show=Outlines display clear
summarize add");
```

Appendix 5. *Box and whisker plot showing median (a) microplastic abundance (items individual^{-1}) and (b) microplastic concentration (items mg dry tissue weight^{-1}) of six freshwater invertebrate families, processed using the standard visual inspection method. Horizontal lines indicate significant differences at p < 0.05 (Dunn-Bonferroni post-hoc test), p <0.05 represented by a single asterisk (*), p<0.01 represented by a double asterisk (**), and p <0.001 represented by a triple asterisk (***).*



Appendix 6. *The habitat, feeding habits and taxa of freshwater fish sampled, with its sample size (n), mean and standard deviation of its body length (mm), microplastic abundance (items individual $^{-1}$), and microplastic concentration (items mg dry tissue weight^{-1}).*

Habitat	**Feeding habits**	**Air-breathing type**	**Taxa**		**Body length (mm)**			**Microplastic abundance (items individual $^{-1}$)**		**Microplastic concentration (Items mg dry tissue weight^{-1})**	
					n	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Benthopelagic	Carnivorous	Non-air	Bagridae	*Hemibagrus*	9	253	61	14	6	.003	.003
	Omnivorous	Non-air	Cichlidae	*Oreochromis*	2	230	28	12	6	.001	.0003
		Non-air	Cyprinidae	*Barbonymus*	1	200	-	13	-	.004	-
Demersal	Carnivorous	Facultative	Butidae	*Oxyeleotris*	1	200	-	6	-	.001	-
		Facultative	Notopteridae	*Notopterus*	1	230	-	45	-	.024	-
	Omnivorous	Continuous	Anabantidae	*Anabas*	1	180	-	1	-	.0003	-
		Continuous	Clariidae	*Clarias*	3	297	30	34	54	.004	.005
		Continuous	Loricariidae	*Hypostomus*	1	335	-	55	-	.005	-

Appendix 7. *The sample size (n), mean of dry tissue weight (mg), microplastic abundance (items individual $^{-1}$), and microplastic concentration (items mg dry tissue weight^{-1}) for different body parts of fish.*

Taxa	Body part	Sample size (n)	Mean dry tissue weight (mg)	Microplastic count (No. of particle)	Microplastic abundance (items individual $^{-1}$)	Microplastic concentration (Items mg dry tissue weight^{-1})
Anabas testudineus	Gills	1	1387.500	0	0.0000	0.0007
	GIT	1	332.100	1	1.0000	0.0030
	Muscle	1	2050.100	0	0.0000	0.0005
Hemibagrus nemurus	Gills	8	2280.563	13	1.6250	0.0035
	GIT	8	2503.013	79	9.8750	0.0032
	Muscle	8	2286.738	13	1.6250	0.0035
Oxyeleotris marmorata	Gills	1	748.500	0	0.0000	0.0013
	GIT	1	2120.800	0	0.0000	0.0005
	Muscle	1	1250.900	6	6.0000	0.0008
Oreochromis mossambicus	Gills	2	2530.200	6	3.0000	0.0008
	GIT	2	8588.100	16	8.0000	0.0002
	Muscle	2	3737.150	2	1.0000	0.0005
Clarias batrachus	Gills	4	1600.525	19	4.7500	0.0025
	GIT	4	2099.725	94	23.5000	0.0019
	Muscle	4	1492.000	7	1.7500	0.0027

Barbonymus schwanenfeldii	Gills	1	934.100	0	0.0000	0.0011
	GIT	1	1171.600	9	9.0000	0.0009
	Muscle	1	982.200	4	4.0000	0.0010
Hypostomus plecostomus	Gills	1	1597.900	2	2.0000	0.0006
	GIT	1	7770.300	50	50.0000	0.0001
	Muscle	1	768.200	3	3.0000	0.0013
Notopterus notopterus	Gills	1	511.200	4	4.0000	0.0020
	GIT	1	429.800	39	39.0000	0.0023
	Muscle	1	899.600	2	2.0000	0.0011