ECOLOGICAL FATE OF MICROPLASTICS IN A TROPICAL MARINE ENVIRONMENT

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6	Miller, M.E., Santana, M.F.M., Carsique, M., Motti, C.A., Hamann, M., & Kroon, F.J. (2022) Temporal patterns of plastic contamination in surface waters at the SS Yongala shipwreck, Great Barrier Reef, Australia. Environmental Pollution. https://doi.org/10.1016/j.envpol.2022.119545	FJK and I conceived the study. FJK organized field logistics, and FJK and MFMS collected and preserved field samples. I processed and analysed samples, produced figures and conducted statistical analysis. MC developed the R script under the supervision of MFMS. I wrote original draft. All co-authors critically discussed ideas, commented on, and reviewed the manuscript.

Permits and Ethics Declaration

All necessary permits and ethics approvals were obtained and used to conduct the work presented in this thesis. The following field work was conducted under the Great Barrier Reef (GBR) Marine Park Authority permit G12/35236.1: fish (*Thalassoma lunare*) collected in Chapters 3 and 4; water, sediment, and organisms (fish; *T. lunare*) collected in Chapter 5; seawater samples collected in Chapter 6.

Fish collection and experimentation for Chapters 3 and 4 were all done in accordance with JCU Animal Ethics Committee approval number A2722. Fish collection for Chapter 5 was carried out in accordance with James Cook University Animal Ethics Committee approval number A2643.

General Abstract

Since the mass production of plastic materials began in the 1950s, the global demand and usage of plastics has been on the rise, with a predicted increase in annual emissions of up to 53 million metric tonnes by 2030, should mitigation and management strategies not change. Marine plastic pollution, including microplastics (plastics < 5mm), is a growing global issue. Microplastics are of particular concern due to their small size and variable buoyancy, making them readily available and continually bioavailable to marine organisms across all trophic levels. The microplastics literature has been exponentially increasing over the past three decades, motivated by scientists, managers, and the general public wanting to know the extent, and mitigate the impact, of microplastic pollution. Despite increased research efforts, there are still major knowledge gaps related to the impact and fate of microplastics in the marine environment. The research presented in this thesis emphasises the persistence, and severity of the ecological issue surrounding plastic, and in particular microplastic, pollution. Estimates for the Great Barrier Reef World Heritage Area (GBRWHA) show that the ecological risk can be substantial, with microplastic contamination widespread and continuous throughout all matrices examined. While bioaccumulation and biomagnification trends were not observed for global reports, local assessments indicate that microplastics are bioconcentrating in coral reef organisms. Laboratory experiments reveal biomagnification is possible, with trophic transfer being a prominent pathway of exposure. Alternative assessments of local *in situ* microplastic ingestion indicate biomagnification up a simple trophic food web is occurring. Microplastic contamination is heterogeneous, with variability across the GBRWHA, yet persistent in nature and subject to increase from physicochemical influences such as wind and extreme weather events. It is the goal of this research to build an empirical base to inform managers, policy makers and the general public about the severity of the issue of plastic pollution, in respect to the ecological fate of microplastics within a tropical marine ecosystem of great traditional, historical and ecological importance (i.e., the GBRWHA Science for Management Strategy).

Table of Contents

List of Tables

List of Figures

Chapter 1: General Introduction

1.1 Plastic history, production, and human use

Natural-based plant materials (e.g., spinifex resin) are among the first materials used for their thermoset plastic properties, first utilised by Aboriginal Australians thousands of years ago as a 'native cementing substance' (Pitman and Wallis, 2012). In recent times (i.e., the 1860s), the first synthesised plastic polymer was considered a semi-synthetic, called *Parkesine*, made from nitrocellulose production (Rasmussen, 2021). Plastics are now produced from monomers, many of which are sourced from fossil fuels, coal, oil, natural gas, and biomass (e.g., cellulose, keratin) (UNEP, 2021) and undergo a process known as polymerisation to form repeating long chain polymers. For example, ethylene, sourced from distillates of natural gas and petroleum, is used to produce polyethylene (PE). Depending on the processing conditions, the density of this polymer can be manipulated. For example, high-density PE (HDPE) is produced under high temperatures, whereas low-density PE (LDPE) is produced by compression. Essentially, modern day plastics, by design, are durable and long-lasting, and have revolutionised everyday human life, and include common-use PE, polypropylene (PP), polyvinyl chloride (PVC), polyurethane (PUR), polystyrene (PS), and polyethylene terephthalate (PET) (GESAMP, 2019; UNEP, 2021). While these plastic types dominate the global market, textile fabrics and fibres now make up about 15% of total polymer production, including polyester (PEST), poly(methyl methacrylate) (PMMA, acrylic), and polyamide (PA, nylon) (Geyer et al., 2017).

Since the mass production of plastic materials began in the 1950s, the global demand and usage of plastics has been on the rise (GESAMP, 2019; Ostle et al., 2019). From the 1950s until 2015, 8.3 billion metric tonnes of plastic have been produced globally (Geyer et al., 2017), with approximately 8% of global oil extraction being used exclusively for manufacturing purposes (Thompson et al., 2009a). In 2015, the associated greenhouse gas emissions (carbon dioxide equivalents, CO_2e) from plastic production were estimated to be approximately 2 Gt CO_2e (Cabernard et al., 2022), contributing directly to global climate change (Avery-Gomm et al., 2019; Lavers et al., 2022). Yet, the persistence of plastics in the natural environment potentially represents a greater threat, with plastics having been identified as contaminants of emerging concern and a growing global threat for ecosystems worldwide (Guzzetti et al., 2018; Kroon et al., 2020).

Plastics, by design, are not biodegradable and when not managed correctly can enter the natural environment and accumulate. A considerable amount of these plastics enters the oceans annually, most originating from inadequate waste management and land-based sources rather than intentional

releases (e.g., littering, illegal dumping) (GESAMP, 2019; UNEP, 2016). However, the contribution of plastic pollution from maritime sources cannot be ignored, with approximately 20% stemming from cargo, recreational and military navigation, fishing activities, aquaculture facilities, oil and gas platforms, and legal and illegal dumping (Čulin and Bielić, 2016). Estimates of global emissions of plastic into waterways (e.g., lakes, rivers, oceans) are as high as 25 million metric tonnes per year (Lau et al., 2020), with a predicted increase in annual emissions of up to 53 million metric tonnes by 2030 should mitigation and management strategies not change (Borrelle et al., 2020; Lavers et al., 2022).

As the climate changes, increases in average sea surface temperature and alterations to the global ocean circulation patterns have been flagged as potential drivers of oceanic plastic pollution (Welden and Lusher, 2017). The scale and severity of storms and weather events are projected to become more frequent (Coumou and Rahmstorf, 2012; Stott, 2016), potentially increasing plastic input, distribution, and accumulation (Welden and Lusher, 2017). With plastic already widespread throughout the environment, it is now considered to be a geological marker of the Anthropocene, an epoch of time defined by the influence of human activities dominating the state, dynamics, and future of the Earth's systems (Villarrubia-Gómez et al., 2018; Zalasiewicz et al., 2016). Strategies to mitigate oceanic plastic pollution rely on empirical knowledge of input, distribution and accumulation and is a key motivation for current plastics research.

1.2 Plastics to microplastics

Plastics are designed to be lightweight, durable, and relatively cheap to produce (Derraik, 2002). To enhance the polymer properties and prolong their life, many plastic polymers are altered at the manufacturing stage to include chemical additives that impart properties such as heat tolerance, flexibility, longevity, and durability of plastics (Hahladakis et al., 2018; Net et al., 2015). One major group of chemical additives is the phthalic acid esters (PAEs), or phthalates, which are primarily used as plasticisers to increase flexibility and durability (Hahladakis et al., 2018; Net et al., 2015). Phthalates are not chemically bonded to plastics, but rather penetrate the interstitial spaces between polymer chains. As such, phthalates can readily migrate into the surrounding environmental matrix, especially in aquatic settings (Gulizia et al., 2022; Liang et al., 2008; Stringer and Johnston, 2001). Leaching is often accelerated by mechanical abrasion (e.g., wave action), ultraviolet light (e.g., prolonged sunlight), and biofouling, causing embrittlement and damage to the integrity of the polymer. Chain breakage occurs resulting in shortened polymers, reducing their molecular weight, and ultimately causing larger plastic items to deteriorate and fragment over time (Arthur et al., 2009; Weinstein et al., 2016).

Research on plastic in the environment typically classes the particles into size-based categories. While there is much debate about the upper and lower limits of plastic pollution size ranges (Frias and Nash, 2019; GESAMP, 2016), many have made recommendations based on the most adopted categories (Arthur et al., 2009; Barnes et al., 2009; GESAMP, 2019; Hartmann et al., 2019). These include: (a) nanoplastics (1 nm to 1 µm), (b) microplastics (MPs; 1 µm to 5 mm), (c) mesoplastics (5 mm to 2 cm) and (d) macroplastics (> 2 cm). In the past decade, the most reported category has been MPs, with thousands of papers published and the field continuing to rapidly expand (Cunningham and Sigwart, 2019; Provencher et al., 2020).

MPs have two forms, predominantly referring to the source of the item: primary and secondary. Primary MPs have been manufactured at deliberately small sizes, such as resin pellets or the microbeads used for cosmetic products (e.g., facewash, toothpaste), whereas secondary MPs occur as a result of fragmentation of larger plastic items (Arthur et al., 2009; GESAMP, 2016). Small synthetic fibres are also categorised as MPs and can be produced from a wide range of materials, including shedding from textile clothing in the washing machine or breaking off from ropes, fishing nets, and moorings (Gago et al., 2018; Hartmann et al., 2019). The fragmentation of plastics, particularly once in the environment, not only complicates mitigation strategies, but produces a gradient of fragment sizes that become bioavailable to an increasing number of life forms.

1.3 Ecological impact and fate of MPs

Marine plastic pollution, including MPs, is a growing global issue (GESAMP, 2020). MPs are of particular concern due to their small size and variable buoyancy, making them continually bioavailable to marine organisms across all trophic levels (Lusher, 2015). Marine organisms can be exposed to MPs through direct ingestion, indirect ingestion via contaminated prey items, or by means of respiration. Given the heterogeneous compositions of polymer, size, colour, shape, and chemical additives, plastics are considered a diverse suite of contaminants and therefore a multiple stressor contaminant (Rochman, 2013). The potential to inflict physical and chemical harm, even on a single species or group of organisms, through ingestion and retention is therefore challenging to assess (Rochman et al., 2019). For marine organisms, examples of adverse impacts include physical retention of plastic particulate matter (Lu et al., 2016), causing gut blockages and internal damage (Pirsaheb et al., 2020), or behavioural changes (Critchell and Hoogenboom, 2018). However, the impacts are not only a result of the plastic particulate matter but can also arise from the absorption of concentrated environmental contaminants in seawater and subsequent transfer, leaching to body tissues across food chains during ingestion and retention of MPs (Teuten et al., 2009).

Some chemical contaminants are incorporated into plastics during the manufacturing process (e.g., chemical additives), and others are adsorbed from the environment, such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), as well as heavy metals (Endo and Koelmans, 2016; GESAMP, 2016; Hahladakis et al., 2018). The chemical leaching and (re)-sorption to MPs is facilitated by the high surface area to volume ratio combined with the non-polar surface of small MPs, with both phenomena resulting in a complex chemical cocktail of monomers, oligomers, and additives (Rochman et al., 2019). These chemical contaminants are inherently toxic given their previous link with immunotoxicity, metabolic toxicity, endocrine toxicity, neurotoxicity, genotoxicity, developmental toxicity, and other adverse effects on marine organisms (Gunaalan et al., 2020; Li et al., 2020; Liang et al., 2008). While knowledge surrounding effects of chemical additives is substantial (Hahladakis et al., 2018), their potential to leach, re-sorb and transfer between matrices via MPs within the marine environment is relatively lacking (see Chapter 2) and cause for concern. Previous impacts have been investigated within laboratory settings, mainly due to difficulty ascertaining the multitude of stressors (e.g., additives and MPs) present in the marine environment (Koelmans et al., 2017). Therefore, adapting endpoints such as bioconcentration, bioaccumulation and biomagnification can assist in improving the understanding of potential ecological effects and fate associated with these contaminants (USEPA, 1992).

Assessing the ecological fate of an environmental contaminant is accomplished by establishing the concentration present in an organism compared to that of its environment and prey items. This not only requires robust sampling of environmental abiotic compartments (i.e., water and sediment) and examination of ecologically important organisms (Santana et al., 2021), but also the assessment of multiple ecologically relevant endpoints (e.g., bioconcentration, bioaccumulation, biomagnification). Bioconcentration is considered the amount of contaminant (i.e., number of MPs) in an organism relative to the concentration in the organism's environment (USEPA, 1997). Bioaccumulation is considered the net uptake of a contaminant from the environment by all possible routes and from any source (e.g., water, sediment, prey) (Spacie et al., 1995). Biomagnification is defined as the increase of a contaminant from lower to higher trophic levels (USEPA, 2008). Traditionally, these endpoints are applied to measure 'dissolved' toxic chemical contamination (Alexander, 1999), yet are now being used to infer particulate MP contamination and determine potential risk (Farrell and Nelson, 2013; Nelms et al., 2018; Zhao et al., 2018). However, the adaptation of these endpoints in MP research (Akhbarizadeh et al., 2019; Rochman et al., 2019) requires in-depth evaluation of the pathways and mechanisms that introduce and transfer MPs through food webs.

1.4 Distribution of plastics and MPs in the marine environment

Globally, plastic polymers can persist in the natural environment. Plastic pollution, and specifically MP pollution, has been documented in all aquatic environmental matrices, including seawater, freshwater, wastewater, sediment, and within a plethora of aquatic organisms (Lusher, 2015; Miller et al., 2017). MPs can be transported large distances, often accumulating in convergence zones, including ocean gyres, or on the seabed in high concentrations (Eriksen et al., 2013b; Law et al., 2010; Maximenko et al., 2020). As a result, plastics are accumulating in every coastal and marine ecosystem, including all six ocean basins and areas with little to no human activity (Peng et al., 2018). More specifically, the presence of plastics has been identified in once-pristine areas of the world including coral reefs (Huang et al., 2021a), polar regions (Bergmann et al., 2019; Waller et al., 2017), remote islands (Lavers et al., 2019), and deep seas (Woodall et al., 2014).

Plastic distribution has been shown to fluctuate both spatially and temporally on a global scale (Barrows et al., 2018b; Law et al., 2010) and correlate to multiple physical mechanisms, including wind speed and direction, surface currents, and river discharge (Brunner et al., 2015; Wichmann et al., 2019). Either in isolation or combined, these mechanisms can generate turbulence, promoting the vertical mixing of plastics within the surface layer (i.e., between 10 and 200 m depth) (Kukulka et al., 2012; Reisser et al., 2013; Veerasingam et al., 2016a), as well as horizontal transport (Zhang, 2017). While imperative to establish the drivers of pollution status and changes in trends, the long-term monitoring of marine plastic pollution, especially alongside physicochemical oceanographic parameters, is seldom done.

At a local scale, investigations over the past decade have established widespread plastic contamination in Australian waters (Reisser et al., 2013). Plastic pollution has been quantified in areas of high economical, ecological, and traditional values such as the Great Barrier Reef World Heritage Area (GBRWHA) (Kroon et al., 2020). This confirms that even well-managed marine ecosystems such as the GBR and associated reef environments are not free from the threat of worldwide plastic pollution (Tan et al., 2020). In the GBR, MP contamination was found to be higher in offshore mid-shelf reef areas compared to inshore reefs, with modelling suggesting non-land-based sources (Jensen et al., 2019). As of 2019, examination of inhabitant species including the commercially important coral trout (Kroon et al., 2018b), planktivorous damselfish (Jensen et al., 2019), green sea turtles (Caron et al., 2018), as well as abiotic compartments including surface and sub-surface waters (Hall et al., 2015; Jensen et al., 2019), has since confirmed organismal intake and persistent contamination of MPs in the GBRWHA (Figure 1.1). The low number of species reported to be contaminated is directly correlated to the small number of studies undertaken. As a result, the understanding of ecological risks associated with MPs in key environmental assets, such as the GBRWHA, is notably lacking and should be an area of research focus.

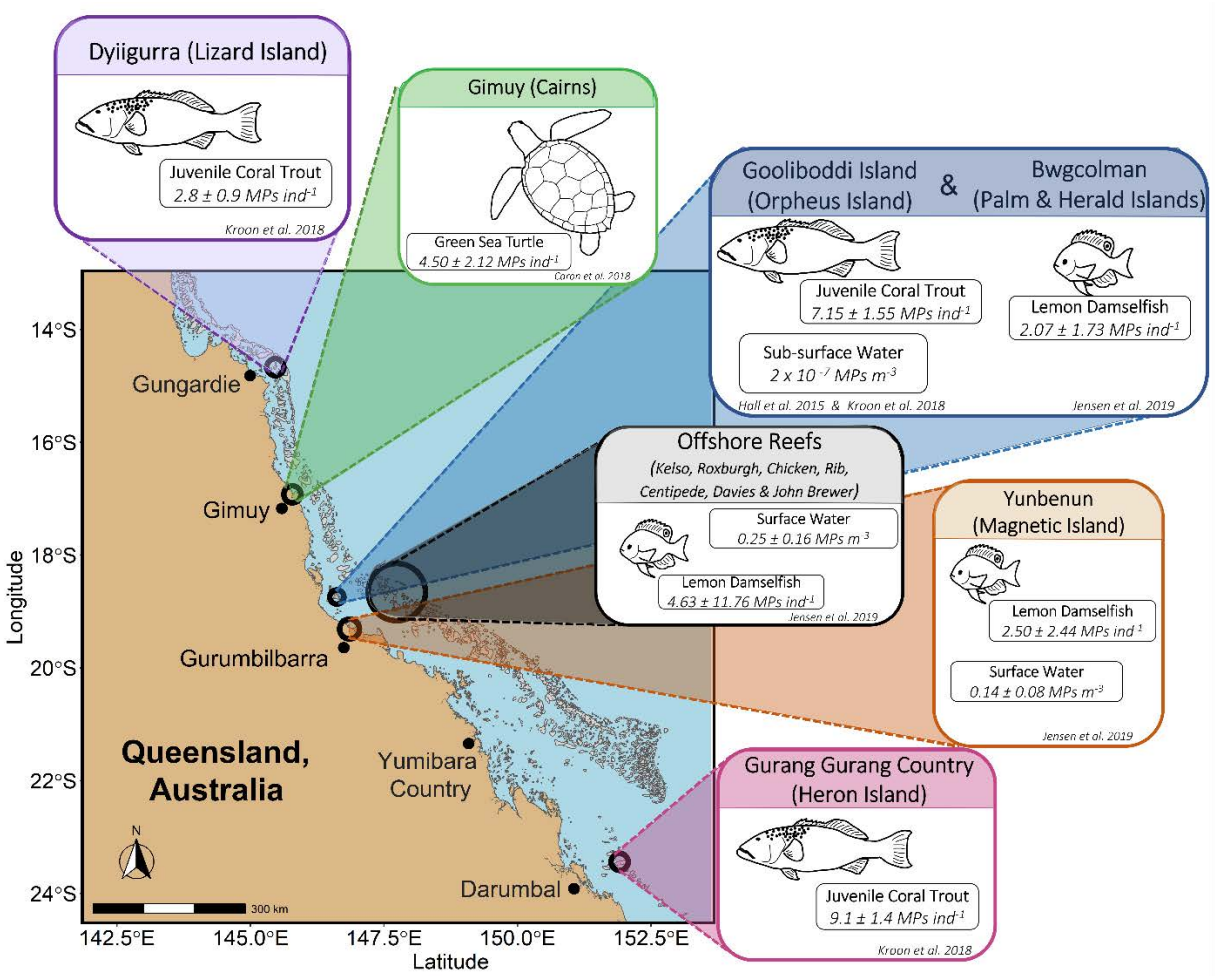

Figure 1.1 Summarised results from studies investigating MP contamination in the Great Barrier Reef World Heritage Area (GBRWHA). Studies were isolated to marine species inhabiting near- and off-shore areas and the surrounding environment, with reports of freshwater and local beach clean-ups excluded. Only reports existing up to and before 2019 (i.e., at the commencement of this thesis) were included, with more recent research in the context of this thesis discussed in Chapter 7.

1.5 Key knowledge gaps

The MPs literature has been exponentially increasing over the past three decades, motivated by scientists, managers, and the public wanting to know the extent and mitigate the impact of plastic pollution. Despite increased research efforts (Avery-Gomm et al., 2019), there are still major knowledge gaps related to the fate of MPs in the marine environment.

A multitude of marine organisms have been shown to ingest MPs (Lusher, 2015), and speculation has been made on the bioaccumulation, biomagnification, and trophic transfer of MPs through marine food webs (Nelms et al., 2018; van Raamsdonk et al., 2020). Bioconcentration, bioaccumulation and biomagnification represent three important metrics for determining ecological risk and are key predictors of exposure to a contaminant (Covernton et al., 2022), yet claims of MP bioconcentration,

bioaccumulation and biomagnification have not been substantiated. Further, it is unclear if general trends of MP contamination within marine organisms support these claims. These endpoints can be assessed with *in situ* data, however, assessment and quantification of true ingestion, retention and trophic transfer of MPs requires controlled laboratory experiments (Athey et al., 2020; Dawson et al., 2018a; Farrell and Nelson, 2013; Santana et al., 2017; Watts et al., 2014), which are seldom done (see Chapter 2 for more details). Quantification of ingestion and retention of MPs at environmentally relevant exposures is ultimately lacking, and dedicated effort is required to assess multiple species within a food web (see Chapter 3 for more details). Equally important, is understanding how these trophic interactions might change as the MP passages through these multiple (i.e., > 2) trophic levels (see Chapter 4 for more details). Establishing the occurrence of MP ingestion and retention by wild-caught marine organisms is necessary to assess whether observations from controlled laboratory experiments are also being detected *in situ* (see Chapter 5) and needs to be assessed in the context of the MP contaminant present in corresponding environmental abiotic compartments (i.e., water and sediment). Finally, robust temporal monitoring of marine plastic contamination is essential to establish baseline contamination levels, identify trends and potential influences on plastic pollution, information that is critical to improve the ecological impact assessment of MPs on marine life. The development of monitoring programs is needed in the GBRWHA but will require significant methodology and validation (see Chapter 6).

This thesis represents a novel contribution to the body of existing work reported in the MP literature. It focusses on three overarching themes to address the knowledge gaps highlighted above and provides meaningful insight to the ecological fate of MPs. These themes are: (a) assessing ecological endpoints in marine organisms globally [Chapter 2] and locally in the GBRWHA [Chapter 5], (b) ingestion and retention of PEST microfibres [Chapter 3] and the trophic transfer [Chapter 4] in marine reef species, and (c) baseline [Chapter 5] and temporal monitoring [Chapter 6] of MP contamination levels in the GBRWHA to predict future risk.

1.6 Thesis outline

The aim of this thesis is to understand the ecological fate and impact of MPs within a tropical marine ecosystem (i.e., the GBR). Contained within is seven chapters outlining and addressing the key knowledge gaps mentioned above:

Chapter 1: Presents an overview of the issues pertaining to MPs in the marine environment and identifies the key knowledge gaps to provide a framework for the research thesis.

Chapter 2: Examines whether current, published findings support the premise that MPs and their chemical additives, bioaccumulate and biomagnify across a typical marine food web.

Chapter 3: Investigates the ingestion and retention of environmentally relevant concentrations of common polyester microfibres by three marine species within a simple food web. Specifically, the exposure of copepods, mysid shrimp and moon wrasse to PEST microfibres (a) alone, (b) pre-adsorbed with bis(2-ethylhexyl) phthalate (DEHP), or (c) dosed alongside DEHP, was conducted to ascertain the influence of DEHP on their ingestion and retention of PEST microfibres.

Chapter 4: Assesses the potential of PEST microfibres to transfer through a typical 3-tier marine food web, comprising of copepods (level 1), mysid shrimp (level 2) and moon wrasse (level 3). The influence of DEHP on the ingestion and transfer of microfibres is also investigated.

Chapter 5: Establishes the MP contamination levels within a typical food web at two Central GBR reefs, Davies and Backnumbers. Copepods, benthic crustaceans (including mysid shrimp) and moon wrasse, as well as the surrounding sediment and water column were examined for the presence of MPs allowing for the assessment of bioconcentration, bioaccumulation and biomagnification of MP contamination.

Chapter 6: Reveals the abundance and distribution of plastic contamination within the surface waters at the SS Yongala Shipwreck, located in the Central GBR. This chapter provides baseline contamination levels as well as details the physicochemical influences on plastic pollution in the local area and provides a foundation for a MPs monitoring program.

Chapter 7: Presents an overview of thesis results and provides context for management of marine MP pollution in the GBR.

Chapter 2: Bioaccumulation and biomagnification of microplastics in marine organisms: A review and meta-analysis of current data

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2.1 Abstract

Microplastic (MP) contamination has been well documented across a range of habitats and for a large number of organisms in the marine environment. Consequently, bioaccumulation, and in particular biomagnification of MPs and associated chemical additives, are often inferred to occur in marine food webs. Presented here are the results of a systematic literature review to examine whether current, published findings support the premise that MPs and associated chemical additives bioaccumulate and biomagnify across a general marine food web. First, field and laboratory-derived contamination data on marine species were standardised by sample size from a total of 116 publications. Second, following assignment of each species to one of five main trophic levels, the average uptake of MPs and of associated chemical additives was estimated across all species within each level. These uptake data within and across the five trophic levels were then critically examined for any evidence of bioaccumulation and biomagnification. Findings corroborate previous studies that MP bioaccumulation occurs within each trophic level, while current evidence around bioaccumulation of associated chemical additives is much more ambiguous. In contrast, MP biomagnification across a general marine food web is not supported by current field observations, while results from the few laboratory studies supporting trophic transfer are hampered by using unrealistic exposure conditions. Further, a lack of both field and laboratory data precludes an examination of potential trophic transfer and biomagnification of chemical additives associated with MPs. Combined, these findings indicate that, although bioaccumulation of MPs occurs within trophic levels, no clear sign of MP biomagnification *in situ* was observed at the higher trophic levels. Recommendations for future studies to focus on investigating ingestion, retention and depuration rates for MPs and chemical additives under environmentally realistic conditions, and on examining the potential of multi-level trophic transfer for MPs and chemical additives have been made.

2.2 Introduction

Contamination of the marine environment with microplastics (MPs; plastics < 5 mm) has been identified as an issue of global concern (GESAMP, 2019), and documented extensively in seawater, marine sediments, and marine biota (Lusher, 2015). MPs are of particular concern as a pollutant in environmental systems because their small size and variable buoyancy makes them readily available for uptake by a wide range of organisms across different trophic levels and feeding strategies (Hermabessiere et al., 2017). Indeed, the uptake of MPs has been confirmed in wild populations of numerous marine organisms across all trophic levels collected from their natural habitat (Lusher, 2015). The prevalence of such reports has resulted in bioaccumulation, and in particular biomagnification of MPs and associated chemical additives, often being inferred in the literature on marine MP contamination (Farrell and Nelson, 2013; Nelms et al., 2018; Zhao et al., 2018). However, limited published evidence appears to exist for trophic transfer and biomagnification of MPs and associated additives within food webs in marine environments.

The ecological risks of MP contamination can be defined as the likelihood of adverse ecological effects occurring as a result of exposure to MPs (GESAMP, 2020; USEPA, 1992). Marine organisms can be exposed through direct ingestion of MPs, through indirect ingestion of MPs via prey items, or by means of respiration. Irrespective of the pathway, MP intake can result in adverse physical and chemical impacts on marine organisms (Barboza et al., 2018b; Gardon et al., 2018; Xu et al., 2017). Examples of potential impacts include lipid accumulation and inflammation from physical retention of MPs in digestive tracts (Lu et al., 2016), and histopathological changes stemming from chemical leaching of plastic additives into tissues (Karami et al., 2016). These impacts are often investigated during controlled laboratory exposures using a variety of endpoints such as growth rate (Chapron et al., 2018; Lo and Chan, 2018), fecundity (Cole et al., 2015), and mortality (Besseling et al., 2013). In wild-caught organisms, however, causality between MP exposure pathways and observed effects is often difficult to ascertain due to the multitude of stressors present in the marine environment (Koelmans et al., 2017). Hence, understanding endpoints such as bioaccumulation and biomagnification can assist in improving understanding of the potential ecological effects associated with different MP exposure pathways in the marine environment (USEPA, 1992).

Bioaccumulation and biomagnification are two critical concepts used in ecological risk assessments to determine the extent of pollutant transport within food webs (Boethling and Mackay, 2000). The classical concept of bioaccumulation and biomagnification usually refers to dissolved chemical contamination (Alexander, 1999), although the terminology has been readily adopted by the MP literature (Akhbarizadeh et al., 2019; Dawson et al., 2018a; Rochman et al., 2019). In this study, bioaccumulation (or body burden) is defined as the net uptake of a contaminant (i.e., MPs or additives)

from the environment by all possible routes (e.g., contact, ingestion, respiration) from any source (e.g., water, sediment, prey) (Gerber, 2009; Maher et al., 2016). In other words, bioaccumulation is occurring when uptake of a contaminant is greater than the ability of an organism to egest a contaminant (Wang et al., 2016). Bioaccumulation and subsequent trophic transfer of a contaminant may result in the biomagnification of these contaminants at higher trophic levels (Kelly et al., 2007). Biomagnification across a food web can thus be defined as the increase in concentration of a contaminant (i.e., MPs or additives) in one organism compared to the concentration in its prey (Gerber, 2009; Maher et al., 2016). An important assumption for this definition is that all contamination in higher trophic levels is a direct result of consumption of prey in lower trophic levels, i.e., trophic transfer is occurring.

This study examines whether current, published findings support the premise that MPs, and their chemical additives, bioaccumulate and biomagnify across a general marine food web. First, following a systematic review of the literature, uptake data on MPs and their additives derived from field observations and laboratory experiments were standardised by sample size for individual marine species. MP reports for translocation are lacking, therefore this study aimed to apply the classical concepts of bioaccumulation and biomagnification to MP contamination found within the gut of marine organisms. For each species, feeding habit was also noted to provide an alternative perspective based on previous findings (Bour et al., 2018a; Mizraji et al., 2017). Second, following assignment of each species to one of five main trophic levels, the average uptake of MPs and of associated chemical additives was estimated across all species within each level. These uptake data within and across the five trophic levels were then critically examined for any evidence of bioaccumulation and biomagnification. If trophic transfer and biomagnification of MPs and associated additives occurs within marine food webs, an increase in average bioaccumulation from lower to higher trophic levels is expected.

2.3 Materials and methods

2.3.1 Systematic review procedures

To conduct a systematic review and meta-analyses of the global literature on MP contamination data for individual marine species, an established protocol was followed (PRISMA (Moher et al., 2009), Figure 2.1). Specifically, a thorough literature search was conducted to evaluate whether bioaccumulation and biomagnification of MPs and associated additives occurs, either *in situ* or under experimental laboratory settings. The search was performed in Google Scholar and Web of Science™, finalised in July 2019, and covered the years 1969 to 2019. The search included 'microplastics OR plastics' with the following terms: ingestion, trophic transfer, toxicity, fish, plastic additives, effects, and

impacts. Additional records were also identified through reference lists in various review studies. Following removal of duplicate records, the remaining publications were screened based on study organisms and contaminants of concern (i.e., MPs and/or associated additives). Records that did not examine MPs and/or associated additives in aquatic species from coastal, pelagic, reef and deep-sea environments were subsequently removed. As the focus of this study was on MPs and associated chemical additives rather than environmental contaminants adsorbed to MPs, plastic additives considered here were limited to those outlined in Hahladakis et al. (2018) and Hermabessiere et al. (2017), and only if directly related to MP contamination. Full text articles were obtained for the remaining records where possible and assessed for eligibility for inclusion in the qualitative and quantitative assessment of evidence for bioaccumulation and biomagnification in a general marine food web. Criteria for exclusion included lack of species-specific information, scientific names not given, inability to assign a trophic level to species, non-aquatic species (i.e., birds), and contaminants were not MPs or associated additives. Lack of polymer assignment of putative MPs with a validated laboratory method, i.e., Fourier Transform Infrared Spectroscopy (FTIR), Raman Spectroscopy, or Gas chromatography–mass spectrometry (GC-MS) (Hermsen et al., 2018), was not used as a criterion as this would have excluded too many reports from the review. Finally, while there has been debate over the larger size limit of MPs being either < 1 mm (Van Cauwenberghe et al., 2013) or < 5 mm (Arthur et al., 2009), the more commonly used threshold of < 5 mm has been utilised when including literature (GESAMP, 2019).

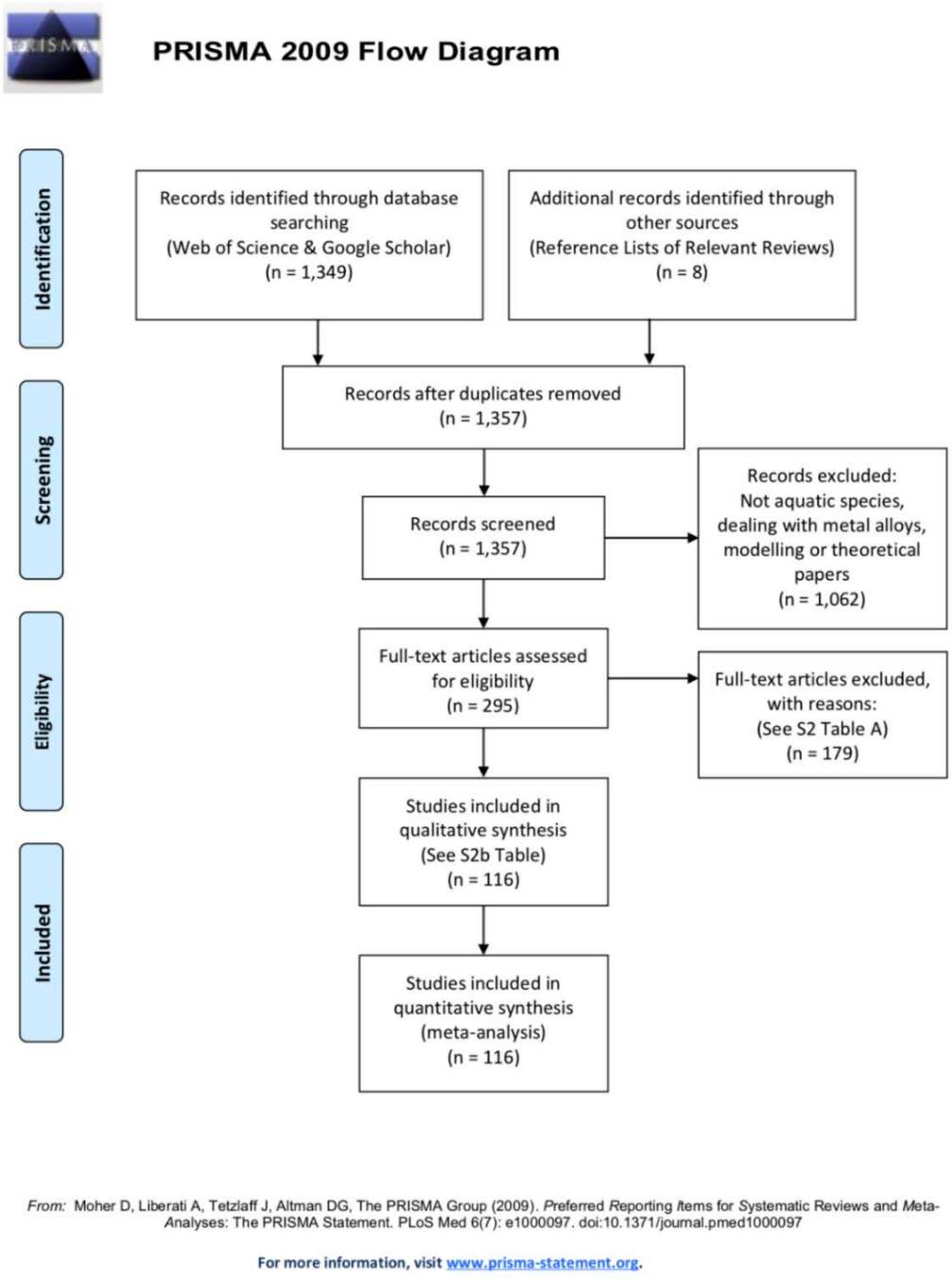

Figure 2.1 PRISMA flowchart providing the steps of data collection for the systematic review of evidence for bioaccumulation and biomagnification in a general marine food web. The review focussed on microplastics and associated chemical additives detected in marine species from coastal, pelagic, reef and deep-sea environments.

2.3.2 Standardisation of contamination data

To enable comparison of contamination data in a consistent format, the findings of eligible reports on MP contamination were collated and standardised into number of MPs per individual organism (MPs individual^{-1}; i.e., body burden) for each individual species (A1.1 Figure). For articles originally reporting in this unit the contamination data was used as is, while for articles reporting in MP g^{-1}, the contamination data was converted to MPs individual^{-1} using the reported individual weights. The unit of MPs individual^{-1} is representative of MPs per number of total organisms in the sample size for a particular species, rather than taken from only the number of organisms that exhibited MP contamination. Moreover, average (± standard deviation, S.D.) MPs individual^{-1} values presented throughout this study include data collated from reports of zero contamination, with concentrations representative of MPs per number of total organisms in the sample size for a particular species, rather than taken from only the number of organisms that exhibited MP contamination. Similarly, concentrations of plastic additives per individual (ng g^{-1}; i.e., body burden) were standardised for each individual species and were based on reported and quantified concentrations of additives in the tissues of the target organisms. All supplementary material available was examined if such data on MPs or additives were not reported in the original article. Any contamination data that could not be standardised given the information presented was removed from analysis. This included data presented as a percentage, without quantifying the number of particles extracted from organisms, as well as data lacking a sample size. Finally, to enable consistency in reviewing, terminology for MP shapes reported in the original article was condensed into four categories, namely fibres (alternatively 'filaments,' 'rope' and 'fishing line'), fragments (alternatively 'particle,' 'irregular' and 'crystal'), films, and spheres (alternatively 'beads' and 'pellets') (Hartmann et al., 2019).

2.3.3 Assignment to trophic level

To enable comparison of contamination data within and across trophic levels, each individual species was assigned to a specific trophic level using FishBase (Froese and Pauly, 2010) for all fish species and SeaLifeBase (Palomares and Pauly, 2010) for all other marine species. These databases use recent information on diet composition and food items, combined with modelling, to obtain a numerical trophic level value for individual species. In short, the trophic level of a given species is estimated using the equation:

$$[Trophic\ Level\ =\ 1 + mean\ trophic\ level\ of\ food\ items]$$

including a weighted mean based on the contribution of the various food items to the overall diet of the species (Froese and Pauly, 2010; Palomares and Pauly, 2010). For the purpose of this study, each species was assigned to one of five main trophic levels, namely: (1) primary producers (i.e.,

autotrophs, level 1); (2) primary consumers (i.e., herbivores, level 2); (3) secondary consumers (levels 2.1 to 2.9), (4) tertiary consumers (levels 3 to 3.9); and (5) quaternary consumers (levels 4 to 4.9).

Prior to assignment, the taxonomic nomenclature for individual species was verified using the World Register of Marine Species (Horton, 2019). Next, individual species were assigned to one of the following five trophic categories as mentioned above. First, primary producers (or autotrophs) are considered to be trophic level 1. While autotrophs produce their own food, primary producers still have the potential to interact with MPs through attachment to outer appendages and may act as an entry point into the food web (Zhang et al., 2017). Second, primary consumers (or herbivores) include a variety of zooplankton, bivalves and reef fishes. Omnivores and carnivores occur throughout levels 2.1 to 4.5 and include a wide variety of organisms (e.g., bivalves, fishes, mammals) with a multitude of feeding strategies. Tertiary and quaternary consumers are often top predators and are an important component to marine food webs. These species are of particular interest due to the potential biomagnification of contaminants resulting from the consumption of lower tropic levels, as well as their eventual use for human consumption (Rummel et al., 2016).

Following trophic assignment, the feeding habit of each individual species was also noted using the information provided by FishBase (Froese and Pauly, 2010) and SeaLifeBase (Palomares and Pauly, 2010). Organisms can exhibit a wide variety of feeding strategies which may affect MP uptake; namely filter feeding, grazing or browsing, selectively feeding on plankton, predator, scavenger and variable. Filter feeding organisms utilise the movement of external or internal appendages to produce a current, drawing particles in (Desforges et al., 2015). Grazers and browsers are herbivorous organisms that feed on algae growing along the substratum, usually by means of scraping (Ogden, 1976). Selectively feeding planktivores and predators use capture-based feeding where prey is obtained in a striking manner (e.g., meroplankton, reef fishes) (Hyatt, 1979). Scavengers are organisms that sift through the benthos opportunistically consuming plant and/or animal matter (Britton and Morton, 1994). Finally, variable indicates that species showcase multiple feeding strategies.

2.3.4 Assessment of bioaccumulation and biomagnification

To assess whether bioaccumulation and biomagnification was evident in a general marine food web, standardised data on MP and chemical additive contamination derived from field observations or laboratory experiments were compared within and across trophic levels. For bioaccumulation, the presence and abundance of MPs and chemical additives for individual trophic levels, and for individual species within each trophic level was examined. To assess bioaccumulation, specific attention was given to field-based reports that provided both estimates of contaminant exposure and quantified contamination within a species, and to laboratory-based reports that provided estimates of

contaminant exposure, as well as uptake and retention of contaminants within a species. For biomagnification, data was examined to determine whether contamination of MPs and chemical additives increased with increasing trophic levels. To assess biomagnification, specific attention was given to field-based studies that quantified levels of contamination across individual trophic levels, and to laboratory-based reports that contained a trophic transfer component.

2.4 Results

2.4.1 Selection of suitable contamination data

The systematic review of the literature identified a total of 1,357 publications (Figure 2.1, A1.1 Table). Following screening of the 1,357 records on study organisms and contaminants of concern (i.e., MPs and/or associated additives), 295 records remained for further assessment of eligibility. Based on eligibility criteria, primarily around inability to standardise contamination data given the information presented in the study (A1.2a Table), a total of 116 publications were included in the qualitative and quantitative assessment (A1.2b Table). These studies include reports on MP contamination in both *in situ* (n = 87) and laboratory-based marine organisms (n = 20), as well as reports on chemical additive uptake as a result of MP uptake in both *in situ* (n = 2 out of 87) and laboratory-based marine organisms (n = 7). Also included in this study are laboratory-based experiments demonstrating the trophic transfer of MPs (n = 2).

Across the 87 studies investigating MP contamination in field collected organisms, a total of 23,049 individuals comprising 411 species across 7 phyla were examined. From these, 537 individual organisms from 94 species exhibited no MP contamination, with the remaining 22,512 individual organisms from 329 species showing contamination with MPs (A1.3a Table). Contamination data for most species could be divided by sample size to obtain estimates of MPs individual^{-1}; data for 11 species (all bivalve molluscs) were transformed from MPs g^{-1} to MPs individual^{-1} to enable inclusion in this study (A1.3b Table). From 2 out of the 87 studies that examined *in situ* organisms, chemical additive uptake linked with MP ingestion was quantified for a total of 8 chemicals in 115 individual organisms from 3 species (A1.4 Table).

Laboratory studies investigating MP uptake (n = 20) included a minimum of 1,610 individuals comprising 21 marine species across 6 phyla (A1.5 Table). Transfer of MPs across two trophic levels was specifically investigated in three laboratory studies (A1.6 Table). In addition, seven laboratory studies investigated the uptake of chemical additives as a result of MP uptake on marine biota (A1.7 Table).

Across these seven studies, 581 individual organisms comprising six species across two phyla were analysed for contamination of five chemical additives.

2.4.2 Contamination in primary producers

MP contamination of primary producers (trophic level 1) in the marine environment was only investigated in one study (A1.3a Table) (Goss et al., 2018). MPs were found within the epiphytic layer of the autotrophic seagrass *Thalassia testudinum* at a quantity of 4.56 MPs individual^{-1} (n = 16; SD not quantifiable) (Figure 2.2a and 2.3). Contamination levels of the surrounding sampling area were not reported. While the shapes of putative MPs, including shapes including fibres, fragments, and spheres, were indicative of MPs (Figure 2.4), their polymer types were not confirmed. Studies on primary producers that examined contamination with chemical additives *in situ*, or contamination with MPs or chemical additives under controlled laboratory exposures were not found.

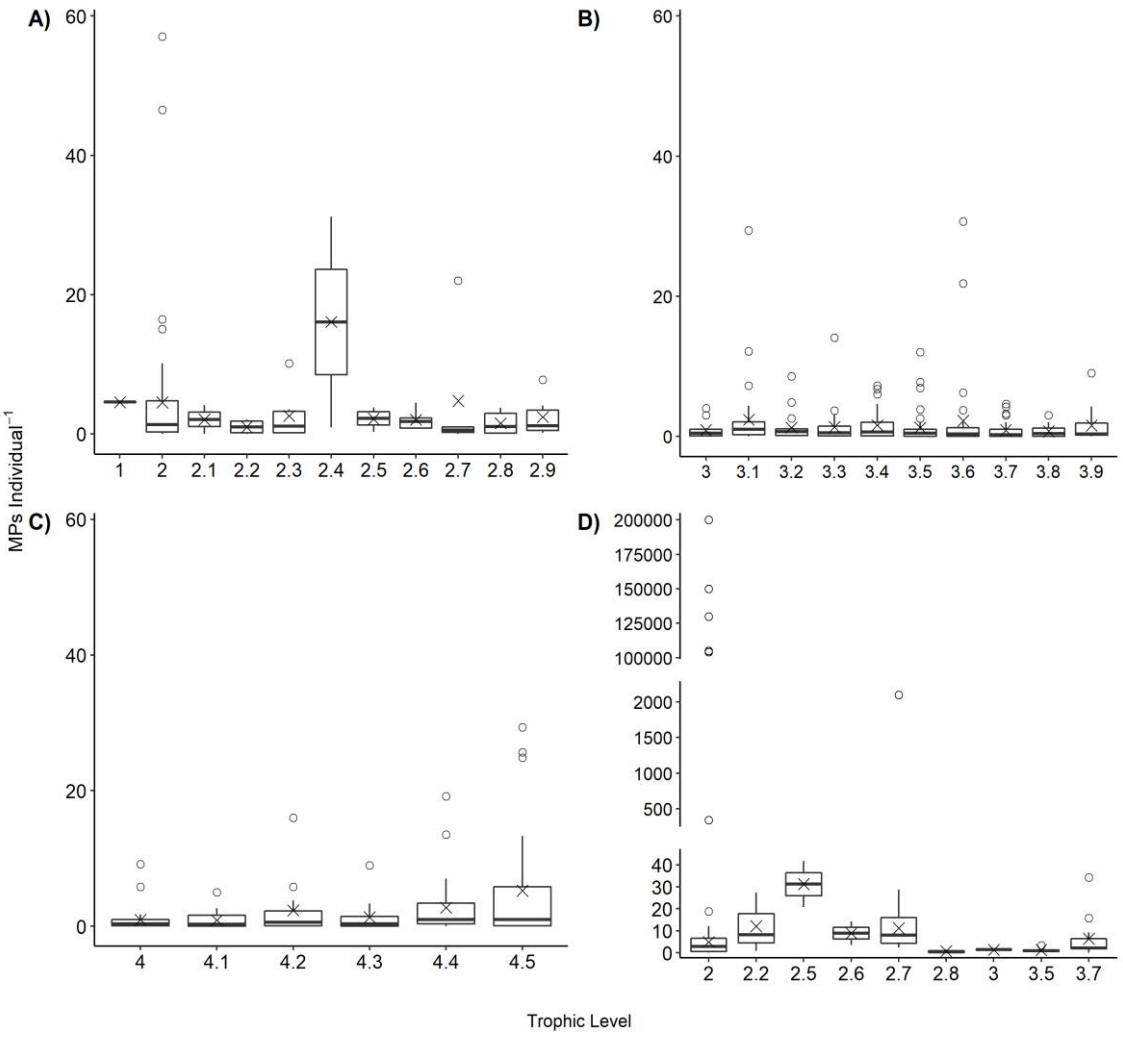

Figure 2.2 Body burden of microplastics individual^{-1} estimated for different trophic levels, based on reports for marine species collected in situ *(a) 1 to 2.9, (b) 3 to 3.9, and (c) 4 to*

4.5, and exposed in laboratory experiments (d) 2 to 3.7. Data have been organised to show the minimum, first quartile, median, mean (X), third quartile, maximum and outliers (°). Trophic levels have been grouped into to a single decimal place (level 4.2 includes 4.21 to 4.29).

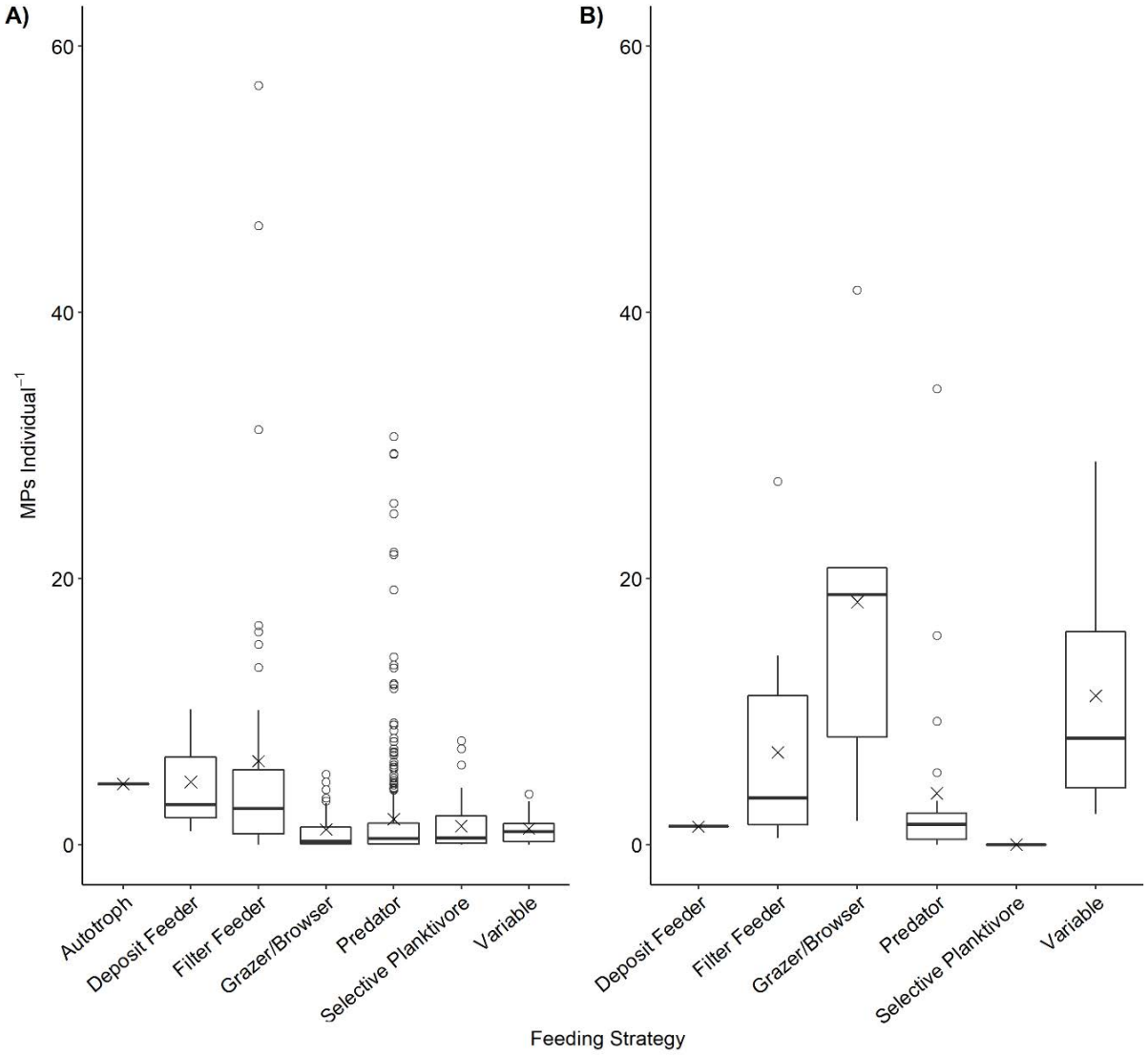

Figure 2.3 Body burden of microplastics individual^{-1} estimated for different feeding strategies, based on reports for marine species collected in situ *(a), and exposed in laboratory experiments (b). Data have been organised into feeding strategies and presented to show the minimum, first quartile, median, mean (X), third quartile, maximum and outliers (°). Note different scales on y-axes. Mean (X) values for (b) laboratory conditions are exclusive of outlier values.*

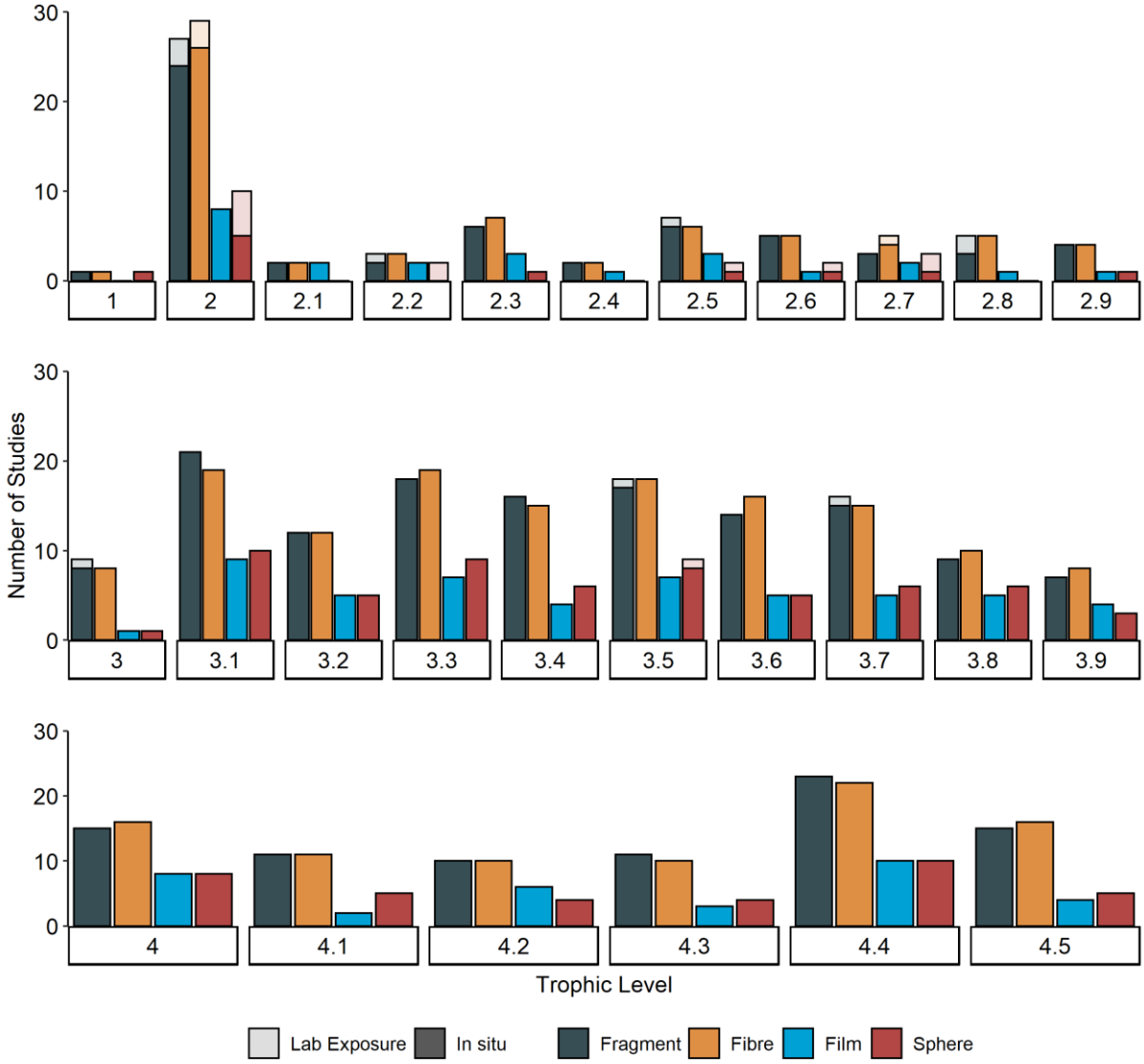

Figure 2.4 Frequency of microplastic (MP) shapes reported in studies on marine species collected in situ *and exposed in laboratory experiments. Data has been organised by trophic level, which are grouped into to a single decimal place, i.e., level 4.2 includes 4.21 to 4.29. Microplastic shapes include those found in organisms collected from field samples (n = 87 studies; dark shaded on bottom) or used in laboratory experiments investigating MP uptake, including those focused on trophic transfer (n = 22 studies; light shaded on top). Details on the number and percentage of studies for each level are provided in A1.8 Table.*

2.4.3 Contamination in primary consumers

A total of 41 publications on marine primary consumers (trophic level 2) contaminated with MPs and/or associated chemical additives were identified. Contamination of herbivores with MPs has been reported in both the field (n = 26 studies; A1.3a Table) and in laboratory experiments (n = 12; A1.5 Table). In contrast, only a few studies report on chemical additive contamination resulting from MP

uptake by herbivores from *in situ* observations (n = 1; A1.4 Table) or laboratory experiments (n = 4; A1.7 Table).

2.4.3.1 Field observations

In situ, 43 herbivorous species have been found to exhibit contamination, comprising 21 families across 4 phyla. On average, herbivores were found to be contaminated with 4.55 ± 8.59 S.D. MPs individual^{-1} (n = 4,993) (Figure 2.2a). MP uptake was greatest in molluscs (6.97 ± 11.22 S.D. MPs individual^{-1}; n = 3,135), followed by annelids (1.65 ± 1.48 S.D. MPs individual^{-1}; n = 18), fishes (0.83 ± 1.68 S.D. MPs individual^{-1}; n = 754), and arthropods (0.44 ± 0.48 S.D. MPs individual^{-1}; n = 1,086). Herbivorous filter feeders demonstrated the highest level of contamination (6.83 ± 11.04 S.D. MPs individual^{-1}; n=21 sp.), followed by browsers/grazers (0.96 ± 1.71 S.D. MPs individual^{-1}; n=17 sp.), scavengers (0.96 MPs individual^{-1}; n = 1 sp.), and selective planktivores (0.12 ± 0.15 S.D. MPs individual^{-1}; n=4 sp.) (Figure 2.3a). Five studies reported environmental MP contamination levels alongside MP uptake in primary consumers (Guven et al., 2017; Karlsson et al., 2017; Kosore et al., 2018; Lourenço et al., 2017; Wang et al., 2019b); however, different reporting units for environmental and organismal contamination makes direct comparisons difficult (Table 2.1). Notwithstanding, the MP body burdens reported for primary consumer species in these studies do not appear to support an accumulation of MPs within organisms compared to MP concentrations in the surrounding environments.

Table 2.1 Environmental contamination and bioaccumulation of microplastics (MPs) for marine organisms (MPs ind^{-1}) collected in situ*. Environmental contamination data on MPs pertain to results reported for locations where marine species were collected; please note different units. Microplastic concentrations reported for marine species were standardised into number of MPs per individual organism (MPs ind^{-1}; i.e., body burden; Section 2.4.1).*

Matrix	Environmental Contamination	Unit (MPs per)	Trophic Level	Associated Species	MP Ind^{-1}	Location	Reference
Surface Water	659.9 ± 520.9	m^{-3}	4.4	*Oncorhynchus tshawytscha*	1.15	Canada	(Collicutt et al., 2019)
Sediment	60.2 ± 63.4	kg^{-1} d.w.					
Surface Water	16,339 - 520,213	km^{-2}	2	*Siganus luridus*	3.13	Turkey	(Guven et al., 2017)
			2.8	*Liza aurata*	3.26		
			3.1	*Mullus barbatus*	1.39		
				Sardina pilchardus	2.14		
			3.4	*Lithognathus mormyrus*	0.63		
				Scomber japonicus	6.71		
				Serranus cabrilla	1.50		
			3.5	*Mullus surmuletus*	1.18		
				Pagellus erythrinus	0.63		
				Upeneus pori	0.69		

			3.6	*Diplodus annularis*	1.96		
				Pelates quadrilineatus	1.48		
				Upeneus moluccensis	0.78		
			3.7	*Sparus aurata*	0.87		
			3.8	*Nemipterus randalli*	1.31		
				Pagellus acarne	1.63		
				Pomadasys incisus	0.79		
				Sciaena umbra	3.00		
				Trachurus mediterraneus	1.77		
			3.9	*Pagrus pagrus*	1.44		
			4	*Chelidonichthys lucerna*	0.75		
			4.1	*Caranx crysos*	5.00		
				Dentex gibbosus	0.29		
			4.3	*Argyrosomus regius*	1.84		
			4.5	*Saurida undosquamis*	1.22		
Surface Water	27	L^{-1}	2	*Mytilus edulis*	1.23	North Sea	(Karlsson et al., 2017)
Sediment	48	kg^{-1} d.w.					
Surface Water	110	m^{-3}	2	*Copepoda spp.*	0.33	Indian Ocean	(Kosore et al., 2018)
Sediment	4.83 ± 2.44	ml^{-1}	2	*Cerastoderma edule*	4.30	Atlantic Ocean	(Lourenço et al., 2017)
				Hediste diversicolor	2.70		
				Pelecyora isocardia	1.50		
				Scolelepis squamata	0.60		
				Scrobicularia plana	3.30		
				Senilia senilis	1.00		
			3	*Diopatra neapolitana*	1.00		
				Glycera alba	3.00		
Sub-surface Water (6 m)	2.4 ± 0.8	m^{-3}	3.1	*Boreogadus saida*	0.22	Artic	(Morgana et al., 2018)
			3.3	*Triglops nybelini*	0.39		
Sub-surface Water	1.39	m^{-3}	3.3	*Callionymus lyra*	0.02	UK	(Steer et al., 2017)
				Microchirus variegatus	0.19		
			3.6	*Anguilla anguilla*	1.00		
			3.7	*Trisopterus minutus*	0.02		
Surface Sediment	560 – 4,205	kg^{-1} d.w.	2	*Acila mirabilis*	5.50	China	(Wang et al., 2019b)
			3.19	*Crangon affinis*	29.40		

The sizes of MPs detected ranges from 10 µm to 4.7 mm, while shapes included fibres, fragments, films and spheres (Figure 2.4). While not all studies confirmed polymer type of putative MPs detected in primary consumers, those that did found a wide range including polyethylene (PE), low-density

polyethylene (LDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyester (PES), viscose (VI; rayon), polyamide (PA; nylon), and others (Figure 2.5).

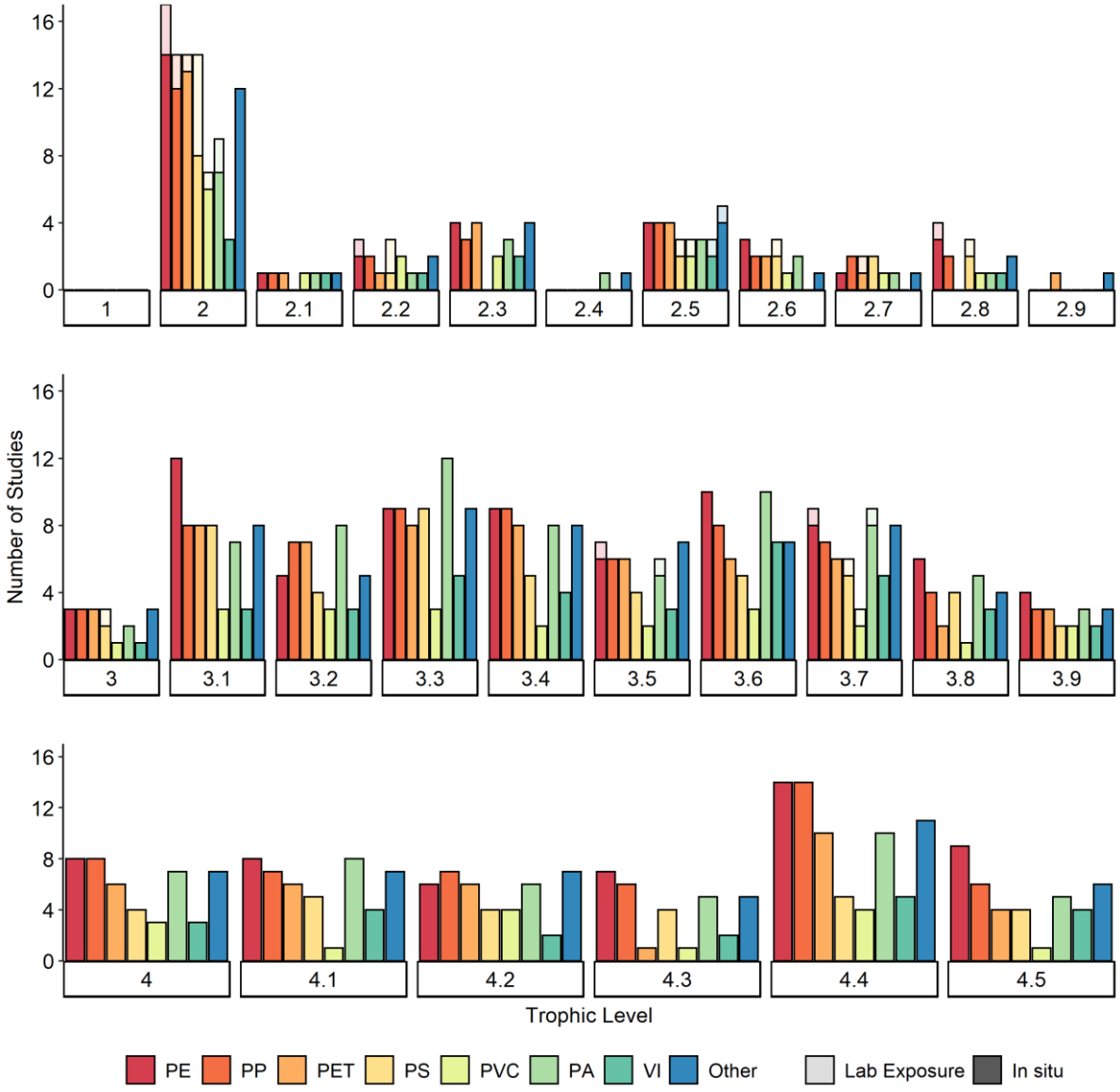

Figure 2.5 Frequency of microplastic (MP) polymer types confirmed in studies on marine species collected in situ *and exposed in laboratory experiments. Data is organised by trophic level and grouped into a single decimal place (level 4.2 includes 4.21 to 4.29). Polymers include those found in organisms collected from field samples (n = 87 studies; dark shaded on bottom) or used in experiments investigating MP intake, including those focused on trophic transfer (n = 22 studies; light shaded on top). Note that not all studies confirmed or reported MP polymer types. 'Other' includes less frequently found polymers such as: PAN, PMMA, CP, PC, ABS, EVA, PVA, PUR, PTFE, ASA, acrylic, alkyd, and epoxy. Some varieties of polymers have been grouped together (i.e., PE includes HDPE, MDPE and LDPE; PET includes PET and PES).*

Only two herbivorous species, namely the bivalves *Mytilus edulis* and *Cerastoderma edule,* have been examined for contamination with chemical additives associated with MPs in their natural habitat (A1.4 Table). Relatively high concentrations of phthalates were reported for *M. edulis* and C. *edule*, 26.36 and 52.36 ng g^{-1}, with concentrations of polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), and polychlorinated biphenyls (PCBs) substantially lower for both organisms (Hermabessiere et al., 2019). Contamination levels in the surrounding environment were not measured.

2.4.3.2 Laboratory exposures

Evidence of MP uptake by herbivores under controlled laboratory exposures has been examined in 11 species and confirmed for 8 species (A1.5 Table). While exposure conditions, such as durations, nominal MP concentrations, polymer types, sizes, and shapes varied, MP uptake ranged from 0 to 200,000 MPs individual^{-1} with an average of 25,596.57 ± 13,511.93 S.D. MPs individual^{-1} (n = 377) (Figure 2.2d). This extraordinary number is mainly a result of *C. helgolandicus*' ingestion rates of 3,278 and 104,100 MPs individual^{-1} (Cole et al., 2015; Procter et al., 2019) and *M. edulis*' ingestion of 105,000 to 200,000 MPs individual^{-1} (Porter et al., 2018). With those extreme values removed, average MP uptake drops to 25.62 ± 14.64 S.D. MPs individual^{-1} (n=241). Measured MP exposure concentrations were not reported in any of these studies. Examination of MP egestion over a depuration period showed that MPs were retained in *A. compressa* for at least 36 hours (Chua et al., 2014), and in *T. gratilla* for fewer than 2 days (Kaposi et al., 2014).

A total of four species have been examined for contamination with chemical additive associated with MPs uptake under controlled laboratory exposures, namely the mussels *M. edulis* (Magara et al., 2018) and *Mytilus spp*. (Paul-Pont et al., 2016), the clam *Scrobicularia plana* (O'Donovan et al., 2018) and the amphipod *Allorchestes compressa* (Chua et al., 2014) (A1.7 Table). Nominal exposure concentrations for PAHs (including fluoranthene and benzo[a]pyrene) and a range of PBDEs ranged from 5 ng to 100 µg l^{-1}, with chemicals dosed alone, alongside MPs, or absorbed to MP particles. Uptake of chemical additives seemed to be highest when dosed alone (2,000 to 117,000 ng g^{-1}) and lowest when absorbed to MPs (0 to 2,710 ng g^{-1}), regardless of the chemical additive or organism used. None of the studies reported on measured exposure concentrations or on retention rates for these chemicals or quantified the MP uptake by the organisms examined.

2.4.4 Contamination in secondary consumers

Overall, a total of 33 publications on marine secondary consumers (trophic level 2.1 – 2.9) contaminated with MPs and/or associated chemical additives were identified. Contamination of secondary consumers with MPs has been reported in both the field (n=26 studies; A1.3a Table) and in

laboratory experiments (n = 7; A1.5 Table). In contrast, only three studies report on chemical additive contamination resulting from MP uptake by secondary consumers from both *in situ* observations (n = 1; A1.4 Table) or laboratory experiments (n = 2; A1.7 Table).

2.4.4.1 Field observations

MP uptake by secondary consumers *in situ* has been investigated in 34 species, with 31 species exhibiting contamination, including various species of bivalves, echinoderms, arthropods, fishes and sea turtles. Overall, MP uptake by secondary consumers averaged 2.99 ± 6.40 S.D. MPs individual^{-1} (n = 2,755; Figure 2.2a). The highest levels of MP uptake are reported for molluscs (7.81 ± 20.67 S.D MPs individual^{-1}; n = 434), arthropods (7.80 ± 10.05 S.D MPs individual^{-1}; n = 900), and echinoderms (6.58 ± 5.06 S.D. MPs individual^{-1}; n = 202) (A1.3a Table). Lower levels were reported for sea turtles (*Chelonia mydas*; 2.3 ± 1.7 S.D. MPs individual^{-1}; n = 53), ascidians (1.78 ± 1.12 S.D. MPs individual^{-1}; n = 15), and fishes (1.39 ± 1.28 S.D. MPs individual^{-1}; n = 1,151). When organised by feeding strategies, species with scavenging behaviours demonstrated the highest levels of MP contamination (6.58 ± 5.06 S.D. MPs individual^{-1}; n = 2), followed by predators (5.44 ± 9.40 S.D. MPs individual^{-1}) and filter feeders (5.27 ± 13.41 S.D. MPs individual^{-1}); much lower levels were reported for species with variable feeding strategies, selective planktivores, and browsers/grazers (Figure 2.3a). Only one study reported environmental MP contamination levels alongside organism contamination (Table 2.1), with MP contamination in surrounding waters appearing to be much higher than levels found in the mullet *Liza aurata* (Guven et al., 2017). The sizes of MPs detected ranged from 8 µm to 5 mm, while shapes included fibres, fragments, films, and spheres (Figure 2.4). Polymer types of MPs detected in secondary consumers were confirmed to include PE, PES, PA, PP, PET, PVC, VI, PS, and others (Figure 2.5).

Only one species, namely the ascidian *Microcosmus exasperates*, has been examined for contamination with chemical additives associated with MPs in their natural habitat. The highest concentrations were reported for bis(2-ethylhexyl) phthalate (DEHP) (range: 4,851 – 4,988 ng g^{-1}; n = 15), followed by dibutyl phthalate (DBP) (range: 1,643 – 2,224 ng g^{-1}; n = 15) (Vered et al., 2019) (A1.4 Table). Contamination levels in the surrounding environment were not measured.

2.4.4.2 Laboratory exposures

Evidence of MP uptake by secondary consumers under controlled laboratory exposures has been documented in a total of 6 species, including bivalves, crustaceans, and fish; all species investigated exhibiting MP uptake (A1.5 Table). While exposure conditions varied, average uptake by secondary consumers was 127.99 ± 853.44 S.D. MPs individual^{-1} (n = 566) (Figure 2.2d). When removing extreme uptake values for *Acanthochromis polyacanthus* (up to 2,102 MPs individual^{-1}; (Critchell and Hoogenboom, 2018)), average MP uptake is reduced to 11.87 ± 12.48 S.D. MPs individual^{-1} (n = 454).

Measured MP exposure concentrations were not reported in any of these studies. Examination of MP egestion over a 48 h depuration period showed that MPs were retained in *Palaemonetes pugio* for an average of 43 h, ranging from approximately 28 to 76 h depending on the MP polymer type (Gray and Weinstein, 2017). Egestion of MPs by the mussel *Mytilus galloprovincialis* was 80% within the first 24 h, and 100% within 8 days (Capolupo et al., 2018).

Only one species, namely the bivalve *M. galloprovincialis,* has been examined for contamination with chemical additive associated with MPs uptake under controlled laboratory exposures (Avio et al., 2015a; Pittura et al., 2018) (A1.7 Table). Nominal exposure concentrations for benzo[a]pyrene and pyrene ranged from 0.15 µg l^{-1} to 15,000 ng g^{-1} and 200 to 260 ng g^{-1}, respectively, with additives either dosed alone or absorbed to MP particles. Uptake was greatest (470 ng g^{-1}) in *M. galloprovincialis* following a 7-day exposure to pyrene absorbed to PE and PS (Avio et al., 2015a). Following a 28-day exposure, uptake of benzo[a]pyrene in the mussel's digestive glands was higher when dosing alone (35 ng g^{-1}) compared to when absorbed with low-density polyethylene (LDPE; 30 ng g^{-1}) (Pittura et al., 2018). Beno[a]pyrene was found to accumulate over time in both the digestive gland and the gills, irrespective of exposure pathway (Pittura et al., 2018). Neither study reported on measured exposure concentrations or on retention rates for these chemicals or quantified the MP uptake by the bivalve examined.

2.4.5 Contamination in tertiary consumers

Marine tertiary consumers (trophic level 3 – 3.9) were investigated and found contaminated with MPs and/or associated chemical additives in 50 publications. Contamination of these consumers with MPs has been reported in both the field (n = 44 studies; A1.3a Table) and in laboratory experiments (n = 4; A1.5 Table). In contrast, only two studies report on chemical additive contamination resulting from MP uptake by tertiary consumers from laboratory experiments (A1.7 Table).

2.4.5.1 Field observations

In situ, evidence of MP uptake by tertiary consumers has been investigated in 224 species across 5 phyla and confirmed in 175 of these species (A1.3a Table). On average, MP uptake by tertiary consumers was 1.47 ± 3.46 S.D. MPs $individual^{-1}$ (n = 10,758) (Figure 2.2b). MP uptake was greatest in arthropods (8.15 ± 16.37 S.D. MPs $individual^{-1}$; n = 300), largely due to high MP uptake by the shrimp *Crangon affinis* (29.40 MPs $individual^{-1}$) (Wang et al., 2019b). In contrast, MP uptake was lower in annelids (2.00 ± 1.41 S.D MPs $individual^{-1}$; n = 5), fishes (1.39 ± 2.97 S.D. MPs $individual^{-1}$; n = 10,256), and sea turtles (1.5 ± 0.80 S.D. MPs $individual^{-1}$; n = 49). Tertiary consumers that exhibit predator (n = 7,897) and selective planktivorous (n = 2,753) behaviour had the highest amount of contamination (1.53 ± 3.82 and 1.36 ± 1.64 S.D. MPs $individual^{-1}$, respectively), with slightly lower contamination levels

in organisms with scavenger (n = 4), variable (n = 94) and filter feeding (n = 10) strategies; Figure 2.3a). Only five studies reported environmental MP contamination levels alongside organism contamination (Table 2.1). Overall, MP contamination in surrounding environments appear to be much higher than in the polychaete worms *Glycera alba* and *Diopatra neapolitana* (Lourenço et al., 2017), the shrimp *Crangon affinis* (Wang et al., 2019b), and in various fish species (Guven et al., 2017; Morgana et al., 2018). Only one study found comparable levels of MP contamination in both surrounding waters (range: 0.26 to 3.79 MPs m^{-3}) and in fish larvae (range: 0.02 to 4.8 MPs $individual^{-1}$, n = 156) (Steer et al., 2017). The sizes of MPs detected range from 10 µm to 5 mm, with the majority being smaller than 2 mm; shapes included fibres, fragments, films, and spheres (Figure 2.4). Polymer types of MPs detected in tertiary consumers were confirmed to include PET, PE, PVC, PP, PA, PS, PES, VI, and others (Figure 2.5).

2.4.5.2 Laboratory exposures

MP uptake by tertiary consumers under controlled laboratory exposures has been reported for 4 species, namely the polychaete worm Arenicola marina and the teleost fishes *Dicentrarchus labrax*, *Seriolella violacea*, and *Sparus aurata* (A1.5 Table). While exposure conditions varied, average uptake by tertiary consumers was 4.76 ± 2.82 S.D. MPs $individual^{-1}$ (n = 617) (Figure 2.2d). The highest average uptake was recorded for the seabream S. *aurata* (6.97 ± 10.13 S.D. MPs individual-1; n = 165), and the lowest for the palm ruff S. *violacea* (0.75 ± 0.15 S.D. MPs $individual^{-1}$; n = 132). Measured MP exposure concentrations were not reported in any of these studies. Examination of MP egestion over a depuration period showed that MPs were retained for fewer than 2 days in D. *labrax* (Mazurais et al., 2015), for an average of 4.4 ± 0.9 days in S. *violacea* (Ory et al., 2018b), and for more than 30 days in S. *aurata* (Jovanovic et al., 2018).

Only one species, namely the lobster *Nephrops norvegicus*, has been examined for contamination with chemical additive associated with MPs uptake under controlled laboratory exposures. Exposure to a nominal exposure concentration of 1.34 µg for PCBs either dosed alone, dosed alongside MPs, or absorbed to MP revealed that uptake of PCBs was highest when exposed to the chemical additive alone (Devriese et al., 2017). The study did not report on measured exposure concentrations or on retention rates for PCBs or quantified the MP uptake by the bivalve examined.

2.4.6 Contamination in quaternary consumers

A total of 42 publications on marine quaternary consumers (trophic level 4 – 4.9) contaminated with MPs and/or associated chemical additives were identified (A1.3a Table). All these studies reported on MP uptake in the field, with no reports on chemical uptake associated with MP contamination *in situ*, or on MP or chemical additive contamination from controlled laboratory exposures.

2.4.6.1 Field observations

MP uptake by quaternary consumers *in situ* has been investigated in a total of 109 species, and confirmed for 85 species, including fish, sea turtles and marine mammals (A1.3a Table). On average, quaternary consumers have ingested 2.42 ± 5.30 S.D. MPs individual^{-1} (n = 4,527; Figure 2.2c). MP uptake was greatest in cetaceans (11.06 ± 8.72 S.D. MPs individual^{-1}; n = 225) , followed by cartilaginous (1.25 ± 0.50 S.D. MPs individual^{-1}; n = 9), and ray-finned fishes (1.04 ± 1.93 S.D. MPs individual^{-1}; n = 4,131) (A1.3a Table). The lowest level of MP uptake was reported for elasmobranchs (0.27 ± 0.10 MPs individual^{-1}; n = 160). The baleen humpback whale, *Megaptera novaeangliae,* was the only filter-feeding quaternary consumer to be investigated, exhibiting the highest MP uptake of 16 MPs individual^{-1} (n = 1). In contrast, quaternary consumers that exhibit predator (n = 4,478) or variable (n = 48) feeding strategies contained substantially less MP contamination (2.34 ± 5.20 and 0.85 ± 0.84 S.D. MPs individual^{-1}, respectively; Figure 2.3a). Two studies reported environmental MP contamination levels alongside organism contamination (Collicutt et al., 2019; Guven et al., 2017) (Table 2.1). In both cases, the MP contamination in the waters surrounding fishes appears to be higher than levels detected in the organisms themselves. The sizes of MPs detected ranges from 10 µm to 5.0 mm with the majority being between 500 µm and 3 mm. Shapes included fragments, films, spheres, and fibres (Figure 2.4). Polymer types of MPs detected in quaternary consumers were confirmed to include PET, PA, PP, PE, PVC, PS, PES, VI, and others (Figure 2.5).

2.4.7 Evidence for biomagnification across a general marine food web

Finally, to assess whether biomagnification was evident in a general marine food web, data was examined to determine whether contamination of MPs and chemical additives increased with increasing trophic level, using the standardised data for each trophic level from the 87 *in situ* reports (A1.3a Table). The two laboratory-based reports that contain a trophic transfer component were also considered (A1.6 Table).

2.4.7.1 Field observations

Across the 5 main tropic levels there was no apparent increase in MP bioaccumulation with increasing trophic level, based on the estimated average MPs individual^{-1} for each of these 5 levels derived from a total of 411 species (22,987 individuals) collected *in situ* (A1.3a Table; Figure 2.2). On average, MP contamination is highest for herbivores (trophic level 2) at 4.55 ± 8.59 S.D. MP ind^{-1} (n = 4,993), and lowest for tertiary consumers (trophic level 3 to 3.9) at 1.47 ± 3.46 S.D. MP individual^{-1} (n = 10,738). Within the 5 tropic levels, the only slight increase in average MP body-burden was observed from trophic level 4 to 4.5 (Figure 2.2c). The slightly higher average MP individual^{-1} in trophic level 4.5 could be largely attributed to relatively high levels of contamination in marine mammals (A1.3a Table).

Notwithstanding, by far the highest average MP individual^{-1} was not in these highest tropic levels, but in secondary consumer trophic level 2.4 (Figure 2.2a) caused by the high bio-burden in the filter-feeding mussel *Perna perna* (Birnstiel et al., 2019). Indeed, rather than biomagnification of MPs across trophic levels, the body burden of MPs in marine species appears to be more influenced by feeding strategy (Figure 2.3a & b). Filter feeders have, on average, higher levels of MP contamination than any of the other feeding strategies, both *in situ* and under laboratory conditions (6.62 ± 11.03 S.D. MP individual^{-1}, n = 3,975; 32,523.89 ± 65,800.44 S.D. MP individual^{-1}, n=319, respectively). However, with outliers removed, laboratory-exposed grazers and browsers demonstrate higher levels of MP contamination on average (18.23 ± 15.24 S.D. MP individual^{-1}; n = 142; Figure 2.3b)

In situ biomagnification of chemical additives as a result of MP uptake in a general marine food web cannot be supported nor refuted based on the current literature. Only three marine species across two trophic levels have been examined for contamination with chemical additives associated with MPs in their natural habitat (Hermabessiere et al., 2019; Vered et al., 2019) (A1.4 Table). The chemical additives examined differ across the two trophic levels, with phthalates (including benzyl butyl phthalate [BBP], diethyl phthalate [DEP], dimethyl phthalate [DMP], diethylhexyl adipate [DEHA], DEHP and DBP), PAHs, PBDEs, and PCBs quantified in two species categorised as primary consumer (Hermabessiere et al., 2019), and only DEHP and DBP quantified in one species categorised as secondary consumer (Vered et al., 2019).

2.4.7.2 Laboratory exposures

Two studies have demonstrated the trophic transfer of MPs between marine species, although neither of these studies quantified MP uptake (A1.6 Table). Two experiments included feeding pre-exposed *Mytilus edulis* mussels (n = 24 to 50) to crabs *Carcinus maenas* (n = 24 to 42) (Farrell and Nelson, 2013; Watts et al., 2014). MP retention times for *C. maenas* ranged from 14 to 21 days but were not estimated for *M. edulis* as these studies assumed the immediate consumption of mussels by crabs. None of the laboratory studies reviewed examined potential trophic transfer of chemical additives associated with MP uptake in marine species.

2.5 Discussion

The aim of this review was to examine whether current, published findings support the premise that MPs, and their chemical additives, bioaccumulate and biomagnify across a general marine food web, a notion often inferred in the literature on marine MP contamination (Farrell and Nelson, 2013; Nelms et al., 2018; Zhao et al., 2018). Following a systematic review of the global literature, data was synthesised from 116 publications that quantified MP contamination for a total of 23,049 individuals

from 411 marine species in their natural habitat (n = 87 articles), and at least 1,610 individuals from 21 marine species in laboratory settings (n = 20 articles). These results corroborate previous studies (Kühn et al., 2015; Provencher et al., 2019) that bioaccumulation of MPs occurs in numerous individual marine species across four main trophic levels representing consumers, with MP contamination of primary producers also reported (Goss et al., 2018). Further, bioaccumulation of chemical additives associated with MP uptake has also been documented, albeit in fewer species (Chua et al., 2014; Devriese et al., 2017; Hermabessiere et al., 2019). Interestingly, in all six species examined, uptake of chemical additives was higher when exposed to the chemical alone compared to exposure alongside or on MPs (Chua et al., 2014; Devriese et al., 2017; Magara et al., 2018; Paul-Pont et al., 2016; Pittura et al., 2018). For most of the studies reporting bioaccumulation of MPs or chemical additives, the concentrations in the surrounding environment were not measured, hindering the elucidation of potential exposure pathways. In contrast to bioaccumulation, biomagnification of MPs across the five main trophic levels is not supported by field-based MP uptake data, although trophic transfer has been reported in two laboratory studies (Farrell and Nelson, 2013; Watts et al., 2014). *In situ* biomagnification of chemical additives as a result of MP uptake cannot be supported nor refuted, due to only a few studies examining different chemical additives (Hermabessiere et al., 2019; Vered et al., 2019). Finally, the body burden of MPs in marine species appears to be more influenced by feeding strategy rather than biomagnification (Bour et al., 2018a; Mizraji et al., 2017), a finding that may well be true for chemical additives as well.

2.5.1 Evidence for bioaccumulation

For this review, bioaccumulation was defined as the net uptake of MPs (or chemical additives) from the environment by all possible routes (e.g., contact, ingestion, respiration) from any source (e.g., water, sediment, prey) (Gerber, 2009; Maher et al., 2016). Results confirm bioaccumulation of MPs in numerous individual marine species constituting a general marine food web, in both field collected and laboratory exposed organisms. On average, however, the body burden for most marine species collected *in situ* could be considered low, with many reports of zero MP uptake for individual species and individuals within species (Baalkhuyur et al., 2018; Pegado et al., 2018; Vendel et al., 2017). Indeed, an apparent low incidence of marine debris (including MPs) uptake has been reported previously, with more than 80% of >20,000 individual coastal, marine and oceanic fish examined not containing any marine debris (Kroon et al., 2018b). The relatively low body burden is likely to reflect the inclusion of all organisms in the quantification of MP individual^{-1} for each species, a more representative estimate of MP bioaccumulation than only including the number of organisms that exhibit contamination (McGoran et al., 2018; Pegado et al., 2018). More broadly, a potential publication bias towards effects (i.e., detecting MP contamination in marine species) versus no effects (i.e., not detecting MP

contamination) may have influenced findings, although the existence and scale of such a bias in the MP literature is currently unknown (Hanson et al., 2018; Harris et al., 2014). Further, the large variety of methodological procedures used to quantify and report on MP contamination in marine organisms (Hidalgo-Ruz et al., 2012; Lusher, 2015; Miller et al., 2017) is likely to have affected the estimates of MP bioaccumulation. For example, polymer type is not always confirmed using spectroscopy or polarised light microscopy (Loder et al., 2015; Shim et al., 2017), a crucial step in the analysis workflow for MP quantification (Kroon et al., 2018a), potentially resulting in over-estimating MP contamination. Conversely, the *a priori* exclusion of microfibres in marine samples as potential contamination (Bour et al., 2018a; Santana et al., 2016) may result in under-estimates of MP bioaccumulation. Combined, while findings are based on the most exhaustive review of the global literature on MP contamination in marine organisms to date, future MP bioaccumulation estimates will likely be more robust with the development of agreed standardized procedures for sample processing and MP characterisation (GESAMP, 2019).

Bioaccumulation of chemical additives associated with MP uptake has been reported upon much less frequently than physical MP bioaccumulation, both *in situ* and in controlled laboratory experiments. Across all three marine species collected from the field, namely the clam *Cerastoderma edule*, the mussel *Mytilus edulis*, and the ascidian *Microcosmus exasperatus*, the concentrations of individual or combined phthalates were highest among the different chemical additives examined (Hermabessiere et al., 2019; Vered et al., 2019). This is not surprising as phthalates are primarily used as plasticisers and commonly detected in the oceanic environment (Hermabessiere et al., 2017). Indeed, other studies have speculated chemical contamination of marine organisms that was indicative of plastic contamination in the marine environment (Fossi et al., 2014; Rochman et al., 2014). Interestingly, phthalate body burden did not increase with MP bioaccumulation across these three marine species suggesting that the two may not be positively correlated. A comparative study examining phthalate and MP body burden within a single species across different levels of environmental contamination would further elucidate uptake of chemical additives associated with MPs *in situ*. Indeed, bioaccumulation of chemical additives was consistently, and often several magnitudes higher, following laboratory exposures of additives only compared to additives on MPs (Chua et al., 2014; Devriese et al., 2017; Magara et al., 2018; Paul-Pont et al., 2016; Pittura et al., 2018). Combined, these results would strongly suggest that environmental exposure to chemical additives *per se* affects bioaccumulation in marine organisms more strongly than exposure to chemical additives associated with MPs (Barboza et al., 2018a; Diepens and Koelmans, 2018; Koelmans et al., 2013).

Comparing MP bioaccumulation to *in situ* MP exposure concentrations revealed that for most, if not all, marine species the reported MP body burdens do not appear to support an accumulation of

MPs within species relative to the surrounding environment. However, different reporting units for organismal and environmental contamination levels makes direct comparisons difficult, an issue identified for marine debris research previously (Provencher et al., 2017). Previous studies detected higher number of MP particles in coastal fish collected from locations with higher MP particles in surrounding seawater and sediment (Guven et al., 2017). While chemical additives have been detected in field-collected marine species, neither of these studies measured their concentrations in the surrounding environment (Hermabessiere et al., 2019; Vered et al., 2019). Repeated field sampling of marine species, in particular from early to mature life history stages, combined with measurements of exposure concentrations will assist in elucidating whether MPs and/or chemical additives accumulate over time. Such studies would also provide critical information for more realistic and comparative laboratory studies, including environmentally relevant exposure characteristics such as concentrations, polymer type, and plastic size, shape and colour, a recommendation raised in previous reviews (Au et al., 2017; Burns and Boxall, 2018; Cunningham and Sigwart, 2019). Many of these characteristics have been demonstrated to affect retention, and thus bioaccumulation of MPs (Graham et al., 2019; Gray and Weinstein, 2017; Xu et al., 2019), but have rarely been examined using environmentally relevant exposures. Currently, comparisons between exposure and uptake of MPs and chemical additives in controlled laboratory studies are further complicated by the absence of measured versus nominal concentrations (Bour et al., 2018b; Cole et al., 2015). Improved quantification of different exposure pathways, such as respiration, direct uptake or indirect uptake via prey, would elucidate their relative importance in bioaccumulation. Such research should also consider that MP uptake by marine organisms can be non-random (Jensen et al., 2019) and highly selective (da Costa et al., 2016; Ory et al., 2017), including active rejection of MPs (Kaposi et al., 2014; Kim et al., 2019). Taking these caveats into account will result in improved estimates of bioaccumulation, subsequent trophic transfer, and potential biomagnification of MPs at higher trophic levels (Provencher et al., 2019).

2.5.2 Evidence for biomagnification

For this study, biomagnification was defined as the increase in concentration of MPs (or chemical additives) due to trophic transfer from lower to higher trophic levels (Gerber, 2009; Maher et al., 2016). The findings on bioaccumulation for different trophic levels do not support *in situ* biomagnification of either MPs or associated additives within a general marine food web. More specifically, there is no evidence based on current, published findings for an increase in average bioaccumulation of MPs and associated additives from lower to higher trophic levels across a general marine food web. In fact, trophic level 2.4 (secondary consumers) exhibited by far the highest average MP bioaccumulation, and trophic level 2 (primary consumers) showed the highest values of MP body burden across the general marine food web. These findings, based on a broad overview, do not negate the notion that trends of

MP biomagnification may differ when taking a targeted approach based on smaller geographic scales, on species-specific food chains, or on future projections of MP contamination. Additionally, the lack of evidence for *in situ* biomagnification of chemical additives as a result of MP uptake is primarily due to a lack of suitable data to support or refute such biomagnification. Such lack of data does not equate to evidence for or against biomagnification, a concept previously addressed for the MP literature (Koelmans et al., 2019), but rather that it remains uncertain based on current, published findings. This highlights the need for more careful inference of potential effects and ecological risks of marine MP contamination based on available evidence. Further, whether leaching of chemical additives from MPs into organisms occurs is currently unclear and requires further investigation for assessments of potential bioaccumulation and biomagnification. In laboratory experiments, trophic transfer has been reported from the mussel *Mytilus edulis* to the crab *Carcinus maenas* (Farrell and Nelson, 2013; Watts et al., 2014); however, it is unclear whether this resulted in biomagnification as MP presence in either prey or predator was not quantified. Importantly, the immediate consumption of contaminated mussels disregards bioaccumulation kinetics of MPs in prey and predator-prey interactions that would occur in the field (Diepens and Koelmans, 2018; Provencher et al., 2019). If trophic transfer of MPs is occurring *in situ*, results of this study imply that MPs ingested via prey items are not being completely retained within the next tropic level. Rather, MPs may become entangled in biological material during digestion by the predator and simply pass through as egested material. One line of evidence for trophic transfer in the field would be to document contaminated prey items from within the digestive tract of a consumer species, a feat not achieved so far for MPs. Only one study which found plastic particles (size not reported) in post-hatchling sea turtle stomachs recovered from fish (Boyle and Limpus, 2008). Finally, bioaccumulation of chemical additives associated with MP ingestion in the field has only been reported from a single trophic level (Hermabessiere et al., 2019; Vered et al., 2019), while trophic transfer has not been examined in controlled laboratory exposures, precluding analysis of potential biomagnification.

Rather than biomagnification through trophic transfer, results of this study corroborate previous studies that MP bioaccumulation is strongly linked with feeding strategies of marine species (Bour et al., 2018a; Mizraji et al., 2017). Field studies support this finding, with MP body burden being higher in pelagic fish species compared to demersal species irrespective of trophic level (Guven et al., 2017). MP bioaccumulation in fish larvae from the English Channel (Steer et al., 2017) were also higher compared to adult fish from the Arctic (Morgana et al., 2018), despite similar levels of MP contamination in surrounding waters. This likely reflects their feeding strategies with fish larvae filter-feeding continuously and unselectively on suspended particulate matter (Lazzaro, 1987; Steer et al., 2017), and adult *Triglops nybelini* and *Boreogadus saida* being selective predators that feed with a striking manner

(Froese and Pauly, 2010). Similarly, omnivorous juveniles of the fish *Girella laevifrons* were shown to have a higher MP body burden (specific quantity not reported) compared to other intertidal fish species categorised as grazing herbivores or selective carnivores (Mizraji et al., 2017). Higher MP contamination has been previously reported in selective predators compared to deposit and filter feeders, although Bour et al. (2018a) suggest caution in these results due to limited sample sizes and the exclusion of fibres. Further, exposure to 50 MP ml^{-1} resulted in higher MP body burdens in the filter feeding mussel *Mytilus edulis* (Porter et al., 2018) compared to the selective-feeding omnivorous shrimp *Palaemonetes pugi* (Gray and Weinstein, 2017), despite a shorter exposure time. Comparative studies examining MP body burden in organisms with varying feeding strategies following uniform exposures will aid quantifying the role of feeding strategies in influencing bioaccumulation of MPs.

The rationale behind assessing whether MP concentrations increased from lower to higher trophic levels stems from the classical concepts of bioaccumulation and biomagnification which is primarily applied to dissolved chemicals (Alexander, 1999). For physical items such as MPs, these end points may not completely suitable as chemicals and physical items would not interact with a marine organism in similar ways. Rather, physical MPs generally only come into contact with body cavities designed to pass material (i.e., gills or gastrointestinal tract). Translocation into other organs may occur via phagocytosis, albeit this is size dependent favouring smaller size classifications (Browne et al., 2008). Conversely, chemicals are readily dissolved and the potential pathways for uptake by the marine organism are greater, including into organs other than gills and gastrointestinal tracts. Therefore, whether the concepts of bioaccumulation and biomagnification are suitable for assessing the ecological risks of MP contamination in marine environments needs further and more detailed consideration.

2.6 Conclusions

Bioaccumulation and biomagnification of MPs, and associated chemical additives, in marine environments are often inferred in the literature on marine MP contamination. This review demonstrates that MP contamination occurs across all five main trophic levels in a general marine food web. Moreover, bioaccumulation of MPs occurs in numerous individual marine species across four main trophic levels representing consumers. The relative importance of different exposure pathways contributing to MP bioaccumulation, however, is not necessarily clear and needs further examination. While chemical additives have been detected in a few marine species collected *in situ*, results from laboratory exposures indicate that environmental exposure to chemical additives *per se* affects bioaccumulation more strongly than exposure to chemical additives associated with MPs. In contrast to MP bioaccumulation, this meta-analysis of *in situ* studies does not support biomagnification of MPs

from lower to higher trophic levels in a general marine food web, even though trophic transfer of MPs has been reported in a few laboratory studies. Indeed, MP bioaccumulation appears to be more strongly linked with feeding strategies, rather than trophic levels, of marine species. Finally, bioaccumulation and biomagnification are two critical concepts used in ecological risk assessments to determine the extent of pollutant transport within food webs. This review highlights the need for targeted field-based and experimental studies to elucidate the possible routes of uptake of MPs (and associated chemicals) and provide confidence in the use of these endpoints in the MP literature.

Chapter 3: Ingestion and retention of polyester microfibres in the presence of associated plasticiser bis(2-ethyl hexyl) phthalate by species inextricably linked within a food web

3.1 Abstract

Microplastics (MPs, < 5 mm) are intrinsically diverse contaminants due to their heterogenous polymeric composition, size, shape, and range of chemical additives. Their chemical, physical and structural properties have promoted their presence and prevalence in the environment, and they now represent an issue of growing concern in marine ecosystems. Numerous research efforts have highlighted the variability in the ingestion (or intake) and retention of MPs by marine organisms, linked to morphological differences between species and potentially to the exposure levels of individuals within a species. Unfortunately, the lack of detailed quantification of MP intake and retention in species inextricably linked within food webs means the variability arising from this exposure pathway cannot be corrected. Further, it is not known whether chemical additives used to manufacture tailored plastics, such as the carcinogenic bis(2-ethylhexyl) phthalate (DEHP), influence MP ingestion and retention. Elucidating the mechanisms that drive MP ingestion and retention is critical to understanding their true ecological impact. This laboratory study details the ingestion and retention of an environmentally relevant polymer, polyester microfibres (PEST), by three selective predators belonging to a multi-level coral reef food web. Copepods (*Parvocalanus crassirostris*), mysid shrimp (*Mysida sp.*) and moon wrasse (*Thalassoma lunare*) were individually exposed to a single dose of a) PEST, (b) PEST pre-adsorbed with DEHP, or (c) PEST added in tandem with DEHP, and assessed for ingestion and retention over 24 or 48 h. All organisms ingested and retained PEST for a least 1 h following exposure, with fish having the longest retention time: fish (48 h +) > mysid shrimp (12 h) > copepod (3 h). Further, MP ingestion was significantly influenced by the presence of DEHP in copepods, albeit less so for mysid shrimp and fish. This study offers insight into the mechanisms of organismal MP contamination for three species within a multi-trophic food web; the different retention times at each consumer level have implications for the trophic transfer of MPs.

3.2 Introduction

Since the mass production of plastic materials began in the 1950s, plastic litter has become a well-documented issue of concern and continues to attract global attention (GESAMP, 2019; Ostle et

al., 2019). Virgin plastic polymers are considered chemically inert; however, in their primary form they are often not fit for purpose. During manufacturing, polymer enhancement with chemical additives (e.g., plasticisers, flame retardants, heat stabilisers, etc.) is often done to impart specific properties such as flexibility and malleability, and to prolong the life of plastics (Hahladakis et al., 2018). Following the entry of plastic into the environment, mechanical abrasion and exposure to ultraviolet (UV) light can damage the integrity of plastic polymers (Zhu 2019), and aquatic environments can facilitate and accelerate the leaching of these chemical additives (Gulizia et al., 2022). These environmental processes can also promote plastic fragmentation over time (Andrady, 2011; Hahladakis et al., 2018) into microplastics (MPs; plastics < 5 mm). MPs that contain highly toxic chemical additives are of particular concern to the marine environment due to their continued uncontrolled introduction (i.e., through waste mismanagement) and subsequent intake by marine organisms across all trophic levels (Au et al., 2017) (Chapter 2). The potential for physical and chemical harm resulting from their ingestion and retention has led to MPs being classified as a diverse contaminant with the potential to act as a multiple stressor (Rochman, 2013; Rochman et al., 2019).

In the marine environment, plastic pollution is associated with two main types of chemical contaminants, either chemicals that are deliberately added to plastic during the manufacturing process (i.e., plastic additives) and/or chemical pollutants adsorbed to the surface of plastics from the surrounding media, i.e., polyhydrocarbons (Endo and Koelmans, 2016). Studies have shown that the digestive fluids of marine organisms can promote the leaching of adsorbed contaminants associated with ingested MP particles (Bakir et al., 2014), indicating the impacts of MP ingestion and retention may potentially pose an even greater risk to marine animals. One major group of chemical additives is the phthalic acid esters (PAEs or phthalates), which are primarily used as plasticisers to increase the flexibility, durability, and longevity of plastics (Hahladakis et al., 2018; Net et al., 2015). Phthalates are not chemically bonded to plastics but fill the free volume between polymer chains, and therefore can eventually migrate into the surrounding environmental matrix (Liang et al., 2008; Stringer and Johnston, 2001). Phthalates are now readily found contaminating the marine environment (Peijnenburg and Struijs, 2006), likely sourced from environmental plastics and MPs (Zhang et al., 2018). Bis(2-ethylhexyl) phthalate, or DEHP, is one of the most frequently used phthalate plasticisers worldwide (Rowdhwal and Chen, 2018). It is a carcinogenic substance and, along with its degradation products, is known to result in endocrine disruption (Crisp et al., 1998; Kamrin, 2009). As a result, DEHP is now considered a priority pollutant. Despite these impacts being well documented (Bergé et al., 2013; Net et al., 2015; Rowdhwal and Chen, 2018), the interactive effects DEHP has on MP ingestion and retention have not been investigated (Chapter 2).

The intake of MPs has been reported for a plethora of marine species, both collected *in situ* and observed under laboratory conditions during exposure experiments (Chapter 2). Yet, detailed quantification of MP ingestion and retention rates is underreported, and rarely considered with other stressors (e.g., the presence of chemical additives). More recently, while there have been efforts to design laboratory-based MP exposure studies to more accurately represent the shape, size, and concentration of MPs found within the natural environment (Bour et al., 2018b; Bucci et al., 2020; Santana et al., 2021), such studies are limited. Ultimately, this lack of information hinders understanding the environmental and ecological relevance of MP contamination (Bucci et al., 2020). To address this shortfall, studies are required to establish the mechanisms that drive MP ingestion and retention, and to accurately assess the implications of MP contamination through trophic transfer, including bioaccumulation and biomagnification of MPs to higher trophic levels (Farrell and Nelson, 2013; Nelms et al., 2018; Zhao et al., 2018).

Coral reefs, including those in the Great Barrier Reef World Heritage Area (GBRWHA), are incredibly important ecosystems with significant traditional and economical importance. They support a high level of biodiversity and productivity; however, like many other natural ecosystems, they are no longer exempt from the widespread presence of MP contamination (Huang et al., 2021a; Kroon et al., 2020; Tan et al., 2020). There are now six reports of MPs contaminating marine biota on the GBR, confirming a diverse range of GBR species are susceptible to MP intake (Caron et al., 2018; Jensen et al., 2019; Kroon et al., 2020; Santana, 2022). While every report of MP ingestion helps clarify the level of MP contamination, focus should be given to elucidating the MP ingestion and retention rates for GBR species at different trophic levels within a food web to assess the full extent of their ecological fate.

The goal of this study was to assess ingestion and retention of an environmentally relevant MP, polyester (PEST) microfibres, under varying exposure conditions by three selective predators belonging to a multi-level coral reef food web. Specifically, copepods (*Parvocalanus crassirostris*), mysid shrimp (*Mysida sp*.) and moon wrasse (*Thalassoma lunare*) were exposed to a single dose of (a) PEST, (b) PEST pre-adsorbed with DEHP (PEST:DEHP), or (c) PEST added in tandem with DEHP (PEST+DEHP). Initial PEST ingestion and retention rates were established, and then compared to those observed in PEST:DEHP and PEST+DEHP treatments to reveal any influence(s) this chemical additive may have. The individual ingestion and retention of MPs by these three trophically interacting species will help elucidate the ecological fate and impact of MPs on ecologically important species within a coral reef food web.

3.3 Methods

3.3.1 Permits and ethics

All experimental aquaria work was conducted at the Australian Institute of Marine Science's (AIMS) SeaSim facilities, located near Gurumbilbarra, on Wulgurukaba and Bindal Country (Townsville, Queensland) in accordance with relevant institutional and national guidelines (Great Barrier Reef Marine Park Authority permit G12/35236.1 and James Cook University (JCU) Animal Ethics Committee Approval Number A2722).

3.3.2 Animal culturing, collection, and husbandry

Water quality parameters, including temperature (°C), pH, salinity (ppt), and dissolved oxygen (DO; mg L^{-1}) were continuously monitored. Light conditions were set to 12h:12h light:dark and achieved using SOL blue LED lights, which were slowly increased to a final level of 80% intensity of 100 Photosynthetic Active Radiation (PAR) over 4 days (20% increase per day). Specific culturing parameters and rearing details for copepods and mysid shrimp can be found in the supporting information (A2.1 Table).

Parvocalanus crassirostris copepods were cultured under laboratory conditions for the purposes of these experiments. Adult *P. crassirostris* were obtained from JCU's Marine and Aquaculture Research Facilities Unit (M.A.R.F.U) and reared in two 200 L conical tanks at SeaSim facilities. Copepods were fed a 200 ml algae mixture of *Tisochrysis lutea* (*T-Iso)* and *Chaetoceros muelleri* in a 1:1 ratio throughout culturing and experimentation. Water changes were conducted twice weekly on stock culture populations.

Mysida sp. mysid shrimp were cultured using a stock population maintained in mesocosm coral reef tanks within SeaSim facilities, originating from local offshore mid-shelf reefs (e.g., Davies Reef). Mysid shrimps were transferred to a 100 L conical tank, and a controlled population was maintained for the duration of the experimental period. Mysid shrimp stock populations were fed a minimum of 25,000 frozen copepods (*P. crassirostris;* Ocean Nutrition Frozen) daily to ensure cannibalism did not occur (Heindler et al., 2017).

Moon wrasse *(T. lunare*, n=72) were collected between January and April 2021 by Cairns Marine using hand and barrier nets at various mid-shelf reefs within the GBRWHA (A2.1 Figure). Upon collection, fish were transported to SeaSim facilities where they underwent acclimation in glass 50 L experimental tanks. Fish were kept individually in tanks, each equipped with an air supply (i.e., titanium rod) and a silica-glazed ceramic structure (13 x 11 cm; hollow) to provide refuge. Fish were fed a minimum of 1.2 mg per g fish weight of frozen mysid shrimp daily (*Mysida sp.;* Ocean Nutrition Frozen).

All fish were observed feeding within 24 h of arrival to SeaSim facilities, an indication of healthy and normal behaviour.

3.3.3 Experimental design

Copepods (n=250 per tank), mysid shrimp (n=6 per tank) and moon wrasse (n=1 per tank) were experimentally exposed to fluorescent yellow PEST microfibres (Birch Polyester Thread Fluoro Yellow 1,000 m, EAN: 9313792079477) and DEHP (Sigma Aldrich, CAS: 117-81-7). PEST was chosen as it is one of the most common MP polymer types found in the GBRWHA (Jensen et al., 2019; Santana, 2022) (Chapters 5 and 6). The fluorescent colour allowed discovery within the individual gastrointestinal tracts (GITs) of copepods and mysid shrimp, without the need to chemically digest all animal tissues (e.g., acidic, caustic), a process which could potentially complicate MP recovery (Miller et al., 2021). Treatments included a single dose of (a) PEST, (b) PEST:DEHP, (c) PEST +DEHP, and (d) controls. Controls consisted of a 1 µm filtered seawater (FSW) control (i.e., food only) and a FSW + dimethylsulfoxide control (DMSO; used as a carrier solvent for DEHP)). All treatments, including the control treatment, were added with food (see details below).

All three species were individually exposed to one of the four treatments for 30 min (copepods and mysid shrimp) or 1 h (fish) and replicates were removed at set depuration timepoints of 0, 1, 3, 6, 12 or 24 h for copepods and mysid shrimp, and 0, 3, 6, 12, 24, or 48 h for fish (Figure 3.1). A total of three tank replicates were conducted for each species timepoint per treatment resulting in 72 replicate tanks for each species. Experimental tank set-up and treatment allocation were randomised using a customised random generator script in R (Version 4.04 with Rstudio, version 1.4.1106).

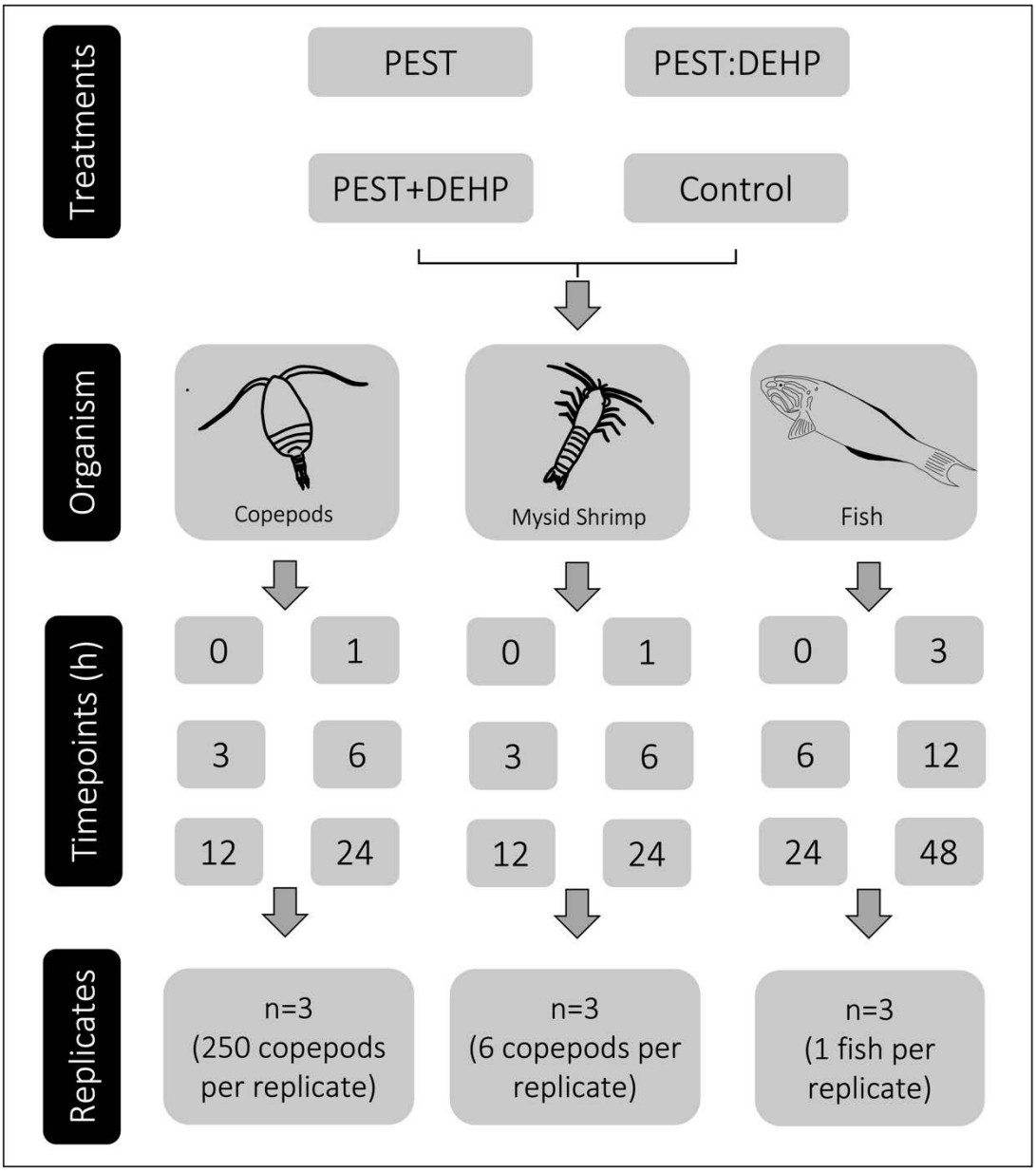

*Figure 3.1. Experimental design for polyester (PEST) exposures of copepods (*Parvocalanus crassirostris*), mysid shrimp (*Mysida sp.*) and fish (*Thalassoma lunare*). Replicates are the number of replicate beakers or tanks per timepoint, number of individual organisms given below in parentheses.*

3.3.4 Treatment preparation

3.3.4.1 Microfibre preparation

PEST microfibres were prepared from the Birch Polyester Thread. Lengths of PEST were prepared to be consistent with that of the natural prey of each species. Microfibres ranged from 10 ± 2 µm for copepods, 350 ± 28 µm for mysid shrimp, and 750 ± 14 µm for fish. Smaller fibres (i.e., 10 ± 2 µm) were prepared using a cryostat. Prior to cryostat cutting, the number of fibres (i.e., monofilaments) contained

within one thread of PEST were counted to ensure the desired concentration would be obtained. Threads were then wrapped around two metal prongs extending from a stainless-steel stage and frozen at -20ºC for > 30 min. The threads were then coated with a water-soluble polyethylene glycol solution (Tissue-Tek®) and placed back in -20ºC until fully frozen (i.e., > 1 h). The stage was removed, and the thread was carefully cut using a scalpel blade within the confines of the cryostat (Leica CM1860 UV cryostat). Cuts were placed upright on the cryostat platforms, re-covered with polyethylene glycol solution, and placed back in the freezer for 1 h. The cryostat was set to various lengths between 8 and 12 µm to get a stock population of fibres that were 10 ± 2 µm and sizes were confirmed throughout the procedure via microscopy (A2.2 Figure). Following cutting procedures, PEST microfibres were filtered over 0.46 µm polytetrafluoroethylene (PTFE, Omnipore) filters and rinsed thoroughly with Milli-Q water (MQ-H_2O) to remove the polyethylene glycol solution. Filters were then backwashed into a (pre-cleaned) 250 ml Schott bottle and filled with FSW. To confirm the desired concentration of PEST in the stock solution, the solution was shaken vigorously to ensure homogeneity and aliquots (1 ml, n=5) taken, filtered over PTFE for visual inspection and counted under magnification (Leica DMI 6000B, with DFC310 FX camera attachment, 10 – 20 x magnifications, I3 filter with 450-490 excitation). This stock solution was used to dose copepods. Larger fibres (i.e., > 300 µm) were cut using a scalpel blade under microscope (Leica M80, 0.75 – 6.0x magnification) and their size was confirmed using a micrometre ruler. These larger fibres were added directly to treatment vials.

3.3.4.2 Dosage

PEST and DEHP doses were prepared to reflect detectable levels within a laboratory setting. The PEST concentration used was 10 PEST microfibres individual^{-1}, representing an order of magnitude higher than the reported levels within *in situ* organisms (Chapter 5). This resulted in the addition of 5,000 PEST L^{-1} for copepods, 120 PEST L^{-1} for mysid shrimp and 0.2 PEST L^{-1} for moon wrasse. DEHP was dosed at a concentration of 1 mg L^{-1}, representing one order of magnitude higher concentration than median levels found globally (Bergé et al., 2013). All treatments were prepared in 20 ml glass scintillation vials and stored in the dark at 4ºC. Treatments included:

a) PEST only. PEST treatments were dosed at a concentration of 10 PEST microfibres individual^{-1}. For copepods, smaller fibres (i.e., 10 µm) were pipetted as 2 ml aliquots of the PEST stock solution (refer above for details) into individual vials to give ~2,500 fibres vial^{-1}. Larger fibres (i.e., > 300 µm; n = 60 for mysid shrimp and n = 10 for fish) were handpicked under magnification (Leica M80, 0.75 – 6.0x magnification) using a long needle and placed into individual vials. All PEST vials were made up to 15 ml with 1 µm FSW.

b) DEHP pre-adsorbed to PEST (PEST:DEHP). Relevant PEST concentrations were added to individual vials. The smaller 10 µm PEST samples were evaporated to dryness under a stream of nitrogen overnight. Hexane (1 ml; Fisher Scientific, CAS Number 110-54-3) was added to each vial followed by DEHP (0.5 mg $vial^{-1}$ for copepods and mysid shrimp, 50 mg $vial^{-1}$ for fish). Vials were capped (aluminium foil lined) and shaken on an orbital shaker (Baxter Multi-Tube Vortexer, Baxter Diagnostics) for 1 h. Hexane was then left to fully evaporate under a continuous nitrogen stream and vials were stored capped (A2 Text and A2.3 Figure).

c) PEST and DEHP added in tandem (PEST+DEHP). These components were dosed from two separate scintillation vials, one containing PEST and the other DEHP. PEST vials were prepared as described above. DEHP vials were prepared by adding DEHP to 1 ml DMSO for a final concentration of 1 mg L^{-1} DEHP in the tank. DEHP is hydrophobic, hence DMSO was used as a carrier solvent to ensure homogeneous distribution of DEHP in the tanks.

d) Controls. Two control treatments consisted of FSW with no PEST or DEHP. One of the control replicates for each species' treatment timepoint also contained DMSO (1 ml) as a solvent control.

Treatments were dosed into tanks and vials rinsed (x3) with FSW. Alongside exposures, a small amount of normal feed for each organism was added: 1,250 cells of T-Iso and *C. muelleri* mixture (1:1) for copepods, 30 frozen copepods for mysid shrimp, and 5 frozen mysid shrimp for the fish. Feed was given in a ratio of 50% of the PEST exposure to promote feeding during the experimental exposure period (Albano et al., 2021), as well as to simulate environmentally realistic conditions. There was no starvation period for any organism during experimentation.

3.3.5 Acclimation and experimental procedures

3.3.5.1 Copepods

Approximately 250 adult copepods were aliquoted from a stock culture population and transferred into 600 ml experimental tanks (i.e., beakers) containing 500 ml FSW one day prior to exposures for acclimation (> 12 h). Tanks were covered with aluminium foil and aerated via titanium rods for the duration of the acclimation and experimental period. Copepods, confirmed to be alive based on observed movement throughout the water column, were subsequently exposed to one of the four treatments. Following a 30 min exposure period, tanks were filtered over a 26 µm stainless steel filter net and rinsed gently with FSW. Copepods were then backwashed into fresh FSW and left for a set depuration period (0, 1, 3, 6, 12 and 24 h). After the depuration period, copepods were emptied over the 26 µm stainless steel filter net and rinsed with FSW. The 26 µm filter net aperture was deliberately chosen as it was larger than the PEST size yet less than the copepods size, ensuring any

free (i.e., not ingested) PEST microfibres are washed away. The filter net was then backwashed into an aluminium foil weigh-boat. At that point, the copepods were collected using a glass pipette and immediately fixed in 70% ethanol (EtOH) for PEST analysis.

3.3.5.2 Mysid shrimp

One day prior to exposures, mysid shrimp (n=6) were removed from their culture tank, transferred into a 600 ml experimental tank (i.e., beaker) containing 500 ml FSW and allowed to acclimate (> 12 h). Following exposure to one of the four treatments for 30 min, tank contents were emptied over a 547 µm stainless steel filter and rinsed with FSW. Cleaned mysid shrimps were then backwashed into fresh FSW and left to depurate for a set period (0, 1, 3, 6, 12 and 24 h). Following the allotted time, mysid shrimp were filtered, rinsed, and sampled using forceps and immediately fixed in 70% EtOH for PEST analysis.

3.3.5.3 Fish

Prior to exposure, fish were left to acclimate in their 50 L glass tanks for a minimum of 7 days with a flow rate of 0.8 L min^{-1}. Under static conditions (i.e., flow halted), fish were exposed to one of the four treatments for 1 h. Following the 1 h exposure period, the flow rate was returned to 0.8 L min^{-1}, debris on the tank bottom was siphoned and fish were left to depurate for a set time (0, 3, 6, 12, 24, and 48 h). At the conclusion of each depuration time, fish were euthanised via ice slurry (ice and FSW mixture) and kept frozen in aluminium foil until dissection.

3.3.6 Contamination control and exposure validation

Filtered water was used throughout to minimise contamination of water-borne MPs, including 0.46 µm filtered MQ-H_2O within the laboratory and 1 µm FSW within SeaSim facilities. All experimental tanks were covered with either glass lids (50 L fish tanks), or aluminium foil (600 ml glass beakers) to reduce the likelihood of contamination by airborne MPs during experimentation. Several 50 ml vials (clear polypropylene cup, yellow high-density polyethylene screw cap) were randomly positioned throughout the experimental room on top of tanks and benches to capture any airborne contamination. These controls were kept closed when lids were on experimental tanks and beakers.

All glassware (except 50 L tanks) was rinsed first with acetone, followed by a rinse with hexane and a final liberal rinse of seawater prior to use in experiments. During the experiment, cotton clothes were always worn, and a lint roller (Scotch-Brite®) was used before to entering the experimental room. Pre-filtered 70% EtOH (0.46 µm) was used throughout to preserve experimental organisms. Hexane was purchased was analytical grade, pre-submicron filtered and therefore assumed no extraneous

contamination existed. As DEHP is hydrophobic and readily adsorbs to plastic, plastic equipment (including nitrile gloves) and utensils were avoided during its preparation where possible.

To validate the nominal concentrations of PEST, tanks and beakers void of organisms were exposed to the different PEST concentrations: n=3 for copepod beakers, n = 3 for mysid shrimp beakers and n= 3 for fish tanks. Vials were dosed in the same fashion as experiments, as described above. Subsequently, each beaker or tank was emptied (beaker) or siphoned (tank) over a 0.46 µm PTFE filter to capture any PEST. Siphoning tanks was followed by flushing with a minimum of 30 s of FSW to ensure any PEST present were not trapped within the siphon hose. Filters were then visually analysed, and contaminating PEST counted using fluorescent microscopy (Leica DMI 6000B, see details below).

3.3.7 PEST analysis

All exposed copepods and mysid shrimp were individually transferred to glass microscopes slide using forceps and compressed with a second slide. Frozen fish were thawed and subsequently measured (standard length, cm), weighed (wet weight (w.w.), g), and dissected to remove the gastrointestinal tract (GIT; stomach and intestines) and gills. Individual fish GITs were chemically digested using a standard 10% potassium hydroxide (KOH, prepared with MQ-H_2O) protocol for 48 h at 40°C (Dawson et al., 2022; Santana et al., 2022). Following digestion, the remains of GITs were filtered (Schlawinsky et al., 2022) over 0.46 µm PTFE filters (diameter = 19 mm) and stored between two glass microscope slides until visual analysis. Additionally, the fish gills were placed between glass microscope slides for analysis.

Slides of copepod, mysid shrimp and fish GIT remnants and gills were visually analysed using fluorescent microscopy (Leica DMI 6000B, with DFC310 FX camera attachment, 10 – 20x magnifications). Images were captured through an I3 filter (excitation range: blue, excitation filter: BP 450-490, dichromatic mirror: 510, suppression filter: LP 515) using LAS X software (version 2.0.0.14332) and analysed to obtain PEST counts and sizes (longest length, mm).

Control vials were filtered over 0.46 µm PTFE filters and were also visually inspected under magnification using a bright-field filter and a fluorescent filter (i.e., I3 filter on Leica DMI 6000B) to determine whether fluorescent microfibres matching the model MP were present.

3.3.8 Data analysis

The I3 fluorescent filter photos were assessed with the aid of ImageJ (FIJI; Version 1.53e). Fluorescing PEST were counted and sized using the freehand line tool in ImageJ against an embedded scale bar on each photo. PEST ingestion and retention was determined individually for copepods, mysid shrimp, and fish as the number of PEST present in the GIT of each organism at a given time point.

Ingestion is considered to be the number of PEST within an organism's GIT immediately following the exposure period (i.e., T_0). Retention is representative of the number of PEST in an organism's GIT throughout the depuration period. Mean ingestion and retention of PEST are presented as average PEST ± standard deviation (S.D.) of replicates per treatment timepoint; in the discussion units are presented as PEST individual^{-1} to enable comparison with literature.

To assess differences in initial ingestion between treatments for each species, multiple general linear models (GLMs) were run with Gaussian distributions. To account for a possible tank effect, tank number was included as a nested variable within each model. Post-hoc analysis was done using the estimated marginal means (EMM) from the model for pairwise comparisons. All statistical analyses were run in R (Version 4.04 with Rstudio, version 1.4.1106).

3.4 Results

3.4.1 General experimental information

Throughout the study, no organism presented any signs of stress or mortality from the control, PEST, PEST:DEHP or PEST+DEHP treatments. Water quality was measured continuously throughout experimentation, with variations in temperature (25.59 ± 0.57 °C), pH (8.15 ± 0.06), salinity (35.53 ± 0.39 ppt), and DO (7.85 ± 0.16 mg L^{-1}) negligible and reflecting *in situ* GBR conditions (i.e., Davies Reef). Ammonia (NH_3) and nitrate (NO_3) levels were consistently below levels considered harmful to marine organisms (i.e., 0 ppm and < 5 ppm, respectively).

3.4.2 Contamination control and exposure validation

No experimental PEST microfibres were found in any organism exposed to the control treatments, confirming cross-contamination did not occur during the experiment. A total of 27 putative MP microfibres were identified from the airborne contamination controls (n=9) and physically characterised (A2.2 Table); however, microscopy revealed none matched fluorescent PEST fibres. While airborne items were not chemically confirmed as MPs (unnecessary, given no fluorescent yellow fibres were recovered), it does highlight the need for controlled environments during laboratory MP exposure experiments to reduce extraneous items in the water that may interfere with ingestion rate determination or analysis of health effects during laboratory studies (Santana et al., 2021).

Measured PEST concentrations were comparable to those of their nominal concentrations, with exposure validation showing measured PEST concentrations varying from 2,475.89 ± 60.55 PEST for

250 copepods, 59.44 ± 1.01 PEST for 6 mysid shrimp, and 9.56 ± 0.73 PEST for 1 fish. This confirms that desired concentrations were achieved and PEST microfibres were available to each organism.

3.4.3 PEST ingestion

All individuals demonstrated immediate PEST ingestion, irrespective of treatment, albeit the initial (i.e., T_0) ingestion rate varied for each organism (Table 3.1; Figure 3.2). No PEST was found within the control treatments. At T_0, copepods ingested < 1% of offered PEST fibres, mysid shrimp consumed between 3.33% and 83.3% and fish between 30% to 100% (A2.3 Table). PEST found within copepods and fish were of similar size ranges to those of the dosed PEST fibres (10.24 ± 2.31 µm and 731.69 ± 62.02 µm, respectively). However, PEST fibres found within mysid GITs were much smaller (i.e., 57.56 ± 27.61 µm), indicating fragmentation of fibres during ingestion.

Table 3.1. Ingestion (T_0) and retention of polyester microfibres (PEST) for copepods, mysid shrimp and fish over time following a single laboratory exposure to PEST under three conditions: PEST, PEST pre-adsorbed with bis(2-ethyl hexyl) phthalate (DEHP; PEST:DEHP), and PEST added simultaneously with DEHP (PEST+DEHP). Data presented as the mean PEST individual^{-1} ± standard deviation present in the gastrointestinal tract at a given time point.

Organism	Retention Time (h)	Number of PEST		
		PEST	PEST:DEHP	PEST+DEHP
Copepod	0	8.08 ± 1.71	8.86 ± 5.25	17.47 ± 1.76
	1	5.65 ± 2.36	6.96 ± 4.13	10.80 ± 3.65
	3	2.01 ± 1.33	3.05 ± 1.96	2.85 ± 2.59
	6	0.00	0.00	0.00
	12	0.00	0.00	0.00
	24	0.00	0.00	0.00
Mysid shrimp	0	18.67 ± 6.08	9.11 ± 4.70	21.33 ± 15.94
	1	18.44 ± 6.77	8.89 ± 5.93	13.56 ± 7.86
	3	14.44 ± 7.73	4.67 ± 6.63	8.22 ± 5.14
	6	7.56 ± 9.68	3.11 ± 4.91	7.56 ± 10.09
	12	2.45 ± 3.97	1.56 ± 3.43	3.78 ± 3.38
	24	0.67 ± 1.41	0.89 ± 2.03	3.33 ± 4.36
Fish	0	8.33 ± 1.53	7.00 ± 1.15	5.67 ± 2.31
	3	5.67 ± 0.58	5.67 ± 1.15	4.33 ± 0.58
	6	4.00 ± 1.41	3.00 ± 0.00	3.00 ± 1.00
	12	2.67 ± 1.15	1.33 ± 0.58	0.33 ± 0.58

24	2. 00 ± 1.00	1.67 ± 1.15	0.33 ± 0.58
48	0.33 ± 0.58	0.33 ± 0.58	0.33 ± 0.74

PEST ingestion by copepods was significantly different for all treatments (GLM; $p < 0.05$; Table 3.2), with the highest ingestion rates recorded for PEST+DEHP, followed by PEST:DEHP and then PEST alone (Figure 3.2). For mysid shrimp, the simultaneous addition of DEHP did not influence PEST ingestion; however, significantly lower ingestion of PEST microfibres pre-adsorbed with DEHP was observed ($p = 0.046$; Table 3.2). For fish, PEST only treatments had the greatest influence on PEST ingestion, with higher ingestion rates at T0 compared to other treatments (Figure 3.2); the presence of DEHP, either added simultaneously or pre-adsorbed, had no influence on PEST ingestion.

*Table 3.2. Differences in ingestion (T_0) rates of polyester microfibres (PEST) for copepods, mysid shrimp, and fish following a single laboratory exposures to polyester fibres under three conditions: PEST, DEHP pre-adsorbed to PEST (PEST:DEHP) and PEST and DEHP added simultaneously (PEST+DEHP). A general linear model using a gaussian distribution with tank replicate as a nested variable (see A2.4 Table) was used for the post-hoc pairs tests. Significant codes: 0.05 **

Organism	Contrast	estimate	SE	df	t.ratio	p.value
Copepod	PEST – PEST+DEHP	-9.39	0.43	189	-21.71	< 0.001 *
	PEST – PEST:DEHP	-1.42	0.51	189	-2.78	0.016 *
	PEST+DEHP – PEST:DEHP	7.97	0.51	189	15.61	< 0.001 *
Mysid shrimp	PEST – PEST+DEHP	2.67	4.82	24.00	0.55	0.846
	PEST – PEST:DEHP	12.22	4.82	24.00	2.54	0.046 *
	PEST+DEHP – PEST:DEHP	9.56	4.82	24.00	1.98	0.138
Fish	PEST – PEST+DEHP	2.67	1.41	6.00	1.89	0.223
	PEST – PEST:DEHP	1.67	1.41	6.00	1.18	0.507
	PEST+DEHP – PEST:DEHP	-1.00	1.41	6.00	-0.71	0.768

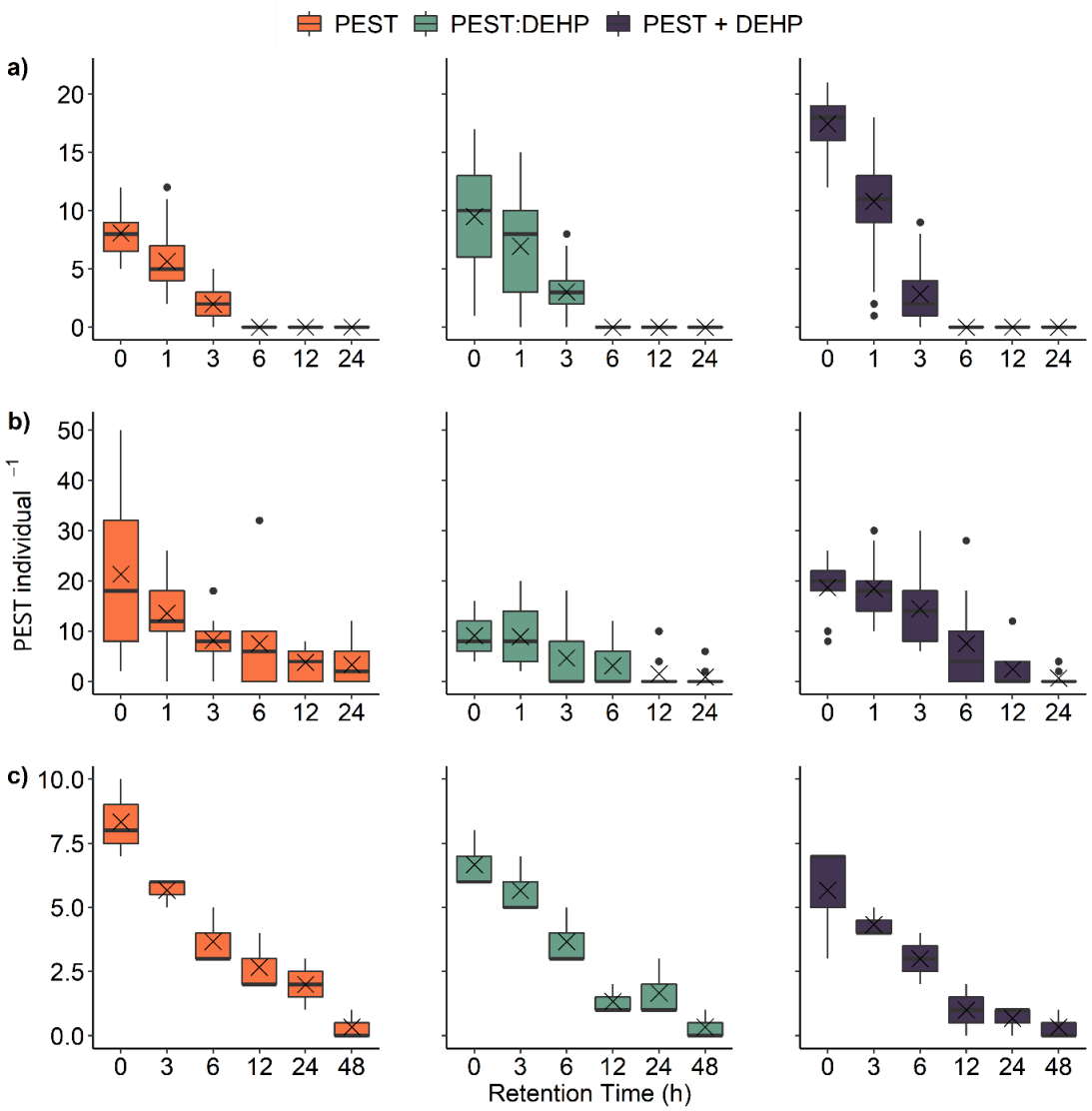

*Figure 3.2. Mean polyester (PEST) ingestion and retention across time, for organisms exposed to PEST, PEST pre-adsorbed with bis(2-ethylhexyl) phthalate, DEHP (i.e., PEST:DEHP), and PEST dosed simultaneously with DEHP (i.e., PEST+DEHP). PEST dosed at a concentration of 10 PEST individual^{-1}; DEHP dosed at a concentration of 1 mg L^{-1}. Data presented as PEST individual^{-1}, for (a) copepods (*Parvocalanus crassirostris*, n= 250 per replicate), (b) mysid shrimp (*Mysida sp.*; n= 6 per replicate) and (c) moon wrasse (*Thalassoma lunare*; n = 1 per replicate). Each time point contained 3 replicates per treatment; however, note that T_{48} was not measured for copepods or mysid shrimp and T_1 was not measured for fish. X is representative of the mean number of MPs at a given timepoint.*

3.4.4 PEST retention

Levels of PEST with the GIT decreased over the depuration period for all exposed species, indicating temporary PEST retention within organisms. At the 1 h post-exposure timepoint, there were signs of full expulsion of PEST; no PEST was observed in some copepods exposed to the PEST:DEHP

treatment. By T_3, levels of PEST contamination in copepods dropped to similar ranges (2.55 ± 2.00 PEST ind^{-1}), regardless of initial ingestion or treatment (Figure 3.2). The majority of PEST were expelled by copepods between T_3 and T_6, with no PEST observed at T_6, indicating that retention of PEST microfibres in *P. crassirostris* is less than 6 h. Irrespective of treatment, individual PEST retention in mysid shrimp ranged from 1 to 24 h, and PEST were retained, on average, for at least 24 h. The first evidence of complete PEST depuration occurred 1 h post-ingestion for those mysid shrimp exposed to PEST only. Mysids exposed to PEST:DEHP treatments did not exhibit full egestion of PEST until T_3, and those exposed to PEST+DEHP treatments until T_6, albeit not all individuals followed this trend (Figure 3.2). PEST were retained by fish for up to 48 h, with all exposed fish containing PEST at the last depuration period. Regardless, there was a steady decrease of PEST in fish GIT across time independent of treatment.

3.5 Discussion

This study revealed three selective predators, the calanoid copepod *P. crassirostris*, mysid shrimp *Mysida sp*. and reef fish *T. lunare*, belonging to different trophic levels within a coral reef food web, all readily ingested and temporarily retained PEST microfibres following acute (i.e., < 1 h) exposure. All three species retained PEST for a least 1 h following exposure, with fish having the longest retention time of > 48 h. As these species comprise a multi-trophic level food web, these findings offer insight into the mechanisms of MP intake and retention at each trophic level and have implications for the trophic transfer of MPs. Further, this study highlights the potential influence the plasticising phthalate, DEHP on these biological processes.

The PEST exposure concentrations used in this study (0.2 to 5,000 PEST L^{-1}) were chosen as they are more representative of environmental contamination compared to previous experimental studies (Capolupo et al., 2018; Gray and Weinstein, 2017; Qu et al., 2018). MP contamination in the GBRWHA is present but considered low, with water column levels averaging at $4.66 \times 10^{-3} \pm 4.24 \times 10^{-3}$ MPs L^{-1} for central mid-shelf reefs (Chapter 5). Often it is standard practice for ecotoxicology studies to use future predicted concentrations to overcome the limitations of using true realistic MP concentrations (Santana et al., 2021). Here, the higher MP concentrations used in laboratory experiments reflect the upper level of GBRWHA contamination, although it should be noted the environmentally relevant concentrations for MPs within smaller size ranges (i.e., < 50 µm) remains uncertain due to sampling technique limitations (Athey et al., 2020; Brander et al., 2020; Covernton et al., 2019). With the continuing decline in the condition of the GBRWHA (GBRMPA, 2019) and model predictions indicating an increase in contaminants of emerging concern (Kroon et al., 2020), including plastic pollution

(Everaert et al., 2020), it is important to understand the impact of higher concentrations of MP contamination may have on marine life.

3.5.1 Copepod ingestion and retention

Zooplankton, the primary consumers in marine food webs, play a crucial role in the marine ecosystem as an important food source for numerous secondary consumers (Turner, 2004). As copepods predominately feed in both surface water and the water column, where the abundance of MPs is high (Barrows et al., 2018a; Choy et al., 2019), there is a higher risk of MP ingestion from the environment (Botterell et al., 2019; Cozar et al., 2014). Confirmation of the ingestion of 10 µm PEST microfibres by *P. crassirostris* here corroborates previous laboratory studies. Calanoid copepods (i.e., *Calanus helgolandicus*) were found to readily consume MPs under laboratory conditions, ingesting anywhere from 3,278 to 104,100 MPs individual^{-1} when exposed to either 20 µm polystyrene fragments for 24 h at a concentration of 75 MPs ml^{-1} (Cole et al., 2015) or 30 µm polyamide fibres for 6 h at a concentration of 80 MPs ml^{-1} (Procter et al., 2019), respectively. MP ingestion has been shown to correlate to the exposure concentration (Santana et al., 2021); the lower exposure concentration (5 PEST ml^{-1}) used here correlates to a lower ingestion rate by *P. crassirostris* (8.08 ± 1.71 to 17.46 ± 1.76 MPs individual^{-1}), following the same trend. Regardless of exposure concentration, the percentage of MPs ingested for both this study, and previous reports (Cole et al., 2015; Procter et al., 2019), is considerably low (i.e., < 1 %).

PEST microfibres were retained in the *P. crassirostris* GIT for up to 3 h, with complete egestion observed by some individuals at 1 h post-exposure. Previously reported effects of MPs on copepods seem to vary, possibly depending on species, exposure time, or MPs used (e.g., polymer type, size, shape, concentration). Prolonged exposure to polystyrene MPs significantly decreased reproductive output in *C. helgolandicus*, but there were no significant differences in egg production rates, respiration, or survival (Cole et al., 2015). Whereas Heindler et al. (2017) observed negative impacts on *P. crassirostris* survival, fertility, population sizes and gene expression after exposure to PET. These impacts may be a consequence of continuous turn-over (i.e., intake, depuration, re-intake) given the > 24 h exposure periods. The short retention time observed here emphasises that if exposure to MPs is not continuous, egestion of fibrous MPs from the GIT can be achieved in less than 3 h. Conversely, as *in situ* MP exposure is continuous, albeit heterogeneous, copepods could potentially continuously ingest and egest MPs, prolonging their exposure. The impacts of confirmed retention of MPs by copepods after exposure were not considered in this study and physiological and behavioural endpoints should be investigated to truly assess risk. Further, plastic shape has been shown to influence both effects (Bucci et al., 2020) and retention times (Santana et al., 2021) in marine organisms. As only one

polymer type and shape (i.e, PEST microfibres) was investigated here, future research should focus on other shapes and polymer types to encompass the diverse suite of heterogeneous MP contamination that exists (Rochman et al., 2019).

3.5.2 Mysid ingestion and retention

Mysid shrimp play an important role in energy transfer as intermediate prey species in marine food webs (Verslycke et al., 2007). Mysid shrimp have been confirmed to ingest MP contamination in the field (Chapter 5) and are routinely used in ecotoxicology studies investigating the effects of MPs under laboratory conditions (Lee et al., 2021; Setala et al., 2014; Wang et al., 2020). While ingestion by organisms is often observed during MP exposure experiments, it is rarely quantified (Chapter 2), with ingestion for mysid shrimp being no exception. This study corroborates previous work reporting MP ingestion, demonstrating ingestion of PEST microfibres (~350 µm) by mysid shrimp at relatively low concentrations (i.e., 0.12 MPs ml^{-1}). The PEST ingestion reported here is slightly lower (9.11 ± 4.70 to 21.33 ± 15.94 PEST $individual^{-1}$) than that observed for polyethylene beads (27 to 32 µm) in *Neomysis sp.,* which ranged from 65.53 ± 63.36 to 266.82 ± 155.30 MPs $individual^{-1}$ depending on exposure concentration (0.2 to 2 mg L^{-1}) (Hasegawa and Nakaoka, 2021). The difference in exposure concentration, MP size, shape and polymer type used by these other studies may be a contributing factor in the difference in ingestion rates observed here.

Small crustaceans, such as mysid shrimp, have powerful mandibles and thick, barbed chitinous spines in their stomach (Dawson et al., 2018b; Friesen et al., 1986) that promote the mechanical breakdown of prey material for digestion. Here, mysid shrimp were found to fragment 350 µm microfibres down to < 90 µm, a process previously observed for MP particles in other small crustaceans including *Neomysis sp*. (Hasegawa and Nakaoka, 2021) and Antarctic krill *Euphausia superba* (Dawson et al., 2018b). If fragmentation of MPs by small crustaceans is common, it could prove to be a route for increased concentrations of smaller-sized MPs. Further investigation of this phenomenon is required to elucidate the interactions between crustaceans and MPs.

On average, the retention time for PEST microfibres in mysid shrimp is at least 24 h; however, some individuals demonstrated full expulsion by 12 h, with a few containing no PEST as early as 1 h post-exposure. These observations match previous ranges (35 min to 13.67 h post-exposure) reported for polystyrene beads (1 and 10 µm) travelling from the gastric mill to the anus of mysid shrimp (*Neomysis awatschensis*) (Lee et al., 2021). MPs have been observed impairing the feeding and swimming behaviour of *N. japonica* (Wang et al., 2020) and inhibiting *N. awatschensis* growth, fecundity, and survival (Lee et al., 2021). Given the similarity in retention time profiles for mysid shrimp and *N. awatschensis*, it is anticipated that similar physiological effects may occur if MP exposure was

continuous. However, this requires further investigation as effects from MPs vary depending on exposure conditions and MP characteristics (Bucci et al., 2020).

3.5.3 Fish ingestion and retention

Reef fish, including wrasse (Family *Labridae*), play an essential role in coral reef ecosystems as tertiary consumers in marine food webs (Holmes et al., 2012; Kramer et al., 2016). Their ability to ingest MPs from environmental exposure has been reported, with MPs shown to bioconcentrate relative to environmental levels (Chapter 5). Evidenced here is the intake of nearly all bioavailable PEST MPs by moon wrasse (*T. lunare*) under laboratory conditions (30 to 100% of offered PEST). Given that *T. lunare* are highly opportunistic selective predators (Holmes and McCormick, 2006), and with no MP contamination observed in their gills, these findings indicate PEST microfibres are being selectively eaten alongside food. However, the accidental ingestion of MPs during prey events, as observed in other fish species, is still possible and warrants further research (Garcia et al., 2021; Vendel et al., 2017).

Retention of PEST in *T. lunare* is at least 48 h, with all individuals still containing PEST at the final time point. This is longer than reports of retention of PP fragments or PET fibres in other reef fish such as *Pomacentrus amboinensis (Santana et al., 2021)*, albeit these species potentially have a quicker gut throughput time given their smaller size compared to *T. lunare* (Welden and Cowie, 2016a). It is not known whether prolonged retention of MPs will have significant impacts on *T. lunare* health. Although given MPs have been demonstrated to impact fish consumption, growth, reproduction, and survival (Foley et al., 2018), there is potential. This highlights the need for research into chronic exposures of MPs and species-specific impacts.

3.5.4 Influence of DEHP

The carcinogenic pollutant DEHP (Kamrin, 2009) has been previously shown to adversely impact *P. crassirostris* (e.g., survival, fecundity, and gene expression; (Heindler et al., 2017)), crustaceans (e.g., growth, moulting, energy metabolism, reproduction, population size, and morphology (Verslycke et al., 2004)), and fish (e.g., decreased fecundity, changed spawning behaviour (Ye et al., 2014)). Here, exposure to PEST treatments containing DEHP demonstrated that copepods ingested and retained higher amounts of PEST. However, the opposite effect was observed for mysid shrimp and fish, with ingestion of PEST higher when exposed to the PEST microfibres alone. This finding suggests that the impact of phthalates on ingestion of PEST is species-specific. Regardless, this study provides evidence that the presence of phthalates can significantly influence the ingestion rate of MPs, potentially compounding their impacts as a marine contaminant (Rochman et al., 2019). In addition, with some individuals of each species exhibiting longer retention of MPs in the GIT, there is a higher likelihood that chemical additives such as DEHP will translocate into their body tissue (Chua et al., 2014) or be

metabolised into toxic by-products (Hu et al., 2017). Further research is needed to elucidate whether chemical additives or contaminants adsorbed to MPs can transfer into organism tissue upon ingestion or be further transferred up the trophic cascade (Foley et al., 2018; Koelmans et al., 2016).

DEHP is hydrophobic, having a relatively high water/octanol partition coefficient (logKow) of 7.5 (ECB, 2008). As such, DEHP is immiscible in FSW, and a non-toxic carrier solvent (i.e., DMSO; 0.2%) was required for dispersal throughout the PEST+DEHP treatment tanks. DMSO is naturally occurring in the environment, produced by multiple biological and photochemical processes (Lee and De Mora, 1999) and is regularly used as a carrier solvent in ecotoxicology studies (Brayton, 1986; Turner et al., 2012). Copepods use chemosensory mechanisms to identify algal prey, and it has been shown that the algal gaseous metabolite dimethyl sulphide (DMS; the precursor to DMSO and a known foraging attractant (Asher et al., 2017)) results in increased MP ingestion (Procter et al., 2019). The elevated ingestion of PEST dosed in tandem with DEHP in DMSO (PEST+DEHP) compared to PEST adsorbed with DEHP (i.e., no DMSO) (PEST:DEHP) indicates DMSO may be acting as a copepod attractant, or that dissolved DMSO is being reduced to DMS. The inclusion of a positive DMSO control with PEST fibres should be considered in the future to elucidate whether ingestion was truly influenced by DMSO rather than DEHP. Regardless, the role of DMSO in the marine environment is poorly understood (Asher et al., 2015) and its use in copepod ecotoxicity studies should be carefully considered. It is also possible the surface area-to-volume ratio of the smaller PEST particles to which copepods were exposed may influence the amount of DEHP that is adsorbed onto the PEST and therefore have a greater influence over ingestion behaviour compared to the larger PEST items mysid shrimp and fish were exposed to (Fred-Ahmadu et al., 2020).

3.6 Conclusions

It has been well established that MPs are ingested by marine organisms, causing a suite of health and behavioural impacts. Quantifying not only the ingestion but also the retention of MPs is becoming increasingly important to help elucidate their longer-term impacts on marine organisms and to assess whether trophic transfer is plausible. This study reports detailed ingestion and retention of environmentally relevant MPs (i.e., PEST microfibres) by the trophically-associated copepod *P. crassirostris*, mysid shrimp *Mysida sp.* and fish *T. lunare*. All three species readily ingested PEST MPs, with retention times increasing in correlation to their size and trophic level (3 h for primary consumers to > 48 h for tertiary consumers). Additionally, this work reports the impact of phthalates on the ingestion of PEST MPs, suggesting that exposure to DEHP, either within the water column or pre-adsorbed to MPs, may influence the intake of MPs. The influence of DEHP on the ingestion of PEST

appears to be species-specific either promoting ingestion by copepods or hindering the intake of PEST in mysid shrimp or fish. Given the high turnover rate of lower trophic level consumption within oceanic food webs, the potential for transfer of MPs, and associated chemical contaminants, to higher tropic levels is probable. While this body of work supports the concept of trophic transfer of MPs and chemical additives in marine food webs, trophic transfer has rarely been confirmed and warrants further investigation.

Chapter 4: Trophic transfer of polyester microfibres confirmed in a simple three-level marine food web

4.1 Abstract

Trophic transfer has been observed as a pathway for the introduction and contamination of marine wildlife with microplastics (MPs; plastics < 5 mm). Ingestion of MPs has the potential to impact animal health, both physically (e.g., gut blockage, decreased feeding) and chemically (e.g., physiological and behavioural changes). Despite the ever-increasing number of MP experimental studies and repeated claims of trophic transfer of MPs, detailed quantification across multiple trophic levels is seldom demonstrated. This study investigated the trophic transfer of an environmentally relevant MP (i.e., microfibres of polyester; PEST) under varying conditions, including with the plasticiser bis(2-ethylhexyl) phthalate (DEHP; a known endocrine disruptor with carcinogenic properties) as a co-contaminant. The uptake and transfer of dosed (a) PEST, (b) PEST pre-adsorbed with DEHP (PEST:DESP) and (c) PEST added in tandem with DEHP (PEST+DEHP), across the three trophic levels: copepods (*Parvocalanus crassirostris*), mysid shrimp (*Mysida sp*.) and moon wrasse fish (*Thalassoma lunare*), was tested in a laboratory setting. Ingestion of MP contaminated prey was observed and quantified at all three trophic levels, with a detected increase in MPs in mysid shrimp compared to direct ingestion from the water column and again in moon wrasse, indicating biomagnification of MPs. There was no significant impact of DEHP, either adsorbed or co-dosed, on the transfer of MPs through this three-level food web. This study is the first to demonstrate the transfer of MPs across three trophic levels belonging to a simplified food web and highlights the continued ecological risk of MPs.

4.2 Introduction

Estimates of global emissions of plastic into waterways (e.g., lakes, rivers, oceans) are as high as 25 million metric tonnes per year (Lau et al., 2020), with a predicted increase in annual emissions of up to 53 million metric tonnes by 2030 (Borrelle et al., 2020). Detrimental impacts of plastic pollution are well documented in marine ecosystems (Almroth and Eggert, 2020), with microplastics (MPs; i.e., plastics between 1 µm and 5 mm) being of greatest concern given their ubiquity worldwide (Guzzetti et al., 2018; Kroon et al., 2020), and the high probability of intentional and unintentional ingestion by marine organisms (Guzzetti et al., 2018; Lusher, 2015; Wright et al., 2013b). The biomagnification and associated trophic transfer of MPs is often speculated within the literature (Farrell and Nelson, 2013;

Nelms et al., 2018; Zhao et al., 2018), yet empirical evidence of its occurrence is needed to appropriately assess the ecological risk of MPs.

MPs are considered a diverse suite of contaminants given their heterogeneous polymer composition, size, colour, and shape (Rochman et al., 2019). This diversity is amplified by the incorporation of chemical additives, such as the phthalic acid esters (PAEs; phthalates) that are integrated into plastics through a process known as plastic compounding to increase the flexibility, durability, and longevity of plastic items (Hahladakis et al., 2018). However, as they are not chemically bonded to plastics, phthalates can migrate into the surrounding environment (Liang et al., 2008), with MPs recently confirmed to be a major source (Cao et al., 2022). This is of particular concern as phthalates have been linked with immunotoxicity, metabolic toxicity, endocrine toxicity, neurotoxicity, genotoxicity, developmental toxicity, and other adverse effects in marine organisms (Li et al., 2020; Yu et al., 2018).

MP contamination is found throughout the marine environment, including the once pristine (GBRMPA, 2014) Great Barrier Reef World Heritage Area (GBRWHA) (Caron et al., 2018; Hall et al., 2015; Jensen et al., 2019; Santana, 2022). Reports of detrimental impacts associated with the intake of MPs and uptake of chemical additives are on the rise (Cunningham and Sigwart, 2019); however, laboratory exposures are often not representative of the most common MPs (e.g., polyethylene or polypropylene fragments, PEST fibres; 100 µm – 1 mm) or additives (e.g., the endocrine disruptor bis(2-ethylhexyl) phthalate; DEHP (Kamrin, 2009)) found within the GBRWHA (Chapters 2, 3, 5) (Hall et al., 2015; Jensen et al., 2019; Santana, 2022). Examining reef trophic models has proven critical to fully understand the mechanism of transfer and downstream ecological effects of various organic and metallic contaminants in coral reef ecosystems (Briand et al., 2018; van Dam et al., 2011). Yet, while the trophic transfer of MPs is often inferred and discussed within the literature (Nelms et al., 2018; Zhao et al., 2018), it is seldom investigated in detail (Chapter 2) and is notably lacking for reef-associated marine species.

Ingestion and retention of MPs and DEHP has previously been shown to occur in three species (copepods, mysid shrimp and moon wrasse fish) within a three-level coral reef food web (Chapter 3). Given the ecological importance of these species and the susceptibility to MPs, it is important to understand whether trophic transfer of MPs to predators through ingestion and retention of MP contaminated prey species (i.e., of the lowest trophic level(s)) occurs, and what the impacts may be at each trophic level. Therefore, the goal of this experimental study was to investigate the transfer of environmentally relevant MPs across three trophic levels representing a simple food web of the GBR. More specifically, the potential for transfer of PEST microfibres from copepods (*Parvocalanus crassirostris*) to mysid shrimp (*Mysida* sp.), to fish *(Thalassoma lunare)* was tested within a controlled

laboratory setting. As MPs are considered a multiple stressor contaminant (Rochman, 2013), additional exposure treatments were chosen to simulate and allow assessment of the effects of adsorbed phthalates on MP ingestion and transfer: (a) PEST, (b) PEST pre-adsorbed with DEHP (PEST:DEHP) and (c) PEST and DEHP added simultaneously (PEST+DEHP). Comparative analysis of findings from this study with previous reports of trophic transfer observed under laboratory conditions was aided by a literature review.

4.3 Methods

4.3.1 Literature review

A literature review describing and documenting the trophic transfer of MPs was conducted in June 2022. Specifically, scientific databases (i.e., Google Scholar and Web of Science™) were searched with combinations of the following keywords: plastic, microplastic, trophic, and transfer. Articles from 1965 to 2022 were screened for relative importance (A3.1 Table). Due to the limited number of studies identified, all studies focusing on aquatic species (i.e., freshwater and marine) were included. Only articles that conducted laboratory experiments investigating the trophic transfer of MPs from a lower to higher trophic level were considered. Methodologies applied and results obtained were extracted from the selected articles for comparison with this study.

4.3.2 Permits and ethics

All experimental aquaria work was conducted at the Australian Institute of Marine Science's (AIMS) National Sea Simulator (SeaSim) facilities, located near Gurumbilbarra, in Wulgurukaba and Bindal Country (Townsville, Queensland), from April to June 2021 in accordance with relevant institutional and national guidelines (Great Barrier Reef Marine Park Authority permit G12/35236.1 and James Cook University (JCU) Animal Ethics Committee Approval Number A2722).

4.3.3 Animal collection and husbandry

Copepods (*P. crassirostris*), mysid shrimp (*Mysida* sp.) and moon wrasse fish (*T. lunare*) were cultured, collected, and maintained as per Chapter 3. In brief, adult copepods were obtained from JCU's Marine and Aquaculture Research Facilities Unit (M.A.R.F.U) and cultured in AIMS SeaSim facilities. Copepods were fed a daily mixture of *Tisochrysis lutea* (*T-Iso)* and *Chaetoceros muelleri* in a 1:1 ratio throughout experimentation. Mysid shrimps were cultured using a stock population maintained in mesocosm coral reef tanks and reared in an aerated conical 100 L tank. Mysid shrimp were fed a daily diet of at least 25,000 copepods (sourced live from the copepod culture tanks, *P. crassirostris*) to ensure

cannibalism did not occur (Heindler et al., 2017). Copepods and mysid shrimp underwent > 12 h acclimation period after transfer to their experimental tanks (i.e., 600 ml glass beakers containing 500 ml FSW and covered with aluminium foil).

Moon wrasse fish (n=32) were collected in April 2021 by Cairns Marine using hand and barrier nets while on SCUBA at various mid-shelf reefs (e.g., Undine, Upolu, Arlington, Sudbury, Flora and Gibson Reefs) off the coast of Gimuy (Cairns, Queensland). Once received at AIMS, fish underwent a minimum acclimation period (> 7 d) in individual 50 L glass flow-through tanks equipped with air supply and covered with a glass lid. A silica-glazed ceramic structure (13 x 11 cm; hollow) was added to the tank to provide refuge. All conditions were as described in Chapter 3, with a 12h:12h light:dark photoperiod using SOL blue LED lights (80% intensity of 100 Photosynthetic Active Radiation (PAR)). Fish were fed a minimum of 1.2 mg per g fish weight of live mysid shrimp daily. All fish were eating within 24 h of arrival to SeaSim facilities, indicating healthy and normal behaviour. Water quality parameters, including temperature (°C), pH, salinity (ppt), and dissolved oxygen (DO; mg L^{-1}), were continuously monitored for all organisms throughout.

4.3.4 PEST and DEHP treatment preparation

PEST microfibres were chosen as they represent one of the most common MPs found in the GBRWHA (Jensen et al., 2019; Santana, 2022) (Chapters 5 and 6), and there is demonstrated evidence of their ingestion and retention by copepods, mysid shrimp and fish (Chapter 3). Fibres were fluorescent yellow in colour to facilitate timely discovery within each species' gastrointestinal tract (GIT). PEST microfibres were prepared using a retail thread (Spotlight; Birch Polyester Thread Fluoro Yellow 1,000 m, EAN: 9313792079477) and cut using an established cryostat method (Chapter 3, Appendix 2) to achieve sizes consistent with that of the copepods natural prey (i.e., 10 ± 2 µm).

Copepods were exposed to concentrations of PEST and DEHP ensuring detectability within a laboratory setting. PEST microfibres were dosed at a concentration of 10 PEST $copepod^{-1}$ to provide each individual copepod an equal opportunity to ingest. DEHP (Sigma Aldrich, CAS Number: 117-81-7) was dosed into tanks at 1 mg L^{-1}, an order of magnitude higher than that of global reported concentrations (Bergé et al., 2013).

Treatments were prepared in 20 ml glass scintillation vials and stored at 4°C until dosed into copepod tanks. Exposure treatments included: (1) PEST, (2) PEST pre-adsorbed with DEHP (PEST:DEHP), (3) PEST and DEHP added in tandem (PEST+DEHP) and (4) control treatments. All preparations for copepod treatments are described in detail in Chapter 3. Briefly, PEST only treatments were prepared by aliquoting 10.4 ml of PEST stock solution into vials. PEST:DEHP treatment vials were prepared by adding 10.4 ml aliquots of PEST stock solution into an exposure vial, allowing it to evaporate under a

nitrogen stream and subsequently adding 1 ml hexane (Fisher Scientific, CAS Number: 110-54-3). Following, DEHP was added (0.5 mg), the vial capped (aluminium foil lined) and shaken on an orbital shaker (Baxter Multi-Tube Vortexer, Baxter Diagnostics) for 1 h to allow for adsorption. The hexane was evaporated under nitrogen flow and vials were stored and sealed without FSW until immediately prior to exposures (See Appendix 2, Text A2). PEST+DEHP treatments were prepared in two vials, one containing 10.4 ml PEST stock solution and one containing 0.5 mg DEHP in 1 ml dimethyl sulfoxide (DMSO; carrier solvent) for a final concentration of 1 mg L^{-1} DEHP in the tank. Control treatments consisted of 1 µm FSW with no MPs or DEHP and 1 µm FSW with DMSO.

Treatments were dosed into tanks via emptying and rinsing (x3) vials using 1 µm FSW. Alongside exposures, copepods were fed a mixed algal feed (6,500 cells of *T-iso* and *C. muelleri*, 1:1). Specifically, treatments were dosed directly into the beaker, rinsing (x3) vials with FSW and the algal feed added to promote feeding. There was no starvation period for any organism during experimentation.

To validate dosed nominal concentrations of PEST, experimental control tanks (600 ml beakers containing 500 ml FSW) were dosed with PEST only treatments (n=3) as described above, and PEST recovery rate was determined. Control beakers were then emptied over 0.46 µm polytetrafluoroethylene (PTFE) filters (Omnipore) and the inside walls and lip rinsed 3x with FSW to ensure all PEST microfibres were captured. Filters were visually inspected, and PEST counted under magnification (Leica DMI6000B; DFC310FX camera attachment; I3 fluorescent filter, excitation BP 450 – 490; 50 – 200x magnification).

4.3.5 Experimental design and procedures

Trophic transfer of PEST microfibres was assessed across three trophic levels, copepods (*P. crassirostris*) representing the primary consumer (level 2.0), mysid shrimp (*Mysid sp*.) representing the secondary consumer (level 2.47), and moon wrasse fish (*T. lunare*) representing the tertiary consumers (level 3.5) (Figure 4.1, (Froese and Pauly, 2010; Palomares and Pauly, 2010)). Prior to experiments, copepods (n= 1,300 per replicate), mysid shrimp (n=8 per replicate) and fish (n=1 per replicate) were transferred to individual experimental beakers or tanks FSW left for acclimation. In total, there were 8 replicates of the trophic transfer set up (i.e., copepods to mysid shrimp to wrasse) for each treatment. High copepod and mysid shrimp populations allowed sub-sampling during the exposure period and ensured enough individuals remained to feed the next trophic level. Experimental tank set up and treatment allocation was randomised using a customised random generator script in R (Version 4.0.4 with Rstudio, version 1.4.1106).

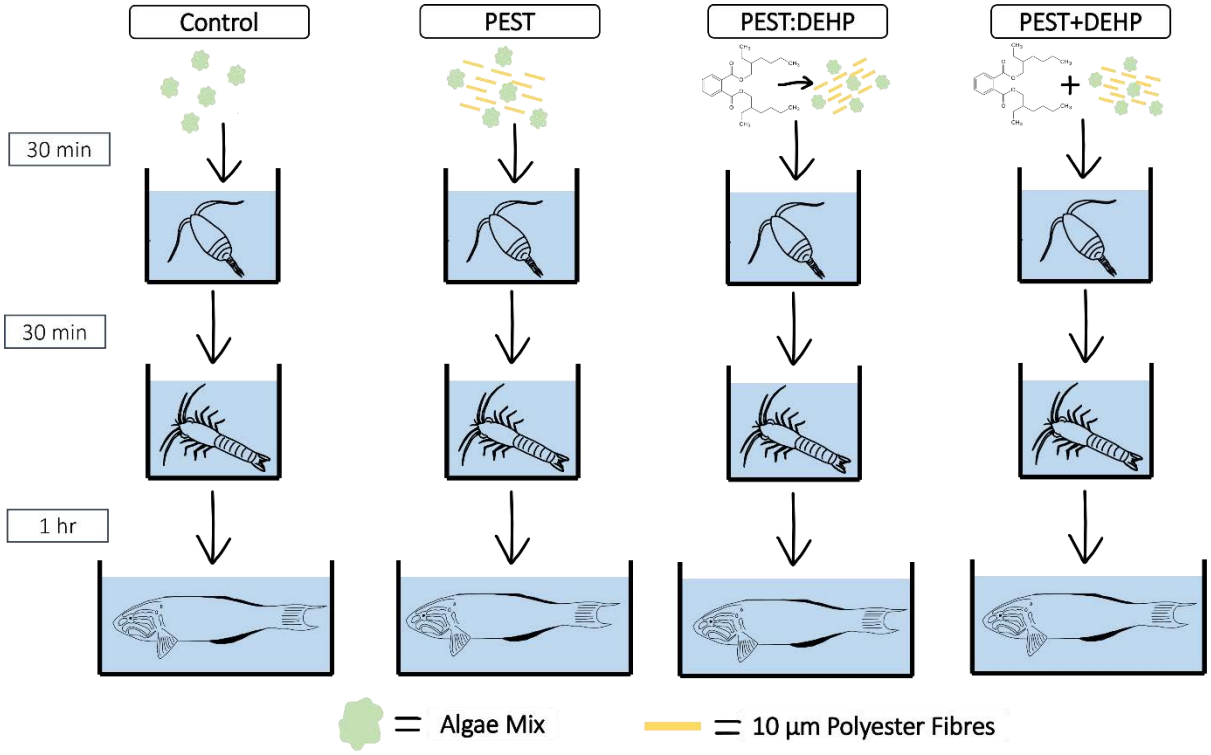

*Figure 4.1. Experimental design to assess the trophic transfer of polyester (PEST) microfibres from copepods (*Parvocalanus crassirostris*) to mysid shrimp (*Mysida sp.*) to moon wrasse fish (*Thalassoma lunare*) within a laboratory setting. Each treatment contained 8 replicates.*

Active copepods observed swimming throughout the water column were subsequently exposed to one of four treatments. Optimal feeding periods were based on previous retention experiments to ensure depuration of plastics did not occur before potential ingestion by the next trophic level organism (Chapter 3). After 30 min of feeding, copepod beaker contents were emptied over a 26 µm stainless steel filter net and rinsed gently with FSW to remove extraneous PEST fibres. Captured live copepods were then backwashed into mysid shrimp experimental beakers and left for 30 min. At the conclusion of this feeding period, mysid shrimp beaker contents were emptied over a 547 µm stainless steel filter net and rinsed with FSW to remove extraneous PEST fibres. Copepod and mysid shrimp net aperture sizes were deliberately selected to ensure any non-ingested PEST were removed. Captured live mysid shrimp were backwashed into the fish tanks and each fish was given 1 h to consume prey. Fish feeding behaviour was recorded using GoPro video cameras (Model Hero5 and above) to confirm consumption of mysid shrimp. GoPro cameras were placed outside fish tanks > 12 h prior to experimentation to allow fish to acclimate to its presence. At the conclusion of each feeding period, sub-samples of 50 copepods (via pipette) and 2 mysid shrimp (using forceps) from each replicate beaker were fixed in 70% ethanol (EtOH) for microscope analysis. At the conclusion of the 1 h feeding period, fish were euthanised via ice slurry (ice and FSW) and kept frozen in aluminium foil until dissection.

4.3.6 PEST quantification

Copepod sub-samples were emptied over 0.46 µm PTFE filters and rinsed with an additional 70% EtOH to remove any adhered extraneous PEST. Retained copepods were transferred to a pre-cleaned glass microscope slide and a cover slide was applied. Mysid shrimps were collected from sub-samples using forceps, positioned on a microscope slide with the dorsal side facing upwards and compressed with a cover slide. Fish were thawed and dissected to extricate the gastrointestinal tract (GIT; stomach and intestines) and the gills. Following an established protocol (Santana, 2022), fish GITs were digested using potassium hydroxide (10% KOH) for 48 h at 40°C. Digestate was filtered (Schlawinsky et al., 2022) onto 0.46 µm PTFE filters (diameter = 19 mm) and rinsed liberally with Milli-Q water (MQ-H_2O). Filters and gills were transferred to a glass microscope slide using forceps and secured in place with a cover slide.

As the PEST were fluorescent, an inverted fluorescent microscope (Leica DMI 6000B; DFC310FX camera attachment) was used to identify contamination at each trophic level. Copepods, mysid shrimp and remnants of digested fish GITs and gills (all secured on glass slides) were observed and photographed under brightfield light (EMP TL-BF) using a blue light filter (I3). To ensure only exposed fluorescent PEST microfibres were counted, the I3 filter channel photos were analysed in ImageJ (FIJI; version 1.53e) using the 'analyse particles' function. Each photo contained an embedded scale bar that was used to calibrate the ImageJ measurements scale. To allow for particle analysis, the photo was transformed into an 8-bit image and a threshold was set individually for each photo, ensuring all fluorescent fibres were selected (A3.1 Figure). Due to the tendency of PEST to cluster within the GIT of mysid shrimp, the area measurements obtained from ImageJ were corrected using the size of the PEST (i.e., 10 x 10 µm or 100 μm^2) to obtain an estimate of PEST.

4.3.7 Contamination control

To minimise contamination resulting from exposure to extraneous water- or airborne MPs, all experimental tanks were covered with either glass lids (50 L fish tanks) or aluminium foil (600 ml glass beakers). Additionally, filtered water was used throughout, including MQ-H_2O within the laboratory and 1 µm FSW within SeaSim facilities. Airborne contamination controls (clear 50 ml polypropylene vial, yellow high-density polyethylene screw cap) were positioned randomly throughout the experimental room on top of tanks and benches to capture any airborne contamination. Airborne contamination control vials were emptied over 0.46 µm filters upon completion of experiments and analysed visually in the same fashion as organisms to determine whether extraneous fluorescent PEST microfibres were present.

Pre-filtered 70% EtOH (0.46 µm) was used throughout to preserve experimental organisms. Plastic materials, tanks and equipment were avoided where possible as DEHP is a hydrophobic chemical that readily adsorbs to plastic. Chemical cleaning of glassware included rinsing with acetone, followed by a rinse with hexane and a final liberal rinse of FSW prior to organism transfer. Hexane was purchased was analytical grade, pre-submicron filtered and therefore assumed no extraneous contamination existed. Nitrile gloves were avoided due to potential phthalate contamination. Cotton clothes were always worn, and a lint roller (Scotch-Brite®) was used prior to entering the experimental room.

4.3.8 Data analysis

PEST ingestion was determined for each trophic level as the number of PEST present within the GIT of each individual organism. Mean ingestion of PEST is reported as average ± standard deviation (S.D.) unless otherwise specified. To determine the impact of species and treatment on PEST ingestion, a general linear model was conducted using a negative binomial distribution due to the non-normality of the data. Post-hoc analysis was done using the estimated marginal means (EMM) from the model for pairwise comparisons. All statistical analysis and graph construction was done in R (Version 4.0.4 with Rstudio, version 1.4.1106), with the '*emmeans'* package for calculating EMMs, the '*pairs'* package to conduct post-hoc pairwise comparisons and the '*ggplot2'* package for graph creations.

4.4 Results and discussion

4.4.1 Trophic transfer of MPs in aquatic species

A total of 35 research articles were identified pertaining to the trophic transfer of MPs in aquatic species (A3.2 Table). After the exclusion of review or commentary articles (n= 8) and those focussed on nano-plastics (n=3) or terrestrial species (n = 1), as well as those only speculating trophic transfer via field studies (n = 8), a total of 14 articles were identified for comparative analysis (Table 4.1). Despite the trophic transfer of MPs being notably observed in all 14 studies, only four quantified MPs at each trophic level (Elizalde-Velázquez et al., 2020; Hasegawa and Nakaoka, 2021; Mateos-Cárdenas et al., 2022; Xu et al., 2022). Three studies investigated interactive effects of chemical contamination adsorbed to MPs, with only one quantifying the impact of a chemical treatment (i.e., dichlorodiphenyltrichloroethane, DDT) on intake of MPs by a secondary consumer (Athey et al., 2020). Additionally, most studies explored the transfer of microbeads (n=11), a plastic shape not detected in the GBRWHA to date (Jensen et al., 2019; Santana, 2022). Therefore, this present study is the first to investigate and report on the trophic transfer of an environmentally relevant MP (i.e., PEST microfibres) across three trophic levels in the presence of a plastic additive (i.e., DEHP).

Table 4.1. Research articles describing the trophic transfer of microplastics (MPs) in aquatic species under controlled laboratory conditions. Despite most studies presenting details of multiple experiments, only information and results related to the trophic transfer aspect of each article are reported below. Colour and/or shape of MPs is reported here if provided in the original article. Ingested MPs are reported as MPs individual^{-1} ± standard deviation, unless otherwise specified; percentages are representative of the number of test individuals that exhibited MP ingestion. NA = not assessed in the study. NR = investigated but data not reported. FL = fluorescent. PE = Polyethylene. LDPE = Low-density polyethylene. PS = Polystyrene. PMMA = Poly(methacrylic acid). HDPE = high-density polyethylene. PEST = Polyester. DDT = Dichlorodiphenyltrichloroethane. BaP = Benzo[a]pyrene. BkF = benzo[k]fluoranthene.

	Test Species		Exposed MPs		Exposure Periods			Ingested MPs	
Reference	Level 1	Level 2	Details	Concentration	1	1 → 2	Additional Treatments	Level 1	Level 2
Athey et al. (2020)	Ciliates (*Favella sp.*)	Larval Fish (*Menidia beryllina*)	10 – 20 µm LDPE beads	500,000 MPs ml^{-1}	1 h	2 h	Adsorbed with DDT	44% of *Favella sp.*	MPs: 81.8 ± 42.0 DDT: 205.4 ± 89.9
Batel et al. (2020)	Brine Shrimp (*Artemia sp.*)	Fish *(Danio rerio)*	1 – 20 µm PE beads	0.5 mg and 2.5 mg	up to 24 h	up to 2 d	Adsorbed with BaP	80 – 95%	NR
Costa et al. (2020)	Copepods (*Tigriopus fulvus*)	Jellyfish (*Aurelia sp.*)	1 – 5 µm FL PE	1 mg L^{-1} and 10 mg L^{-1}	6 h	24 h	NA	NR	NR
da Costa Araújo et al. (2020)	Fry (*Poecilia reticulata*)	Fish (*Danio rerio*)	35.46 ± 18.17 µm FL PE fragments	60 mg L^{-1}	48 h	10 d	NA	NR	38.08 PE MPs mg^{-1}
Elizalde-Velázquez et al. (2020)	Zooplankton (*Daphnia magna*)	Fish (*Pimephales promelas*)	5.48 ± 0.06 µm FL PS Fragments	20 MPs ml^{-1} and 2,000 MPs ml^{-1}	4 h	5 d	NA	Low: 0.72 ± 0.08 High: 50.7 ± 22.46	Low: 4.7 ± 1.88 High: 546 ± 149.5
Farrell and Nelson (2013)	Mussels *(Mytilus edulis)*	Crabs (*Carcinus maenas*)	0.5 µm FL PS beads	~411 million	1 h	up to 4 h	NA	NR	163,111 ± 34,140
Hanslik et al. (2020)	Zooplankton *(Daphnia magna)* and Fly Larvae (*Chironomus riparius*)	Fish *(Danio rerio)*	48 µm PMMA beads	50 mg L^{-1}	24 h	48 h	Adsorbed with BkF	> 80% *D. magna*	NR

								> 60% C. riparius	
Hasegawa and Nakaoka (2021)	Shrimp (*Neomysis sp.*)	Fish (*Myoxocephalus brandtii*)	27 – 32 µm FL PE beads	0.2 mg L^{-1} 2 mg L^{-1}	24 h	24 h	NA	Low: 65.53 ± 63.36 High: 266.82 ± 155.39	Low: 250 ± 250* High: 700 ± 675*
Mateos-Cárdenas et al. (2022)	Duckweed *(Lemna minor)*	Amphipod (*Gammarus deubeni)*	30.28 ± 2.78 µm FL PE beads 1 µm PS FL beads	50,000 MPs ml^{-1}	72 h	24 or 96 h	NA	PE: 42.22 ± 8.25 ** PS: 175.9 ± 7.11 **	PE 24h: 13.5 ± 5.7 PE 96h: 9.4 ± 10.8 PS 24h: 0 PS 96h: 19.5 ± 16.3
Setala et al. (2014)	Copepods (*Acartia sp., Eurtermora affinis, Limnocalanus macrurus*) and Polychaeta (Marenzelleria sp.)	Shrimp (*Mysida sp.*)	10 µm FL PS beads	2,000 MPs ml^{-1}	3 – 12 h	3 h	NA	3% of copepods 86% of Marenzelleria sp	80%
Stienbarger et al. (2021)	Ciliates *(Favella sp.)*	Larval fish *(Centropristis striata)*	10 – 20 µm LDPE beads	1 x 10^4 MPs L^{-1} and 1 x 10^6 MPs L^{-1}	1 h	2 h	Adsorbed with phenanthrene	Low: NR High: 2	Low: NR High: > 2
Tosetto et al. (2017)	Amphipods (*Platorchestia smithi*)	Fish (*Bathygobius krefftii*)	38 – 45 µm biofouled PE beads	3.8% of sediment	72 h	Every 2 d for 7 d	NA	NR	NR
Uy and Johnson (2022)	Brine Shrimp (*Artemia salina)*	Fish *(Leuresthes tenuis)*	1 – 5 µm FL PE beads	8 x 10^6 MPs L^{-1}	NR	NR	NA	79.10%	53.90%
Xu et al. (2022)	Mussel *(Brachidontes variabilis)*	Snail *(Reishia clavigera)*	6408 ± 605 μm^2 Blue PP fragments & 6246 ± 535 μm^2 Orange PP fibres	10 items L^{-1} and 1,000 items L^{-1}	Up to 8 d	7 or 14 d	NA	0.22 ± 0.09 fibres, 1.07 ± 0.28 fragments	Low: 0 High: 1.4 ± 1.6 fibres, 0.8 ± 0.8 fragments

*Data extrapolated from graphs – raw data not given; **Attached to surface of whole *L. minor*

4.4.2 Contamination control and exposure validation

A total of 16 putative MP fibres were isolated from airborne contamination controls, albeit none matched the fluorescent PEST microfibre used for the experiment. Therefore, putative MP items were not further characterised past shape and colour (primarily black and blue fibres; A3.3 Table). However, this finding does highlight the need to control for contamination in exposure studies, especially those investigating common place non-fluorescent fibres, as extraneous MPs may influence results (Santana et al., 2021).

A critical step in (eco)toxicology studies is the measurement of doses applied to the experimental system to confirm nominal concentrations. Measured PEST concentrations were similar to the nominal concentration of 10 PEST copepod^{-1} (i.e., 13,000 PEST) with an average of 12,972 ± 19.55 PEST for 1,300 copepods. This confirmed the desired concentration (i.e., ~26,000 MPs L^{-1}) was achieved. PEST exposure concentrations used here are lower than those used in other MP trophic transfer experiments (Table 4.1) and in MP exposure experiments in general (Bucci et al., 2020), albeit still higher than concentrations found in the water column of GBRWHA mid-shelf reefs (4.66 x 10^{-3} ± 4.24 x 10^{-3} MPs L^{-1}) (Chapter 5) and globally (i.e., 11.8 ± 24.0 MPs L^{-1}; (Barrows et al., 2018a)). However, the increased concentration use within a laboratory setting is standard practice for ecotoxicology studies and still necessary due to the limitations of using true realistic MP concentrations (Santana et al., 2021).

4.4.3 Water quality and animal health

Variations in temperature (25.82 ± 0.42 °C), pH (8.18 ± 0.05), salinity (35.28 ± 0.29 ppt), and DO (7.99 ± 0.08 mg L^{-1}) were negligible and reflected *in situ* water quality conditions for the GBR (i.e., Davies Reef) at the time of experimentation. Ammonia (NH_3) and nitrate (NO_3) levels were consistently below levels considered harmful to marine organisms (i.e., 0 ppm and < 5 ppm, respectively).

Copepods, mysid shrimp and fish showed no signs of stress (i.e., abnormal swimming) or mortality resulting from exposure to PEST or DEHP. In addition, treatments containing PEST and DEHP did not prohibit or limit feeding of live prey, with all organisms exhibiting feeding immediately following exposure. Here, DEHP exposure concentrations were substantially below previously reported lethal concentrations (i.e., concentration at which 50% of the population is killed) for copepods (7 to 10 mg L^{-1}; (Seo et al., 2006)), mysid shrimp (> 1 mg L^{-1}; (Biddinger and Reinert, 1997)) and fish (between 5.41 and 37.95 mg L^{-1}; (Qin et al., 2011; Zhang et al., 2015; Zhao et al., 2014)), and findings indicate this acute low level exposure has no immediate effect. This corroborates previous exposure experiments applying similar concentrations and treatment regimens (Chapter 3).

4.4.4 PEST ingestion and transfer

PEST microfibres were found in the GIT of all individual organisms, regardless of treatment or trophic level (Figure 4.2). As the secondary and tertiary predators were exposed to contaminated prey only (i.e., copepods as the initial consumer of PEST, and mysid shrimp as the consumer of contaminated copepods), and controls were confirmed to be free of dosed PEST, PEST ingestion reported here is a direct result of trophic transfer. This study is the first to successfully validate and quantify MP ingestion across three trophic levels as a direct result of transfer from contaminated copepods (primary consumer, level 2.0) to mysid shrimp (secondary consumer, level 2.47) to moon wrasse fish (tertiary consumer, level 3.5) under laboratory conditions (Figure 4.3). Further, this study demonstrates that ingestion of MPs through trophic transfer (mysid shrimp = 30.13 ± 20.09 PEST ind^{-1}; fish = 148.29 ± 74.01 PEST ind^{-1}) is substantially higher compared to exposure to prey-sized MPs in the water column (mysid shrimp = 16.37 ± 11.18 PEST ind^{-1}; fish = 6.89 ± 1.90 PEST ind^{-1}) (Chapter 3), a phenomenon previously observed for mysid shrimp and fish (Hasegawa and Nakaoka, 2021). Additionally, this is the first study to investigate the influences of phthalates on the transfer of an environmentally relevant MP (i.e., PEST microfibres) through a simplified trophic food web.

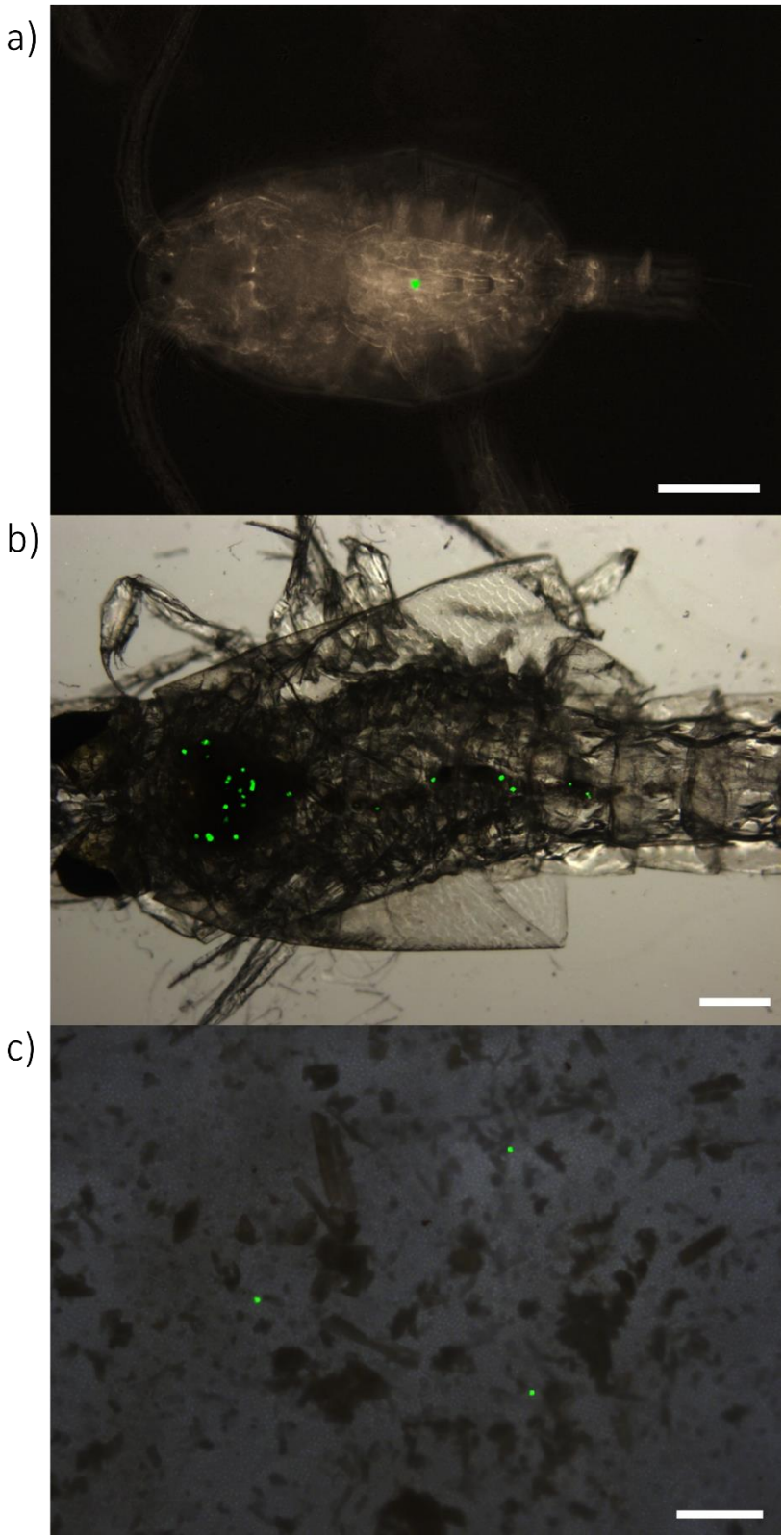

Figure 4.2. Fluorescent polyester microfibres (10 µm PEST) identified in (a) copepod, Parvocalanus crassirostris*, (b) mysid shrimp,* Mysida sp.*, and (c) in the gut contents of moon wrasse fish,* Thalassoma lunare*. PEST in mysid shrimp and fish are a direct result of trophic transfer. The white scale bar equates to 100 µm.*

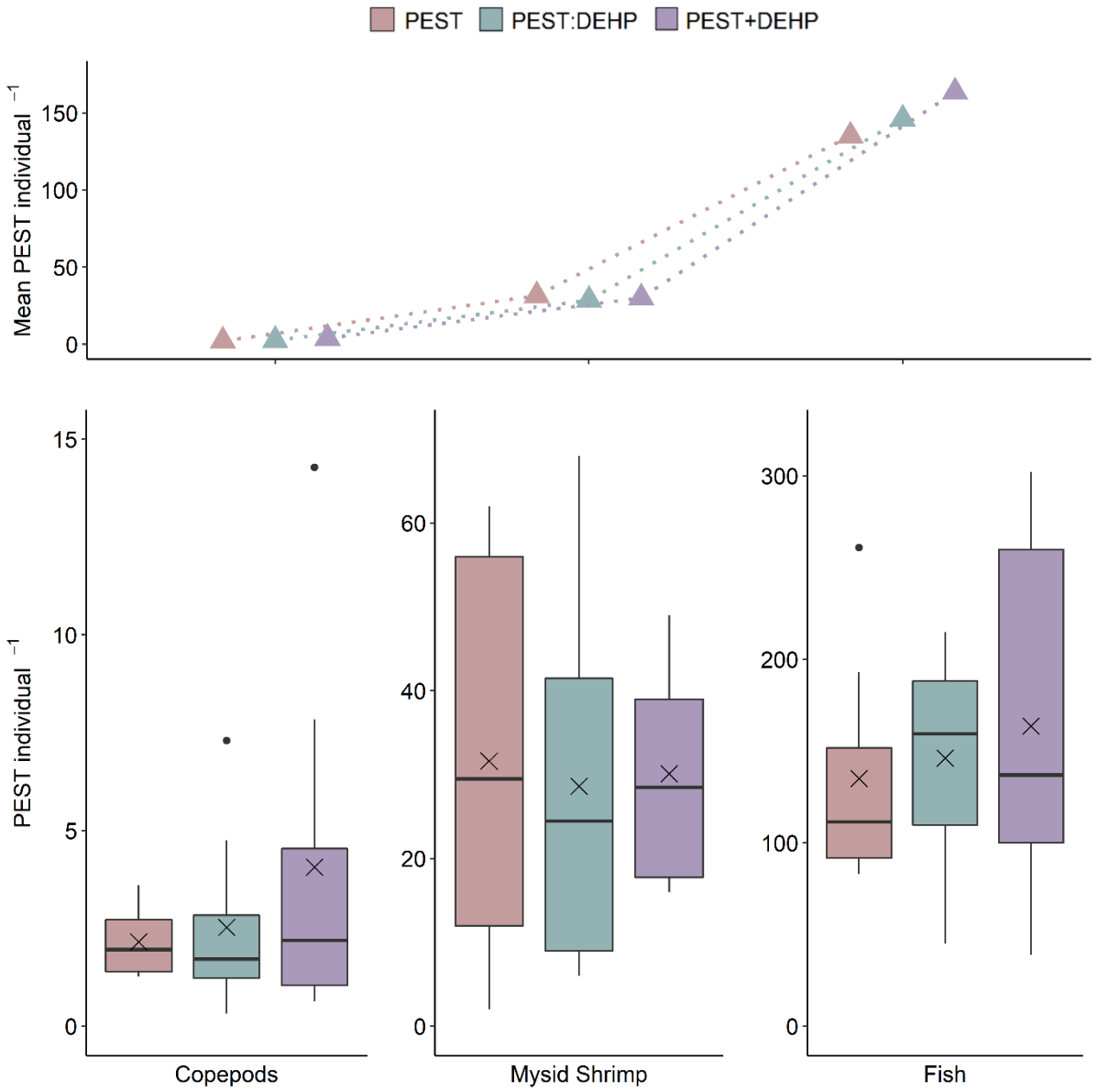

*Figure 4.3. Mean polyester microfibres (PEST) per individual copepod (*Parvocalanus crassirostris*), mysid shrimp (*Mysida sp.*) and moon wrasse fish (*Thalassoma lunare*), exposed through a trophic transfer laboratory experiment (top). A statistical description of each dataset (middle line = median, X = mean, boxes = interquartile range (IQR), and whiskers = 1.5 times IQR) is presented (bottom). Copepods were exposed to four treatments: 10 µm PEST, PEST pre-adsorbed with bis(2-ethyl hexyl) phthalate (PEST:DEHP), PEST dosed simultaneously with DEHP (PEST+DEHP) and controls (i.e., no PEST or DEHP). No PEST were recovered from control individuals.*

Microscopy of mysid shrimp revealed that after a 30 min exposure to PEST contaminated copepods, PEST microfibres were present (28.63 ± 22.83 PEST ind^{-1} to 31.63 ± 24.95 PEST ind^{-1}, dependant on treatment) and no intact copepods were observed. Mysid shrimp use their mandibles to break apart the copepod as they enter the mouth; they are not swallowed whole (Friesen et al., 1986).

Previously, PEST (> 300 µm in size) ingested from the water column by mysid shrimp were found to be fragmented, having an average length of 57.56 ± 27.61 µm) (Chapter 3); these findings corroborate other MP ingestion studies (Dawson et al., 2018b). Here, PEST found within mysid shrimp were of the same size as the original microfibres (i.e., 10 ± 2 µm), indicating that they were not impacted by the initial intake and seemingly protected from mechanical fragmentation. This finding does suggest fragmentation of MPs by crustaceans may be dependent on MP size. However, mysid shrimp have been reported to fragment small (27 to 32 µm) polyethylene MP beads to roughly 6.41 µm (Hasegawa and Nakaoka, 2021), and Antarctic krill to less than 1 µm (Dawson et al., 2018b). An additional rationale is that incorporating MPs in copepod biomass may limit the impact of mastication processes on MPs, minimising any mechanical stress. Similar to this study, (Setala et al., 2014) did not report fragmentation following mysid shrimp exposure to copepods and polychaete worms contaminated with 10 µm polystyrene beads. To establish whether smaller PEST microfibres (e.g., 10 µm) taken directly from the water column are mechanically impacted by mysid shrimp ingestion, further experimental work is required. This information will also provide further insight into the impacts of crustacean consumption on MP abundance and size distribution in the marine environment.

Analysis of the fish GITs demonstrated ingestion via trophic transfer from mysid shrimp contaminated prey, irrespective of treatment (135.13 ± 62.41 PEST ind^{-1} to 163.64 ± 99.15 PEST ind^{-1}). Investigation of the gills revealed no PEST, indicating that all contamination found in fish results from trophic transfer from lower levels. Fibres found matched the original dose, further highlighting the lack of impact ingestion processes have on small-sized (10 µm) PEST fibres. Ingestion found here was substantially higher (148.29 ± 74.01 PEST ind^{-1}) than when fish were exposed to environmental PEST, such as within the water column (6.89 ± 1.90 PEST ind^{-1}; Chapter 3).

An increase in PEST contamination was observed from the primary trophic level to the tertiary level, with PEST contamination significantly different between the three species (all p-values < 0.001; A3.2 Table). This follows the same trend observed in other studies, demonstrating trophic transfer across two levels (Table 4.1). More specifically, a 2.6- to 3.8-fold increase has been observed in MPs transferred from shrimp (*Neomysis sp*.) to sculpin fish (*Myoxocephalus brandtii*), depending on exposure concentration (Hasegawa and Nakaoka, 2021). Similarly, a 6.5- to 10.8-fold increase in MP ingestion from freshwater zooplankton (*Daphnia magna*) to the fathead minnow (*Pimephales promelas*) was observed (Elizalde-Velázquez et al., 2020). Increases in concentrations between species in the present study are greater, with an overall trend of 20.7-fold increase of PEST from copepods to mysids and an 8.7-fold increase from mysids to fish (Table 4.2). From trophic levels 2.0 to 3.5 (i.e., copepods through to fish), there was a 112-fold increase in PEST concentration. As MP ingestion has been shown to be dependent on MP exposure concentrations (Hasegawa and Nakaoka, 2021; Santana

et al., 2021), an investigation into how varying exposure concentrations may impact the subsequent transfer of MPs to higher trophic levels is needed.

*Table 4.2. Percent (% ± standard deviation) increases in ingested polyester microfibres (PEST) in the trophic chain comprising primary consumer = copepods (*Parvocalanus crassirostris*) secondary consumer = mysid shrimp, (*Mysida sp.*) and tertiary consumer = moon wrasse fish (*Thalassoma lunare*). Exposure treatments include PEST alone, PEST pre-adsorbed with DEHP (PEST:DEHP), and PEST added in tandem with DEHP (PEST+DEHP).*

Treatment	Copepods → Mysid shrimp	Mysid shrimp → Fish	Copepods → Fish
PEST	1,839.42 ± 1,669.00	1,215.25 ± 1,503.16	7,859.77 ± 6,193.75
PEST:DEHP	2,409.13 ± 3,926.92	850.82 ± 758.25	14,745.20 ± 21,623.00
PEST+DEHP	1,945.02 ± 2,170.30	538.49 ± 252.49	11,037.55 ± 13,588.20
Overall Trend	2,064.52 ± 2,653.52	867.85 ± 980.75	11,214.17 ± 14,779.39

4.4.5 Influence of phthalates

The influence of phthalates, specifically DEHP, has previously been shown to impact ingestion of PEST microfibres directly from the seawater for copepods (positively), mysid shrimp and moon wrasse (both negatively) (Chapter 3). The present trophic transfer study has demonstrated a similar result, with highest ingestion rates for copepods observed when exposed to the PEST+DEHP treatment, albeit not significantly so (all p-values > 0.05; A3.4 Table). Direct water column exposure to either PEST:DEHP or PEST+DEHP was shown to negatively impact ingestion of PEST by mysid shrimp and moon wrasse (Chapter 3); however, the presence of DEHP in this study had no significant impact on the transfer of PEST from copepods to mysid shrimp or mysid shrimp to fish (all p-values > 0.05; A3.4 Table). It may be that the amount of DEHP uptake by copepods was not substantial enough to have an impact on mysid shrimp ingestion of copepod prey. Further, the dilution of DEHP as it transfers through the trophic levels may be large enough that an influence on prey ingestion is not occurring. However, this does not negate the potential impacts of DEHP uptake within these organisms individually, and investigations into the biomagnification factor of DEHP should be prioritised. DEHP is a known endocrine disruptor (Crisp et al., 1998), previously shown to influence *P. crassirostris* survival, fecundity, and gene expression (Heindler et al., 2017), alter crustacean energy metabolism, reproduction, and morphology (Verslycke et al., 2004), and impact gonadal histopathology of fish (i.e., medaka, *Oryzias melastigma*; (Ye et al., 2014). The impact of phthalates such as DEHP within a trophic transfer scenario has yet to be

thoroughly investigated and quantifying the levels of DEHP within each trophic level to ascertain if the amount is substantial enough to adversely impact higher trophic level organisms is of high importance.

4.5 Conclusions

The trophic transfer of MPs in marine ecosystems is often referred to within the scientific literature, with multiple laboratory experiments observing its potential. Despite the increase in research efforts, ingestion is seldom quantified and trophic levels are mostly capped at two and use processed feed rather than live contaminated prey. This study is the first to demonstrate the transfer of MPs across three trophic levels belonging to a simplified marine food web: copepods, mysid shrimp and moon wrasse fish. Ingestion was observed and detailed at every trophic level, with a detected increase in MP ingestion by the secondary (mysid shrimp) and tertiary (moon wrasse fish) predators. Phthalates, including DEHP, have previously been shown to influence individual ingestion and retention of PEST microfibres. Yet the presence of DEHP had no significant impact on the transfer of PEST microfibres through this food web. Future research should focus on understanding the mechanisms driving MP transfer, including those related to associated chemical additives, varying retention times and prolonged exposure.

Chapter 5: Microplastic bioconcentration, bioaccumulation and biomagnification in a simple coral reef food web

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5.1 Abstract

Microplastics (MPs, < 5 mm) are a ubiquitous marine contaminant and with irrefutable experimental evidence of MP ingestion, retention, and trophic transfer there is an urgent need to accurately assess the ecological risk they pose. The critical step toward this is to establish the relationship between MP contamination in marine organisms and with their surrounding local environment. Prior laboratory experiments have confirmed members of a three-tier marine food web, copepods, mysid shrimp and moon wrasse fish, all ingest and retain MPs from their local environment, and that ingestion of MP-contaminated live prey by higher-level predators supports the trophic transfer of these MPs. There remains, however, little evidence of this occurring *in situ*. Investigated here is the MP contamination in wild caught taxa from two mid-shelf reefs in the central Great Barrier Reef, Australia, associated with this same food web (zooplankton, benthic crustaceans, and reef fish), as well as in the distinct environmental matrices which they inhabit (i.e., mid-column water and sediment). Applying rigorous protocols modified from the literature, MP contamination was detected in both environmental matrices and all three trophic levels. MPs were found to bioconcentrate, with similar MP concentrations, polymer type, size, shape, and colour at each trophic level compared to their surrounding environment. In contrast, MP contamination varied across the three trophic levels and bioaccumulation and biomagnification was not evident across the simple food web examined. Given the heterogeneity of MPs abundant within the marine environment, it is impossible to ignore trophic transfer as a prominent pathway of exposure from lower to higher trophic levels.

5.2 Introduction

Plastic, and more specifically microplastic (MPs; plastics < 5 mm), is ubiquitous throughout the marine environment and is considered a contaminant of emerging concern (GESAMP, 2020; Kroon et al., 2020; Lusher, 2015). Globally, the presence of these contaminants in diverse areas of the world such as coral reefs (Huang et al., 2021a), polar regions (Bergmann et al., 2019; Waller et al., 2017),

remote islands (Lavers et al., 2019) and deep seas (Woodall et al., 2014) has highlighted the pervasiveness of MP contamination. Furthermore, MP contamination has been documented in over 325 species of benthic and pelagic marine organisms caught across the world's oceans (Chapter 2). The ecological risk MP contamination poses to marine organisms, however, is still unclear (Anbumani and Kakkar, 2018; Khalid et al., 2021).

Bioconcentration, bioaccumulation, and biomagnification are three critical concepts used in ecological risk assessments to determine the extent of pollutant transport within food webs (Beek et al., 2000; Chormare and Kumar, 2022; Feijtel et al., 1997). Bioconcentration, bioaccumulation and biomagnification are three critical concepts used in ecological risk assessments to determine the extent of pollutant transport within food webs (Beek et al., 2000; Chormare and Kumar, 2022; Feijtel et al., 1997). Bioconcentration is classified as an increase in contamination (e.g., MPs) in an organism relative to the concentration in the organism's environment (USEPA, 1997). Bioaccumulation is considered the net uptake of a contaminant (e.g., MPs) from the environment by all possible routes from any source (e.g., water, sediment, and prey) (Spacie et al., 1995). Biomagnification is defined as the increase of a contaminant (e.g., MPs) in an organism compared to the concentration found within its prey (USEPA, 2008). These endpoints are traditionally applied to measure dissolved chemical contamination (Alexander, 1999) and are now being developed to assess particulate MP contamination (Akhbarizadeh et al., 2019; Covernton et al., 2022; Rochman et al., 2019). Bioconcentration, bioaccumulation and biomagnification of MPs in marine organisms are often inferred in the literature (Koelmans et al., 2019; Nelms et al., 2018; Zhao et al., 2018) but have not been unequivocally proven. Unfortunately, the inadequacy of sampling regimes (i.e., no contemporaneous assessment of the surrounding environment or appropriate prey items) hinders the elucidation of potential exposure pathways (Chapter 2). A systematic review found that while MPs may bioaccumulate within all trophic levels across a general marine food web, no clear evidence of biomagnification was observed at the higher trophic levels (Chapter 2). More recent marine field studies investigating commercially important benthic species in the Persian Gulf (Akhbarizadeh et al., 2019), bivalves, echinoderms, arthropods and fish in coastal British Columbia, Canada (Covernton et al., 2022), further support this, with no evidence of biomagnification or trophic transfer of MPs. While these studies find no evidence of biomagnification, further assessments are needed to elucidate if these trends are widespread. To accurately evaluate trophic transfer within a marine food web, a combined assessment of all possible intake routes (e.g., contact, ingestion, respiration) from any source (e.g., water, sediment, prey) is required and should be linked to multiple biological endpoint measurements able to establish the occurrence of bioconcentration, bioaccumulation and biomagnification within each trophic level (Alexander, 1999).

Coral reef ecosystems, such as the Great Barrier Reef World Heritage Area (GBRWHA) (GBRMPA, 2019), are a source of high marine biodiversity (Fisher et al., 2015; Knowlton et al., 2010) and hold significant economic and cultural importance (Burke et al., 2011). The abundance and distribution of MPs in coral reef systems is reported on globally (Huang et al., 2021a). On the GBR, MPs have been found contaminating a variety of abiotic matrices, including surface waters (Jensen et al., 2019; Reisser et al., 2013; Santana, 2022) (Chapter 6), sub-surface waters (Hall et al., 2015), beach sand (Bauer-Civiello et al., 2019; Critchell and Hoogenboom, 2018), and benthic sediment (Santana, 2022). Moreover, MP contamination has been reported for a variety of GBR species, including hard corals, sponges, sea cucumbers and sea squirts (Santana, 2022), green sea turtles (Caron et al., 2018), planktivorous damselfish (Jensen et al., 2019; Santana et al., 2021), Australian sharpnose sharks (Schlawinsky et al., 2022), and commercially important fish species (Dawson et al., 2022; Kroon et al., 2018b).

The apparent prevalence of MP contamination in both abiotic and biotic matrices of the GBR provides a unique opportunity to examine bioconcentration, bioaccumulation, biomagnification, and trophic transfer in a simple coral reef food web. In coral reef environments, zooplankton represent a vital food source for secondary consumers (e.g., invertebrates such as crustaceans) due to their high nutritional value (Kramer et al., 2016). Benthic crustaceans, including crabs, isopods, amphipods, shrimps, prawns, and lobsters, are the dominant prey for approximately 50% of all fish species on coral reefs (Froese and Pauly, 2010; Kramer et al., 2015), including wrasses (Family: *Labridae*) (Holmes et al., 2012; Kramer et al., 2016). For example, the highly abundant moon wrasse (*Thalassoma lunare*) is a tertiary predator and feeds primarily on benthic crustaceans, as well as fish and gastropod eggs (Kramer et al., 2015; Sano et al., 1984). Benthic crustaceans represent secondary predators, feeding primarily on zooplankton. As such, zooplankton, benthic crustaceans, and moon wrasse comprise a simple three-tier coral reef food web that is suitable to assess possible routes of MPs intake from different sources into multiple biological endpoints.

The goal of this study was to measure bioconcentration, bioaccumulation and biomagnification of MPs by assessing the MP contamination within individual trophic levels of a simple coral reef food web against contamination in the surrounding environments. To achieve this, zooplankton (i.e., copepods), benthic crustaceans (i.e., crabs, lobsters, isopods, amphipods, and shrimps), and a benthic-feeding reef fish (i.e., moon wrasse, *T. lunare*) were collected along with mid-column water and benthic sediment samples from two coral reefs in the central GBR, adjacent to Gurumbilbarra (Townsville, Queensland, Australia). MP contamination in these abiotic and biotic matrices were characterised (i.e., MP shape, colour, polymer, and size) and quantified following established and rigorous protocols (Kroon et al., 2018a; Santana, 2022). Finally, MP concentrations and physicochemical characteristics

were compared across all matrices, and the occurrence of MP bioconcentration, bioaccumulation and biomagnification estimated at each of the three trophic levels.

5.3 Methods

5.3.1 Solution preparation

Analytical grade reagents including ethanol (EtOH, absolute), nitric acid (HNO_3, 70%), potassium iodide (KI) and potassium hydroxide (KOH pellets) were purchased from Univar®, Australia. Absolute EtOH and Milli-Q water (MQ-H_2O) were used to prepare 95% and 70% EtOH solutions. Brine KI solution was prepared to achieve a final density of 1.69 g cm^{-3}. A 10% KOH solution was prepared by combining 10 g KOH and 90 g MQ-H_2O. Clove oil (eugenol) solution was created in a 1:3:3 solution of clove oil:95% EtOH:MQ-H_2O to give a final concentration of 400 mg L^{-1}. This final solution was kept in the dark to limit degradation prior to use in the fish collection. All solutions were prepared using MQ-H_2O (0.22 µm filtered) and subsequently filtered over 0.46 µm polytetrafluoroethylene (PTFE; Millipore) filters to remove any potential MP contamination prior to sample handling. Solution preparation was conducted within a pre-cleaned fume hood, with all equipment and work surfaces cleaned thoroughly with MQ-H_2O and filtered 70% EtOH between each filtration (Chapter 6).

5.3.2 Sample collection

Marine organisms and environmental samples were collected from Backnumbers (-18.520, 147.132) and Davies (-18.831, 147.634) reefs located in the central GBR off the coast of Gurumbilbarra (Townsville, Australia; Figure 5.1) between October and December 2019 on the RV Apollo and RV Cape Ferguson (A4.1 Table). During the collections, water temperatures varied between 25.8 and 27.5°C, with wind speeds ranging from 5 to 10 knots. All sample collections were done in accordance with the GBR Marine Park Authority permit G12/35236.1.

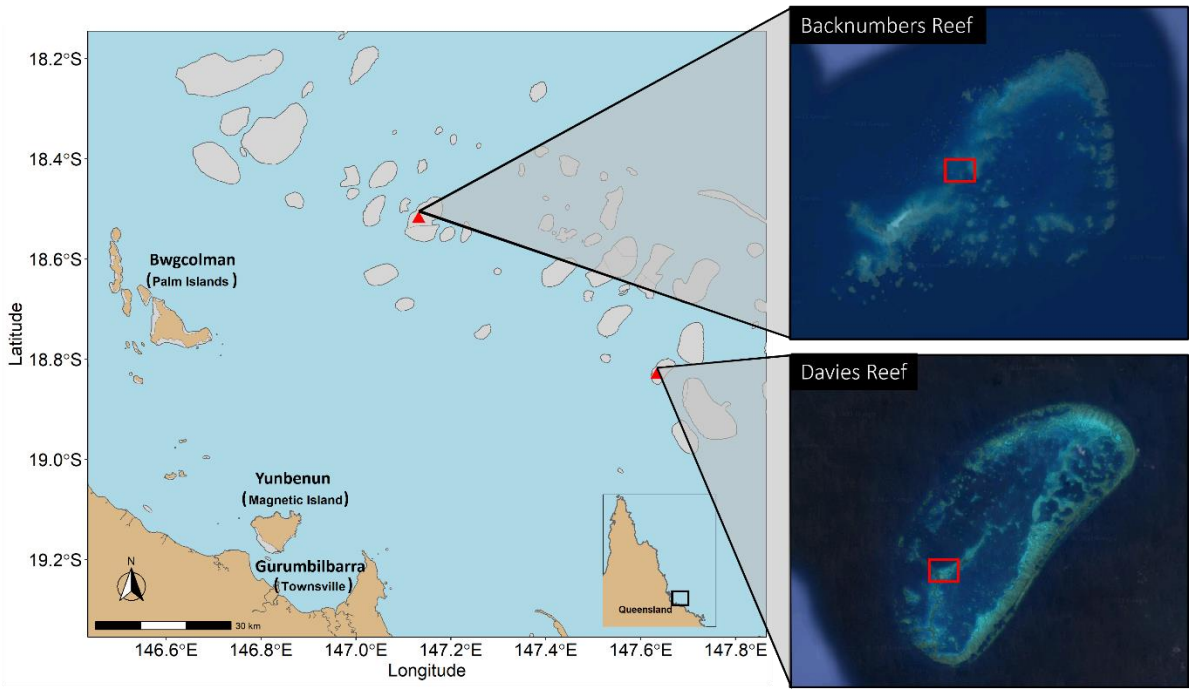

*Figure 4.1 Sample collection locations at Backnumbers Reef and Davies Reef on the Great Barrier Reef in relation to Gurumbilbarra (Townsville, Queensland), Australia. The red boxes show collection areas for seawater, sediment, copepods, benthic crustaceans, and fish (*Thalassoma lunare*) in 2019. Satellite images obtained from Google Maps©.*

5.3.2.1 Water column and zooplankton samples

Water column and zooplankton samples were collected using a submerged seawater pump connected to a plankton net. This method allowed sampling of a larger volume of water than what can be achieved by plankton net alone. The pump was positioned ~ 2 m above the benthos (depth of 6.5 m) and attached to a hose (blue polyvinyl chloride, PVC), allowing transport of water to the surface (Santana et al, 2022) and into the plankton net. The pumping device consisted of a battery-driven submersible bilge pump (Johnson L4000 – 4000 GPH 24V). The hose end was connected to white PVC piping allowing the flow of water to enter a 75 µm plankton net (0.5 m diameter, 1 m length, nylon netting, and polyester, PEST, canvas) fitted with a cod end (400 ml clear polypropylene, PP, jars) attached. The plankton net was positioned over the vessel's side using an on-board davit system, ensuring the net and cod end did not enter the water. The pump was left running outside of the plankton net for 2 min (204 L min^{-1}) to flush the system prior to sample collection for 10-min. Three replicate samples were taken from each reef. Between each replicate, the pump was turned off and the net rinsed from the outside with MQ-H_2O to assist the transfer of material into the cod end. After, the cod end was removed, filled with 70% EtOH to preserve zooplankton, capped with a lid, and kept on ice for transfer to the laboratory (i.e., < 3 h). The net was then cleaned with MQ-H_2O from the outside before attaching a new cod end and repeating for the next replicate.

5.3.2.2 Sediment samples

Sediment samples were collected on SCUBA with a minimum of 15 m between replicates. Samples were collected on sandy bottoms next to coral reef and rubble habitat (6.1 ± 2.0 m depth) using a 0.5 m^2 quadrat, metal hand shovel and large 15 L low-density polyethylene (LDPE) plastic bags. The quadrat was placed randomly on top of the sediment with the opened bag positioned at one end. Using the hand shovel, the top layer of sediment (~ 2 cm) was scraped slowly towards the plastic bag and placed inside with extreme caution to avoid losing and sediment. Once the entire top layer was transferred, the bag was gently pressed to remove excess water and sealed using zip ties. Three replicate samples were taken from each reef. Bags were stored on ice for transfer to the laboratory.

5.3.2.3 Benthic crustaceans

Benthic crustaceans were collected from the coral rubble substrate within ~30 m of sediment collections; a minimum of 15 m was maintained between replicates. While on SCUBA, a 0.5 m^2 quadrat was placed randomly onto coral rubble and using a metal hand shovel, the rubble, and a small layer (~ 1 cm) of sediment was scraped into clear acrylic boxes (40 x 40 x 35 cm; A4.1 Figure) that were pressed slightly into the benthos. Three replicate samples were taken from each reef. Each replicate, contained within its own acrylic box, was then winched on board the RV Apollo using a stainless-steel frame attached to the on-board davit system.

Once onboard the RV Apollo, boxes were drained through holes lined with 315 µm stainless steel mesh. Individual boxes were then emptied over 2 cm and 315 µm stacked stainless steel sieves (40 x 40 cm). Large rubble pieces trapped on the top sieve (> 2 cm) were rinsed using the seawater pump and hose (blue PVC) to detach organisms from the rubble. Freed organisms were washed into the lower sieve and the large 'cleaned' rubble was subsequently returned to the reef environment. The organisms trapped on the bottom sieve were further rinsed with seawater, concentrated into sample containers (500 ml, PP), preserved with 70% EtOH and stored on ice for transport to the laboratory.

5.3.2.4 Fish

Collection, anesthetisation and euthanasia of moon wrasse, *T. lunare*, were done in ordinance with the James Cook University Animal Ethics Committee (Approval Number A2643). Moon wrasse fish were captured on SCUBA by deploying a barrier net (pink nylon) with a weighted chain along the bottom and floats along the top positioned between coral bommies and often within coral rubble substrate. Fish were gently herded towards the barrier net and corralled fish captured with hand nets, using caution to avoid bycatch. Fish were transferred into a small, perforated bucket equipped with a flip-top and anesthetised by spraying with clove oil solution (400 mg L^{-1}) prepared in squirt bottles to reduce stress during transfer to the RV Cape Ferguson (Kroon, 2015). Twenty fish were collected from each

reef. Once on board, fish were removed from the bucket, rinsed with MQ-H_2O, transferred into individual LDPE bags, and placed on ice for euthanasia. Once operculum movement ceased for a minimum of 5 mins, fish were transferred to individual pre-cleaned PP containers (500 or 120 ml) containing 70% EtOH and sealed with lid and Parafilm® for transport to the laboratory.

5.3.3 Laboratory processing

5.3.3.1 Water column and zooplankton samples

Water column samples were filtered over a clean 40 µm sieve (blue PVC pipe with nylon netting). The retentate was backwashed into 50 ml vials (SARSTEDT Australia #L0239; HDPE) using 70% EtOH to preserve biological material. Samples were stored at 4^{o}C until further processing.

Preserved samples were filtered under vacuum onto individual 26 µm stainless steel filters using a custom-built stainless steel filtration system (Schlawinsky et al., 2022). Vials were rinsed thrice with MQ-H_2O to ensure complete sample transfer. Filters were gently backwashed with MQ-H_2O into a pre-cleaned Bogorov chamber to allow sorting of zooplankton under microscope (Leica M165C, 7.3 to 120x magnification). Sorted zooplankton for each sample replicate were placed into a pre-cleaned and pre-weighed test tube (10 ml) using a glass pipette and metal forceps. Post sorting, water in the Bogorov chamber was recovered by filtering under vacuum onto the second set of 26 µm stainless steel filters. Both sets of filters, i.e., backwashed and second filters, were assessed for MP contamination in the water column.

Sorted zooplankton reef replicates were dried overnight under an aluminium foil cover. Each sample replicate was digested overnight in concentrated HNO_3 using a method adapted from Miller et al. (2021). Due to their small size, zooplankton were grouped by taxa from each reef replicate and digested whole to allow processing. Briefly, 20 ml of 70% HNO_3 (15.8 M) per 1 g wet weight of copepods was added to a glass test tube and digested overnight (i.e., 17 hrs) at room temperature, 22 ± 1ºC. To minimise contamination, digestions were conducted in a clean fume hood and loosely covered with aluminium foil. Digestions were neutralised with 10% KOH (final concentration of 7:1 KOH:HNO_3) and filtered under vacuum onto 26 µm stainless steel filters. Filters, while still under vacuum, were rinsed thoroughly with MQ-H_2O three times to ensure complete transfer of material.

5.3.3.2 Sediment samples

Bagged sediment samples were emptied into large pre-cleaned aluminium foil trays, covered with aluminium foil, frozen at -18^{o}C and lyophilised (DynaVac) to obtain dry weights. Before further processing, a visual inspection was done on the sediment to confirm no benthic organisms existed. For each site replicate, subsamples (n = 10) were randomly taken following the manual homogenisation of

dry samples using a metal scoop. A total of 1.5 kg (150 g per subsample) for each site replicate was processed by density floatation using KI brine solution (Miller et al., 2021; Santana, 2022). Briefly, 150 g subsamples were transferred into 500 ml pre-cleaned beakers. KI solution (~400 ml) was added to fill the beaker to ~1 cm below the edge. Subsamples were sealed tightly with Parafilm®, mechanically shaken (Baxter Multi-Tube Vortexer, Baxter Diagnostics) at 175 rpm for 5 minutes, and left to settle for 3 h. The Parafilm® was carefully removed and with aid of a squirt-bottle filled with filtered KI, the top ~ 50 ml of supernatant was decanted into a second, pre-cleaned 250 ml beaker. The original beaker was filled with KI and the density floatation process was repeated twice more. For these final two iterations, the settlement period was reduced to 1 h and the top layer of supernatant decanted into the 250 ml beaker. KI was added to the combined supernatant to give a final volume of 200 ml and left for another 1 h to settle. Finally, the top 50 ml of supernatant was filtered under vacuum onto 263 and 26 µm stacked stainless steel filters (Schlawinsky et al., 2022). MQ-H2O was subsequently poured over filters to dissolve excess salt residue, and to rinse the filtration system to ensure complete transfer of material.

5.3.3.3 Benthic crustaceans

Crustaceans were isolated under a microscope (Leica M165C, 7.3 to 120x magnification) and identified to the lowest taxonomic level possible. Sorted crustaceans were carefully rinsed with MQ-H_20 and stored grouped by taxa in pre-weighed scintillation vials with ~2 ml MQ-H_2O at 4^oC. Prior to processing, vials representing a single taxa per sample replicate were left to evaporate fully under aluminium foil cover. Dried samples were weighed, and larger crustaceans were cut using dissection scissors to promote quick digestion in HNO_3. All vials were subject to the same HNO_3 protocol as described above for the zooplankton. Whole crustaceans were digested due to their size preventing dissection. Following digestion, replicates were neutralised with 10% KOH and filtered under vacuum onto 26 µm stainless steel filters (Schlawinsky et al., 2022). Filters, while still under vacuum, were rinsed with MQ-H_2O three times to ensure complete transfer of material.

5.3.3.4 Fish

Moon wrasses were removed from 70% EtOH, rinsed with MQ-H_2O and measured (standard length, cm; wet weight, g). The gastrointestinal tracts (GIT; stomach and intestine) were excised by sharp dissection, the outer tissue rinsed with MQ-H_2O and then placed into pre-weighed test tubes. Fish GITs were subject to 10% KOH digestion (20 ml 10% KOH to 1 g GIT w.w.) for 48 h at 40^oC (Santana, 2022) after which digestates were transferred into 100 ml beakers and KI solution was added. Test tubes were rinsed three times with KI to ensure the transfer of all material. Beakers were covered with Parafilm® and shaken (Baxter Multi-Tube Vortexer, Baxter Diagnostics) at 175 rpm for 5 minutes,

followed by a 3 h settlement period. The supernatant was decanted directly over a 26 µm stainless steel filter under vacuum (Schlawinsky et al., 2022) and rinsed with MQ-H_2O and 70% EtOH.

5.3.4 Identification and characterisation of MPs

Filters were visually examined under a microscope (Leica MZ16A, 0.73x – 12.0x magnification; Leica DFC 500 camera attachment) and putative MPs were identified based on criteria established by Hidalgo-Ruz et al. (2012). Putative MPs were photographed and their physical characteristics, including colour and size (FIJI, ImageJ) were measured as per Hidalgo-Ruz et al. (2012) and Chapter 6. Putative MPs were sorted into three main categories based on their shape, namely fragments, fibres, and beads (Hartmann et al., 2019).

All putative MPs were chemically characterised using Fourier transform infrared spectroscopy (FTIR) to confirm polymer composition following the workflow established by Kroon et al. (2018a). In short, putative MPs were analysed using a Perkin Elmer Spotlight 200 microimaging Attenuated Total Reflectance FTIR (µATR-FTIR) in transmittance mode (background scans acquired prior to each item). For all putative MPs analysed, spectra were collected with 10 scans at 4 cm^{-1} resolution between wavenumbers 4,000 and 650 cm^{-1}. The final chemical assignment of putative MPs was confirmed as per Kroon et al. (2018a), using a custom-built R script protocol (See Chapter 6). This process included the comparison of sample spectra to an in-house project-specific contaminant library comprising of all plastic sampling materials and laboratory equipment, as well as MPs derived from airborne contamination controls and processing blanks (see below). This library was developed to eliminate extraneous MPs resulting from the processing (A4.2 Table). When items returned a composite blend (i.e., mix of two or more polymers) match, items were assigned the polymer which comprised the majority of the MP. For example, a 60:40 PEST:rayon blend was sorted into 'PEST Blends,' whereas a 70:30 rayon:PEST blend was classified as a 'Rayon Blend.' Based on the final spectral assignment, anthropogenic items were then categorised into one of three main groups: (i) synthetic, (ii) semi-synthetic, and (iii) naturally derived (Kroon et al., 2018b).

5.3.5 Contamination prevention

5.3.5.1 Field and laboratory procedures

Laboratory and field equipment was rinsed initially with reverse osmosis (RO, 20 µm filtered) water once, then thrice with MQ-H_2O, followed by 70% EtOH. Laboratory equipment was left to dry under an aluminium foil cover. Field equipment was stored in pre-cleaned plastic bags or covered plastic bins (Nally™) until used. Acrylic boxes used in sample collection were cleaned thoroughly with

MQ-H_2O and sealed with lids until sample collection. Where possible, separate stainless-steel filtration systems (Schlawinsky et al., 2022) were used for each sample replicate to prevent cross-contamination.

Work surfaces were cleaned with 70% EtOH and wiped down with a 'lint-free' purple cloth (Vileda Actifibre©; Polyester:Viscose). Blue nitrile gloves and a green 100% cotton lab coat were worn for all samples. Gloves were pulled above the cuffs of the lab coat and taped using duct tape (silver polyethylene, PE) to reduce the possibility of textile fibres becoming airborne while working.

5.3.5.2 Airborne contamination

Controls (i.e., petri dishes filled with MQ-H_2O) were placed around the working area on the vessel to account for airborne contamination during seawater sample collection (n=4). Separate laboratory controls were placed around the lab bench and workspace during all remaining sample processing (n=4 per sample processing batch, 30 total). Additionally, procedural blanks (i.e., a sample that does not contain any of the matrices) were processed alongside samples (n = 1 per sample matrix) to account for any potential contamination occurring due to sample handling. All controls and procedural blanks were individually filtered over 26 µm filters and visually analysed as per procedures described below. All samples, controls and procedural blanks were covered with either glass or aluminium foil when not being handled. Counts of MPs found within samples were corrected (i.e., subtracted) based on contamination found, albeit only when the polymer type, colour, and shape of MPs found in a sample matched those in the appropriate contamination control (Jensen et al., 2019; Kroon et al., 2018a).

5.3.6 Data analysis

MP abundance (counts per individual) was converted to MP concentrations (calculated by weight) for each matrix analysed, to facilitate comparisons to the literature. Water column (MPs m^{-3}), sediment (MPs kg^{-1}) and biota (MPs g^{-1}) concentrations are presented as an average estimated between the two coral reefs (i.e., average ± standard deviation, S.D.) unless otherwise specified. MP concentrations in biota were calculated based on gram wet weight (w.w.) of the entire organism (A4 Text). To allow comparison, sizes are presented as the longest length (Hartmann et al., 2019).

Comparisons of physical (i.e., shape, colour) and chemical (i.e., polymer) characteristics of MPs isolated from organisms to those found within their surrounding environment were made using Fisher's exact tests. For these tests, ratios of these characteristics in organisms were compared to those found within their respective environment: zooplankton to water column, benthic crustaceans to sediment and reef fish to both sediment and water column due to their foraging strategy (i.e., sifting through sediment and rubble for prey). For each matrix, these ratios were calculated by combining the raw data from both sites. Due to the limitation of using Fisher's exact tests with continuous data, a general linear model (GLM) with a negative binomial distribution was used to compare size distributions (i.e., longest

length) across matrices (formula: Length ~ Matrix). Post-hoc analysis for size distributions was done using the estimated marginal means (EMM) from the model for pairwise comparisons. All statistical analyses were conducted using Rstudio (Version 1.4.1106) and R (Version 4.0.4).

To estimate bioconcentration (BCF), bioaccumulation (BAF) and biomagnification factors (BMF), data (per weight) was required to be transformed into comparable units, given below. The following equations were used:

Bioconcentration Factor:

$$BCF = \frac{C_o}{C_w + C_s}$$

Bioaccumulation Factor:

$$BAF = \frac{C_o}{C_w + C_s + C_p}$$

Biomagnification Factor:

$$BMF = \frac{C_o}{C_p}$$

Where C_o is the concentration of MPs in the organism (i.e., copepod, crustacean, or fish; MP kg^{-1}), C_w is the concentration of MPs in the water column (MPs L^{-1}), C_s is the concentration of MPs in the sediment (MPs kg^{-1}) and C_p is the concentration of MPs in the prey of the organism. BCF, BAF, and BMF values were calculated using data from both reefs as replicates. For zooplankton, where prey was not investigated, only BCF and BAF values could be calculated which in essence are the same. BCF, BAF and BMF values > 1 indicate MP bioconcentration, bioaccumulation, or biomagnification is occurring.

5.4 Results

5.4.1 Taxonomic Assessment

Zooplankton and benthic crustaceans collected across both reefs were identified into six different taxa (Table 5.1, Figure 5.2). Zooplankton comprised of both Harpacticoida (n=80) and Calanoida (n=260) copepods. Benthic crustaceans included isopods (Family Cirolanidae; n=4), squat lobsters (Family Galatheidae; n=76), shrimps (Family Penaediae and Infraorder Caridea; n=38), crabs (Families Xanthidae, Majidae, and Portunidae; n=31), and amphipods (Order Amphipoda; n=54). All fish

were confirmed to be moon wrasse (*T. lunare*). The distribution of taxa across the two study sites, as well as the combined weight and size ranges, are reported in Appendix 4 (A4.1b Table).

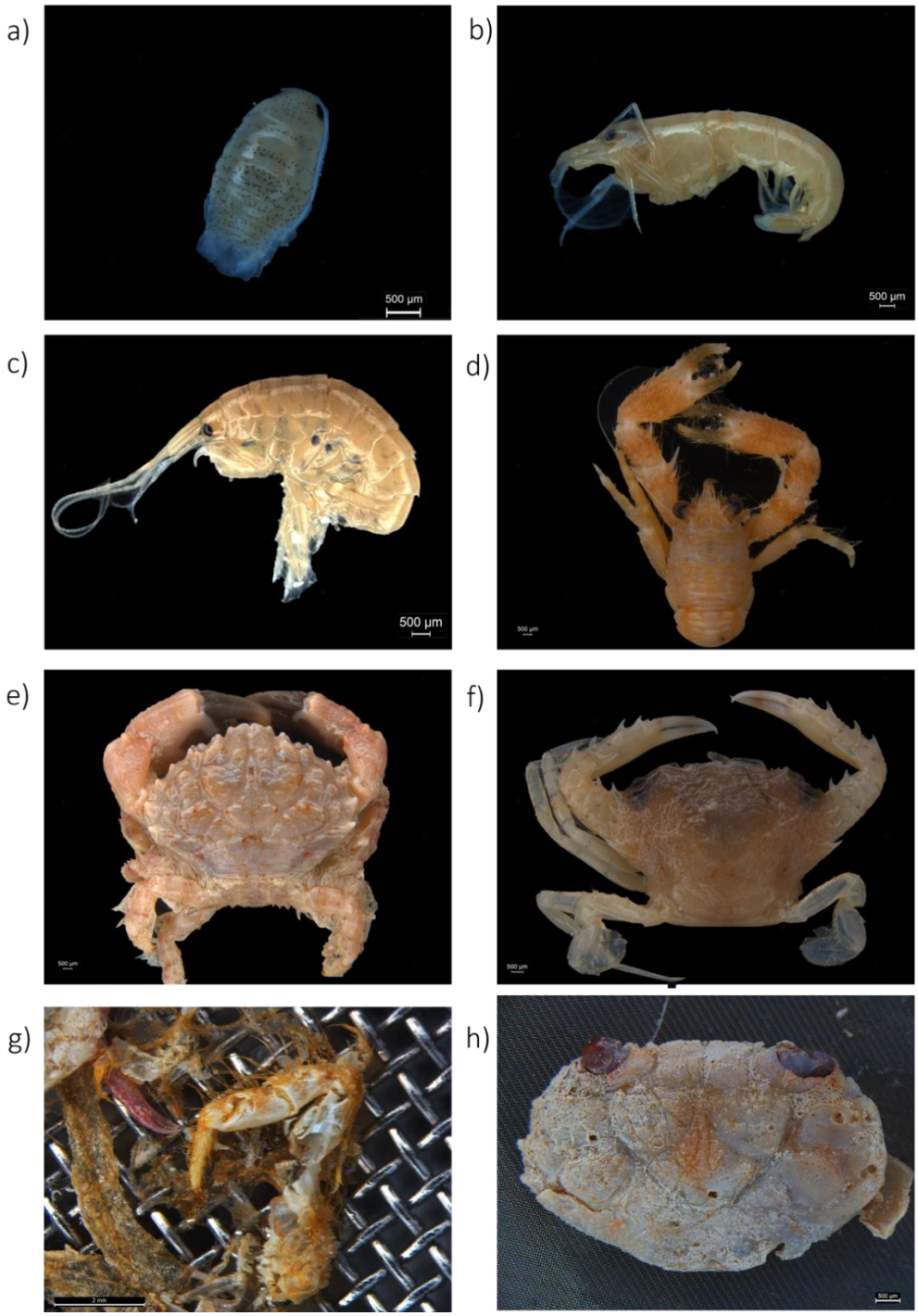

*Figure 5.2. Examples of benthic crustacean taxa collected and processed for microplastic contamination, including a) an isopod (Family Cirolanidae) from Backnumbers Reef, b) shrimp (Infraorder Caridea) from Backnumbers Reef, c) amphipod (Order Amphipoda) from Davies Reef, d) squat lobster (*Galathea australiensis*) from Davies Reef, e) rubble crab (*Etisus frontalis*) from Davies Reef, and f) swimmer crab (*Thalamita spinimana*) from Backnumbers Reef. Unknown crustacean parts (g and h) retrieved from dissection of two separate fish (*Thalassoma lunare*) collected at Davies Reef are also shown. All scale bars are representative of 500 µm, except g) which is representative of 2 mm.*

5.4.2 Airborne contamination

Across all field and laboratory processing, a total of 39 control filters were generated and analysed for airborne and procedural contamination (A4.2 Table). Across all filters, a total of 35 MPs were detected, comprising airborne contaminants from field (n=4) and laboratory (n=30) controls, and from procedural blanks (n=1). Four of these 35 items, detected on laboratory control filters, matched polymer type, colour and shape to MPs isolated from field samples. Specifically, two items matched MPs isolated from copepods, and two from fish; these four items were excluded from final analysis.

5.4.3 MP contamination of water and sediment

5.4.3.1 Water Column

Six water column samples were collected representing a total of 12,240 L (12.24 m^3), averaging 6,120 L per reef. From these, 69 putative items were isolated under microscope, and all were successfully characterised via FTIR: 48 putative items from Backnumbers reef and 21 from Davies reef. Following the exclusion of 12 items of natural origin (e.g., keratin, cotton), 57 items were confirmed to contain plastic (n=50 synthetic plastics and n=7 semi-synthetic plastic blends). All semi-synthetic items were fibres, while synthetic items consisted of both fibres (n=26) and fragments (n=24). No primary microbeads were isolated from water column samples at either reef. MP concentrations ranged from 2.94 to 12.74 MPs m^{-3} for Backnumbers reef, and 1.47 to 3.92 MPs m^{-3} for Davies reef (Table 5.1; Figure 5.3), with an average across both reefs of 4.66 ± 4.24 MPs m^{-3}.

Table 5.1. Microplastic (MP) contamination of various sample matrices (i.e., water column, sediment, and organisms) collected from Backnumbers Reef and Davies Reef, Great Barrier Reef. Organism sample sizes (n) are across three replicates for each site. Polymers and colours reported are the most prevalent and are not inclusive of all identified. MP concentrations reported in MPs m^{-3} for the water column, MPs kg^{-1} for sediment, and MPs g^{-1} for all organisms. PEST = polyester, PE = polyethylene.

Site	Matrix	n	Fragments	Fibres	MP Concentration	Polymer	MP Size Range (mm)	Colour
Backnumbers Reef	Water Column	3	20	23	7.03 ± 5.10	PEST	0.069 – 4.77	White & Transparent*
	Sediment	3	1	1	0.44 ± 0.38	PEST & PE*	0.208 – 1.10	Blue & Orange*
	Zooplankton *Copepods*	167	-	4	4.81 ± 1.90	PEST	0.342 – 1.05	Transparent
	Benthic Crustaceans							
	Isopods	3	-	-	-	-	-	-
	Lobsters	25	-	-	-	-	-	-
	Shrimps	26	-	1	0.57 ± 75.93	PEST:rayon	1.69	Transparent
	Crabs	11	1	1	4.76 ± 6.73	PEST:rayon	2.38 – 5.52	Blue & Transparent*
	Amphipods	27	-	2	3.92 ± 6.79	nylon:acrylic & PEST:rayon	3.84 – 3.97	Black & Orange*
	Fish							
	Moon wrasse	20	6	24	0.09 ± 0.15	PEST	0.081 – 5.98	Blue
Davies Reef	Water Column	3	4	10	2.29 ± 1.41	PEST	0.088 – 3.86	Blue
	Sediment	3	7	20	2.40 ± 6.0	PEST	0.128 – 2.83	Blue
	Zooplankton *Copepods*	173	-	2	2.64 ± 2.29	acrylic	0.442 –0.548	Transparent
	Benthic Crustaceans							
	Isopods	1	-	1	9.09	PEST:rayon	0.939	Blue
	Lobsters	51	-	8	5.95 ± 7.97	PEST	0.656 – 1.87	Pink
	Shrimps	12	-	5	45.68 ± 75.93	PEST	0.482 – 1.62	Transparent
	Crabs	20	2	14	5.18 ± 3.64	PEST:rayon	0.091– 2.74	Black
	Amphipods	27	-	-	-	-	-	-
	Fish							
	Moon wrasse	20	7	32	0.22 ± 0.26	PEST	0.075 – 2.39	Blue

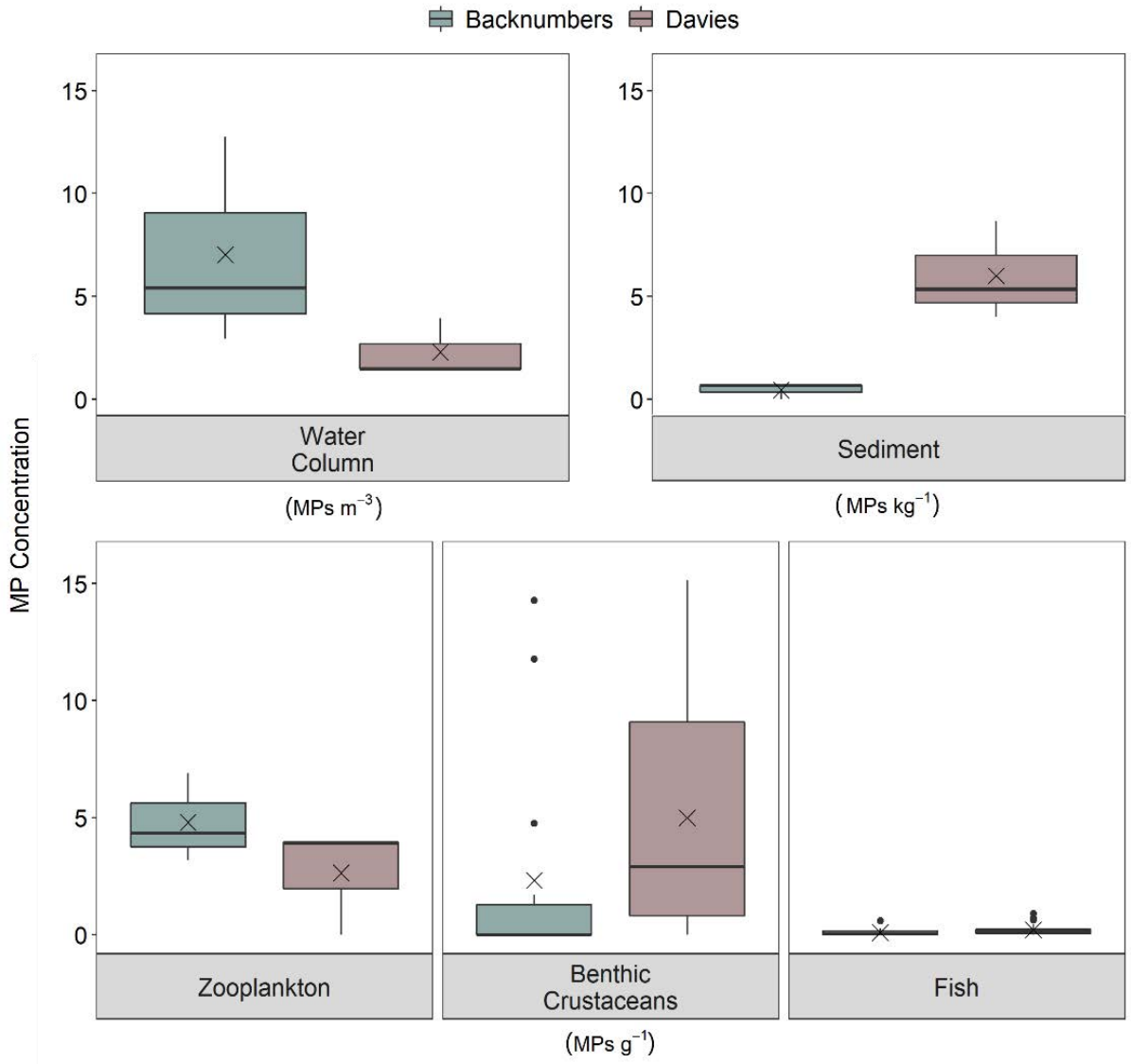

Figure 5.3 Microplastic contamination in sediment (MPs kg^{-1}), water column (MPs m^{-3}) and reef organisms (MPs g^{-1}) collected at Backnumbers Reef and Davies Reef, located in the central Great Barrier Reef World Heritage Area. Reef organisms include zooplankton (n=340), benthic crustaceans (n=203), and Thalassoma lunare *fish (n=40).*

The most common polymer found within the water column was PEST (n=15 for Backnumbers; n=5 for Davies), followed by PP (n=14) for Backnumbers reef and PE (n=4) for Davies reef (Figure 5.4). All seven semi-synthetic items consisted mostly of rayon or rayon blends (i.e., rayon:PEST). Other polymers found included PVC, polystyrene (PS), and acrylic. Size length distribution was similar at both sites, ranging from 87.70 µm to 4.77 mm for fibres and 69.66 µm to 837.52 µm for fragments, and an average of 800.70 ± 1,094 µm for all confirmed MPs. Across both reefs, colour abundance was similar, with transparent MPs being the most abundant (24.5%), followed by blue (22.8%) and white (17.5%) MPs.

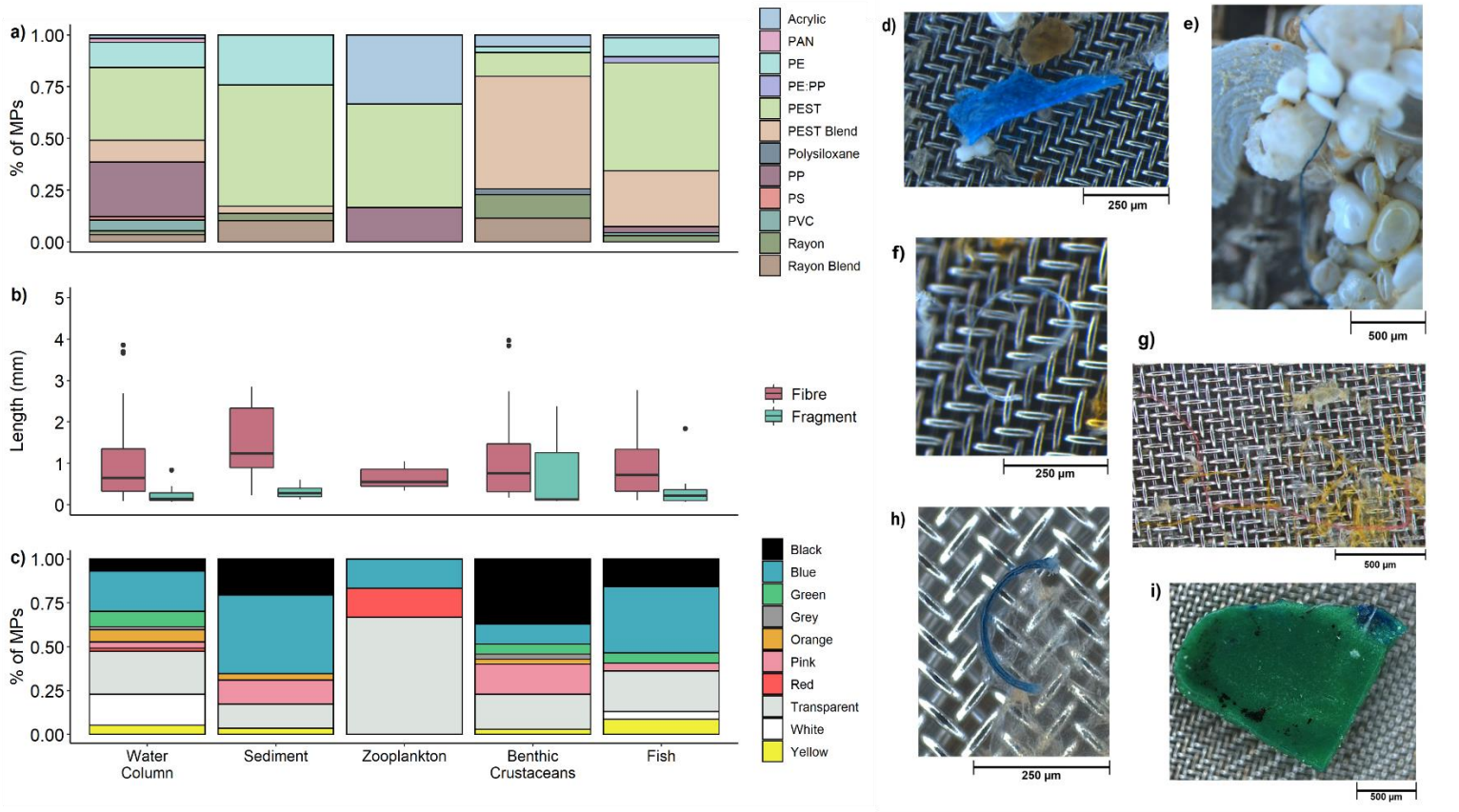

Figure 5.4. Chemical and physical characteristics of microplastics (MPs) isolated from the environment (i.e., water column and sediment) and organisms (i.e., zooplankton [n=340], benthic crustaceans [n=203], and fish, Thalassoma lunare *[n=40]), including a) polymer type, b) size in length and c) colour, from Backnumbers and Davies Reefs, Great Barrier Reef. Polymer type and colour data presented as percent frequency (% of MPs); length is representative of the longest length (mm). Examples of MPs isolated include: d) blue polyethylene (PE) fragment from Davies water column, e) blue polyester (PEST) fibre from Davies sediment, f) transparent polypropylene (PP) fibre isolated from a Backnumbers copepod, g) pink acrylic fibre isolated from a Davies benthic crustacean, h) blue PEST fibre and (i) green polyethylene terephthalate (PET) fragment, both from two* T. lunare *(Backnumbers). Additional polymer abbreviations: PAN = polyacrylonitrile, PS = polystyrene, PVC = polyvinyl chloride. PEST blends include PEST:epoxy, PEST:acrylic, PEST:rayon, PEST:nylon; Rayon blends include rayon:nylon, rayon:PEST:acrylic, rayon:PEST, rayon:PE and rayon:acrylic.*

5.4.3.2 Sediment

Six sediment samples were collected totalling 3 kg of sediment. A total of 73 putative MPs were isolated using microscopy, and 29 were confirmed by FTIR to be MPs. More than half (n=39) were identified as filamentous algae based on a match to cellulose, highlighting the importance of FTIR confirmation of putative MPs. Sediment MP loadings at Backnumbers reef ranged from 0 to 0.67 MPs kg^{-1}, and at Davies reef from 4 to 8.67 MPs kg^{-1} (Table 5.1). Across both reefs, an average of 3.22 ± 3.41 MPs kg^{-1} was found, comprising of both fragments (n=8) and fibres (n=21). The only two polymers found at Backnumbers reef were one PE fragment and one PEST fibre (Figure 5.4). The most abundant polymers found at Davies Reef were PEST (n=15) and PE (n=6), followed by rayon blends (i.e., rayon:PEST, rayon:nylon). Size length distributions ranged from 128.21 µm to 2.85 mm, with an average of 1.22 ± 0.93 mm for all confirmed MPs. Blue MPs were most abundant (44.8%), followed by black (20.6%), transparent (13.7%) and pink (13.7%) items.

5.4.4 MP contamination in coral reef organisms

5.4.4.1 Zooplankton

A total of 340 individual copepods were isolated from seawater collected at both reefs, with 33 putative MPs isolated from these individuals and analysed via FTIR. Following the exclusion of natural items (n=25) and airborne contaminants (n=2), a total of 6 MPs were detected across the 340 copepods (Table 5.1; A4.3 Table). Copepods contained an average of 3.72 ± 3.74 MPs g^{-1} across both reefs, with higher ingestion found at Backnumbers reef (4.81 ± 1.90 MPs g^{-1}) compared to Davies reef (2.64 ± 2.29 MPs g^{-1}). These six MPs included synthetic fibres of PEST (n=2), acrylic (n=2), PP (n=1), and PEST:acrylic (n=1) (Figure 5.4). Fibre lengths ranged from 341.87 µm to 1.05 mm with an average of 624.42 ± 270.17 µm. Clear fibres were most abundant (66.7%), followed by blue (16.7%) and red (16.7%). No fragments or primary microbeads were found in any of the copepods.

5.4.4.2 Benthic crustaceans

A total of 203 crustaceans, representing five taxa, were collected at both reefs, with 37 putative MPs isolated from these individuals and analysed via FTIR. Two putative MPs were removed due to their natural origin (i.e., aragonite and keratin). The remaining 35 items included semi-synthetic (n=26) and synthetic (n=9) MPs which were isolated from up to 124 crustaceans, with the remaining replicates (up to 79 individuals) containing no MPs. Crustaceans were found to be more contaminated at Davies reef (4.99 ± 5.37 MPs g^{-1}) compared to Backnumbers reef (2.32 ± 4.74 MPs g^{-1}), with an average of 3.56 ± 5.12 MPs g^{-1} across both reefs (Table 5.1; A4.3 Table), with crabs containing the most (5.83 ± 5.14

MPs g^{-1}), followed by shrimps (3.75 ± 5.57 MPs g^{-1}), isopods (3.03 ± 5.42 MPs g^{-1}), squat lobsters (2.98 ± 6.00 MPs g^{-1}) and amphipods (1.96 ± 4.80 MPs g^{-1}).

MPs ingested by crustaceans were primarily fibres (n=32; 91.4%) and comprised mostly of PEST (n = 13; 37.1%) and PEST:Rayon blends (n=19; 54.3%) (Figure 5.4). MP fragments were present in substantially lower numbers (n=3) and were comprised of polysiloxane (n=1), polyethylene terephthalate (PET, n=1) and PE (n=1) polymers. For all crustacean taxa, fibres averaged a length of 1.20 ± 1.25 mm, ranging from 173.65 µm to 3.97 mm. Black MPs were most prevalent (n=13; 37.1%), followed by transparent (n=7; 20%) and pink (n=6; 17.1%) items. Similar to zooplankton, no primary microbeads or pellets were found in crustaceans.

5.4.4.3 Fish

A total of 115 putative MPs were identified from the GITS of 40 moon wrasse. Following FTIR analysis, 44 items of natural origin (i.e., cotton, aragonite-based) and 2 airborne contaminants (i.e., 1 yellow rayon fibre, 1 transparent acrylic fibre), were excluded from the dataset. The remaining 69 MPs included PEST (n=28), PEST:rayon (n=14), PET (n=9), and PE (n=7) polymers (Table 5.1). Other polymer types included rayon, PEST:acrylic, PP, PVC and PEST:nylon albeit in much lower abundance.

Ten out of the 40 fish collected did not contain any MPs (n=7 at Backnumbers; n=3 at Davies). Overall, *T. lunare* contained an average of 0.16 ± 0.22 MPs g^{-1}, with contamination at Davies slightly higher (0.22 ± 0.26 MPs g^{-1}) than Backnumbers (0.09 ± 0.18 MPs g^{-1}). MP contamination included fibres (n=57) and fragments (n=13), but no primary microbeads (Figure 5.4). Mean MP length was 859.1 ± 853.3 µm, with fibres ranging from 109 µm to 5.08 mm (mean 973.8 ± 882.1 µm) and fragments ranging from 75.1 µm to 1.84 mm (av. 356.2 ± 468.2 µm). Blue MPs were most abundant within fish GITs (n=26; 37.6%), followed by transparent (n=16; 23.2%), black (n=10; 14.5%) and yellow (n=6; 8.69%) MPs. Pink, white, and green items were also found but were less common.

5.4.5 Concentration, shape, colour, polymer and size comparisons

When converted to comparable units, MP concentrations were substantially lower in the water column (0.005 ± 0.004 MPs L^{-1}) compared to the sediment (3.22 ± 3.41 MPs kg^{-1}). Furthermore, MP concentrations in the three trophic levels included 3,751.20 ± 2,229.57 MPs kg^{-1} in zooplankton, 3,556.75 ± 5,123.92 MPs kg^{-1} in benthic crustaceans and 155.28 ± 220.12 MPs kg^{-1} in moon wrasse; all drastically higher than MP concentrations found within their immediate environment. Refer to A4.4 Table for reef-specific concentrations.

Fibres were the most prevalent shape for both water column and sediment samples, with ratios of fibres to fragments not significantly different (Fisher's, p-value = 0.241). Further, across the three

trophic levels, fibres were more prevalent and the ratio of MP shapes (i.e., fibre vs. fragment) did not differ significantly (Fisher's, p-value = 0.284). When comparing MP shapes in individual trophic levels to their surrounding environment, only reef fish had a significantly different ratio of fibres:fragments (4:1 in fish compared to 2:1 in sediment and water column combined; Fisher's, p-value = 0.013). Conversely, zooplankton and benthic crustaceans demonstrated similar trends with more fibres present (1:0 fibres:framgents and 9:1 fibres:fragments, respectively; compared to 3:2 fibres:fragments in the water column and 7:3 fibres:fragments in the sediment), yet this difference was not significant (Fisher's, p-values = 0.074 and 0.054, respectively).

The water column had significantly higher ratios of blue, transparent, and white coloured MPs, with sediment containing higher ratios of blue and black items (Fisher's, p-value = 0.009). The ratios of MP colours differ significantly between zooplankton, benthic crustaceans, and reef fish (Fisher's, p-value = 0.001), with a substantially higher ratio of black items in benthic crustaceans. However, the proportions of MP colours did not differ significantly when comparing zooplankton, benthic crustaceans, or fish to their respective environments (Fisher's, p-values = 0.457, 0.063 and 0.286, respectively).

PEST is the most prevalent polymer in all samples. However, the ratios of the different polymer types for the sediment and water column differed significantly (Fisher's, p-value = 0.006); the water column contained a higher ratio of PP MPs. Polymer distributions were also significantly different among the three trophic levels (Fisher's, p-value = 0.007). The distribution of polymers found in zooplankton was similar to that found in the water column (Fisher's exact test, p-value = 0.236), whereas for benthic crustaceans and fish they differed significantly from those in their environments (Fisher's exact test, p-values = 0.050 and 0.016, respectively).

Size distributions of MPs found within all five matrices were not significantly different from one another (GLM; all p-values > 0.05; A4.5 Table). Irrespective of the matrix examined, all MPs were smaller than 5 mm, and both fibres and fragment lengths were skewed to the smaller size classes with 68.3% of MPs < 1 mm.

5.4.6 Bioconcentration, bioaccumulation and biomagnification factors

While differences in MP contamination between matrices across the two reef locations occurred (Figure 5.3), when converted to comparable units (i.e., MPs kg^{-1} and MPs L^{-1}), these became negligible, revealing the same trend of BCF, BAF and BMF values. Therefore, a generalised trend of these endpoints, is presented using the two reef locations combined. Copepods were found to have very large (i.e., > 1) BCF and BAF values, whereas calculation of a BMF value was not possible due to their trophic level placement (Table 5.2). Benthic crustaceans and fish were also found to have large BCF

values. However, when taking into consideration potential MP uptake via prey (BAF and BMF), values are reduced to < 1, indicating there is little if any bioaccumulation or biomagnification of MPs across this simple marine food web.

*Table 5.2. Bioconcentration (BCF), bioaccumulation (BAF) and biomagnification (BMF) factors for zooplankton, benthic crustaceans, and fish (*Thalassoma lunare*) collected on the Great Barrier Reef, Australia. BMF for zooplankton is not possible due to the trophic level placement of these organisms (i.e., no prey analysed).*

Organism	BCF	BAF	BMF
Zooplankton	1053.87	1053.87	-
Benthic Crustaceans	1102.23	0.95	0.95
Fish	48.12	0.04	0.02

5.5 Discussion

This study is the first comprehensive assessment of bioconcentration, bioaccumulation and biomagnification of MPs in a simple coral reef food web. The results corroborate previous studies that MP contamination is prevalent in the GBR, and in coral reef ecosystems in general. MP contamination was found in both abiotic and biotic matrices collected at two mid-shelf reefs in the central GBR. Specifically, this study is the first to report MP bioconcentration from the environment into three individual trophic levels of planktonic zooplankton (i.e., copepods), benthic crustaceans (i.e., crabs, lobsters, isopods, amphipods, and shrimps), and benthic-feeding moon wrasse (*T. lunare*). MP concentrations, polymer type, size, shape, and colour varied depending on the trophic level, but appeared to be similar to that of their immediate environment. However, there was no clear indication of bioaccumulation or biomagnification across this simple coral reef food web observed here.

5.5.1 Presence and abundance of MPs

MP contamination was found in all abiotic and biotic matrices, although the concentrations of MPs varied widely across the water column, sediment and all three trophic levels. MPs were prevalent in the water column at both reefs, with MP concentrations similar to, albeit lower, than those reported for coral reefs at Dyiigurra (0.036 to 0.044 MPs L^{-1}; (Santana, 2022)) and substantially lower compared to the Xisha Islands coral reefs (0.2 to 45.2 MPs L^{-1}; (Ding et al., 2019a)). MP contamination of sediment was similar to or lower than those reported for other coral reef environments both globally (Huang et al., 2021a) and within the GBR (Santana, 2022). Current findings match both global (Cozar et al., 2014;

Mai et al., 2020; Song et al., 2018; Van Cauwenberghe et al., 2015) and local (Santana, 2022) reports of benthic sediments acting as a sink for MPs, once in comparable units. Many properties of MPs are known to influence the vertical distribution of MPs, including biofouling, shape, and polymer type of the MP itself (Liu et al., 2020; Reisser et al., 2015), as well as environmental influences on MPs such as wind, currents, and tides (Frere et al., 2017) (Chapter 6). These results highlight that while MP concentrations may be lower than other coral reef environments globally, MPs are ubiquitous throughout the abiotic environment of the GBRWHA (Kroon et al., 2020).

Comparisons of the three trophic levels revealed the variability in MP uptake across different taxa despite similar environmental exposure (Guven et al., 2017; Lourenço et al., 2017; Santana et al., 2021). Copepods were the most contaminated taxa when analysed based on MP contamination by weight, followed by benthic crustaceans and moon wrasse. This corroborates previous reviews and meta-analyses that have shown lower trophic levels are more susceptible to MP contamination (Walkinshaw et al., 2020) (Chapter 2). However, when comparing MP contamination by individual organisms to facilitate comparisons to other studies (Chapter 2; A4 Text), this trend is reversed with fish containing a higher concentration of MPs (1.5 to 1.95 MPs individual^{-1}) than either benthic crustaceans (0.03 to 1 MP individual^{-1}) or copepods (0.01 to 0.02 MPs individual^{-1}) (A4.3 Table). MP uptake in copepods is similar to previous studies with concentrations ranging from 0.03 to 0.33 MPs individual^{-1} for Calanoida copepods in the Pacific and the Indian Oceans, respectively (Desforges et al., 2014; Kosore et al., 2018). Benthic crustaceans show lower MP contamination compared to similar species globally, such as isopods (0.96 MPs individual^{-1}; (Karlsson et al., 2017), amphipods (2.1 ± 1.7 MPs individual^{-1}; (Jamieson et al., 2019), crabs (1 and 21.99 MPs individual^{-1} (Waite et al., 2018), prawns (7.80 MPs individual^{-1};(Abbasi et al., 2018)) and shrimp (1 to 29.40 MPs individual^{-1};(Devriese et al., 2015; McGoran et al., 2018; Wang et al., 2019c)). Finally, MP uptake by individual moon wrasse is higher compared to other wrasse (*T. rueppellii*; 0.08 MPs individual^{-1}; (Baalkhuyur et al., 2018)) from the Red Sea, but similar to or lower than MP contamination in other reef fish collected in the GBR (Jensen et al., 2019; Kroon et al., 2018b; Santana, 2022).

The assessment of MP contamination between trophic levels and their environment is non-trivial, requiring extensive field sampling, and is further complicated by inconsistency in units reported, which limit direct comparisons (Chapter 2). When standardised by weight, concentrations were significantly higher in individual trophic levels than in their relative environments. This positive correlation between environmental contamination and uptake by marine biota has been observed previously at Dyiigurra (Santana et al., 2021). Here, copepods, benthic crustaceans, and moon wrasse also showed increased uptake where MP concentrations in the immediate surrounding environment was higher. Specifically, the average MP concentration in the water column at Davies reef was lower

than at Backnumbers reef, and this correlates to lower MP contamination in copepods from Davies reef. Conversely, the average MP concentration in the sediment at Davies reef was higher than at Backnumber reef, and this correlates with higher levels of MP contamination observed in benthic crustaceans at Davies Reef. The higher levels of MPs observed in moon wrasse was also positively correlated to the higher levels of MPs in Davies Reef sediment.

5.5.2 Bioconcentration, bioaccumulation and biomagnification

Results indicate bioconcentration in copepods, benthic crustaceans, and *T. lunare* on two coral reefs of the central GBR. However, bioaccumulation of MPs was only observed for copepods. In this simple food web, copepods are the base trophic level, with MP contamination a direct result of environmental exposure as their phytoplankton prey is unable to ingest MPs. MP contamination in the two higher trophic levels is lower than expected given the quantities of MPs in their prey, indicating that MPs are not bioaccumulating in either benthic crustaceans or in *T. lunare*. Further, biomagnification of MPs in the two higher trophic levels does not appear to be occurring, corroborating previous findings for a generalised marine food web (Chapter 2), and for coastal ecosystems in the Persian Gulf (Akhbarizadeh et al., 2019) and in British Columbia, Canada (Covernton et al., 2022). While biomagnification has been reported from the surface of macroalgae (*Pelvetiopsis limitata* and *Endocladia muricata*) to the herbivorous snail (*Tegula funebralis*), macroalgae do not ingest the MPs and the definition of biomagnification has not been met (Saley et al., 2019). Therefore, currently, no evidence for MP biomagnification exists for marine species. The lack of bioaccumulation and biomagnification may be due to the complexity of food webs, with benthic crustaceans not exclusively consuming copepods, and *T. lunare* not exclusively consuming benthic crustaceans (Kramer et al., 2015). These two trophic levels have been shown to feed opportunistically on a wide variety of available prey with intricate trophic relationships (Fulton and Bellwood, 2002; Kramer et al., 2016). Therefore, further evaluation of MP bioaccumulation and biomagnification may be required, including assessment of additional prey and their surrounding environments, before excluding their occurrence.

While bioaccumulation or biomagnification did not appear to be prevalent in this simple food web, the similarity in MP shape, colour, polymer type and size across all three trophic levels means that trophic transfer cannot be ruled out as a potential route of exposure. Fibres were the most prevalent shape of MP (75.5%) found in all organisms and environmental matrices, despite fish having a lower number of fragments compared to what was expected given environmental observations. This same trend has been seen previously both in the GBR (Jensen et al., 2019; Santana, 2022), and globally (Barrows et al., 2018a; Dris et al., 2015; Gallagher et al., 2016). Blue and transparent coloured MPs were most frequently found within all matrices and organisms, yet all three trophic levels had different

proportions of colours when compared to one another. In the abiotic matrices of other coral reef systems (Huang et al., 2021a), as well as in the GBR (Jensen et al., 2019; Santana, 2022), the distribution of colours is skewed toward blue, transparent, black, and white items. The most abundant MP polymer type was PEST, yet the proportions differed not only between the three trophic levels but also between organisms and their environment. The density of PEST (1.38 g cm^{-3}) dictates it will sink in the marine environment, potentially increasing exposure to organisms that reside and forage within the water column or amongst the sediment. Other common polymers found include PE, PP and PVC; these polymers, along with PET and PEST, represent about 63% of global plastic production, making their presence in the marine environment expected (GESAMP, 2019), therefore the presence of these are expected in marine environments. Contrary to this, all size distributions were skewed towards the smaller size classes (i.e., < 1 mm), irrespective of matrix, a similar finding in other areas of the GBRWHA (Chapter 6) and globally (Auta et al., 2017; Ding et al., 2019a). The present study used a lower size limit of 26 µm, and hence smaller MPs (i.e., <26 µm) were not considered. Given the size distribution observed here, processing techniques targeting smaller MPs should be explored in marine matrices. The similarities of shape, colour, polymer, and size across all matrices confirm both environmental interaction and trophic transfer could be significant routes of exposure of synthetic MPs. The differences in proportions of MP colours and polymers across the three trophic levels indicate potential preferential selectivity of MPs based on colour (Rochman et al., 2019; Xiong et al., 2019) or polymer (Sheng et al., 2021) by marine organisms. Additionally, the varying individual retention rates of MPs may contribute to these differences as well as the lack of accumulation and magnification past this point. Regardless, the ecological efficiency of coral reefs, based on the transfer of energy between trophic levels, is under threat by the presence of MPs that have the potential to enter the food web at the lowest level and interfere in these processes.

5.6 Conclusions

This study serves as the first comprehensive assessment of bioconcentration, bioaccumulation and biomagnification of MPs in a simplified coral reef food web. As an emerging contaminant of concern, understanding the ecological fate of MPs is increasingly important. To accurately do this, assessments of MPs in organisms and their surrounding environment in comparable units is crucial. Further, determining bioconcentration, bioaccumulation and biomagnification factors for coral reef organisms can assist in improving understanding of potential effects associated with MPs and elucidate potential MP exposure pathways. Overall, contamination within copepods, benthic crustaceans and moon wrasse fish is similar to that of their surrounding environment (i.e., sediment and water column) in the central GBRWHA. However, species-specific physiology (e.g., gut length, digesting mechanisms)

and physicochemical properties of the MPs (e.g., size, polymer, colour, shape) have an influence on the intake and potential retention of MPs. Evidence shows that when compared to their environment, MPs are concentrating in all organisms investigated. However, a comparison of MP contamination found within their prey items shows bioaccumulation and biomagnification are not occurring in the higher trophic levels. As such, environmental exposure is the primary source of MP contamination for copepods, benthic crustaceans, and moon wrasse (*T. lunare*) on the GBR. Yet, given the heterogeneity of MPs in the marine environment, it is impossible to ignore trophic transfer as a prominent pathway of exposure from lower to higher trophic levels.

Chapter 6: Temporal patterns of plastic contamination in surface waters at the SS Yongala shipwreck, Great Barrier Reef, Australia

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6.1 Abstract

Plastic pollution is ubiquitous within the marine environment, including surface waters, water column and benthic sediments. Marine plastic contamination is expected to increase if future projections of increased plastic production eventuate. Conversely, national, and international efforts are aiming to reduce marine plastic contamination. In this context, scientists, managers, and the general public are increasingly interested in understanding the status and temporal trends of plastic contamination in the marine environment. Presented here is the first temporal assessment of plastic contamination in surface waters of the Great Barrier Reef (GBR), Australia. Specifically, duplicate surface seawater samples (n = 66) were collected at the SS Yongala shipwreck (Central GBR) monthly from September 2016 to September 2019 and analysed for plastic presence and abundance. The processing workflow involved density separation, followed by filtration, visual identification and sizing of putative plastics using stereomicroscopy, and chemical characterisation using Fourier transform infrared spectroscopy. A total of 533 plastic items were identified across all tows, consisting of macro-, meso- and microplastic fragments and fibres, with polypropylene and polyethylene being the most common polymers. Plastic contamination was detected in every replicate tow, bar one. Plastic concentrations fluctuated and spiked every three months, although contamination did not significantly alter across the three-year period. Wind speed, salinity, and river discharge volume, but not surface current speed nor sea surface temperature, had a significant influence on the levels of plastic contamination. This study reveals, for the first time, the chronic presence of plastic debris in the surface waters of the GBR highlighting the need for long-term and on-going monitoring of the marine environment for plastic contamination.

6.2 Introduction

Anthropogenic impacts are circuitously linked to ecosystem function, with a multitude of threats (e.g., climate change, ocean acidification, overfishing, and pollution) impacting the health of oceans (Crain et al., 2009; Gelcich et al., 2014). Marine pollution stems from a range of land and sea-based sources (Kroon et al., 2020) and includes a variety of chemical contaminants (e.g., nutrients, pesticides, oils) as well as marine debris (e.g., plastics) (Eriksen et al., 2014; Thushari and Senevirathna, 2020). To assess the risks and potential effects of marine pollution, it is important to establish the baseline status of pollutants and examine temporal trends of concentrations through robust monitoring programs (Lindenmayer and Likens, 2010). Given incremental changes and variability in marine pollution, long-term data sets are required to detect trends in pollutants of interest and improve understanding of associated effects on the health of a marine ecosystem (Lavers et al., 2021; Lynch et al., 2014; Thompson et al., 2011).

Globally, plastic pollution is pervasive throughout the marine environment (Everaert et al., 2020). To date, plastics have been found in every marine matrix investigated, including seawater, marine sediment, ice cores, and a multitude of organisms at every trophic level (Chapter 2). Of particular concern are microplastics (MPs, < 5 mm; (Arthur et al., 2009)), given their small size and associated ease of ingestion by a large range of marine organisms (Ceccarelli, 2009; Kühn et al., 2015; Lusher, 2015; Wright et al., 2013b). Based on current plastic usage, predictions estimate that by 2030 there will be a yearly input of between 20 and 53 million metric tonnes of plastic into aquatic ecosystems (Borrelle et al., 2020). The associated risks will likely increase by roughly 50% in some marine environments, such as the Mediterranean and Yellow Seas (Everaert et al., 2020). Mitigation strategies to reduce marine plastic pollution are being implemented at regional, national, and international levels; however, growth in plastic waste is still expected to exceed these management efforts (Borrelle et al., 2020). To inform such mitigation strategies, scientists, managers and the general public are increasingly interested in understanding the status and trends of plastic contamination in the marine environment (Gacutan et al., 2022; Ryan et al., 2020a).

Plastic polymers can persist in the marine environment, with items potentially transported large distances and often accumulating in convergence zones, including ocean gyres, or on the seabed in high concentrations (Eriksen et al., 2013b; Law et al., 2010; Maximenko et al., 2019). The distribution of plastic contamination has been shown to fluctuate both spatially and temporally on a global scale (Barrows et al., 2018a; Law et al., 2010) and is driven by multiple mechanisms, including wind speed and direction, surface currents, and river discharge (Brunner et al., 2015; Kukulka et al., 2012). These mechanisms, either in isolation or combined, generate turbulence, and can promote vertical mixing of plastic debris within the sea surface layer between 10 and 200 metres depth (Kukulka et al., 2012;

Reisser et al., 2013; Veerasingam et al., 2016b). Combining monitoring of marine plastic pollution with that of physicochemical oceanographic parameters thus provides useful information on what drives status and trends in plastic pollution, as well as on their potential sources.

The present study reports baseline status and for the first-time temporal trends of plastic contamination in the Great Barrier Reef World Heritage Area (GBRWHA), Australia, over a three-year period (i.e., 2016 to 2019). Marine plastics, including MPs, were recently identified as a contaminant of emerging concern for the GBR marine ecosystems (GBRMPA, 2019), and associated targeted monitoring on status and trends recommended (Kroon et al., 2020). For this study, sampling was conducted at the SS Yongala shipwreck, located in the central GBRWHA. The SS Yongala is one of several National Reference Stations (NRS) managed by the Integrated Marine Observing System (IMOS) that provide fundamental baseline information on physical and biogeochemical oceanic properties in Australian coastal seas (Lynch et al., 2014). Specifically, this study (i) identifies and quantifies macro- (i.e., > 2 cm), meso- (i.e., 5 mm – 2 cm) and microplastics (i.e., 1 µm – 5 mm) present in surface waters, (ii) determines any temporal trends in plastic pollution, and (iii) examines the influence of underlying physicochemical forces (e.g., wind speed, current speed, sea surface temperature, salinity, and river discharge volume) on temporal trends of plastic pollution. Based on the abundance and temporal variability of plastic contamination at the SS Yongala NRS, as well as the influence of physicochemical forces, recommendations are made for a marine plastic monitoring program in the GBR and Australian coastal waters more broadly.

6.3 Methods

6.3.1 Study location

Surveys were conducted at the SS Yongala NRS, established by IMOS in 2007. The IMOS is a coordinated system of oceanographic equipment established to collect *in situ* oceanographic data in Australia's open ocean and coastal waters. The observing system currently includes seven NRS sites as part of the Australian National Mooring Network (ANMN) (Lynch et al., 2014). This network was established to provide *in situ* observations of high-quality physical, chemical, and biological data, aimed at capturing large-scale, long-term variability in Australian coastal waters and understanding coastal trends and their impacts on ecosystems (Lara-Lopez et al., 2016; Lynch et al., 2014). The SS Yongala NRS is moored 10.8 nautical miles off the east coast of Cape Bowling Green, Queensland, Australia (Fig. 1), and is located at the mid-continental shelf, between the outer reef and the coastal region of the central GBR lagoon (Lynch et al., 2014; Thompson et al., 2011). The NRS is adjacent to the culturally significant SS Yongala Shipwreck (27 – 30 m depth) and rests within a no-take marine reserve. Being

one of the largest, intact shipwrecks in the world, it serves as a major tourist attraction with more than 10,000 divers visiting each year and is protected under the Australian Historic Shipwrecks Act of 1976 (Stieglitz, 2012). The SS Yongala NRS is an area of high flow, with the surrounding seascape being primarily flat seabed (apart from the shipwreck). Hydrodynamically, the station is influenced by the south-eastward lagoonal branch of the East Australian Current and the opposing south-easterly trade wind forced current (Brinkman et al., 2002; Burrage et al., 1991). The station is also influenced by the Burdekin River, the largest river in Queensland and Australia's 4th largest river by volume. The climate of the region is considered tropical and characterised by distinct dry (May to October) and wet (November to April) seasons.

6.3.2 Sample collection

Seawater surface samples were collected by neuston tows adjacent to the SS Yongala NRS following Kroon et al. (2018a) and in accordance with the GBR Marine Park Authority permit G12/35236.1. Monthly collections (n = 2 replicates) were conducted for three years, from September 2016 to September 2019, from the Research Vessel (RV) Cape Ferguson or RV Apollo. Briefly, horizontal tows sampling at the air-sea interface were conducted using a plankton net (polyester (PEST), length 254 cm, 355 µm aperture with an open area of 50% and 150 mm thread thickness) on a neuston frame (stainless-steel, 74.5 cm diameter, 30.0 cm height). The net was positioned off the starboard side of the vessel and each tow was conducted at < 4 knots for ten minutes with the start and finish location of each tow recorded (Global Positioning System, GPS, Garmin GPSMap78; Figure 6.1). At the conclusion of each tow, the net was rinsed from the outside using the onboard seawater pump and hose, and all collected material concentrated into the cod end (transparent 750 ml polypropylene (PP) jar). The cod end was then carefully removed, sealed with a transparent PP lid, and transported to the laboratory on ice. Between tow replicates, the net was rinsed using seawater pumped from the ocean via the onboard hose system, and a new, pre-cleaned cod end attached. In the laboratory, samples were volume reduced through a 40 µm sieve (polyamide (PA) netting, PP frame), concentrated into 50 ml vials (transparent PP cup, yellow high-density polyethylene (HDPE) screw cap) and preserved in 70% ethanol (EtOH). When biological loads were high, cod end samples were split evenly over multiple preservation vials. Samples were sealed with Parafilm M® and stored in the dark at 4ºC until further processing.

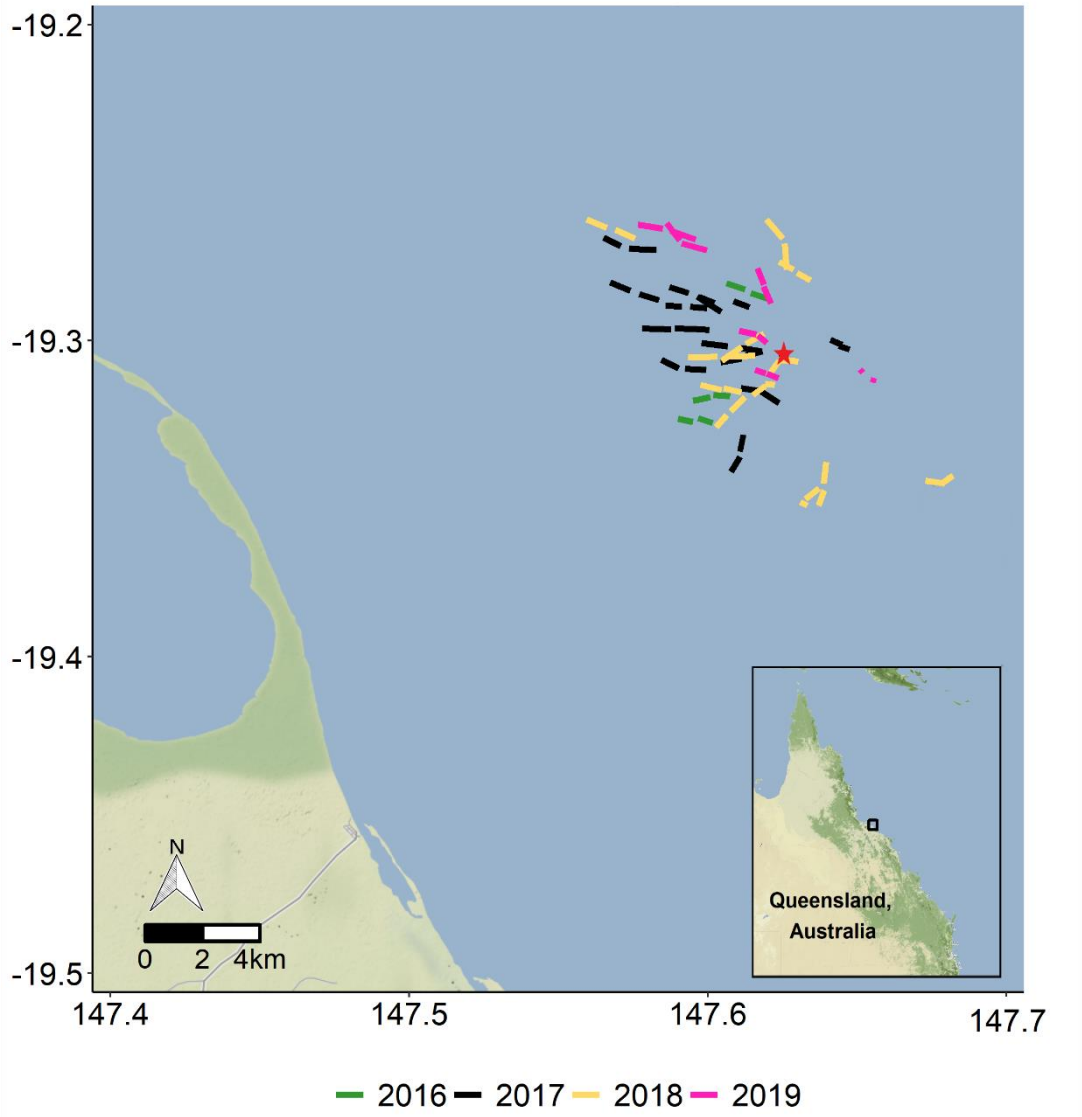

Figure 6.1 Paired trajectories of 66 individual seawater surface tows conducted monthly at the SS Yongala National Reference Station (NRS) located in the Great Barrier Reef World Heritage Area, Australia, from September 2016 to September 2019. The red star represents the NRS location. Inset is the location in relation to Queensland, Australia.

6.3.3 Physicochemical parameters

The potential influence of five physicochemical parameters, namely wind speed, current speed, sea surface temperature (SST), salinity, and river discharge volume, on the status and trends of marine plastic pollution was examined. These five parameters were chosen based on previous reports regarding their influence on plastic contamination (Brunner et al., 2015; Hitchcock, 2020; Kukulka et al., 2012; Welden and Lusher, 2017). Publicly available data on wind speed, current speed, SST and salinity data were obtained from the Australian Ocean Data Network (AODN; portal: imos.aodn.org.au/webportal) (Lynch et al., 2014; Thompson et al., 2011). High temporal resolution data on these parameters were obtained from moored sub-surface (~1 m) sensors, surface meteorology and

real-time sensor data (collected where logistically feasible). Specifically, for the period September 2016 to 2019, data on wind speed (knots; weather stations WXT520, Viasala, Helsinki, Finland), current speed (knots; bottom mounted, upward-looking 600 kHz acoustic doppler current profilers (ADCPs)), salinity (parts per thousand (ppt)) and SST (°C; both measured using a SBE 16+, Seabird, Seattle, WA, USA Water Quality Monitor) were extracted. Additionally, the discharge volume of the Burdekin River, the main river influencing the SS Yongala location, was obtained for the Clare Station from the Queensland Government Water Monitoring Information Portal (WMIP; https://water-monitoring.information.qld.gov.au/).

To assess potential influence on temporal trends of plastic pollution, wind speed, salinity and SST data were averaged for the 6 h prior to the sample tow, accounting for the varying lag times between the physical process event and the response in the plastic abundance and distribution. This averaged data represents the almost instantaneous effect of these physicochemical factors on the vertical mixing of the surface mixed layer (Klein and Coste, 1984; Kukulka et al., 2012). Current speed was averaged from 2 weeks prior to the sample tow collection to appropriately remove the tidal current fluctuations and reveal any major underlying current influences. Similarly, river discharge volume was averaged for the 2 weeks prior to account for a delay in transport time of plastic output to the sample location (Critchell et al., 2015).

6.3.4 Laboratory processing

Samples were processed using a recommended density flotation method adapted from Miller et al. (2021). Briefly, preserved samples were transferred from their 50 ml vials into clean 250 ml glass beakers (Beaker #1) and the vial walls and lid rinsed with hypersaline sodium chloride solution (342 g L^{-1} NaCl, prepared with 0.22 µm filtered Milli-Q water (MQ-H_2O) in a squeeze bottle (Polytetrafluoroethylene, PTFE or Polypropylene, PP). Hypersaline solution was added to Beaker #1 to a final volume of 200 ml. Samples were covered with Parafilm M® and swirled on mechanical shaker for 5 min (Orbital; 125 rpm, Reciprocal mode on and Vibro mode off), and left to settle overnight (approx. 17 h). Following settlement, the surface supernatant (~50 ml) was decanted into a second pre-cleaned beaker (Beaker #2) and the sides of Beaker #1 rinsed carefully with NaCl solution to ensure any material adhering to the glassware was transferred. The residual settled material was processed twice more as per above, with a reduction in settlement time to 1 h each (Quinn et al., 2017). All three decanted surface supernatants were combined, made up to 200 mL with extra NaCl solution and left to settle for 1 h, after which the total volume was filtered through a stainless-steel filtration apparatus (Schlawinsky et al., 2022) onto pre-cleaned large (547 µm) and small (26 µm) aperture stainless-steel filters. Beaker #2 and the filtration apparatus was rinsed thoroughly with MQ-H_2O and 70% EtOH to ensure transfer

of all material onto the filters. Filters were stored under aluminium foil cover and left to dry for a minimum of 2 h at room temperature (22 ± 1ºC) prior to further analysis.

6.3.5 Visual identification and characterisation of putative plastics

To identify putative plastics, stainless-steel filters were examined visually by microscopy (Leica MZ16A, 0.73x – 12.0x magnification; Leica DFC 500 camera attachment). Putative plastics were identified using the criteria established by Hidalgo-Ruz et al. (2012), photographed and physically characterised. All putative plastics were sorted into three main categories of shape (fragments, fibres, and beads), then further characterised by assigning a colour (Santana, 2022). The size of each item was measured using the image analysis software Image J (FIJI, version 1.53e). Fragments and beads were measured to obtain the longest calliper length (mm), shortest calliper length (considered width; mm), and surface area (mm^2). Fibres were measured to obtain the length and width, with 3 width measurements randomly taken along the length of the fibre to account for twisting and bending (reported as an average ± standard deviation (S.D.)). Putative plastics were categorised into macro- (i.e., > 2 cm), meso- (i.e., 5 m – 2 cm) and microplastics (i.e., 1 µm – 5 mm), based on longest dimension (i.e., length) measurement (Hartmann et al., 2019).

6.3.6 Chemical characterisation of putative plastics

Putative plastics were chemically characterised using Attenuated Total Reflectance Fourier transform infrared (ATR-FTIR) spectroscopy and their polymer composition confirmed following the workflow established by Kroon et al. (2018a). Putative plastics identified on the larger 547 µm filters were handpicked, manually transferred to the ATR-FTIR and analysed in ATR mode (PerkinElmer Spectrum 100; 1 mm ATR window, pressure gauge = 150, atmospheric (CO_2/H_2O) suppression and atmospheric vapor compensation, and background scans acquired hourly). Putative plastics identified on the smaller 26 µm filters were analysed directly using a PerkinElmer Spotlight 200 microimaging FTIR (µATR-FTIR, background scans acquired prior to each item). All spectra were collected between 4,000 and 650 cm^{-1} with 10 scans at 4 cm^{-1} resolution. In some instances, larger putative plastics captured on the smaller 26 µm filters (i.e., surface area > 200 μm^2) were analysed using ATR-FTIR. Due to the heterogeneity of plastic polymer types and the restricted reliability of shape and colour as diagnostics, all putative plastics were subjected to FTIR. For final chemical assignment, FTIR spectra of samples were searched against commercially available FTIR spectral libraries (Jung et al., 2018b) (NICODOM IR libraries (ATR): Polymers and Additives, Coatings, Fibres, Dyes and Pigments, Petrochemicals; NICODOM Ltd., Czech Republic), and further interrogated by comparison against all other sample spectra. To exclude potential extraneous plastic contamination, FTIR spectra of samples were also searched against a project specific in-house contaminant library (see below). Putative plastics with a >

90% match to a contaminant, as well as having the same shape and colour were excluded from further analysis. Based on final spectral assignment, anthropogenic items were then categorised into one of three main groups following definitions in Kroon et al. (2018b), namely (i) synthetic, (ii) semi-synthetic, and (iii) naturally derived.

6.3.7 Contamination prevention

The scientific literature on collection, processing, and analysis of plastics in marine samples, including prevention of contamination, was rapidly expanding during the three years of this study (Hermsen et al., 2018; Prata et al., 2019). Hence, contamination protocols were continuously updated to reflect recommendations in the most recent publications in combination with the authors' own learnings (Kroon et al., 2018a; Miller et al., 2021). Presented here are the procedures adopted throughout the study.

Aqueous-based solutions were prepared with either RO (filtered to 20 µm) or MQ-H_2O (filtered to 0.22 µm). Hypersaline NaCl solutions were prepared using MQ-H_2O exclusively. Prior to March 2018, 50 ml subsamples of prepared solutions of NaCl and 70% EtOH were transferred into a Bogorov chamber and visually checked for contamination by microscopy (Miller et al., 2021). From March 2018, prepared solutions were filtered over 0.46 µm Millipore PTFE filters. All laboratory equipment and work surfaces were cleaned throughout processing using RO or MQ-H_2O and filtered 70% EtOH. Specifically, all laboratory equipment was initially rinsed once with tap water, twice with RO, and finally with 70% EtOH. Equipment was covered by aluminium foil to minimise airborne contamination and left to air dry. Prior to sample processing, work surfaces were cleaned with 70% EtOH and either wiped down with paper towels and then covered with aluminium foil or wiped using a 'lint-free' purple cloth (Vileda Actifibre©; PEST:Viscose). Between samples the stainless-steel filtration apparatus was rinsed with MQ-H_2O and 70% EtOH (Schlawinsky et al., 2022). Prior to January 2019, blue nitrile gloves and white lab coats (50% PEST, 50% cotton) were worn throughout processing; from January 2018 white lab coats were replaced with green-dyed 100% cotton lab coats.

During all processing steps, laboratory blanks (i.e., four clean petri dishes filled with MQ-H_2O) were placed on work surfaces to determine the extent of airborne contamination. The petri dishes were covered with clean glass lids at times when samples were covered and opened when samples were exposed to air. Laboratory blanks were replaced before each new batch of samples was processed. These blanks were filtered directly onto 26 µm stainless-steel filters and analysed as per samples (i.e., stereomicroscopy and FTIR spectroscopy). Information on the contaminants was compiled into a project specific in-house contaminant library database, along with spectra of all potential plastic materials from equipment used in field collection and laboratory processing (A5.1 Table). This in-house

contaminant library was subsequently used to screen for and identify potential contaminants in the seawater surface samples.

6.3.8 Data analysis

Confirmation of chemical assignment of putative plastics was completed using an R script (R Studio, Version 1.4.1106), designed specifically to apply all criteria mentioned above to each sample spectrum. This was accomplished by collating sample specific data (e.g., ID, GPS, sizes, colour) and original spectra data for each putative plastic item. The script allowed for semi-automation of data analysis, substantially minimising manual data entry, and improving data quality. Primarily, the tidyverse and R.utils packages for data manipulation, and the tcltk package to make it intuitive, were used during development. In short, steps include assignment of polymers based upon the 70% threshold (Kroon et al., 2018a), as well as exclusion of items based on either a poor-quality spectrum (i.e., match hits < 60%) or a spectral match > 90% to a contaminant having the same shape and colour. Due to the continuous collection and processing of surface tow samples throughout the three-year period, putative plastics in samples were only ever compared to contamination found in corresponding processing blanks conducted at the same time, as well as items in the in-house contaminant library (i.e., sample or processing equipment, see A5.1 Table). Spectra with match hits between 60% and 70% were automatically assigned as 'to check' to facilitate further interrogation of spectra. Manual confirmation of spectra occurred for all spectra requiring further investigation.

Given that the focus of this study is on marine plastic pollution, only those items that were identified as synthetic and semi-synthetic following FTIR (Kroon et al., 2018a) and subsequent assignment were included in further analysis. Plastic data are presented as an average between the tow replicates (i.e., average ± standard deviation, S.D.), both as count abundances (i.e., plastics per tow), and as concentration (i.e., plastics per m^3). The average count of plastic items per replicate surface seawater tow as a function of volume of water sampled was calculated as per Kroon et al. (2018a). To determine whether the replicate tow (paired) or season (i.e., wet vs. dry; non-paired) had any impact on plastic concentrations, Wilcoxon signed-rank tests with continuity correction were applied. To analyse group variances between years, Kruskal-Wallis rank sum test was conducted due to the lack of homogeneity and normality in the data. A general linear model (GLM) incorporating four physicochemical parameters (i.e., wind speed, current speed, salinity, and SST) was run with a Quasi-Poisson distribution to examine their influence on plastic levels. To account for varying replicate tow volumes, an offset using the natural log volume (m^3) was applied. Sampling month was considered a nested variable, and replicate tow a random effect. Additionally, plastic count was compared against a 2-week average of river discharge water volume from the closest source location (i.e., Clare Station,

Burdekin River) using a second GLM, including the same volume offset, nested variables, and random effect consideration. All statistical analyses were conducted in R (version 4.0.4).

6.4 Results

6.4.1 General collection information

Replicate samples were collected monthly for three years from September 2016 to September 2019, except for December 2016, January 2019, April 2019 and June 2019 due to inclement weather (Figure 6.1). In total, 66 replicate tows (representing 33 sampling months) were conducted. The average tow duration was 10.01 min ± 0.01 S.D., with all tows occurring in the hours around mid-day (11:00 to 13:30 AEST), except for the two replicate tows collected on the RV Cape Ferguson in March 2017, which were collected at 16:12 and 16:25 AEST. Seawater surface tows covered an average distance of 678.18 m ± 210.27 S.D., which equates to an average of 75.79 m^3 ± 23.50 S.D. of seawater.

6.4.2 Contamination prevention

Of the 37 stainless-steel filters (26 µm) used in the processing of laboratory blanks, 11 were free of plastics while the remaining 26 contained a total of 113 items (4 particles and 109 fibres, A5.1 Table). All, apart from one item were chemically characterised using FTIR. None of the airborne contaminants captured in the laboratory blanks were found to match the polymer type, shape, and colour of any of the plastics found in the environmental samples. Additionally, when environmental samples were compared to the in-house contaminant library, no items were found to match in polymer type, shape, or colour. Similarly, no contaminants were observed in aliquots of the NaCl or 70% EtOH solutions inspected in the Bogorov chamber prior to March 2018. Therefore, contamination resulting from exogenous sources was determined to be negligible and no contamination corrections were applied on plastics found in the environmental samples.

6.4.3 Putative plastic pollution

Putative plastics (n=845) were observed in all 66 seawater surface samples. Of these, 53 items were either lost during processing (n=12) or their poor orientation on the stainless-steel filter (n=41) prohibited comprehensive spectral analysis (Table 6.1; Schlawinsky et al. 2022). The other 792 were chemically characterised using FTIR and categorised into three main groups based on final spectral assignment following definitions in Kroon et al. (2018b). Most of the 792 items (n=448) were synthetic plastic polymers, with the remainder comprising of naturally derived items (n=259; e.g., inorganic, biological or cotton) and semi-synthetic items (n=85). Semi-synthetics included composite blends of

synthetic polymers and the chemically modified cellulosic material rayon (rayon:polyester, rayon:nylon and rayon:nylon:polyester), as well as rayon itself (Hartmann et al., 2019; Kroon et al., 2018a). Only items characterised as synthetic and semi-synthetic (as defined here) were considered in further analysis.

Table 6.1 Categorisation of putative plastic items isolated from 66 individual seawater surface tows collected monthly at the SS Yongala National Reference Station located in the Great Barrier Reef World Heritage Area, Australia, from September 2016 to September 2019. Naturally derived includes items chemically characterised as biological, inorganic and cotton; semi-synthetics include composite blends (e.g., rayon:polyester, cotton:nylon), as well as 100% rayon.

Category	Number of items	Fragments	Fibres	Beads	Progressed through the analysis workflow
Not analysed	53	12	39	2	No
Naturally derived	259	95	161	3	No
Semi-synthetic	85	6	79	-	Yes
Synthetic	448	338	110	-	Yes
Total	**845**	**451**	**389**	**5**	

6.4.4 Physical and chemical characteristics of confirmed plastics

A total of 533 plastic (i.e., synthetic, and semi-synthetic) items were detected across all tows, with plastic items in every tow sampled from September 2016 to September 2019 (Figure 6.2) except for one (tow #2, August 2018). The mean level of plastic contamination per two replicate tows was 8.08 ± 6.88 S.D. (0.13 ± 0.17 S.D. plastics m-3 or 0.005 ± 0.004 plastics km^2), ranging from 1 to 37 plastics tow-1 (0.01 to 0.95 plastics m^{-3}). Plastic concentrations did not differ significantly between replicate tow samples (Wilcoxon signed-rank test; V = 254.5, p-value = 0.866; A5.1a Figure). Across all tow samples synthetic polymers were more prevalent than semi-synthetic ones. Polyethylene (PE, n=207) and polypropylene (PP, n=123) were the most common polymers found (Figure 6.3a), the majority being fragments (n=190 and 106, respectively). Fibres (n=189) were mostly 100% rayon (n=38; representing 20.1% of all synthetic and semi-synthetic fibres combined, and 48.1% of semi-synthetic fibres) and polyester (PEST, n=29; 15.3%, respectively), or composite blends of the two (n=74, 39.1 %) (Figure 6.3a).

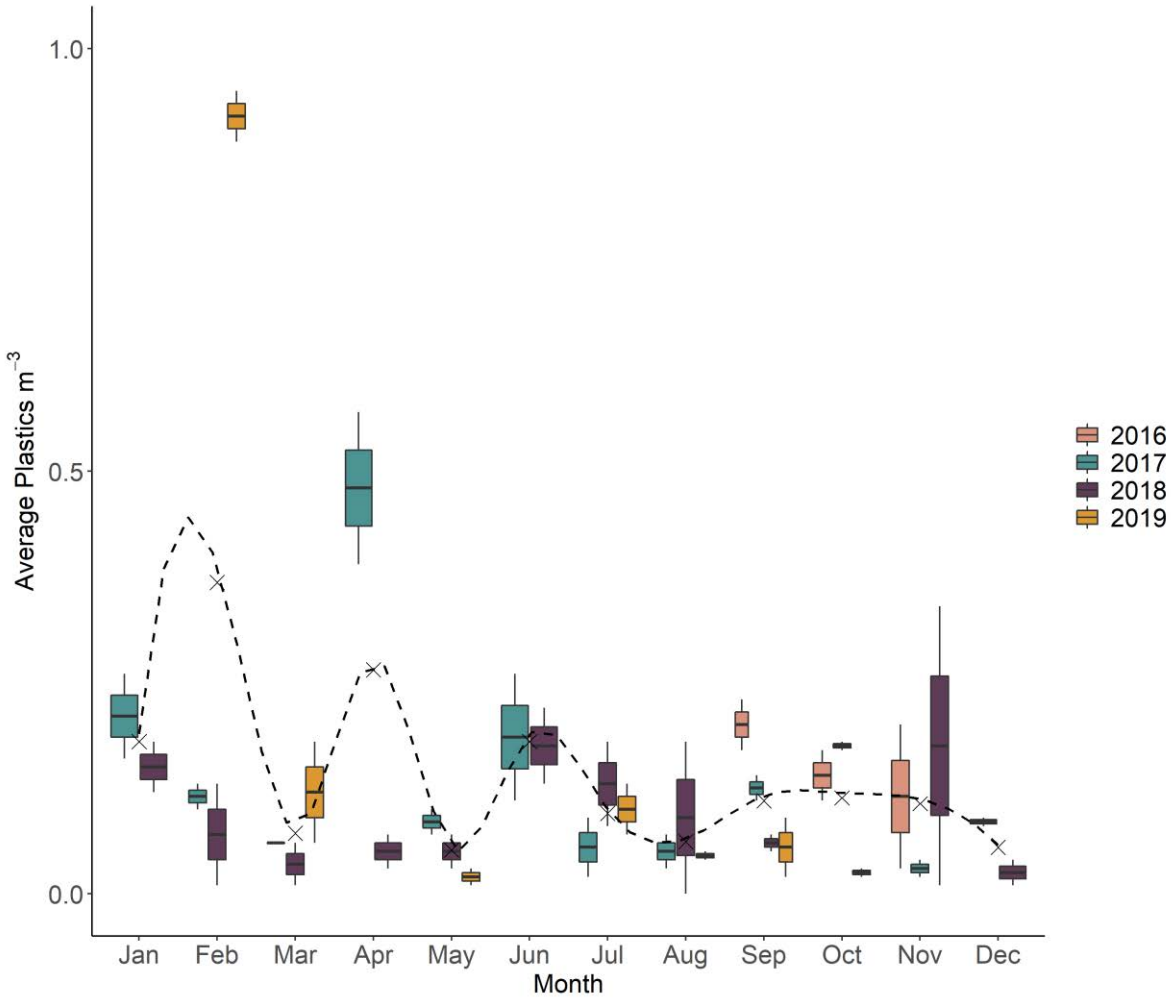

Figure 6.2 Mean plastic concentration (m^{-3}) averaged across two replicate seawater surface tows, conducted monthly at the SS Yongala National Reference Station from September 2016 to September 2019. X represents the mean plastic concentration for each month for all years sampled. The dashed line is representative of a fitted spline presenting a smoothed trend of the mean plastic concentrations. Sampling did not occur in December 2016, and January, April, and June 2019 due to inclement weather.

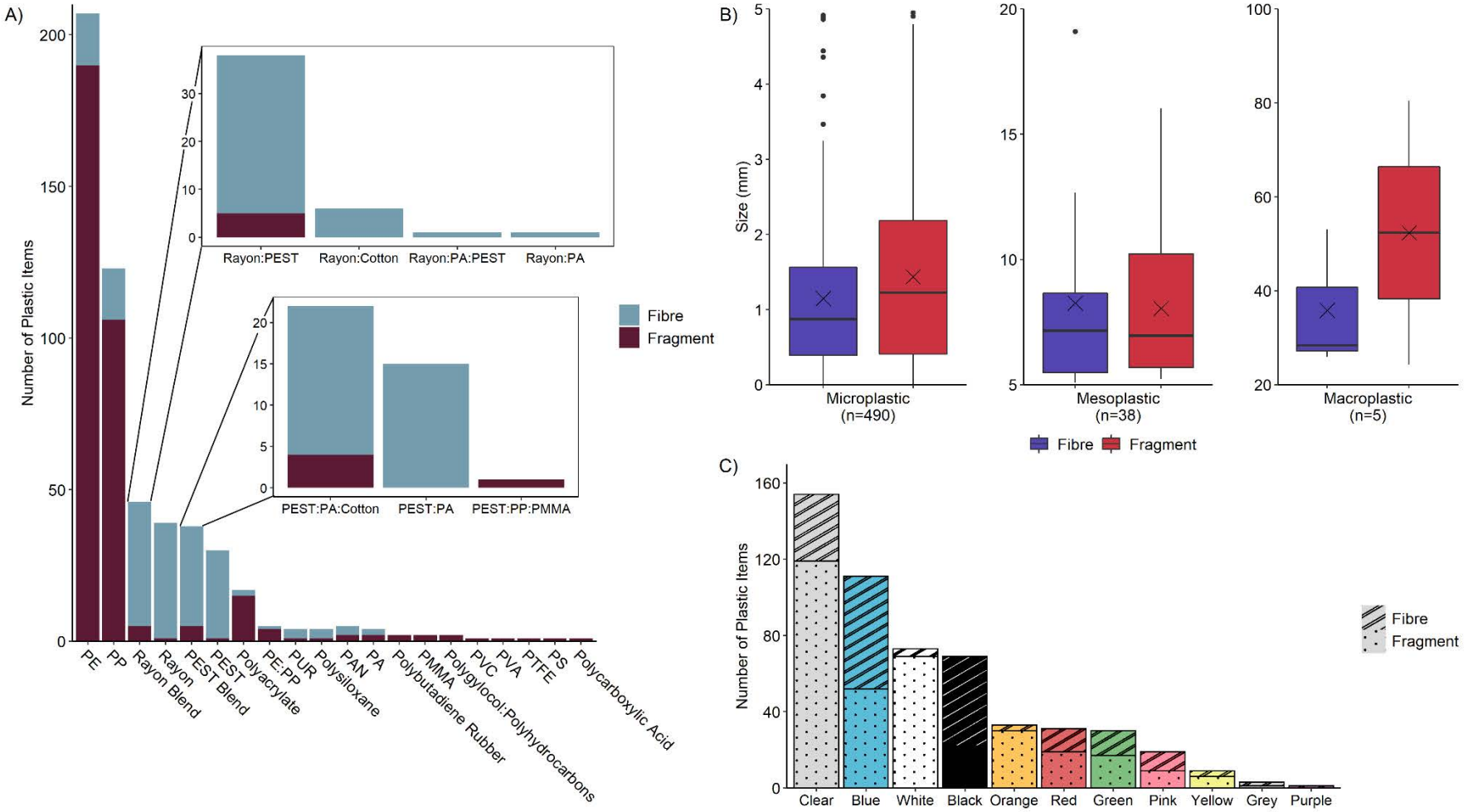

Figure 6.3 Physical and chemical characterisation of synthetic and semi–synthetic plastics isolated from 66 individual seawater surface tows, conducted at the SS Yongala National Reference Station from September 2016 to September 2019. Plastic characteristics are presented by (a) polymer type, (b) size, and (c) colour. Size is presented as the longest length measurement only, with X representing the mean length value. PE: polyethylene; PP: polypropylene; PEST: polyester; PUR: polyurethane; PAN: polyacrylonitrile; PA: polyamide; PMMA: poly(methyl methacrylate); PVC: polyvinyl chloride; PVA: polyvinyl alcohol; PTFE: polytetrafluoroethylene; PS: polystyrene.

Most plastic items were fragments (n=344, 64.5%), all appearing to be secondary plastics (i.e., from the breakdown of larger items); no plastic beads were observed (Figure 6.3a-c; A5.1 Figure). Fragments were found in all tow samples that contained plastic items. Plastic fibres (n=189, 35.5%) were detected in all but the four tow samples collected in April and October 2018.

The mean length and surface area for fragments was 3.55 mm ± 25.82 S.D., and 1.33 mm^2 ± 17.42 S.D., respectively. Length ranged from 1.21 µm to 8.05 cm, while surface area ranged from 512 μm^2 to 32.2 mm^2 (Figure 6.3b). For fibres, the mean lengths and widths were 2.01 mm ± 4.16 S.D. (ranging from 6.93 µm to 12.61 mm) and 0.91 mm ± 2.99 S.D. (ranging from 1.68 µm to 1.71 mm), respectively. Based on the longest length, most fragments and fibres were MPs (n=490, 92%), followed by mesoplastics (n=38, 7%) and macroplastics (n=5, 1%) (Figure 6.3b).

Plastic items were mostly transparent (n=154, 28.9%), with 119 transparent fragments and 35 transparent fibres. Other abundant colours observed included blue (n=111, 20.8%), white (n=71, 13.3%), and black (n=67,12.6%) (Figure 6.3c).

6.4.5 Temporal trends in plastic pollution

Plastic contamination in seawater surface samples varied with an evident increase in mean plastic concentrations every two to three months (Figure 6.2). Highest plastic concentrations (0.57 plastics m^{-3} and 0.92 plastics m^{-3}) were measured during April 2017 and February 2019, respectively. Despite these high concentrations occurring in the wet season, overall, the mean plastic contamination was not significantly affected by season (Wilcoxon signed-rank test, W = 505.5, p-value = 0.661; A5.2b FIgure). Furthermore, the mean plastic concentration did not significantly change over the three-year period sampled (Kruskal-Wallis rank sum test, $\chi 2$ = 5.69, p-value = 0.128; A5.2c Figure).

6.4.6 Physicochemical forces influencing plastic pollution

Average wind speed and salinity at the Yongala NRS in the 6 h prior to the sample tows showed a significant effect on plastic concentration (GLM; p-value = 0.008 and 0.014, respectively; A5.2a Table). More specifically, plastic concentrations decreased with an increase in either wind speed or salinity (Figure 6.4). In contrast, neither average SST in the 6 h prior, nor the average current speed in the 2 weeks prior to the sample tows significantly affected mean plastic concentrations (A5.2a Table). Plastic contamination at the Yongala NRS increased significantly with the average volume of the Burdekin River discharge in the 2 weeks prior to the sample tows (GLM, p-value = 0.042; Figure 6.4e, A5.2b Table). When analysed without the outliers of the two major weather events (i.e., April 2017 and February 2019), the influence of salinity and river discharge volume on plastic concentration is reduced (p-value

= 0.151 and 0.457, respectively, A5.3a,b Tables). The influence of wind speed, however, is still close to being significant (p-value = 0.053, A5.3a Table), with plastic concentration increasing with wind speed.

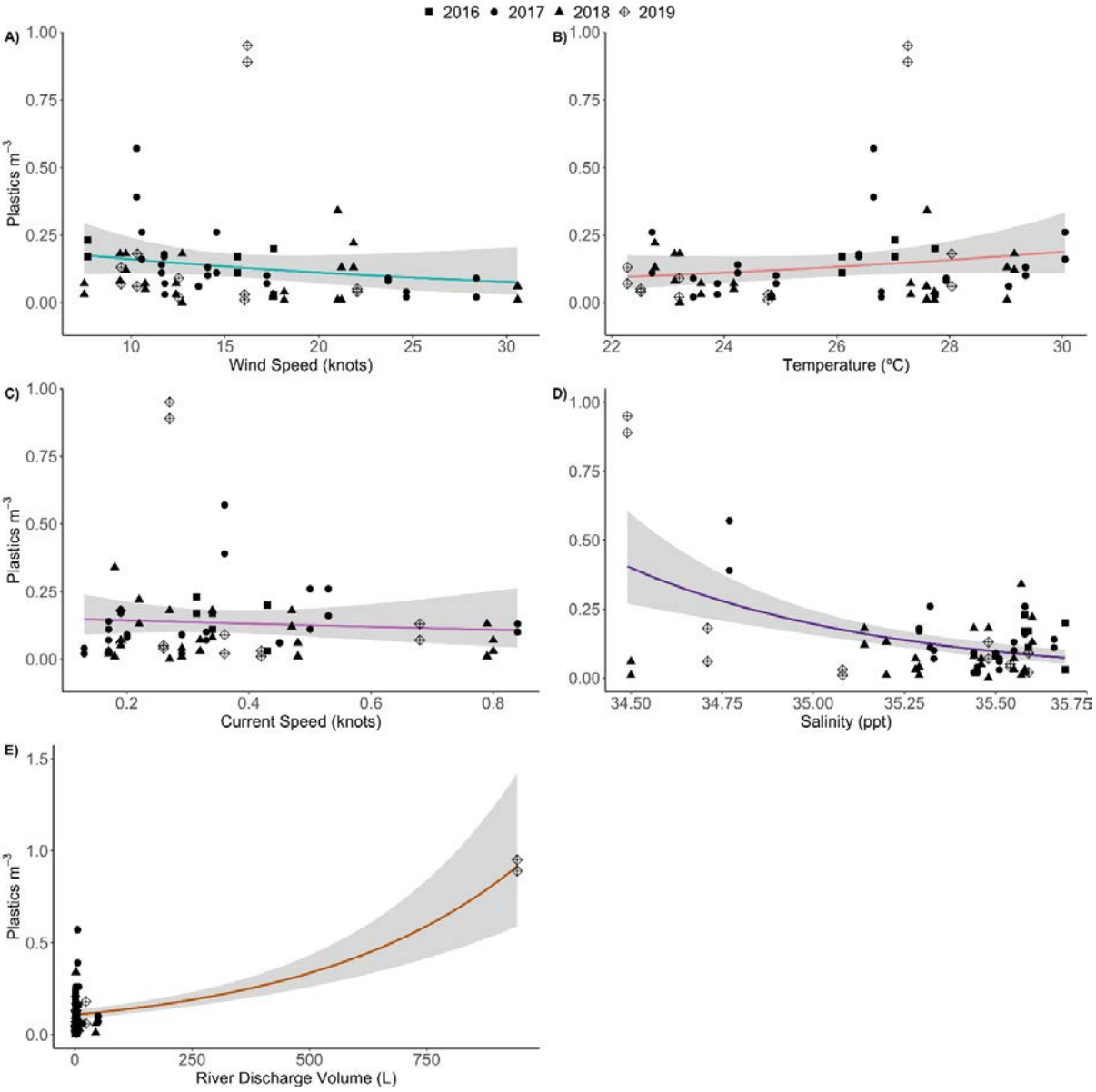

Figure 6.4 Mean plastic concentrations (m^{-3}) in relation to four individual physicochemical parameters measured at the SS Yongala National Reference Station from September 2016 to September 2019: (a) wind speed (knots), (b) temperature (degrees Celsius, °C), (c) current speed (knots), (d) salinity (parts per thousand, ppt), and (e) river discharge volume (litres, L). Data was modelled using a general linear model regression line with a Quasi-Poisson distribution and a log link fitted to each trend.

6.5 Discussion

Increased reporting on plastic pollution in the marine environment and associated adverse impacts to marine life highlights the necessity and urgency to implement plastic monitoring programs

(Maximenko et al., 2019). This study is the first temporal assessment of plastic pollution in the surface waters of the GBRWHA, Australia. Specifically, it details the physical and chemical characteristics of the plastics found at the SS Yongala National Reference Station (NRS) between September 2016 and September 2019, reveals temporal trends in the levels of plastics observed, and identifies the potential physicochemical parameters that influence the observed variability in plastic concentrations.

Combining three years of data, plastic items were identified across all surface seawater tows conducted at the SS Yongala NRS except one (98%), which is comparable to reports of plastic pollution in surface tows conducted in the South Pacific Oceans (96%) (Eriksen et al., 2013b), and in other Australian waters (between 80% and 100% of surface tows sampled) (Jensen et al., 2019; Reisser et al., 2013). Estimates of mean plastic loading at the SS Yongala NRS (0.13 ± 0.17 S.D. plastics m^{-3}) are comparable to previous findings in surface waters (0.04 to 0.48 MPs m^{-3}) in both the central (Jensen et al., 2019) and northern (Santana, 2022) sections of the GBR Marine Park. While they are the same order of magnitude as plastic concentrations reported for the northwest coast of Australia (0.01 to 0.41 MPs m^{-3}; Kroon et al., 2018a), they are substantially lower than averages across other Australian waters (4,256.4 ± 757.8 S.E. plastics km^{-2}; Reisser et al., 2013). Additionally, plastic contamination at the SS Yongala NRS is substantially higher than those reported for sub-surface tow conducted at Orpheus Island, QLD (2 x 10^{-7} plastics m^{-3}; Hall et al., 2015); however, this may be due to the fact that sub-surface sampling tend to report significantly lower abundances (Kroon et al., 2018a). In comparison to global estimates calculated from surface grab samples (11,800 ± 24,000 S.D. plastics m^{-3}; (Barrows et al., 2018a)), levels of plastic contamination at SS Yongala NRS are substantially lower.

The physical and chemical characteristics of plastic items found in surface waters at the SS Yongala NRS share similar characteristics to plastic items previously reported contaminating marine waters around Australia (Reisser et al., 2013), and specifically in the GBR (Hall et al., 2015; Jensen et al., 2019). Plastic items found were all secondary plastics (i.e., characterised as fragments of larger pieces broken down over time (GESAMP, 2016)) and predominantly made of polyolefins (PE and PP). Further, this study supports previous reports that primary MPs (i.e., microbeads or pellets manufactured to be < 5 mm) are either absent or at least below detectable levels in GBR waters (Hall et al., 2015; Jensen et al., 2019) and thus pose less risks to this environment than secondary plastic items. Secondary MPs as a major contributor to anthropogenic debris in the environment (Ajith et al., 2020; Andrady, 2015; Barrows et al., 2018a; Hale et al., 2020), except for a few places like the Great Lakes, USA (Eriksen et al., 2013a). Similar to these results, most MPs previously detected in both GBR waters and coral reef fishes have been identified as microfibres of textile origin, (Jensen et al., 2019; Kroon et al., 2018b), likely derived from clothing and furnishings given their width of less than 50 µm (Sinclair, 2015). While 100% rayon fibres have been traditionally included in MP studies as semi-synthetic items (Cai et al.,

2019; Hartmann et al., 2019; McGoran et al., 2021), the classification of rayon as such may warrant future investigation due to the source material being cellulose (Stark, 2019).

Size ranges of plastic items found in this study skew towards the smaller size classes, with the majority of items (83.6%) < 3 mm in length, which agrees with findings for Australian-based and global estimates of MP sizes (Barrows et al., 2018a; Jensen et al., 2019; Kroon et al., 2018a; Reisser et al., 2013). Neuston towing is widely accepted as the standard method for MP sampling of the air-sea interface (GESAMP, 2019; Isobe et al., 2021). However, the relatively large mesh size of the neuston net (355 µm) will inherently result in an underestimation of smaller MPs, including fibres that are often < 50 µm in width (Covernton et al., 2019; Ryan et al., 2020b), hence the skew towards the smaller MPs in this study could be even more pronounced. The recovery of smaller-sized MP fibres from GBR waters is especially important given their preferential uptake by and longer retention in marine organisms (Santana et al., 2021), which has the potential to adversely impact health (Mishra et al., 2019; Rebelein et al., 2021). As the vast majority of current studies on MP concentrations in surface waters of aquatic environments employ the neuston tow sampling technique, these results are directly comparable with the literature and meet the globally accepted reporting requirements (Cowger et al., 2020; GESAMP, 2019). Despite this, with fibres representing 20.1% of MPs chemically analysed here, the design of sampling techniques specific for the collection of microfibres of the lower size range should be explored to improve world-wide data accuracy.

Transparent and white MP fragments constitute the majority of plastics in this study (64.5%), with transparent and blue PEST fibres also present in relatively high numbers (25.9%) compared to other colours and polymer types at the SS Yongala NRS. These data match a similar finding for surface waters at Lizard Island in the northern GBR (Santana, 2022), but differ from those in the central GBR (Jensen et al., 2019), highlighting the variety and variability in MP size, shape, colour and polymer types found throughout the GBRWHA. This variability is likely associated with adjacent local land use and differences in the oceanographic and hydrodynamic features at each site, and further supports the need to conduct scientifically robust monitoring of marine plastics at multiple locations of interest to understand baseline contamination status, potential sources and longer-term trends.

The potential influences of environmental physicochemical factors (i.e., wind speed, current speed, SST, salinity and river discharge volume) on plastic transport, accumulation and distribution have been previously modelled and discussed (Brunner et al., 2015; Critchell and Lambrechts, 2016; Hitchcock, 2020; Kukulka et al., 2012; Welden and Lusher, 2017). Modelled simulations (Brunner et al., 2015; Critchell and Lambrechts, 2016) have identified wind speed as a key driver of increased turbulence at the sea surface, which acts to submerge plastics and result in lower estimates in surface plastic concentrations. At the SS Yongala NRS, plastic concentrations in surface waters decreased with

increased average wind speed with plastic concentrations being higher at wind speeds < 15 knots compared to those at wind speeds > 25 knots. These findings also concur with an Australia-wide field investigation that observed lower wind speeds were linked to an increase in marine plastic concentrations (Reisser et al. (2015). As passive particles in the ocean, plastic items are subject to the physics of surface water mixing within the ocean surface boundary layer. Thus, lower wind speeds likely allow plastics to congregate at the sea surface, while increased wind facilitates both vertical and horizontal dispersion of these contaminants (Kukulka et al., 2012). While previous studies have documented the influence of ocean circulations (Neumann et al., 2014) and weather conditions (Moore et al., 2002; Welden and Lusher, 2017) on plastic aggregations, neither current speed nor SST influenced plastic concentrations at the SS Yongala NRS. This may be in part because the complex hydrodynamics associated with coral reef systems and the coastal adjacent seas (Lambrechts et al., 2008) limits the impact of current speed and SST at a local scale. Further research on the role of different physicochemical parameters in influencing plastic accumulation at different geographical scales will be important to elucidate potential sources of plastic pollution and inform mitigation strategies. It would also be of benefit to correct for the influences of physiochemical parameters, and specifically wind, on plastic levels (Kukulka et al., 2012), further improving estimates of plastic contamination levels.

The relative densities of plastics, ranging from 0.02 (i.e., expanded Polystyrene, ePS) to 2.2 g cm^{-3} (i.e., PTFE), also influence their transportation and distribution in the marine environment. For example, high salinity levels can promote higher accumulation of plastics at the ocean surface layer (GESAMP, 2019). In contrast, at the SS Yongala NRS plastic concentrations were found to increase significantly when salinity levels decreased (< 35 ppt), a relationship strongly influenced by two outliers (April 2017 and February 2019). An assessment of weather conditions at each of these times indicated a significant influx of freshwater to the site, explaining the drop in salinity. In March 2017, three weeks prior to the April 2017 sample collection Severe Tropical Cyclone Debbie passed over and made landfall just south of the SS Yongala NRS, with heavy rainfalls recorded (Bureau of Meteorology; www.bom.gov.au/cyclone/history/debbie17.shtml). In 2019, North Queensland experienced another extreme rainfall event causing the biggest flooding event in the last 100 years (Bureau of Meteorology; www.bom.gov.au/qld/flood/townsville.shtml) and a subsequent increased outflow of plastic debris from river-based sources (Bauer-Civiello et al., 2019) immediately preceding sampling. These two spikes in plastic loading occurred after both major weather events and corroborates the observation of higher concentrations of marine plastics in GBR surface waters between Shoalwater Bay and Townsville in February 2013, which again were preceded by major flooding events (Hardesty et al., 2014) associated with Ex-Tropical Cyclone Oswald. Plastic concentrations at the SS Yongala NRS reverted to baseline

levels after 1 to 2 months confirming the impact of major weather events on plastic concentrations (Hitchcock, 2020). Further, the removal of these two outliers from analysis highlights the consistent influence of wind speed (but not salinity) on plastic concentrations, and further elucidates the unique influence of extreme weather events on river discharge volume and plastic pollution in the GBRWHA. This highlights the importance of collecting long-term datasets on marine plastic contamination to capture and understand events that are major drivers of plastic pollution.

Recent studies have recommended further monitoring to determine temporal variation in marine plastic pollution (Maximenko et al., 2019; Ryan et al., 2020a), with this study being one of the first to report longer-term trends in marine surface waters. Specifically, this study showed temporal variations in plastic concentrations at SS Yongala NRS, in the Central GBR, with notable fluctuations within each year. However, plastic concentrations consistently returned to lower levels following large spikes in contamination, with no obvious increase or decrease during the three-year period from 2016 to 2019. This suggests that the area around the SS Yongala NRS is not a plastic accumulation zone on the surface but is continuously exposed to relatively low-level input of plastic contamination, suggesting chronic exposure to resident marine organisms. The plastic concentrations reported here act as a useful baseline for future monitoring to detect a projected increases in plastic production and waste, as well as examine the effectiveness of management strategies to reduce plastic waste. Temporal trends in plastic contamination reported in previous studies seem to vary based on location and matrix sampled. Law et al. (2010) found no significant differences in plastic contamination collected via surface tows over a 22-year timeframe throughout the North Atlantic Ocean. Additionally, Beer et al. (2018) saw no increase of MP contamination in seawater samples over a 30-year period (i.e., 1986 to 2015) from the Baltic Sea. These data suggests that levels of marine plastic contamination have stabilised (Cole et al., 2011). Contrary, Thompson et al. (2004) indicated significant changes in contamination within seawater samples between the 1960s and 1970s when compared to samples from the 1980s and 1990s collected in the North Sea and Northwest Atlantic. Similarly, predictive models of plastic pollution in accumulation zones and various sediment and biotic matrices, indicate plastic contamination levels will continue to increase over time (Claessens et al., 2011; Everaert et al., 2020; Matsuguma et al., 2017). Such discrepancies in temporal trends in turn emphasise the need for broader spatial scales and environmental matrices for plastic monitoring programs. In Australian waters, this could be established in association with other IMOS NRS locations, as this oceanographic observing system provides complementary physical, chemical and biological information across multiple Australian-wide sites for a comprehensive assessment of potential sources and impacts of plastic contamination on Australian marine ecosystems. Furthermore, the combination of physicochemical and plastic monitoring and

associated analyses as conducted here will expand the knowledge surrounding marine plastic pollution and elucidate possible factors influencing its prevalence.

6.6 Conclusions

Robust monitoring programs that establish baseline and trends of marine plastic can provide comprehensive assessment of ecological risk, inform appropriate mitigation measures, and assess effectiveness and promote adaptive management. This study provides baseline information of marine plastic contamination at the SS Yongala NRS and, for the first time, temporal trends of such contamination on the GBR, Australia. Plastic items were identified across all 66 surface seawater tows bar one, conducted monthly from September 2016 to September 2019. Estimates of plastic concentrations were similar to those previously reported for the GBRWHA and northwest Australia, but substantially lower than those for other Australian waters and global estimates of surface contamination. The 533 synthetic and semi-synthetic items identified comprised mostly of polyolefins (PE and PP), were all secondary plastics, with fragments more common than fibres. The inherent underestimation of MP fibre contamination through the use of neuston tows warrants further attention. Regardless, plastic concentrations were found to be significantly influenced by extreme weather events and associated increases in river discharges, resulting in outflow of plastic debris from river-based sources, and by wind speed outside of these events. Despite peaking immediately following extreme weather events, the overall trend of plastic contamination did not change over the three years of study, suggesting continued and chronic risks of plastic exposure to marine organisms. Finally, these results provide a foundation for longer-term plastic monitoring at the SS Yongala NRS, other IMOS NRS locations in Australian coastal waters, and coastal locations around the world, to determine future levels of contamination with projected increases in plastic waste, as well as assess the effectiveness of plastic waste management strategies.

Chapter 7: General Discussion

Microplastics (MPs) are ubiquitous throughout the marine environment and, as evidenced here, throughout the Great Barrier Reef World Heritage Area (GBRWHA). Since the mass production of plastic materials began in the 1950s, the global demand and usage of plastics has been on the rise (GESAMP, 2019; Ostle et al., 2019), with a predicted increase in annual emissions of up to 53 million metric tonnes by 2030 should mitigation and management strategies not change (Borrelle et al., 2020). Plastic, given its widespread distribution in the environment, is now considered a geological marker of the Anthropocene, an epoch of time defined by the influence of human activities dominating the state, dynamics, and future of the Earth's systems (Villarrubia-Gómez et al., 2018; Zalasiewicz et al., 2016). Strategies to mitigate oceanic plastic pollution are reliant on empirical knowledge of input, distribution and accumulation and is a key motivation for current MPs research.

The goal of this PhD thesis was to understand the ecological fate of MPs in a tropical marine ecosystem (i.e., the GBRWHA). Three overarching themes were explored to address key knowledge gaps identified within the literature: (a) bioaccumulation and biomagnification in marine organisms globally (Chapter 2) and locally in the GBRWHA (Chapter 5), (b) ingestion and retention of MPs (polyester, PEST, microfibres) (Chapter 3) and their trophic transfer (Chapter 4) in marine reef species, and (c) baseline (Chapter 5) and temporal monitoring (Chapter 6) of MP contamination levels in the GBRWHA to establish current and predict future risk.

7.1 Bioconcentration, bioaccumulation and biomagnification of MPs

The classical concepts of bioconcentration, bioaccumulation and biomagnification, which are primarily applied to dissolved chemicals (Alexander, 1999), are now being considered to assess the ecological fate and risk of plastics (Covernton et al., 2022; Rochman et al., 2019). In brief, bioconcentration refers to a higher concentration of a contaminant (e.g., MPs) within an organism compared to that of its environment (USEPA, 1997), bioaccumulation is considered the net intake of a contaminant, irrespective of its source (e.g., respiration, ingestion, trophic transfer) (USEPA, 2008), and biomagnification is defined as an increase in a contaminant from lower to higher trophic levels (Spacie et al., 1995). The recent adaptation of these concepts to assess the ecological risk of MPs (Akhbarizadeh et al., 2019; Covernton et al., 2022) has led many to infer incidence in marine environments (Farrell and Nelson, 2013; Nelms et al., 2018; Zhao et al., 2018). However, to accurately measure these endpoints

for MPs, the evaluation of the pathways and mechanisms that introduce and transfer MPs through food webs is needed and these were explored here (Chapter 2 and Chapter 5).

Following a systematic review of the global marine MPs literature, there is evidence of MPs bioaccumulating within each trophic level, as per the definitions detailed in Chapter 2. Yet, no evidence in the literature exists to support bioaccumulation or biomagnification of MPs or associated chemical additives from lower to higher trophic levels across a generalised marine food web. In fact, lower trophic levels (i.e., primary and secondary consumers [levels 2 – 2.9] of primary producers [level 1]), represented primarily by filter feeding organisms, exhibited by far the highest average MP accumulation across a global marine food web, a notion previously observed (Bour et al., 2018a; Mizraji et al., 2017). Global reports of MP ingestion do not appear to support a concentration or accumulation of MPs within species relative to their surrounding environment, yet reporting in non-comparable units (i.e., MP units standardised by body weight versus per individual) make these conclusions difficult to ascertain. Further, there is no evidence of biomagnification of MPs through trophic levels on a global scale (Chapter 2). These findings, based on a broad overview, do not negate the notion that trends of MP bioaccumulation or biomagnification may differ when using a targeted approach focused on smaller geographic scales, on species-specific food chains, or on future projections of MP contamination. For example, *in situ* assessments of MP contamination of a multi-level trophic food web within two central GBR reefs (i.e., Backnumbers and Davies) reveal an increase in MP concentrations relative to the surrounding water column and sediment (Chapter 5). However, like global trends, when considering alternative exposure pathways (i.e., predator-prey interactions), bioaccumulation and biomagnification are not supported by *in situ* findings.

The body burden of a pollutant is traditionally reported as the weight of pollutant per gram weight of the analysed tissue (Thornton et al., 2002) which is an important component in (eco)toxicology assessments. Quantifying the weight of MPs ingested is not routinely done due primarily to difficulties associated with handling heterogenous particulate matter less than 5 mm (Rivers et al., 2019). Therefore, the literature has adapted the classical definition of MP body burden to be representative of the number of MPs per gram weight of organism (Jensen et al., 2019; Kroon et al., 2018b; Santana et al., 2021) to account for the potential difference in impacts particulate MPs may have on smaller compared to larger organisms. Yet, it remains standard practice in MP field-based research to report MP contamination as MPs individual^{-1} (Chapter 2; (Uddin et al., 2020)) because plastics are, in essence, heterogenous particulate matter varying in shape, size, and polymer type (Andrady, 2011) and measuring these physicochemical properties is important to understand the associated ecological and physiological risks MPs pose (Lambert et al., 2017; Rochman et al., 2019). To allow appropriate conclusions to be made with such data and to facilitate comparisons with previous

and future studies (Cowger et al., 2020), it is important that MP contamination of organisms be reported in both units.

Problems in data interpretation arise when transforming MP contamination from per individual to per gram weight of the matrix analysed (i.e., water, sediment, organism) to enable comparisons between organisms and their environment. For example, when assessing data as MPs individual^{-1}, both experimental (Chapter 4) and *in situ* (Chapter 5) findings reveal the occurrence of MP biomagnification from lower trophic levels (i.e., copepods, level 2.0) through to higher levels (i.e., reef fish – level 3.5). However, when standardised by body weight, biomagnification is no longer observed. Instead, the trend matches closer to that of global *in situ* data, with lower trophic levels containing higher levels of MPs and a decrease in contamination levels as trophic level increases. Global calculations of these endpoints were acquired using MPs individual^{-1} (Chapter 2), necessitated by the majority of MP reports employing these units and not reporting the body weight of organisms investigated, prohibiting traditional calculations. Yet, it can be argued that as MPs are heterogeneous particulate matter, analysing ingestion reports in a similar way to dissolved chemical contamination (i.e., per gram body weight) may not be appropriate for assessing ecological endpoints. To measure an endpoint that is comparing an organism to its environment (i.e., assessing bioconcentration and bioaccumulation), the reporting of all matrices in comparable units is paramount (Hartmann et al., 2019; Rochman et al., 2019). Hence, standardising by weight is necessary. However, to determine endpoints associated with an organism's prey (i.e., assessing biomagnification through trophic transfer), understanding the numeric quantity of MPs per individual will establish whether this contamination is retained and increases in quantity over time or trophic level.

Overall, *in situ* results indicate there is a trend of bioconcentration of MPs occurring in organisms (i.e., copepods, benthic crustaceans, and reef fish) in the central GBR compared to the surrounding water column and sediment, yet when considering prey interactions, the bioaccumulation and biomagnification of MPs is not supported by traditional assessments of these endpoints. When considering the complexity of analysing physical particulate matter in marine organisms, assessing by MPs per individual reveals there is biomagnification from the lower to higher trophic levels, confirmed under controlled laboratory conditions (Chapter 4) and observed *in situ* on the GBR (Chapter 5). These seemingly opposite findings, which both provide information imperative to guide ecological risk assessments, highlight the need for consensus among researchers to clarify which unit is most important for assessing environmental risks of MPs. Additional investigations are also needed to understand how species-specific retention times impact the bioaccumulation and biomagnification potential of MPs through the food web.

7.2 Ingestion, retention and trophic transfer of MPs through a simple marine food web

It is well established that many marine organisms are susceptible to MPs ingestion (Egbeocha et al., 2018; Lusher, 2015; Ryan, 2019) causing a suite of health and behavioural impacts (Aragaw and Mekonnen, 2021; Auta et al., 2017; Gola et al., 2021). Therefore, quantifying both the ingestion and retention of MPs is increasingly important to help elucidate the impact on various marine organisms and assess whether trophic transfer is plausible. This work is the first to report detailed ingestion, retention, and trophic transfer of environmentally relevant MPs (i.e., PEST microfibres) for copepods (*Parvocalanus crassirostris*), mysid shrimp (*Mysida sp.*) and moon wrasse (*Thalassoma lunare*).

7.2.1 MP ingestion by primary, secondary and tertiary consumers

Zooplankton (specifically copepods), mysid shrimp and reef fish (Family Labridae) play crucial roles in energy transfer processes in coral reef ecosystems, i.e., as an important food source and as intermediate prey species and predators, respectively (Holmes et al., 2012; Kramer et al., 2015; Turner, 2004; Verslycke et al., 2007). As all these organisms predominately feed within the water column or forage through benthic sediments for prey, both environments in which MPs are abundant, they are at risk of MP ingestion (Barrows et al., 2018a; Botterell et al., 2019; Choy et al., 2019; Cozar et al., 2014).

Experimental work revealed that consumers representing the three different trophic levels (2.0, 2.47 and 3.5) all readily ingested and temporarily retained PEST microfibres following a short-term (i.e., < 1 h) exposure (Chapter 3). A comparison of the MP concentrations at each level (Chapter 3) corroborates previous reviews and meta-analyses that have shown that lower trophic level organisms are more susceptible to MP contamination (Walkinshaw et al., 2020), with the highest concentration per body size found in copepods. This was further supported by *in situ* findings (Chapter 5), where all three trophic levels demonstrated the ability to ingest MPs from environmental exposure (Chapters 2 and 5), and that MPs were found to bioconcentrate compared to environmental levels (Chapter 5).

The GBRWHA is not considered a pristine environment (GBRMPA, 2019), yet MP contamination is substantially lower than reported for other marine environments, with water column levels averaging at 0.005 ± 0.004 MPs L^{-1} for central coral reefs (Chapter 5). Experimental exposure concentrations applied here (Chapter 3) (0.05 to 5,000 MPs L^{-1}), while still higher than observed, are more representative of environmental contamination compared to previous experimental studies (Capolupo et al., 2018; Gray and Weinstein, 2017; Qu et al., 2018). Although the higher concentrations applied in laboratory experiments are necessary due to the limitations of using true realistic MP concentrations (Santana et al., 2021), they do enable assessment of the impacts of predicted increases in environmental MPs, a standard practice for ecotoxicology studies. Under laboratory conditions,

copepods and mysid shrimp exhibited lower ingestion concentrations compared to that reported for the calanoid copepod *Calanus helgolandicus* or the mysid shrimp *Neomysis sp*. (Cole et al., 2015; Hasegawa and Nakaoka, 2021; Procter et al., 2019), possibly a result of the lower exposure (and more environmentally realistic) concentration (0.12 to 5 MPs ml^{-1}) applied here. Yet, all organisms (copepods, mysid shrimp and reef fish) exhibited higher ingestion under experimental conditions compared to *in situ* findings, providing further evidence that ingestion is correlated to exposure concentration.

Mysid shrimps were found to fragment larger ingested PEST microfibres (i.e., ~350 µm, Chapter 3), yet smaller (i.e., 10 µm) fibres offered within pre-contaminated prey (Chapter 4) remained intact. This process has previously been observed for other small crustaceans, including *Neomysis sp*. (Hasegawa and Nakaoka, 2021) and Antarctic krill *Euphausia superba* (Dawson et al., 2018b). Small crustaceans have developed mandibles, as well as chitinous and thick barbed spines in their stomach (Dawson et al., 2018b; Friesen et al., 1986), which promote the mechanical breakdown of prey material for digestion. Here, mechanical fragmentation of MPs is more likely to be size-dependent. The Antarctic krill digestive system is suited to grinding hard phytoplankton cell structures (Dawson et al., 2018b). Therefore, the fragmentation of 31.5 µm MPs to < 1 µm mimics this process. While mysid shrimp have similar digestive mechanisms to krill, their larger size, omnivorous diet, and preferential consumption of prey larger than 10 µm in size (Friesen et al., 1986) may preclude fragmentation of MPs less than this size. Further, the incorporation of MPs in copepod biomass may reduce the efficiency of mastication processes and limit the mechanical breakdown of smaller MPs, as Setala et al. (2014) similarly did not report fragmentation following mysid exposure to pre-treated copepods and polychaete worms. If fragmentation of larger MPs (i.e., > 10 µm) by small crustaceans is common, the by-product may pose hazards to higher trophic level organisms (Hasegawa and Nakaoka, 2021) and could be a potential route for increased MP concentrations.

7.2.2 MP retention in primary, secondary and tertiary consumers

MPs are ubiquitous in the marine environment and hence *in situ* MP exposure is unavoidable. As such, marine organisms are prone to continuous ingestion and egestion of MPs, potentially prolonging their exposure. Previously observed impacts of MPs on copepods (Heindler et al., 2017), mysid shrimp (Lee et al., 2021) and reef fish (Foley et al., 2018) are likely a consequence of continuous turn-over (i.e., MP intake, depuration, re-intake) resulting from > 24 h exposure periods, yet limited knowledge of ingestion and retention rates makes it difficult to evaluate impact and risk. Here, recovery and expulsion of fibrous MPs from the gastrointestinal tract (GIT) after a single exposure (up to 1 h) was observed in less than 48 h for most individuals of these three species (Chapter 3). Currently, it is not known whether the retention times observed here (i.e., 3 h to > 48 h) will negate potential impacts that can stem from

continuous MP ingestion and retention within the gut. The physical characteristic of shape has been reported to influence both effects (Bucci et al., 2020) and retention times (Santana et al., 2021) of MPs in marine organisms, and assessing the impacts of various shapes, as well as textures and polymer types, should be made a priority to encompass the wide suite of heterogeneous MP contamination that exists (Rochman, 2013). Regardless, this work offers insight into how MPs are ingested and retained within each level and is critical information needed to assess implications for the trophic transfer of MPs as environmental contamination increases.

7.2.3 Trophic transfer of MPs through a simple marine food web

The detailed systematic review of the literature revealed a total of 35 research articles pertaining to the trophic transfer of MPs in aquatic species (Chapters 2 and 4). Despite trophic transfer of MPs being notably observed in all studies, this work is the first to investigate and report on the trophic transfer of an environmentally relevant MP (i.e., PEST microfibres) across three trophic levels under various plastic additive treatment conditions.

MPs were confirmed to be ingested by copepods and present in all individuals of the two higher-level organisms offered contaminated prey (Chapter 4). An increase in MP contamination was observed from the primary trophic level through to the tertiary level, with MP contamination differing significantly between the three species. This follows the same trend as observed in other studies, providing further corroborating evidence for trophic transfer (Chapter 4). The overall trend was a 20.7-fold increase of MPs from copepods to mysids and an 8.7-fold increase from mysids to fish. From trophic levels 2 to 3.5 (i.e., copepods through to fish), there was a 112-fold increase in MP concentration. As MP ingestion has been shown to be dependent on MP exposure concentrations (Hasegawa and Nakaoka, 2021; Santana et al., 2021), investigation into how varying exposure concentrations may impact the subsequent transfer of MPs to higher trophic levels is needed.

In situ investigations (Chapter 5) established MP contamination in each of the three trophic levels, and physicochemical comparisons of the MPs revealed trophic transfer as a prominent pathway given similarities in MP polymer type, colour, shape, and size found throughout all matrices. Yet, the low bioaccumulation and biomagnification factors (i.e., < 1), imply that MPs ingested via contaminated prey items are unlikely to be completely retained within the next trophic level (Chapter 5). MP interactions within GIT of organisms may be more complex; rather than passaging as an individual item through the predator's gut, MPs may become integrated in larger masses of biological material during digestion and simply pass through at a different and possibly faster rate, thereby affecting (i.e., limiting) the time of retention. In addition, this body of work demonstrated that ingestion of MPs via trophic transfer (Chapter 4) is substantially higher than when predators are experimentally exposed to prey-

sized MPs only (Chapter 3), a phenomenon previously observed for mysids and fish (Hasegawa and Nakaoka, 2021). More specifically, mysids ingested 1.3x to 3.3x more MPs and fish ingested 16x to 25x more MPs when exposed via trophic transfer (Chapter 4). Therefore, the trophic transfer of MPs may be a significant route of contamination exposure in the natural environment, despite bioaccumulation and biomagnification not always observed in the higher trophic levels.

7.2.4 Influence of phthalate plasticiser on MP ingestion and transfer

Bis(2-ethylhexyl) phthalate (DEHP) is a priority pollutant with carcinogenic properties known to result in endocrine disruption (Kamrin, 2009) and has previously been shown to impact *P. crassirostris* (e.g., survival, fecundity, and gene expression; (Heindler et al., 2017)), crustaceans (e.g., growth, moulting, energy metabolism, reproduction, population size, and morphology (Verslycke et al., 2004)), and fish (e.g., decreased fecundity, changed spawning behaviour (Ye et al., 2014)). Under experimental conditions, the uptake of chemical additives is reported to be diluted by the contemporaneous presence of MPs in the water column during exposure, most likely due to the immediate adsorption of chemical additives when added to an aquatic media (Gulizia et al., 2022; Liang et al., 2008; Stringer and Johnston, 2001), or if pre-adsorbed to the MPs (Chua et al., 2014; Devriese et al., 2017; Magara et al., 2018; Paul-Pont et al., 2016). This is the first body of work to describe and detail the influence of phthalates on the transfer of MPs through the food web (Chapters 3 and 4). Findings show DEHP, either within the water column or pre-adsorbed to MPs, can influence the intake of MPs. The impact phthalates have on MP ingestion of microplastics is species-specific, with phthalates significantly influencing the ingestion rate of microplastics (Chapters 3 and 4), potentially compounding their impacts as a marine contaminant (Rochman, 2013). However, DEHP had no significant impact on the transfer of MPs from copepods to mysid shrimp or mysid shrimp to fish. This result does not necessarily negate the potential impacts of DEHP uptake within these organisms individually, and investigations into the biomagnification and bioaccumulation factors of DEHP should be prioritised. For example, the longer the ingested MPs are retained within an organism's gut, the higher the likelihood that the phthalate additives will translocate into the body tissue and have a further impact on animal health (Chua et al., 2014). In addition, further research is needed to elucidate whether contaminants adsorbed to MPs may transfer into the organism upon ingestion or further transfer up the trophic levels (Foley et al., 2018; Koelmans et al., 2016). Given the high turnover rate of lower trophic level consumption within oceanic food webs, the potential for transfer of MPs, and associated chemical contaminants, to higher tropic levels is probable. Albeit the concept of trophic transfer of MPs and chemical additives is still underdeveloped and warrants further investigation.

7.3 Baseline contamination and monitoring of MPs

Establishing the baseline occurrence of MP contamination throughout the abiotic and biotic matrices within an ecosystem is necessary to assess whether observations from controlled laboratory experiments are also being detected *in situ* (see Chapter 5). Further, robust temporal monitoring of marine plastic contamination is essential to establish baseline contamination levels, identify trends and potential influences on plastic pollution (Chapter 6), information that is critical to improve the ecological risk assessment of MPs on marine life. This work is the first baseline assessment of MPs in copepods, benthic crustaceans and moon wrasse (*T. lunare*), including their surrounding environmental matrices (i.e., water column and sediment), as well as the first temporal assessment of MPs in surface waters of the central GBRWHA.

7.3.1 Baseline contamination of the central Great Barrier Reef

Coral reef ecosystems, such as the GBRWHA (GBRMPA, 2019), are not only crucial to marine biodiversity (Fisher et al., 2015; Knowlton et al., 2010) but hold significant economic and cultural importance (Burke et al., 2011). The abundance and distribution of MPs has previously been reviewed for coral reef systems globally (Huang et al., 2021a), with contamination reported in seawater (Connors, 2017; Portz et al., 2020; Tan et al., 2020), sediment (Patterson et al., 2020; Patti et al., 2020; Zhang et al., 2019), and marine organisms (Ding et al., 2019a; Rotjan et al., 2019). In recent years, the research efforts in the GBRWHA have increased with the addition of several reports published since the commencement of this thesis (Figure 7.1). Additional to previous work mentioned in Chapter 1, MPs have now been found contaminating abiotic matrices, such as surface waters (Chapter 6; Santana et al., 2022), sub-surface waters (Chapter 5; Santana et al., 2022), and benthic sediment (Chapter 5; Santana et al. 2022) across the GBRWHA. Further, MP contamination has now been reported for a wide range of different biological taxa collected on the GBR, including hard corals, sponges, sea cucumbers and sea squirts (Santana, 2022), planktivorous damselfish (Jensen et al., 2019; Santana et al., 2021), moon wrasse (Chapter 5), Australian sharpnose sharks (Schlawinsky et al., 2022), and commercially important fish species (Dawson et al., 2022). All work, including the current research thesis, contributes to understanding the baseline contamination of MPs in the GBRWHA to better inform managers and policy surrounding the management of the reef.

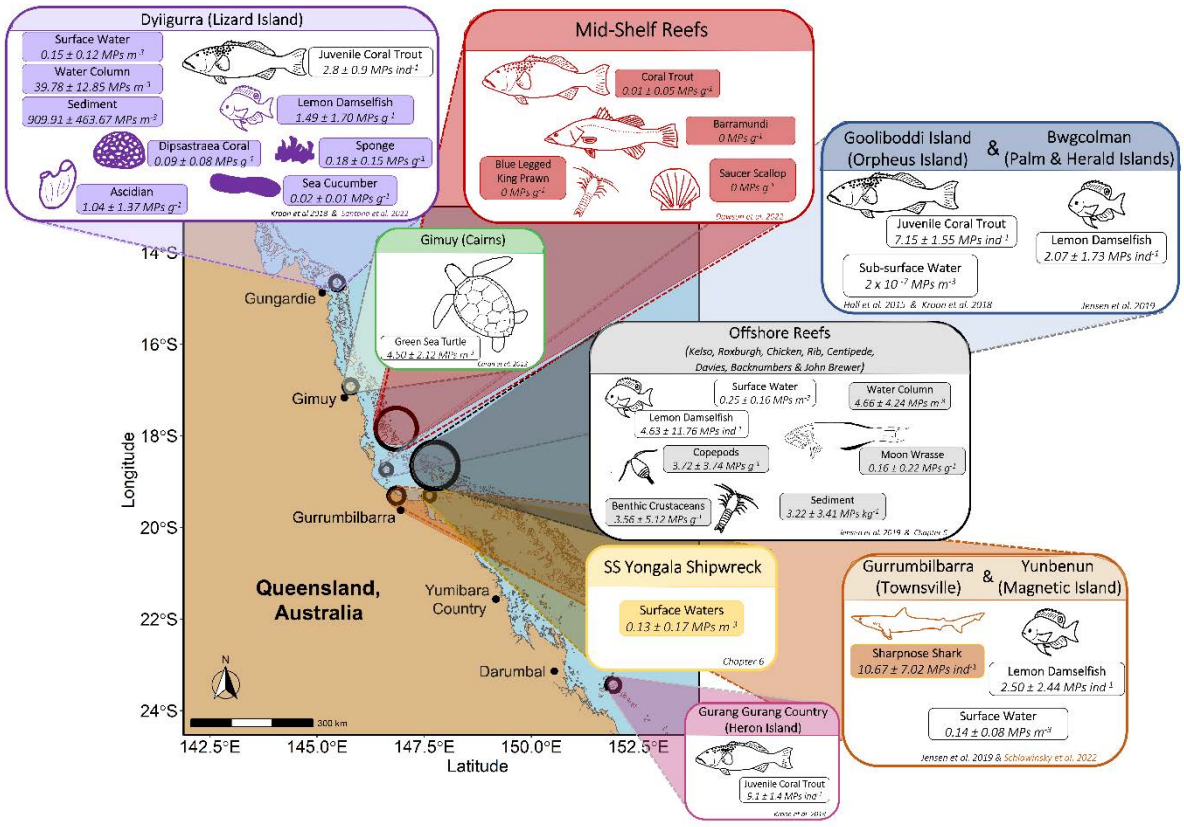

Figure 7.1 Summarised results from studies investigating MP contamination in the Great Barrier Reef World Heritage Area (GBRWHA). Only studies investigating marine species inhabiting near-shore and off-shore areas and their surrounding environment are included, with reports of freshwater and local beach clean-ups excluded. Most recent reports, since 2019 (i.e., the commencement of this thesis), have been added and highlighted in colour, with older reports (i.e., pre-2019, see Figure 1.1) shown in black.

It is well-known that MPs are ubiquitous throughout the marine environment, with contamination globally estimated at a magnitude of 11.8 ± 24.0 MPs L^{-1} established using surface level grab samples (Barrows et al., 2017). While the GBRWHA has substantially lower reports of MPs comparatively, it is not free from the threat of worldwide plastic pollution. MP contamination in central GBRWHA locations, both among shallow coral reef habitats (Backnumbers and Davies reefs) (Chapter 5) and at more coastal surface waters at the SS Yongala Integrated Marine Observing System (IMOS) National Reference Station (NRS) (Chapter 6), follows similar trends found at Dyiigurra (Santana, 2022), with sediments acting as a sink for MPs (3.22 ± 3.41 MPs kg^{-1}; Chapter 5), and surface waters having the least amount of MP contamination (1.0 x 10^{-5} to 9.5 x 10^{-4} MPs L^{-1}; Chapter 6) compared to the water column (0.005 ± 0.004 MPs L^{-1}; Chapter 5). Globally, marine sediments have been demonstrated to be a sink for MPs (Cozar et al., 2014; Liu et al., 2020; Song et al., 2018; Van Cauwenberghe et al., 2015), with reports here providing further evidence to support this trend. MPs were prevalent in the

water column at centrally located reefs, with MP concentrations similar to, albeit lower, than those reported for other shallow coral reefs at Dyiigurra (0.036 to 0.044 MPs L^{-1}; (Santana, 2022)) or the Xisha Islands (0.2 to 45.2 MPs L^{-1}; (Ding et al., 2019a)). The variation in MP distribution between locations, even those that are part of the same reef ecosystem, is potentially due to the strength and interactions of multiple physical mechanisms, including wind speed and direction, surface currents, and river discharge (Brunner et al., 2015; Kukulka et al., 2012). In-depth coral reef hydrodynamic modelling is needed to provide further insight into the potential sources, pathways, and fate of MPs within a complex shallow coral reef system, such as the GBRWHA.

Comparisons of species representing three trophic levels in Chapter 5 revealed the variability in MP intake across different taxa despite similar environmental exposure (Guven et al., 2017; Lourenço et al., 2017; Santana, 2022). Here, *in situ* MP intake by representative organisms was similar to previous studies for copepods (Desforges et al., 2015; Kosore et al., 2018), lower for related benthic crustacean species (Abbasi et al., 2018; Devriese et al., 2015; Jamieson et al., 2019; Karlsson et al., 2017; McGoran et al., 2017; Waite et al., 2018; Wang et al., 2019b), and higher for other reef wrasse species (Baalkhuyur et al., 2018) (See Appendix 1 for details). Locally, MP contamination in reef moon wrasse (*Thalassoma lunare*; Chapter 5) was similar to or lower than MP contamination in other reef fish collected in the GBR (Jensen et al., 2019; Kroon et al., 2018b; Santana, 2022). Copepods were the most contaminated taxa when analysed based on MP contamination by weight, followed by benthic crustaceans and moon wrasse. This corroborates previous reviews and meta-analyses that have shown that lower trophic levels are more susceptible to MP contamination (Walkinshaw et al., 2020). However, when comparing MP contamination by individual organism to facilitate comparisons to other studies (Chapter 2), the trend is reversed with fish containing a higher concentration of MPs (1.5 to 1.95 MPs $individual^{-1}$) than either benthic crustaceans (0.03 to 1 MP $individual^{-1}$) or copepods (0.01 to 0.02 MPs $individual^{-1}$). As biomagnification was only observed in the trophic web when assessing MP contamination per individual, rather than body weight, this further highlights the need for a MP research community conversation to reach a consensus on reporting units (Hartmann et al., 2019; Rochman et al., 2019).

7.3.2 Monitoring of plastics, physicochemical trends, and potential influences

Increased reporting on plastic pollution in the marine environment and associated adverse impacts to marine life highlights the necessity and urgency to implement plastic monitoring programs (Maximenko et al., 2019). Given incremental changes and variability in marine pollution, long-term data sets are required to detect trends in pollutants of interest and improve understanding of associated effects on the health of a marine ecosystem (Lavers et al., 2021; Lynch et al., 2014; Thompson et al.,

2011). Robust monitoring programs that establish baseline and trends of marine plastic contamination can provide comprehensive assessment of ecological risk, inform appropriate mitigation measures, assess effectiveness, and promote adaptive management. Recent studies have recommended further monitoring to determine temporal variation in marine plastic pollution (Maximenko et al., 2019; Ryan et al., 2020a), with Chapter 6 being one of the first to report longer-term trends in marine surface waters of the GBR, Australia.

Monitoring marine plastic pollution allows insight into how the physicochemical characteristics of MPs change temporally and spatially, and whether they are potential drivers of these trends. Quality-controlled monitoring data can also provide a more robust and accurate assessment of ecological risk to marine organisms. Three years of data collected at the SS Yongala NRS has provided invaluable information regarding temporal variations of MP concentration, polymer types, colours, shapes, and sizes. The majority of these MPs were transparent, white or blue small secondary plastics (i.e., 83.6% < 3 mm in length) comprised of mostly polyolefins (PE and PP) or PEST, with fragments more common than fibres. The suite of MPs identified in the nearby shallow coral reef environment (i.e., Davies and Backnumbers Reefs; Chapter 5) highlight the heterogeneity of MP contamination, yet there is considerable overlap of the most prevalent physicochemical characteristics (i.e., small [< 1 mm] blue or transparent fibres comprised of PEST). These data match a similar finding for surface waters at Dyiigurra in the northern GBR (Santana, 2022), but differ from those in other areas of the central GBR (Jensen et al., 2019), further highlighting the variety and variability in MP size, shape, colour, and polymer types found throughout the GBRWHA. In other coral reef systems (Huang et al., 2021a), the distribution of colours is similarly skewed towards blue and clear items, as well as black, and white items, potentially contributed to by these being the most commonly used colours in plastics manufacture globally (GESAMP, 2016). Other common plastic polymers found in the GBRWHA include PE, PP and PVC; these polymers, along with PET and PEST, represent about 63% of global plastic production, making their presence in the marine environment expected (GESAMP, 2019). Global trends of shapes isolated from marine environments highlight the increasing prevalence of fibres (Barrows et al., 2018a; Dris et al., 2015; Gallagher et al., 2016) yet results here (Chapter 6) emphasise this may not be a universal trend. This variability is likely associated with adjacent local land use and differences in the oceanographic and hydrodynamic features at each site or result from collection method limitations. Regardless, these data support the need to develop robust collection methods suited to capture MPs of all shapes and sizes, as well as conduct scientifically robust monitoring of marine plastics at multiple locations of interest to accurately measure baseline contamination status, potential sources, and longer-term trends.

The potential influence of environmental physicochemical factors (i.e., wind speed, current speed, sea surface temperature, salinity and river discharge volume) on plastic transport, accumulation

and distribution have been previously modelled and discussed (Brunner et al., 2015; Critchell and Lambrechts, 2016; Hitchcock, 2020; Kukulka et al., 2012; Welden and Lusher, 2017). Plastic concentrations at the SS Yongala NRS were found to be significantly influenced by extreme weather events and associated increases in river discharges, resulting in the outflow of plastic debris from river-based sources, and by wind speed independent of these events. Despite peaking immediately following extreme weather events, the overall trend of plastic contamination did not change over the three years of monitoring suggesting marine organisms in the GBRWHA face sustained and chronic risk of plastic exposure. Moving forward, the concurrent monitoring of physicochemical oceanographic parameters should be implemented alongside monitoring of marine plastic pollution to better elucidate any influences on plastic trends. Finally, Chapter 6 provides a foundation for longer-term plastic monitoring at the SS Yongala NRS, other IMOS NRS locations in Australian coastal waters, and coastal locations around the world, enabling future levels of contamination to be predicted and compared based on projected increases in plastic waste, and a baseline against which the effectiveness of plastic waste management strategies can be assessed.

7.4 Contributions to the MP research field

The research presented in this thesis emphasises the persistence and severity of the ecological issue surrounding plastic pollution, and in particular MPs. Estimates for the GBRWHA indicate the ecological risk is substantial, with MP contamination widespread and omnipresent throughout all matrices examined. While bioaccumulation and biomagnification trends were not observed in global reports (Chapter 2) or *in situ* assessments of the GBR (Chapter 5), laboratory experiments (Chapter 4) revealed that MPs do bioconcentrate and that biomagnification is possible, with trophic transfer being a prominent pathway of exposure (Chapters 3 and 4). The long-term monitoring of SS Yongala NRS surface waters reinforced microplastic contamination is heterogeneous and variable across the GBRWHA, yet persistent in nature and subject to fluctuation from physicochemical influences such as extreme weather events (Chapter 6), factors that are likely to have a significant impact on inhabiting organisms.

This body of work contributes novel research to better understand the ecological fate and impact of MPs in a tropical marine ecosystem. Appraisal of this research in the context of the burgeoning literature has highlighted further knowledge gaps and areas of focus for future work (Figure 7.2).

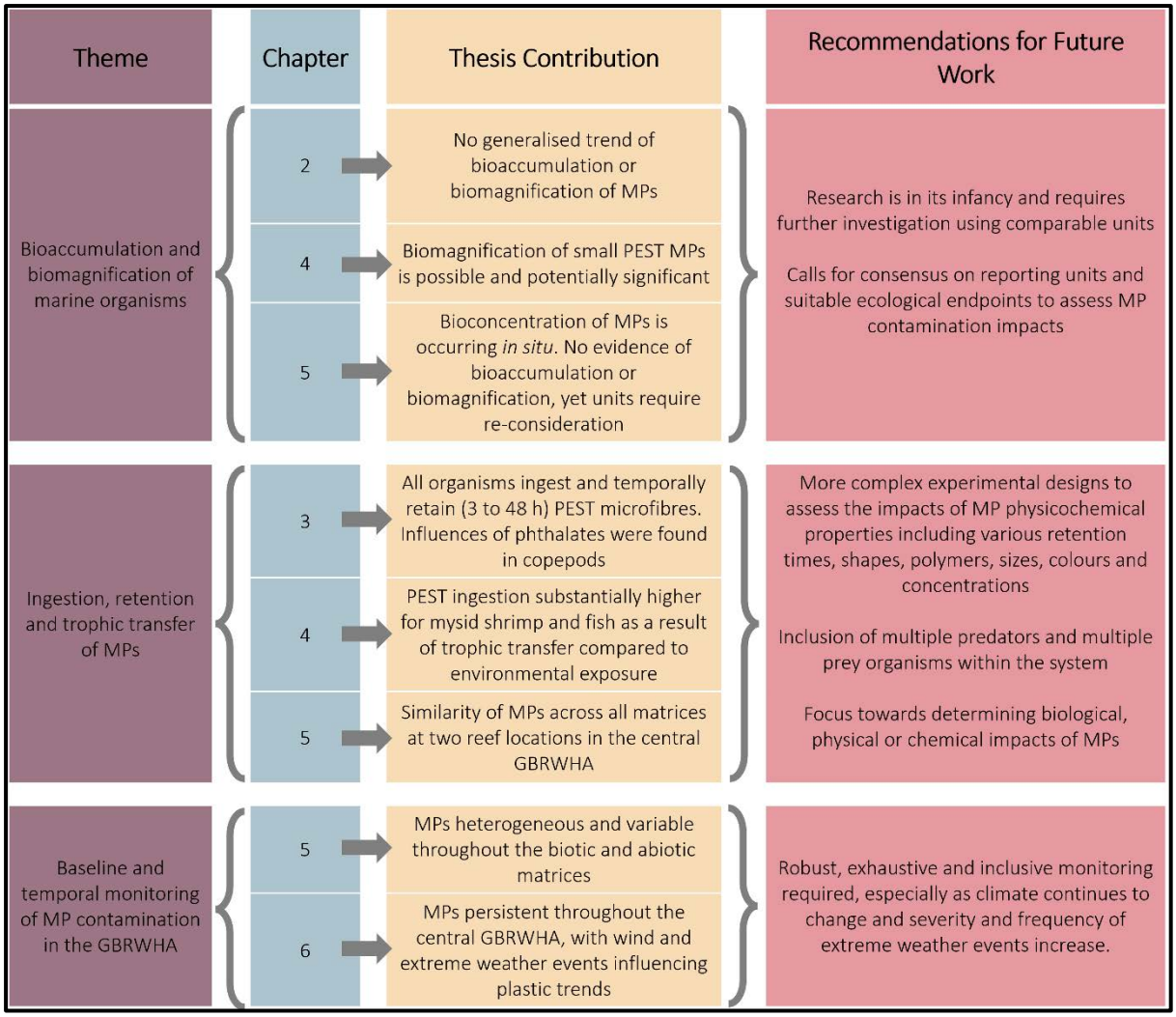

Figure 7.2 Key themes addressed by the PhD thesis, summarising contributions from each data chapter and recommendations for future work.

If the blight of MPs in the marine environment is to be successfully mitigated, scientific research to understand the extent and impact MPs pose and to develop technologies to limit these, needs be a collaborative, constructive effort. Therefore, presented below are the major lessons learnt throughout the PhD thesis, so that others may build upon this work.

Lesson 1: Reporting units matter. The observed trends of bioaccumulation and biomagnification differed depending on the units used to assess MP contamination, i.e., standardised by individual or by body weight. A consensus has been called for (Hartmann et al., 2019; Rochman et al., 2019) previously, yet researchers often choose whichever best suits the research question posed. Both units of measurement are important to ecological risk assessments given MP heterogeneity inherently proliferates the modes of interaction with marine organisms, i.e., it is a multiple stressor contaminant (Rochman, 2013). As such, there is no one-unit-fits-all. It is recommended moving forward that data be

reported in both units and made available to others, so that appropriate risk assessments and trends can be analysed. Calls for open access data to be a priority (Cowger et al., 2020), as well as the harmonisation of MP collection and processing, are also supported.

Lesson 2: Suitable endpoints are necessary. Traditionally, endpoints of bioconcentration, bioaccumulation and biomagnification are applied to dissolved chemical contaminants (Alexander, 1999). The body of work presented here highlights that when these endpoints are applied to a physical particulate contaminant (i.e., MPs) complications in assessments arise. Even with open access data and a consensus on reporting units, in-depth evaluation on how and when to apply these units to assess the endpoints of bioconcentration, bioaccumulation and biomagnification is needed. Currently, MPs (particulate or physical items) need to be reported in comparable units of concentration per biotic body weight to enable direct comparison with MPs in the organism's environment. Yet, to understand if MP concentrations are increasing or to determine the incidence of prey to predator MP transfer, individual numeric quantities are a must. With no single reporting protocol available that comprehensively addresses the ecological risk of MPs, applying these endpoints as described here is the best approach thus far.

Lesson 3: Trophic transfer of MPs cannot be ignored. The similarity in polymer type, colour, shape, and size of MPs found across all trophic levels in Chapter 5 and abiotic matrices in Chapters 5 and 6, warrants further discussion as to whether trophic transfer is a route of exposure. *In situ* assessment prohibits anything further than speculation (Chagnon et al., 2018; Nelms et al., 2018; Renzi et al., 2018a; Welden et al., 2018) and claims of trophic transfer without empirical evidence need to cease. Further, controlled laboratory experiments are required to elucidate its potential occurrence. The work presented in Chapter 4 (showing a 112-fold increase from copepods to fish) is a foundational groundwork to be built upon. Additionally, as well as expanding assessments to include other MP polymers, shapes, and sizes, investigations are needed to understand the impact of varying duration, concentration, and frequency of MP exposure on marine organisms, both in isolation and as part of a more complex food web, are critical. This area of research should be a priority moving forward.

Lesson 4: MPs should be treated as co-contaminants. The current research provides evidence that the presence of phthalates can significantly influence the ingestion rate of MPs, potentially compounding their impacts as a marine contaminant (Rochman et al., 2019). Moving forward, it is essential to consider MPs as a multiple stressor contaminant (i.e., plastic polymer and additives). Marine organisms are exposed to MPs amongst a cocktail of abiotic chemistries that may also include contaminants (e.g., PAEs, PAHs, PCBs) (Koelmans et al., 2016; Rochman et al., 2013), and multidisciplinary and holistic approaches are imperative to accurately assess the ecological risk MPs pose. Further research is needed to elucidate whether contaminants adsorbed to MPs can transfer into

the organism upon ingestion or further transfer up the trophic food web (Foley et al., 2018; Koelmans et al., 2016).

Lesson 5: Robust, exhaustive, inclusive monitoring is required. Assessment of the abiotic and biotic matrices of the GBRWHA in Chapters 5 and 6 highlights the variability and heterogeneous nature of MP contamination. Temporal analyses revealed that multiple physicochemical oceanographic parameters (i.e., wind, river discharge) influence plastic trends. In particular, the major influence that extreme weather events have on plastic pollution cannot be ignored. As the climate continues to change, the scale and severity of storms and weather events are projected to intensify and become more frequent (Coumou and Rahmstorf, 2012; Stott, 2016), potentially increasing plastic input, distribution, and accumulation (Welden and Lusher, 2017). Monitoring programs that are spatially and temporally robust, standardised, and inclusive of physicochemical parameters are paramount to effectively investigate baseline trends and observe changes in marine plastic pollution.

7.5 Concluding remarks

The mere existence of anthropogenic products (i.e., plastics) within an environment they do not naturally exist is cause for concern. The distribution and concentrations found throughout both abiotic and biotic matrices of the GBRWHA is alarming and has been identified as an issue for environmental managers (Kroon et al., 2020). It has been suggested by some (Stafford and Jones, 2019) that a focus on plastic research is a distraction from other issues facing the global environment (i.e., climate change, biodiversity loss). However, it is important not to downplay the importance and extent of the plastic pollution issue. It is unclear how plastic pollution can be treated as separate from these other global issues, as plastic production and manufacturing is responsible for 8% of global oil extraction (Thompson et al., 2009b). This, in itself, causes substantial greenhouse gas emissions (approx. 2 Gt carbon dioxide equivalents, CO_2e) (Cabernard et al., 2022), thereby contributing directly to global climate change. Combined with the immense number of megafaunal species (at least 914 species; (Kühn et al., 2015)) directly impacted by plastic, either as a result of ingestion or entanglement, this contributes to biodiversity loss. While it is important to keep global threats in perspective, the luxury of tackling environmental issues one at a time does not exist (Avery-Gomm et al., 2019). The gravity of the situation that is global plastic pollution is not lost on the MP research community and should be addressed with the urgency it deserves.

Research efforts must move beyond proving the existence, persistence, and observation of MP pollution, and transition into elucidating impacts (i.e., physical, chemical, biological) and developing feasible mitigation strategies or alternatives to alleviate the issue. Mitigation efforts are critical to

addressing the issue of plastic pollution. Yet clean-up actions are often not feasible in many (remote) areas of the global environment where plastic accumulates or are not generally applicable to MPs. A preventative approach to stopping plastic pollution is required, rather than attempting to address the issue downstream when plastic pollution has already entered the marine environment and fragmented into smaller items. While social campaigns often target the consumer to choose a 'zero-waste' lifestyle, this approach stems from a place of privilege and naivety given the personal monetary cost and current lack of infrastructure allowing these choices to be convenient for all. It's a perception that operates in a reality where personal choices have the potential to make these large-scale changes, which in essence is impossible without a 100% collaborative effort, and often not feasible given growing global human populations. Instead, the focus should be shifted to the major contributors to the plastic waste issue, as 100 corporations produce 90% of global plastic and are responsible for 70% of total global CO_2 emissions (Charles et al., 2021).

The omnipresence of plastics, and in particular MPs, in the marine environment is feeding the community's growing concern about plastic pollution, and the building momentum has already led to ground-breaking, global agreements, such as plastic straw and bag bans, united efforts for beach clean-ups, and even the United Nations Environment Program (UNEP) Global Plastic Treaty. These efforts should be commended as massive strides in the right direction. Yet, it is impossible to expect change without an institutional overhaul of plastic manufacturing practices and adoption of a circular economy and non-petroleum-based alternatives (i.e., glass, bioplastics). It is the goal of this research to inform managers, policy makers and the general public about the severity of the issue of plastic pollution with respect to the ecological fate of MPs within a tropical marine ecosystem of great traditional, historical and ecological importance (i.e., the GBRWHA). It is my hope that this research thesis, in part, influences change to reduce the issue of global plastic pollution.

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Appendix 1: Chapter 2

Table A9.1 PRISMA Checklist for systematic review and meta-analysis.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	11
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	11
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	11
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	11
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	11-12
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	12
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	12
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	12

Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	12
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	12 – 14
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	12 - 14
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	N/A
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	14 - 15
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	16
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	13 & A1.2 table
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	N/A
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	16
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	N/A
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	N/A
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	N/A
DISCUSSION			

Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	28 – 29
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	28 – 32
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	33 - 34
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	iv

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097. For more information, visit: www.prisma-statement.org.

Table A9.2 Full-text articles excluded from (a) and included in (b) the present study. Reasons for exclusion, primarily around inability to standardise contamination data given the information presented, are given. Main categories around field or laboratory-based studies for included papers have been provided.

(a) Publications excluded

Reference	Reason
Akhbarizadeh et al. (2018)	Inability to convert units
Allen et al. (2017)	Inability to convert units
Asmonaite et al. (2018)	Does not mention ingestion
Barboza et al. (2018b)	No quantification
Barboza et al. (2018c)	No quantification
Barboza et al. (2018d)	No quantification
Beiras and Tato (2019)	No quantification
Beiras et al. (2018)	No quantification
Bessa et al. (2018)	Not enough information
Besseling et al. (2017)	No quantification
Bjorndal et al. (1994)	No size measurements
Bordbar et al. (2018)	Percentage only
Bour et al. (2018a)	Percentage only
Boyle and Limpus (2008)	No size measurements
Brate et al. (2016)	Not enough information
Browne et al. (2008)	No quantification
Budimir et al. (2018)	Not enough information
Bussolaro et al. (2019)	Nanoplastics only
Cannon et al. (2016)	Percentage only
Carreras-Colom et al. (2018)	Percentage only
Caruso et al. (2018)	No quantification
Chagnon et al. (2018)	Not microplastics
Chapron et al. (2018)	No quantification
Cole and Galloway (2015)	No quantification
Cole et al. (2016)	No quantification
Compa et al. (2018)	Not enough information

Courtene-Jones et al. (2017a)	Inability to convert units
Courtene-Jones et al. (2017b)	Inability to convert units
Courtene-Jones et al. (2019)	No trophic level
Cunha et al. (2019)	No quantification
Dantas et al. (2019)	Percentage only
Davarpanah and Guilhermino (2015)	No quantification
Davison and Asch (2011)	No species-specific information
Dawson et al. (2018b)	No quantification
Dawson et al. (2018a)	Inability to convert units
de Orte et al. (2019)	Inability to convert units
de Sa et al. (2015)	Inability to convert units
Detree and Gallardo-Escarate (2018)	No quantification
Ding et al. (2018)	Range only
Ding et al. (2019b)	MPs not from entire sample size
Duncan et al. (2018)	No species-specific information
Egbeocha et al. (2018)	Review paper
Espinosa et al. (2018)	No quantification
Fang et al. (2018)	Range only
Fernandez and Albentosa (2019)	Inability to convert units
Ferreira et al. (2016a)	Percentage only
Ferreira et al. (2016b)	No quantification
Ferreira et al. (2019)	No species-specific information
Floren and Shugart (2017)	Focus on marine birds
Fonte et al. (2016)	No quantification
Fossi et al. (2014)	MPs not investigated
Fossi et al. (2016)	Quantity not given
Fossi et al. (2017)	Quantity not given
Franzellitti et al. (2019)	No quantification
Galgani et al. (2018)	No quantification
Gardon et al. (2018)	No quantification
Garrido et al. (2019)	No quantification
Gaspar et al. (2018)	No quantification
Gassel and Rochman (2019)	Quantity not given
Gebhardt and Forster (2018)	No quantification
Goncalves et al. (2019)	No quantification
Granby et al. (2018)	No quantification
Green (2016)	No quantification
Green et al. (2016)	No quantification
Green et al. (2017)	No quantification
Green et al. (2019)	No quantification
Guebert-Bartholo et al. (2011)	Categories only
Gutow et al. (2016)	No quantification
Gutow et al. (2019)	No quantification
Guven et al. (2018)	No quantification
Hall et al. (2015)	Inability to convert units
Halstead et al. (2018)	Percentage only
Hankins et al. (2018)	Inability to convert units
Hermabessiere et al. (2018)	Method development only
Hermsen et al. (2017)	No species-specific information
Horn et al. (2019)	Not enough information
Jacob et al. (2019)	No quantification
Jamieson et al. (2019)	No trophic level
Jeong et al. (2017)	No quantification
Jung et al. (2018a)	No species-specific information
Jung et al. (2018b)	Method development only
Karami et al. (2018)	Not enough information
Khan and Prezant (2018)	Inability to convert units
Khan et al. (2017)	No quantification
Kolandhasamy et al. (2018)	Not enough information
Kühn et al. (2017)	Method development only
Kumar et al. (2018)	Percentage only
La Beur et al. (2019)	Not enough information
Leung and Chan (2018)	No quantification
Li et al. (2016a)	No quantification
Li et al. (2018a)	Range only
Liboiron et al. (2016)	Not only microplastics
Liboiron et al. (2019)	Not only microplastics
Lo and Chan (2018)	No quantification
Long et al. (2017)	No quantification
Luan et al. (2019)	No quantification
Luis et al. (2015)	No quantification
M'Rabet et al. (2018)	No quantification
Macali et al. (2018)	Quantity not given

Mao et al. (2018)	No quantification
Martinez-Gomez et al. (2017)	No quantification
Mathalon and Hill (2014)	No ingestion (fecal casts)
Mecozzi et al. (2016)	Method development only
Messinetti et al. (2018)	Inability to convert units
Messinetti et al. (2019)	No quantification
Miranda and de Carvalho-Souza (2016)	Range only
Naidoo et al. (2017)	Percentage only
Naidu (2019)	Quantity not given
Naidu et al. (2018)	Quantity not given
Naji et al. (2018)	Not enough information
Nel and Froneman (2018)	No ingestion (tube structures)
Nobre et al. (2015)	No quantification
Okubo et al. (2018)	No quantification
Oliviero et al. (2019)	No quantification
Ory et al. (2018a)	Percentage only
Peda et al. (2016)	No quantification
Peters et al. (2018)	No species-specific information
Pham et al. (2017)	Not only microplastics
Phuong et al. (2018b)	Method development only
Pozo et al. (2019)	Percentage only
Prata et al. (2018)	No quantification
Rebolledo et al. (2013)	Not only microplastics
Reichert et al. (2018)	Area investigated not mentioned
Reichert et al. (2019)	No quantification
Remy et al. (2015)	Not microplastics
Renzi et al. (2018a)	Not only microplastics
Renzi et al. (2018b)	Not enough information
Ribeiro et al. (2017)	No quantification
Rist et al. (2019)	No quantification
Rivera-Hernandez et al. (2019)	No quantification
Roch and Brinker (2017)	MPs not from entire sample size
Rochman et al. (2014)	MPs not investigated
Rochman et al. (2015)	Not only microplastics
Romeo et al. (2016)	Not only microplastics
Rosas-Luis (2016)	Not only microplastics
Santana et al. (2016)	Percentage only
Santana et al. (2017)	No quantification
Santana et al. (2018)	Inability to convert units
Savoca et al. (2019)	Percentage only
Schuyler et al. (2012)	No size measurements
Schuyler et al. (2014)	Review paper
Seuront (2018)	No quantification
Silva et al. (2016)	No quantification
Sjollema et al. (2016)	No quantification
Smith (2018)	Not only microplastics
Sun et al. (2017)	Inability to convert units
Sun et al. (2018b)	Inability to convert units
Sun et al. (2018c)	No species-specific information
Sun et al. (2018a)	No quantification
Sundbaek et al. (2018)	Inability to convert units
Sussarellu et al. (2016)	Inability to convert units
Syakti et al. (2019)	Inability to convert units
Syberg et al. (2017)	No quantification
Tang et al. (2018)	No quantification
Thiagarajan et al. (2019)	No quantification
Thushari et al. (2017)	Inability to convert units
Tosetto et al. (2017)	No quantification
Van Cauwenberghe and Janssen (2014)	Inability to convert units
van Franeker et al. (2018)	Not only microplastics
von Moos et al. (2012)	No quantification
Wang et al. (2017)	Method development only
Wang et al. (2019c)	No quantification
Wang et al. (2019a)	Range only
Watts et al. (2015)	No quantification
Watts et al. (2016)	No quantification
Welden and Cowie (2016a)	Percentage only
Welden and Cowie (2016b)	No quantification
Wesch et al. (2016)	Not only microplastics
White et al. (2018)	No species-specific information
Wieczorek et al. (2018)	Not enough information
Wieczorek et al. (2019)	No quantification
Wilcox et al. (2018)	Not only microplastics
Wojcik-Fudalewska et al. (2016)	Not microplastics

Woods et al. (2018)	Inability to convert units
Wright et al. (2013a)	No quantification
Yu et al. (2018)	Inability to convert units
Zhang et al. (2017)	No quantification
Zhu et al. (2019b)	No species-specific info
Zhu et al. (2019c)	No quantification

(b) Publications excluded

Reference	Reason
Abbasi et al. (2018)	Field MP Uptake
Alomar and Deudero (2017)	Field MP Intake
Alomar et al. (2017)	Field MP Intake
Arias et al. (2019)	Field MP Intake
Avio et al. (2015b)	Field MP Intake
Avio et al. (2017a)	Field MP Intake
Avio et al. (2015a)	Lab Chemical
Azad et al. (2018)	Field MP Intake
Baalkhuyur et al. (2018)	Field MP Intake
Bellas et al. (2016)	Field MP Intake
Bernardini et al. (2018)	Field MP Intake
Besseling et al. (2015)	Field MP Intake
Besseling et al. (2013)	Lab MP Intake
Birnstiel et al. (2019)	Field MP Intake
Boerger et al. (2010)	Field MP Intake
Bonello et al. (2018)	Field MP Intake
Bour et al. (2018b)	Lab MP Intake
Brate et al. (2018)	Field MP Intake
Brennecke et al. (2015)	Lab MP Intake
Capolupo et al. (2018)	Lab MP Intake
Cardozo et al. (2018)	Field MP Intake
Caron et al. (2018)	Field MP Intake
Catarino et al. (2017)	Field MP Intake
Catarino et al. (2018)	Field MP Intake
Cheung et al. (2018)	Field MP Intake
Cho et al. (2019)	Field MP Intake
Chua et al. (2014)	Lab MP Intake & Lab Chemical
Cole et al. (2016)	Lab Trophic Transfer
Cole et al. (2015)	Lab MP Intake
Collard et al. (2015)	Field MP Intake
Collard et al. (2017)	Field MP Intake
Collicutt et al. (2019)	Field MP Intake
Courtene-Jones et al. (2019)	Field MP Intake
Critchell and Hoogenboom (2018)	Lab MP Intake
Davidson and Dudas (2016)	Field MP Intake
Desforges et al. (2015)	Field MP Intake
Devriese et al. (2015)	Field MP Intake
Devriese et al. (2017)	Lab Chemical
Digka et al. (2018)	Field MP Intake
Donohue et al. (2019)	Field MP Intake
Duncan et al. (2018)	Field MP Intake
Farrell and Nelson (2013)	Lab Trophic Transfer
Ferreira et al. (2018)	Field MP Intake
Foekema et al. (2013)	Field MP Intake
Garnier et al. (2019)	Field MP Intake
Giani et al. (2019)	Field MP Intake
Goldstein and Goodwin (2013)	Field MP Intake
Goss et al. (2018)	Field MP Intake
Gray and Weinstein (2017)	Lab MP Intake
Guven et al. (2017)	Field MP Intake
Hamer et al. (2014)	Lab MP Intake
Hermabessiere et al. (2019)	Field MP Intake & Field Chemical
Hernandez-Gonzalez et al. (2018)	Field MP Intake
Herrera et al. (2019)	Field MP Intake
Jabeen et al. (2017)	Field MP Intake
Jeong et al. (2016)	Lab MP Intake
Jovanovic et al. (2018)	Lab MP Intake
Kaposi et al. (2014)	Lab MP Intake
Karami et al. (2017)	Field MP Intake
Karlsson et al. (2017)	Field MP Intake
Kosore et al. (2018)	Field MP Intake
Kroon et al. (2018a)	Field MP Intake
Kühn et al. (2018)	Field MP Intake
Li et al. (2015)	Field MP Intake
Li et al. (2016b)	Field MP Intake
Li et al. (2018b)	Field MP Intake
Lourenço et al. (2017)	Field MP Intake
Lusher et al. (2013)	Field MP Intake
Lusher et al. (2015)	Field MP Intake

Lusher et al. (2016)	Field MP Intake
Magara et al. (2018)	Lab Chemical
Mancuso et al. (2019)	Field MP Intake
Markic et al. (2018)	Field MP Intake
Mazurais et al. (2015)	Lab MP Intake
McGoran et al. (2018)	Field MP Intake
Mohsen et al. (2019)	Field MP Intake
Morgana et al. (2018)	Field MP Intake
Murphy et al. (2017)	Field MP Intake
Nadal et al. (2016)	Field MP Intake
Naidoo et al. (2016)	Field MP Intake
Nelms et al. (2018)	Field MP Intake
Nelms et al. (2019b)	Field MP Intake
Neves et al. (2015)	Field MP Intake
O'Donovan et al. (2018)	Lab Chemical
Ory et al. (2018b)	Lab MP Intake
Paul-Pont et al. (2016)	Lab Chemical
Pegado et al. (2018)	Field MP Intake
Pellini et al. (2018)	Field MP Intake
Perez-Venegas et al. (2018)	Field MP Intake
Peters et al. (2017)	Field MP Intake
Phuong et al. (2018a)	Field MP Intake
Pittura et al. (2018)	Lab Chemical
Porter et al. (2018)	Lab MP Intake
Procter et al. (2019)	Lab MP Intake
Qu et al. (2018)	Lab MP Intake
Romeo et al. (2015)	Field MP Intake
Rummel et al. (2016)	Field MP Intake
Setala et al. (2016)	Lab MP Intake
Silva et al. (2018)	Field MP Intake
Steer et al. (2017)	Field MP Intake
Su et al. (2019)	Field MP Intake
Sun et al. (2019)	Field MP Intake
Tanaka and Takada (2016)	Field MP Intake
Taylor et al. (2016)	Field MP Intake
Teng et al. (2019)	Field MP Intake
Vandermeersch et al. (2015)	Field MP Intake
Vendel et al. (2017)	Field MP Intake
Vered et al. (2019)	Field MP Intake & Field Chemical
Vroom et al. (2017)	Lab MP Intake
Wagner et al. (2017)	Field MP Intake
Waite et al. (2018)	Field MP Intake
Wang et al. (2019b)	Field MP Intake
Watts et al. (2014)	Lab Trophic Transfer
Xiong et al. (2018)	Field MP Intake
Xu et al. (2017)	Lab MP Intake
Zhao et al. (2018)	Field MP Intake
Zhu et al. (2019a)	Field MP Intake

Table A9.3 Bioaccumulation of (a) microplastics (MPs) for marine organisms collected in situ *and (b) relevant data calculations. MP concentrations per individual (i.e., body-burden) were estimates for each species, with concentrations representative of MPs per number of total organisms in the sample size for a particular species, rather than taken from only the number of organisms that exhibited MP ingestion. Also included are data calculations transforming MPs gram weight^{-1} to MPs individual^{-1}, for* in situ *marine organisms exhibiting MP contamination.*

(a) MP contamination for marine organisms *in situ*

Trophic Level	Class	Family	*Species*	Location	Area Investigated	n	MP Ind^{-1}	Reference
1	Magnoliopsida	Hydrocharitaceae	*Thalassia testudinum*	Belize	WO	16	4.56	Goss et al. (2018)
2	Actinopterygii	Acanthuridae	*Acanthurus lineatus*	South Pacific	GIT	24	0.25	Markic et al. (2018)
			Acanthurus sohal	Red Sea	GIT	3	0	Baalkhuyur et al. (2018)
			Ctenochaetus striatus	South Pacific	GIT	56	0.30	Markic et al. (2018)
		Gobiidae	*Boleophthalmus pectinirostris*	China	GIT	9	5.30	Su et al. (2019)
		Hemiramphidae	*Hyporhamphus unifasciatus*	Brazil	GIT	209	0.14	Vendel et al. (2017)
		Kyphosidae	*Kyphosus sandwicensis*	South Pacific	GIT	39	4.72	Markic et al. (2018)
		Mugilidae	*Mugil curema*	Brazil	GIT	186	0.01	Vendel et al. (2017)
			Mugil curvidens	Brazil	GIT	9	0	Vendel et al. (2017)
			Mugil hospes	Brazil	GIT	28	0	Vendel et al. (2017)
			Mugil incilis	Brazil	GIT	5	0	Vendel et al. (2017)
			Mugil liza	Brazil	GIT	2	0	Vendel et al. (2017)
		Scaridae	*Nicholsina usta*	Brazil	GIT	2	0	Vendel et al. (2017)
			Scarus niger	South Pacific	GIT	30	0.27	Markic et al. (2018)
			Scarus oviceps	South Pacific	GIT	45	0.31	Markic et al. (2018)
			Scarus psittacus	South Pacific	GIT	30	0.17	Markic et al. (2018)
		Siganidae	*Siganus luridus*	Turkey	GIT	15	3.13	Guven et al. (2017)
			Siganus punctatus	South Pacific	GIT	29	0.24	Markic et al. (2018)
			Siganus spp.	French Polynesia	GIT	33	0.15	Garnier et al. (2019)
	Bivalvia	Acrcidae	*Senilia senilis*	Atlantic Ocean	Soft tissue	20	1.00	Lourenço et al. (2017)
			Scapharca subcrenata	China	Soft tissue	6	46.52	Li et al. (2015)
			Tegillarca granosa	China	Soft tissue	18	5.16	Li et al. (2015)
		Cardiidae	*Cerastoderma edule*	Atlantic Ocean	Soft tissue	10	4.30	Lourenço et al. (2017)

			France	Soft tissue	50	5.72	Hermabessiere et al. (2019)
	Mytilidae	*Modiolus modiolus*	UK	Soft tissue	6	3.50	Catarino et al. (2018)
		Mytilus edulis	China	Soft tissue	1100	3.94	Li et al. (2016b)
			Europe	Whole animal	125	0.73	Vandermeersch et al. (2015)
			France	Soft tissue	50	5.88	Hermabessiere et al. (2019)
			France	Soft tissue	120	0.60	Phuong et al. (2018a)
			North Sea	Soft tissue	17	1.23	Karlsson et al. (2017)
			South Korea	Soft tissue	60	0.68	Cho et al. (2019)
			UK	Soft tissue	18	4.20	Catarino et al. (2017)
			UK	Soft tissue	162	6.69	Li et al. (2018b)
			USA	GIT	35	0.37	Zhao et al. (2018)
		Mytilus spp.	Norway	Soft tissue	332	1.50	Brate et al. (2018)
			UK	Soft tissue	36	3.20	Catarino et al. (2018)
	Nuculidae	*Acila mirabilis*	China	Soft tissue	20	5.50	Wang et al. (2019b)
	Ostreidae	*Alectryonella plicatula*	China	Soft tissue	18	10.12	Li et al. (2015)
		Crassostrea gigas	France	Soft tissue	60	2.10	Phuong et al. (2018a)
			Italy	Soft tissue	100	0.18	Bonello et al. (2018)
			South Korea	Soft tissue	60	0.77	Cho et al. (2019)
		Crassostrea spp.	China	Soft tissue	306	3.98	Teng et al. (2019)
		Crassostrea virginica	USA	Soft tissue	90	16.47	Waite et al. (2018)
	Pectinidae	*Mizuhopecten yessoensis*	China	Soft tissue	6	57.04	Li et al. (2015)
			South Korea	Soft tissue	60	1.21	Cho et al. (2019)
	Pharidae	*Sinonovacula constricta*	China	Soft tissue	6	15.06	Li et al. (2015)
	Semelidae	*Scrobicularia plana*	Atlantic Ocean	Soft tissue	10	3.30	Lourenço et al. (2017)
	Veneridae	*Cyclina sinensis*	China	Soft tissue	30	4.76	Li et al. (2015)
		Meretrix lusoria	China	Soft tissue	18	9.79	Li et al. (2015)
		Pelecyora isocardia	Atlantic Ocean	Soft tissue	20	1.50	Lourenço et al. (2017)
		Ruditapes philippinarum	Canada	Soft tissue	54	9.00 [a]	Davidson and Dudas (2016)
			China	Soft tissue	24	5.53	Li et al. (2015)
			South Korea	Soft tissue	60	1.15	Cho et al. (2019)
Gastropoda	Littorinidae	*Littorina littorea*	North Sea	Soft tissue	28	1.42	Karlsson et al. (2017)

	Hexanauplia	Calanidae	*Neocalanus cristatus*	Pacific Ocean	Whole animal	960	0.03	Desforges et al. (2015)
			Copepoda spp.	Indian Ocean	WO	110	0.33	Kosore et al. (2018)
	Malacostraca	-	*Isopoda spp.*	North Sea	Soft tissue	16	0.96	Karlsson et al. (2017)
	Polychaeta	Nereidae	*Hediste diversicolor*	Atlantic Ocean	Soft tissue	10	2.70	Lourenço et al. (2017)
		Spionidae	*Scolelepis squamata*	Atlantic Ocean	Soft tissue	8	0.60	Lourenço et al. (2017)
2.1	Actinopterygii	Engraulidae	*Cetengraulis edentulus*	Brazil	GIT	5	0	Vendel et al. (2017)
		Kyphosidae	*Girella tricuspidata*	South Pacific	GIT	20	4.15	Markic et al. (2018)
2.2	Actinopterygii	Acanthuridae	*Naso unicornis*	South Pacific	GIT	30	0.23	Markic et al. (2018)
				Red Sea	GIT	2	0	Baalkhuyur et al. (2018)
		Mugilidae	*Ellochelon vaigiensis*	South Pacific	GIT	33	2.06	Markic et al. (2018)
	Ascidiacea	Pyuridae	*Herdmania momus*	Isreal	Soft tissue	15	1.78	Vered et al. (2019)
2.3	Actinopterygii	Acanthuridae	*Naso lituratus*	South Pacific	GIT	28	0.25	Markic et al. (2018)
		Poeciliidae	*Poecilia vivipara*	Brazil	GIT	75	0.09	Vendel et al. (2017)
2.32	Malacostraca	Euphausiidae	*Euphausia pacifica*	Pacific Ocean	Whole animal	413	0.06	Desforges et al. (2015)
2.37	Holothuroidea	Stichopodidae	*Apostichopus japonicus*	China	GIT	200	10.15	Mohsen et al. (2019)
			Holothurian spp.	Indian Ocean	Internal cavities	2	3.00	Taylor et al. (2016)
2.38	Reptilia	Cheloniidae	*Chelonia mydas*	Australia	GIT	2	3.5	Caron et al. (2018)
				Global	GIT	51	1.09	Duncan et al. (2019)
2.4	Actinopterygii	Gerreidae	*Diapterus auratus*	Brazil	GIT	29	0.97	Vendel et al. (2017)
2.48	Bivalvia	Mytilidae	*Perna perna*	Brazil	Soft tissue	10	31.20	Birnstiel et al. (2019)
2.5	Actinopterygii	Mugilidae	*Liza haematocheila*	China	GIT	18	3.30	Jabeen et al. (2017)
				China	GIT	17	1.20	Su et al. (2019)
			Mugil cephalus	China	GIT	60	2.25	Cheung et al. (2018)
				China	GIT	18	3.10	Jabeen et al. (2017)
				South Africa	GIT	70	3.80	Naidoo et al. (2016)
				South Pacific	GIT	22	0.27	Markic et al. (2018)
2.58	Maxillopoda	Lepadidae	*Lepas spp.*	Pacific Ocean	GIT	385	1.35	Goldstein and Goodwin (2013)
2.6	Actinopterygii	Clupeidae	*Sardinella albella*	Thailand	Stomach	14	2.30	Azad et al. (2018)
2.69	Bivalvia	Mytilidae	*Mytilus galloprovincialis*	China	Soft tissue	18	4.48	Li et al. (2015)
				Europe	Whole animal	300	0.76	Vandermeersch et al. (2015)
				Ionian Sea	Soft tissue	80	0.85	Digka et al. (2018)

				Italy	Soft tissue	26	1.77	Avio et al. (2017a)
2.7	Actinopterygii	Mugilidae	*Planiliza subviridis*	Malaysia	Internal cavities	30	0.53	Karami et al. (2017)
		Pomacanthidae	*Pygoplites diacanthus*	Red Sea	GIT	5	0	Baalkhuyur et al. (2018)
		Pomacentridae	*Abudefduf sexfasciatus*	Red Sea	GIT	5	0.20	Baalkhuyur et al. (2018)
		Scombridae	*Rastrelliger brachysoma*	Thailand	Stomach	3	1.00	Azad et al. (2018)
2.71	Malacostraca	Panopeidae	*Panopeus herbstii*	USA	Soft tissue	90	21.99	Waite et al. (2018)
2.8	Actinopterygii	Acanthuridae	*Acanthurus gahhm*	Red Sea	GIT	10	0.10	Baalkhuyur et al. (2018)
		Clupeidae	*Anodontostoma chacunda*	Thailand	Stomach	14	2.00	Azad et al. (2018)
		Mugilidae	*Liza aurata*	Turkey	GIT	39	3.26	Guven et al. (2017)
		Pomacentridae	*Dascyllus trimaculatus*	Red Sea	GIT	2	0	Baalkhuyur et al. (2018)
		Sparidae	*Boops boops*	Mediterranean	GIT	337	3.75	Nadal et al. (2016)
				Portugal	Stomach	32	0.09	Neves et al. (2015)
2.9	Actinopterygii	Clupeidae	*Sardinella gibbosa*	Thailand	Stomach	3	0.30	Azad et al. (2018)
			Sardinella jussieu	Thailand	Stomach	8	1.30	Azad et al. (2018)
		Engraulidae	*Anchoa januaria*	Brazil	GIT	194	0.15	Vendel et al. (2017)
		Leiognathidae	*Eubleekeria splendens*	Thailand	Stomach	10	1.00	Azad et al. (2018)
			Photopectoralis bindus	China	GIT	18	4.10	Jabeen et al. (2017)
2.92	Malacostraca	Penaeidae	*Penaeus semisulcatus*	Persian Gulf	WO	12	7.80	Abbasi et al. (2018)
3	Actinopterygii	Belonidae	*Strongylura marina*	Brazil	GIT	4	0	Vendel et al. (2017)
		Engraulidae	*Coilia nasus*	China	GIT	18	4.00	Jabeen et al. (2017)
				China	GIT	36	0.69	Su et al. (2019)
		Gerreidae	*Diapterus rhombeus*	Brazil	GIT	31	0.03	Vendel et al. (2017)
		Hemiramphidae	*Hyporhamphus roberti roberti*	Brazil	GIT	35	0.03	Vendel et al. (2017)
		Myctophidae	*Benthosema glaciale*	Atlantic Ocean	GIT	27	0.33	Lusher et al. (2016)
			Myctophidae spp.	Global	Stomach	13	0.38	Wagner et al. (2017)
		Sternoptychidae	*Maurolicus muelleri*	Atlantic Ocean	GIT	282	0.03	Lusher et al. (2016)
	Anthozoa	Actiniidae	*Actinia equina*	North Sea	Soft tissue	7	0.41	Karlsson et al. (2017)
	Gastropoda	Turritellidae	*Turritellidae spp.*	North Sea	Soft tissue	10	0.53	Karlsson et al. (2017)
	Polychaeta	Glyceridae	*Glycera alba*	Atlantic Ocean	Soft tissue	1	3.00	Lourenço et al. (2017)
		Onuphidae	*Diopatra neapolitana*	Atlantic Ocean	Soft tissue	4	1.00	Lourenço et al. (2017)
3.07	Malacostraca	Varunidae	*Hemigrapsus sanguineus*	North Sea	Soft tissue	9	0.99	Karlsson et al. (2017)

3.1	Actinopterygii	Ammodytidae	*Ammodytes personatus*	China	GIT	50	0.54	Sun et al. (2019)
		Cepolidae	*Cepola macrophthalma*	UK	GIT	62	2.25	Lusher et al. (2013)
		Clupeidae	*Lile piquitinga*	Brazil	GIT	2	0	Vendel et al. (2017)
			Sardina pilchardus	Ionian Sea	GIT	36	1.80	Digka et al. (2018)
				Mediterranean	GIT	99	1.78	Avio et al. (2015b)
				North Sea	Stomach	3	3.33	Collard et al. (2015)
				Turkey	GIT	7	2.14	Guven et al. (2017)
			Rhinosardinia bahiensis	Brazil	GIT	179	0.19	Vendel et al. (2017)
		Engraulidae	*Anchoviella commersonii*	China	GIT	30	0.40	Sun et al. (2019)
			Engraulis encrasicolus	Mediterranean	Livers	10	0.90	Collard et al. (2017)
				North Sea	Stomach	3	4.30	Collard et al. (2015)
			Engraulis japonicus	China	GIT	80	0.39	Sun et al. (2019)
				Japan	GIT	64	2.30	Tanaka and Takada (2016)
		Gadidae	*Boreogadus saida*	Artic	Stomach	72	0.03	Kühn et al. (2018)
				Artic	GIT	85	0.22	Morgana et al. (2018)
		Lateolabracidae	*Lateolabrax japonicus*	China	GIT	18	2.10	Jabeen et al. (2017)
		Monacanthidae	*Meuschenia scaber*	South Pacific	GIT	19	0.74	Markic et al. (2018)
		Mullidae	*Mullus barbatus*	Ionian Sea	GIT	25	1.50	Digka et al. (2018)
				Mediterranean	GIT	11	1.57	Avio et al. (2015b)
				Mediterranean	GIT	132	0.21	Giani et al. (2019)
				Spain	GIT	128	1.75	Bellas et al. (2016)
				Turkey	GIT	207	1.39	Guven et al. (2017)
		Myctophidae	*Benthosema pterotum*	Red Sea	GIT	10	0.10	Baalkhuyur et al. (2018)
			Symbolophorus californiensis	Pacific Ocean	Stomach	74	7.20	Boerger et al. (2010)
		Pholidae	*Pholis fangi*	China	GIT	79	0.48	Sun et al. (2019)
		Phosichthyidae	*Vinciguerria mabahiss*	Red Sea	GIT	10	0	Baalkhuyur et al. (2018)
		Sciaenidae	*Micropogonias furnieri*	Argentina	GIT	20	12.10	Arias et al. (2019)
				Brazil	GIT	6	0	Pegado et al. (2018)
				Brazil	GIT	1	0	Vendel et al. (2017)
			Cynoscion leiarchus	Brazil	GIT	2	1.00	Pegado et al. (2018)
				Brazil	GIT	9	0	Vendel et al. (2017)

		Sternoptychidae	*Maurolicus mucronatus*	Red Sea	GIT	10	0.10	Baalkhuyur et al. (2018)
3.19	Malacostraca	Crangonidae	*Crangon affinis*	China	Soft tissue	10	29.40	Wang et al. (2019b)
			Crangon crangon	North Sea	Whole animal	165	1.23	Devriese et al. (2015)
				UK	GIT	116	1.00	McGoran et al. (2018)
	Reptilia	Cheloniidae	*Eretmochelys imbricata*	Pacific	GIT	1	2	Duncan et al. (2019)
3.2	Actinopterygii	Alepocephalidae	*Xenodermichthys copei*	Atlantic Ocean	GIT	5	1.20	Lusher et al. (2016)
		Atherinopsidae	*Atherinella brasiliensis*	Brazil	GIT	405	0.04	Vendel et al. (2017)
		Callionymidae	*Callionymus planus*	China	GIT	18	4.80	Jabeen et al. (2017)
		Carangidae	*Trachurus novaezelandiae*	South Pacific	GIT	31	0.03	Markic et al. (2018)
		Engraulidae	*Coilia mystus*	China	GIT	9	0.33	Su et al. (2019)
		Gerreidae	*Eucinostomus argenteus*	Brazil	GIT	98	0.01	Vendel et al. (2017)
		Hemiramphidae	*Hyporhamphus ihi*	South Pacific	GIT	24	0	Markic et al. (2018)
		Myctophidae	*Notoscopelus kroyeri*	Atlantic Ocean	GIT	417	0.16	Lusher et al. (2016)
			Hygophum reinhardtii	Pacific Ocean	Stomach	47	1.30	Boerger et al. (2010)
			Loweina interrupta	Pacific Ocean	Stomach	27	1.00	Boerger et al. (2010)
		Myliobatidae	*Rhinoptera bonasus*	Brazil	GIT	1	0	Pegado et al. (2018)
		Paralepididae	*Arctozenus risso*	Atlantic Ocean	GIT	14	0.29	Lusher et al. (2016)
		Pleuronectidae	*Glyptocephalus cynoglossus*	UK	GIT	23	1.00	McGoran et al. (2018)
			Pleuronectes platessa	Scotland	GIT	62	0.89	Murphy et al. (2017)
				UK	GIT	99	2.52	McGoran et al. (2018)
		Scombridae	*Rastrelliger kanagurta*	Malaysia	Internal cavities	30	0.10	Karami et al. (2017)
		Soleidae	*Solea solea*	Adriatic Sea	GIT	533	8.57	Pellini et al. (2018)
				UK	GIT	18	1.00	McGoran et al. (2018)
		Zoarcidae	*Enchelyopus elongatus*	China	GIT	20	0.80	Sun et al. (2019)
	Elasmobranchii	Narcinidae	*Narcine brasiliensis*	Brazil	GIT	6	0.50	Pegado et al. (2018)
3.3	Actinopterygii	Achiridae	*Achirus declivis*	Brazil	GIT	7	0.14	Vendel et al. (2017)
			Trinectes paulistanus	Brazil	GIT	3	0	Vendel et al. (2017)
		Argentinidae	*Argentina silus*	Scotland	GIT	15	0.07	Murphy et al. (2017)
		Ariidae	*Sciades herzbergii*	Brazil	GIT	57	0.07	Vendel et al. (2017)
		Callionymidae	*Callionymus lyra*	UK	GIT	50	1.80	Lusher et al. (2013)
			C. lyra larvae	UK	GIT	86	0.02	Steer et al. (2017)

		Carangidae	*Trachurus picturatus*	Portugal	Stomach	29	0.03	Neves et al. (2015)
		Chaetodontidae	*Chaetodon austriacus*	Red Sea	GIT	10	0.10	Baalkhuyur et al. (2018)
		Cottidae	*Triglops nybelini*	Artic	GIT	71	0.39	Morgana et al. (2018)
		Engraulidae	*Stolephorus waitei*	Malaysia	Internal cavities	30	0.03	Karami et al. (2017)
		Gobionellinae	*Ctenogobius boleosoma*	Brazil	GIT	27	0.04	Vendel et al. (2017)
		Leiognathidae	*Aurigequula fasciata*	Thailand	Stomach	3	1.30	Azad et al. (2018)
			Leiognathus berbis	Thailand	Stomach	8	0.90	Azad et al. (2018)
		Pleuronectidae	*Platichthys flesus*	North Sea	GIT	36	0.06	Rummel et al. (2016)
				Scotland	GIT	47	1.02	Murphy et al. (2017)
				UK	GIT	126	3.10	McGoran et al. (2018)
		Sciaenidae	*Johnius belangerii*	Malaysia	Internal cavities	30	0.53	Karami et al. (2017)
		Sciaenidae	*Stellifer brasiliensis*	Brazil	GIT	1	0	Vendel et al. (2017)
		Scombridae	*Scomberomorus brasiliensis*	Brazil	GIT	1	0	Pegado et al. (2018)
		Sillaginidae	*Sillago sihama*	China	GIT	18	2.80	Jabeen et al. (2017)
				Persian Gulf	WO	17	14.10	Abbasi et al. (2018)
		Soleidae	*Buglossidium luteum*	UK	GIT	50	1.25	Lusher et al. (2013)
		Soleidae	*Microchirus variegatus*	UK	GIT	51	1.50	Lusher et al. (2013)
			M. variegatus larvae	UK	GIT	16	0.19	Steer et al. (2017)
		Sparidae	*Spondyliosoma cantharus*	Italy	GIT	5	3.65	Avio et al. (2017a)
		Stromateidae	*Pampus argenteus*	China	GIT	18	3.00	Jabeen et al. (2017)
				China	GIT	9	1.10	Su et al. (2019)
				China	GIT	10	0.20	Sun et al. (2019)
3.38	Reptilia	Cheloniidae	*Lepidochelys olivacea*	Pacific	GIT	1	1	Duncan et al. (2019)
3.39	Reptilia	Cheloniidae	*Natator depressus*	Pacific	GIT	4	1.5	Duncan et al. (2019)
3.4	Actinopterygii	Atherinopsidae	*Atherinella blackburni*	Brazil	GIT	2	0	Vendel et al. (2017)
		Carangidae	*Alepes melanoptera*	Thailand	Stomach	8	1.30	Azad et al. (2018)
			Decapterus maruadsi	China	GIT	78	0.41	Sun et al. (2019)
				South Pacific	GIT	25	1.56	Markic et al. (2018)
		Cheilodactylidae	*Nemadactylus macropterus*	South Pacific	GIT	23	0.30	Markic et al. (2018)
		Clupeidae	*Clupea harengus*	North Sea	Stomach	3	4.00	Collard et al. (2015)
				North Sea	GIT	566	0.03	Foekema et al. (2013)

		Engraulidae	*Anchoa marinii*	Brazil	GIT	22	0.04	Vendel et al. (2017)
			Anchoa tricolor	Brazil	GIT	6	0	Vendel et al. (2017)
			Anchovia clupeoides	Brazil	GIT	10	0.10	Vendel et al. (2017)
		Gerreidae	*Eugerres brasilianus*	Brazil	GIT	64	0.04	Vendel et al. (2017)
			Eucinostomus melanopterus	Brazil	GIT	21	0	Vendel et al. (2017)
		Gobiidae	*Ctenogobius stigmaticus*	Brazil	GIT	3	0	Vendel et al. (2017)
		Haemulidae	*Orthopristis chrysoptera*	Gulf of Mexico	Stomach	157	0.54	Peters et al. (2017)
		Hemiramphidae	*Hyporhamphus intermedius*	China	GIT	18	3.70	Jabeen et al. (2017)
		Malacanthidae	*Branchiostegus japonicus*	China	GIT	18	4.60	Jabeen et al. (2017)
		Monacanthidae	*Thamnaconus septentrionalis*	China	GIT	18	7.20	Jabeen et al. (2017)
				China	GIT	9	0.67	Su et al. (2019)
		Myctophidae	*Myctophum aurolanternatum*	Pacific Ocean	Stomach	462	6.00	Boerger et al. (2010)
		Oxudercidae	*Tridentiger barbatus*	China	GIT	8	4.50	Su et al. (2019)
		Pleuronectidae	*Limanda limanda*	North Sea	GIT	89	0.05	Rummel et al. (2016)
				Scotland	GIT	19	0.68	Murphy et al. (2017)
				UK	GIT	308	3.25	McGoran et al. (2018)
		Pleuronectidae	*Cleisthenes herzensteini*	China	GIT	36	0.44	Sun et al. (2019)
		Pristigasteridae	*Opisthopterus tardoore*	Thailand	Stomach	3	2.00	Azad et al. (2018)
		Sciaenidae	*Paralonchurus brasiliensis*	Brazil	GIT	6	0	Pegado et al. (2018)
		Scombridae	*Scomber japonicus*	China	GIT	9	0.78	Su et al. (2019)
				Portugal	Stomach	35	0.57	Neves et al. (2015)
				Turkey	GIT	7	6.71	Guven et al. (2017)
		Scorpaeniformes	*Agonus cataphractus*	UK	GIT	3	0	McGoran et al. (2018)
		Serranidae	*Serranus cabrilla*	Turkey	GIT	6	1.50	Guven et al. (2017)
		Sparidae	*Lithognathus mormyrus*	Turkey	GIT	46	0.63	Guven et al. (2017)
		Tetradontidae	*Sphoeroides testudineus*	Brazil	GIT	55	0.04	Vendel et al. (2017)
3.5	Actinopterygii	Achiridae	*Achirus lineatus*	Brazil	GIT	10	0.20	Vendel et al. (2017)
		Ariidae	*Bagre marinus*	Brazil	GIT	4	7.75	Pegado et al. (2018)
			Cathorops spixii	Brazil	GIT	2	1.00	Vendel et al. (2017)
		Carangidae	*Alepes apercna*	Thailand	Stomach	3	2.00	Azad et al. (2018)
			Alepes kleinii	Thailand	Stomach	4	0.80	Azad et al. (2018)

	Decapterus macrosoma	South Pacific	GIT	25	0.32	Markic et al. (2018)
Cynoglossidae	*Cynoglossus abbreviatus*	China	GIT	18	6.90	Jabeen et al. (2017)
		Persian Gulf	WO	11	12.00	Abbasi et al. (2018)
Drepaneidae	*Drepane longimana*	Thailand	Stomach	3	0.30	Azad et al. (2018)
Elopidae	*Elops saurus*	Brazil	GIT	1	0	Vendel et al. (2017)
Gobiidae	*Gobionellus oceanicus*	Brazil	GIT	6	0	Vendel et al. (2017)
	Microgobius meeki	Brazil	GIT	1	0	Vendel et al. (2017)
Haemulidae	*Genyatremus luteus*	Brazil	GIT	8	0	Pegado et al. (2018)
Holocentridae	*Myripristis spp.*	French Polynesia	GIT	33	0.27	Garnier et al. (2019)
Labridae	*Thalassoma rueppellii*	Red Sea	GIT	12	0.08	Baalkhuyur et al. (2018)
Macrouridae	*Coryphaenoides rupestris*	Scotland	GIT	5	0	Murphy et al. (2017)
Mullidae	*Upeneus pori*	Turkey	GIT	78	0.69	Guven et al. (2017)
	Mullus surmuletus	Mediterranean	GIT	417	0.50	Alomar et al. (2017)
		Portugal	Stomach	4	1.75	Neves et al. (2015)
		Turkey	GIT	51	1.18	Guven et al. (2017)
Muraenesocidae	*Cynoponticus savanna*	Brazil	GIT	1	0	Pegado et al. (2018)
Nemichthyidae	*Nemichthys scolopaceus*	Atlantic Ocean	GIT	1	1.00	Lusher et al. (2016)
Nemipteridae	*Parascolopsis eriomma*	Red Sea	GIT	5	0.60	Baalkhuyur et al. (2018)
Paralichthyidae	*Citharichthys macrops*	Brazil	GIT	7	0	Vendel et al. (2017)
	Etropus crossotus	Brazil	GIT	1	0	Vendel et al. (2017)
Pholidae	*Pholis gunnellus*	UK	GIT	1	0	McGoran et al. (2018)
Sciaenidae	*Bairdiella ronchus*	Brazil	GIT	4	0	Pegado et al. (2018)
	Dendrophysa russelii	Thailand	Stomach	3	0.30	Azad et al. (2018)
	Johnius carouna	Thailand	Stomach	20	3.80	Azad et al. (2018)
	Menticirrhus americanus	Gulf of Mexico	Stomach	150	0.57	Peters et al. (2017)
		Brazil	GIT	1	0	Pegado et al. (2018)
		Brazil	GIT	1	0	Vendel et al. (2017)
Sparidae	*Pagellus erythrinus*	Ionian Sea	GIT	19	1.90	Digka et al. (2018)
	Pagellus erythrinus	Turkey	GIT	54	0.63	Guven et al. (2017)
	Dentex macrophthalmus	Portugal	Stomach	1	1.00	Neves et al. (2015)
Terapontidae	*Terapon theraps*	Thailand	Stomach	5	0.80	Azad et al. (2018)

		Tetradontidae	*Sphoeroides greeleyi*	Brazil	GIT	31	0	Vendel et al. (2017)
	Elasmobranchii	Dasyatidae	*Dasyatis zugei*	Thailand	Stomach	3	0.30	Azad et al. (2018)
3.53	Reptilia	Cheloniidae	*Lepidochelys kempii*	Atlantic	GIT	10	0.5	Duncan et al. (2019)
3.54	Reptilia	Cheloniidae	*Caretta caretta*	Global	GIT	33	2.52	Duncan et al. (2019)
3.6	Actinopterygii	Anguillidae	*Anguilla anguilla*	UK	GIT	2	2.00	McGoran et al. (2018)
			A. anguilla larvae	UK	GIT	1	1.00	Steer et al. (2017)
		Carangidae	*Caranx hippos*	Brazil	GIT	3	30.67	Pegado et al. (2018)
		Cottidae	*Taurulus bubalis*	UK	GIT	3	2.00	McGoran et al. (2018)
		Cynoglossidae	*Cynoglossus robustus*	China	GIT	9	0.67	Su et al. (2019)
			Symphurus tessellatus	Brazil	GIT	26	0.04	Vendel et al. (2017)
		Diodontidae	*Chilomycterus spinosus spinosus*	Brazil	GIT	1	0	Vendel et al. (2017)
		Engraulidae	*Setipinna taty*	China	GIT	20	0.35	Sun et al. (2019)
		Gempylidae	*Thyrsites atun*	South Pacific	GIT	28	0.61	Markic et al. (2018)
		Haemulidae	*Anisotremus surinamensis*	Brazil	GIT	1	0	Pegado et al. (2018)
				Brazil	GIT	1	0	Vendel et al. (2017)
			Anisotremus virginicus	Brazil	GIT	1	0	Pegado et al. (2018)
			Conodon nobilis	Brazil	GIT	8	0	Pegado et al. (2018)
			Haemulopsis corvinaeformis	Brazil	Stomach	44	1.25	Silva et al. (2018)
			Orthopristis ruber	Brazil	GIT	2	0	Pegado et al. (2018)
			Pomadasys corvinaeformis	Brazil	GIT	4	0	Vendel et al. (2017)
		Holocentridae	*Neoniphon sammara*	Red Sea	GIT	5	0.20	Baalkhuyur et al. (2018)
			Sargocentron spiniferum	Red Sea	GIT	5	0	Baalkhuyur et al. (2018)
		Mullidae	*Upeneus moluccensis*	Turkey	GIT	18	0.78	Guven et al. (2017)
		Oxudercidae	*Synechogobius ommaturus*	China	GIT	17	3.70	Su et al. (2019)
		Paralichthyidae	*Citharichthys spilopterus*	Brazil	GIT	12	0	Vendel et al. (2017)
		Platycephalidae	*Platycephalus indicus*	Persian Gulf	WO	12	21.80	Abbasi et al. (2018)
		Priacanthidae	*Heteropriacanthus cruentatus*	South Pacific	GIT	10	0.30	Markic et al. (2018)
		Sciaenidae	*Collichthys lucidus*	China	GIT	18	6.20	Jabeen et al. (2017)
				China	GIT	26	1.20	Su et al. (2019)
		Scombridae	*Scomber scombrus*	North Sea	GIT	51	0.03	Rummel et al. (2016)
				Portugal	Stomach	12	0.46	Neves et al. (2015)

				UK	GIT	31	0.58	Nelms et al. (2018)
		Sparidae	*Diplodus annularis*	Turkey	GIT	48	1.96	Guven et al. (2017)
			Pagrus auratus	South Pacific	GIT	22	0.05	Markic et al. (2018)
		Tetraodontidae	*Colomesus psittacus*	Brazil	GIT	2	0	Pegado et al. (2018)
				Brazil	GIT	5	0	Vendel et al. (2017)
		Terapontidae	*Pelates quadrilineatus*	Turkey	GIT	135	1.48	Guven et al. (2017)
	Elasmobranchii	Triakidae	*Mustelus canis*	Brazil	GIT	2	0	Pegado et al. (2018)
			Mustelus higmani	Brazil	GIT	3	0	Pegado et al. (2018)
3.7	Actinopterygii	Apogonidae	*Apogon lineatus*	China	GIT	20	0.40	Sun et al. (2019)
		Batrachoididae	*Batrachoides surinamensis*	Brazil	GIT	1	0	Pegado et al. (2018)
		Carangidae	*Trachurus trachurus*	North Sea	GIT	100	0.01	Foekema et al. (2013)
				Portugal	Stomach	44	0.07	Neves et al. (2015)
				Scotland	GIT	5	0	Murphy et al. (2017)
				UK	GIT	56	1.50	Lusher et al. (2013)
			Selene setapinnis	Brazil	GIT	1	0	Pegado et al. (2018)
			Alepes vari	Thailand	Stomach	3	1.70	Azad et al. (2018)
		Engraulidae	*Lycengraulis grossidens*	Brazil	GIT	146	0.18	Vendel et al. (2017)
		Gadidae	*Trisopterus minutus*	UK	GIT	50	2.00	Lusher et al. (2013)
				UK	GIT	1	0	McGoran et al. (2018)
			T. minutus larvae	UK	GIT	53	0.02	Steer et al. (2017)
			Trisopterus luscus	UK	GIT	7	1.00	McGoran et al. (2018)
		Gobiidae	*Bathygobius soporator*	Brazil	GIT	6	0	Vendel et al. (2017)
		Haemulidae	*Haemulon steindachneri*	Brazil	GIT	5	0	Pegado et al. (2018)
		Lethrinidae	*Gnathodentex aureolineatus*	South Pacific	GIT	29	0.07	Markic et al. (2018)
		Polynemidae	*Polydactylus oligodon*	Brazil	GIT	1	3.00	Pegado et al. (2018)
			Polydactylus virginicus	Brazil	GIT	13	0	Pegado et al. (2018)
				Brazil	GIT	14	0	Vendel et al. (2017)
		Sciaenidae	*Johnius borneensis*	Thailand	Stomach	3	1.00	Azad et al. (2018)
			Larimichthys crocea	China	GIT	18	4.60	Jabeen et al. (2017)
			Larimichthys polyactis	China	GIT	30	0.97	Sun et al. (2019)
		Scomberesocidae	*Cololabis saira*	Pacific Ocean	Stomach	54	3.20	Boerger et al. (2010)

		Sebastidae	*Sebastiscus marmoratus*	China	GIT	18	4.20	Jabeen et al. (2017)
		Serranidae	*Epinephelus areolatus*	Red Sea	GIT	5	0.20	Baalkhuyur et al. (2018)
		Sparidae	*Sparus aurata*	Turkey	GIT	110	0.87	Guven et al. (2017)
		Triglidae	*Chelidonichthys kumu*	China	GIT	177	0.45	Sun et al. (2019)
				South Pacific	GIT	27	0.07	Markic et al. (2018)
			Trigla lyra	Portugal	Stomach	31	0.26	Neves et al. (2015)
3.8	Actinopterygii	Carangidae	*Trachurus mediterraneus*	Turkey	GIT	98	1.77	Guven et al. (2017)
			Oligoplites saliens	Brazil	GIT	2	0	Vendel et al. (2017)
		Haemulidae	*Haemulon plumierii*	Brazil	GIT	13	0	Pegado et al. (2018)
			Pomadasys incisus	Turkey	GIT	29	0.79	Guven et al. (2017)
		Hexagrammidae	*Hexagrammos otakii*	China	GIT	40	0.38	Sun et al. (2019)
		Lethrinidae	*Gymnocranius grandoculis*	Red Sea	GIT	10	0.20	Baalkhuyur et al. (2018)
		Lethrinidae	*Lethrinus microdon*	Red Sea	GIT	10	0.20	Baalkhuyur et al. (2018)
		Lutjanidae	*Lutjanus alexandrei*	Brazil	GIT	1	0	Vendel et al. (2017)
			Lutjanus synagris	Brazil	GIT	2	0	Vendel et al. (2017)
				Brazil	GIT	2	0.50	Pegado et al. (2018)
			Pristipomoides multidens	Red Sea	GIT	10	0.20	Baalkhuyur et al. (2018)
		Nemipteridae	*Nemipterus randalli*	Turkey	GIT	135	1.31	Guven et al. (2017)
		Sciaenidae	*Cynoscion jamaicensis*	Brazil	GIT	3	0	Pegado et al. (2018)
			Sciaena umbra	Turkey	GIT	1	3.00	Guven et al. (2017)
		Serranidae	*Epinephelus merra*	French Polynesia	GIT	33	0.39	Garnier et al. (2019)
			Rypticus randalli	Brazil	GIT	1	0	Vendel et al. (2017)
		Sparidae	*Pagellus acarne*	Portugal	Stomach	1	1.00	Neves et al. (2015)
		Sparidae	*Pagellus acarne*	Turkey	GIT	52	1.63	Guven et al. (2017)
		Triglidae	*Chelidonichthys cuculus*	UK	GIT	55	2.00	Lusher et al. (2013)
				UK	GIT	6	0	McGoran et al. (2018)
			Prionotus punctatus	Brazil	GIT	1	0	Vendel et al. (2017)
	Elasmobranchii	Rajidae	*Raja asterias*	Portugal	Stomach	7	0.57	Neves et al. (2015)
			Raja clavata	UK	GIT	7	1.00	McGoran et al. (2018)
		Scyliorhinidae	*Scyliorhinus canicula*	Portugal	Stomach	20	0.40	Neves et al. (2015)
				Spain	GIT	72	1.20	Bellas et al. (2016)

				UK	GIT	8	1.50	McGoran et al. (2018)
3.9	Actinopterygii	Butidae	*Oxyeleotris marmorata*	China	GIT	18	4.20	Jabeen et al. (2017)
		Carangidae	*Megalaspis cordyla*	Thailand	Stomach	29	1.60	Azad et al. (2018)
		Exocoetidae	*Cheilopogon pitcairnensis*	South Pacific	GIT	21	0.10	Markic et al. (2018)
		Lethrinidae	*Lethrinus obsoletus*	South Pacific	GIT	30	0.17	Markic et al. (2018)
		Lutjanidae	*Lipocheilus carnolabrum*	Red Sea	GIT	7	0.29	Baalkhuyur et al. (2018)
			Lutjanus analis	Brazil	GIT	3	0.33	Pegado et al. (2018)
			Lutjanus kasmira	Red Sea	GIT	12	0.17	Baalkhuyur et al. (2018)
		Sciaenidae	*Ctenosciaena gracilicirrhus*	Brazil	GIT	11	0	Pegado et al. (2018)
			Macrodon ancylodon	Brazil	GIT	13	0.15	Pegado et al. (2018)
		Scombridae	*Scomber colias*	Canary Islands	GIT	120	2.17	Herrera et al. (2019)
		Scorpaeniformes	*Myoxocephalus scorpius*	UK	GIT	5	0	McGoran et al. (2018)
		Sparidae	*Pagrus pagrus*	Turkey	GIT	9	1.44	Guven et al. (2017)
		Terapontidae	*Terapon jarbua*	China	GIT	18	2.00	Jabeen et al. (2017)
	Elasmobranchii	Sphyrnidae	*Sphyrna tiburo*	Brazil	GIT	2	9.00	Pegado et al. (2018)
4	Actinopterygii	Achiridae	*Achirus achirus*	Brazil	GIT	3	0	Vendel et al. (2017)
		Ariidae	*Bagre bagre*	Brazil	GIT	7	9.14	Pegado et al. (2018)
			Notarius grandicassis	Brazil	GIT	4	0	Pegado et al. (2018)
		Carangidae	*Caranx papuensis*	South Pacific	GIT	32	1.03	Markic et al. (2018)
		Centrolophidae	*Psenopsis anomala*	China	GIT	18	1.10	Jabeen et al. (2017)
				China	GIT	10	0.20	Sun et al. (2019)
		Clupeidae	*Alosa fallax*	Portugal	Stomach	1	1.00	Neves et al. (2015)
		Exocoetidae	*Cheilopogon simus*	French Polynesia	GIT	34	0.24	Garnier et al. (2019)
		Gadidae	*Melanogrammus aeglefinus*	North Sea	GIT	97	0.08	Foekema et al. (2013)
				UK	GIT	6	5.83	McGoran et al. (2018)
		Haemulidae	*Plectorhinchus gaterinus*	Red Sea	GIT	6	0.33	Baalkhuyur et al. (2018)
		Lethrinidae	*Lethrinus amboinensis*	South Pacific	GIT	26	0.38	Markic et al. (2018)
		Ophichthidae	*Ophichthus cylindroideus*	Brazil	GIT	1	0	Pegado et al. (2018)
				Brazil	GIT	1	0	Vendel et al. (2017)
		Pleuronectidae	*Hippoglossus hippoglossus*	Scotland	GIT	14	0	Murphy et al. (2017)
		Priacanthidae	*Priacanthus arenatus*	Brazil	GIT	122	1.72	Cardozo et al. (2018)

		Rachycentridae	*Rachycentron canadum*	Brazil	GIT	1	0	Pegado et al. (2018)
		Sciaenidae	*Micropogonias undulatus*	Gulf of Mexico	Stomach	383	0.87	Peters et al. (2017)
			Cynoscion microlepidotus	Brazil	GIT	16	0.25	Pegado et al. (2018)
			Cynoscion virescens	Brazil	GIT	7	0.43	Pegado et al. (2018)
		Scophthalmidae	*Phrynorhombus norvegicus*	UK	GIT	1	0	McGoran et al. (2018)
		Serranidae	*Epinephelus chlorostigma*	Red Sea	GIT	3	0.33	Baalkhuyur et al. (2018)
			Epinephelus epistictus	Red Sea	GIT	5	0.20	Baalkhuyur et al. (2018)
			Epinephelus radiatus	Red Sea	GIT	7	0.14	Baalkhuyur et al. (2018)
		Stomiidae	*Stomias boa boa*	Atlantic Ocean	GIT	5	0.80	Lusher et al. (2016)
			Astronesthes indopacificus	Pacific Ocean	Stomach	7	1.00	Boerger et al. (2010)
		Triglidae	*Chelidonichthys lucerna*	Mediterranean	GIT	3	1.00	Avio et al. (2015b)
				Turkey	GIT	24	0.75	Guven et al. (2017)
4.1	Actinopterygii	Aploactinidae	*Erisphex pottii*	China	GIT	120	0.32	Sun et al. (2019)
		Bramidae	*Brama brama*	Portugal	Stomach	3	0.67	Neves et al. (2015)
		Carangidae	*Caranx crysos*	Turkey	GIT	1	5.00	Guven et al. (2017)
				Brazil	GIT	3	0	Pegado et al. (2018)
		Carangidae	*Scomberoides tol*	Thailand	Stomach	3	2.20	Azad et al. (2018)
		Gadidae	*Gadus morhua*	North Sea	GIT	80	0.14	Foekema et al. (2013)
				North Sea	GIT	81	0.01	Rummel et al. (2016)
				UK	GIT	3	0	McGoran et al. (2018)
			Micromesistius poutassou	Scotland	GIT	20	0	Murphy et al. (2017)
				UK	GIT	27	2.00	Lusher et al. (2013)
		Lophiidae	*Lophius litulon*	China	GIT	20	0.25	Sun et al. (2019)
		Lutjanidae	*Lutjanus gibbus*	South Pacific	GIT	29	0.41	Markic et al. (2018)
		Muraenidae	*Gymnothorax ocellatus*	Brazil	GIT	1	0	Pegado et al. (2018)
		Pleuronectidae	*Hippoglossoides platessoides*	UK	GIT	104	2.67	McGoran et al. (2018)
		Sciaenidae	*Cynoscion acoupa*	Brazil	GIT	552	1.91	Ferreira et al. (2018)
		Serranidae	*Epinephelus itajara*	Brazil	GIT	2	0	Pegado et al. (2018)
		Sparidae	*Dentex gibbosus*	Turkey	GIT	14	0.29	Guven et al. (2017)
		Trichiuridae	*Eupleurogrammus muticus*	China	GIT	15	0.33	Sun et al. (2019)
4.2	Actinopterygii	Carangidae	*Caranx latus*	Brazil	GIT	57	0	Vendel et al. (2017)

			Seriola lalandi	South Pacific	GIT	15	0.27	Markic et al. (2018)
		Centrolophidae	*Schedophilus velaini*	South Pacific	GIT	14	1.43	Markic et al. (2018)
		Centropomidae	*Centropomus ensiferus*	Brazil	GIT	1	0	Vendel et al. (2017)
			Centropomus undecimalis	Brazil	GIT	2	0	Vendel et al. (2017)
		Gadidae	*Gadus macrocephalus*	China	GIT	40	0.43	Sun et al. (2019)
		Lutjanidae	*Pristipomoides typus*	Red Sea	GIT	5	0	Baalkhuyur et al. (2018)
		Sciaenidae	*Cynoscion arenarius*	Gulf of Mexico	Stomach	139	0.79	Peters et al. (2017)
		Synodontidae	*Harpadon nehereus*	China	GIT	18	3.80	Jabeen et al. (2017)
				China	GIT	18	2.50	Su et al. (2019)
	Elasmobranchii	Pentanchidae	*Galeus melastomus*	Mediterranean	Stomach	21	0.34	Alomar and Deudero (2017)
4.24	Reptilia	Cheloniidae	*Dermochelys coriacea*	Atlantic	GIT	2	1.5	Duncan et al. (2019)
4.25	Actinopterygii	Serranidae	*Plectropomus spp.**	Australia	GIT	20	5.80	Kroon et al. (2018a)
4.27	Mammalia	Balaenopteridae	*Megaptera novaeangliae*	Netherlands	GIT	1	16.00	Besseling et al. (2015)
4.3	Actinopterygii	Ariidae	*Cathorops agassizii*	Brazil	GIT	1	0	Vendel et al. (2017)
		Carangidae	*Oligoplites palometa*	Brazil	GIT	8	0	Vendel et al. (2017)
			Oligoplites saurus	Brazil	GIT	22	0.05	Vendel et al. (2017)
			Selene vomer	Brazil	GIT	2	1.00	Pegado et al. (2018)
				Brazil	GIT	2	0	Vendel et al. (2017)
		Gadidae	*Pollachius pollachius*	Scotland	GIT	5	0	Murphy et al. (2017)
		Liparidae	*Liparis tanakae*	China	GIT	245	0.36	Sun et al. (2019)
		Phycidae	*Phycis phycis*	Italy	GIT	7	3.38	Avio et al. (2017b)
		Sciaenidae	*Argyrosomus regius*	Portugal	Stomach	5	0.80	Neves et al. (2015)
				Turkey	GIT	51	1.84	Guven et al. (2017)
		Scombridae	*Scomberomorus guttatus*	Thailand	Stomach	5	0.60	Azad et al. (2018)
			Thunnus alalunga	Mediterranean	Stomach	31	0.13	Romeo et al. (2015)
		Scophthalmidae	*Lepidorhombus whiffiagonis*	Scotland	GIT	10	0.10	Murphy et al. (2017)
4.36	Mammalia	Delphinidae	*Grampus griseus*	UK	GIT	1	9.00	Nelms et al. (2019a)
4.37	Mammalia	Delphinidae	*Lagenorhynchus albirostris*	UK	GIT	1	3.00	Nelms et al. (2019a)
4.4	Actinopterygii	Coryphaenidae	*Coryphaena hippurus*	South Pacific	GIT	10	0.40	Markic et al. (2018)
		Gadidae	*Merlangius merlangus*	North Sea	GIT	105	0.10	Foekema et al. (2013)
				UK	GIT	50	1.75	Lusher et al. (2013)

				UK	GIT	29	4.67	McGoran et al. (2018)
		Lotidae	*Molva molva*	Scotland	GIT	5	0	Murphy et al. (2017)
		Lutjanidae	*Lutjanus jocu*	Brazil	GIT	4	0	Vendel et al. (2017)
		Merlucciidae	*Merluccius merluccius*	Mediterranean	GIT	3	1.33	Avio et al. (2015b)
				Mediterranean	GIT	97	0.38	Giani et al. (2019)
				Mediterranean	Stomach	67	0.46	Mancuso et al. (2019)
				Portugal	Stomach	12	0.35	Neves et al. (2015)
				Spain	GIT	12	1.00	Bellas et al. (2016)
		Muraenesocidae	*Muraenesox cinereus*	China	GIT	18	2.40	Jabeen et al. (2017)
		Salmonidae	*Oncorhynchus tshawytscha*	Canada	GIT	74	1.15	Collicutt et al. (2019)
		Scombridae	*Katsuwonus pelamis*	South Pacific	GIT	26	0.35	Markic et al. (2018)
			Thunnus albacares	South Pacific	GIT	68	0.68	Markic et al. (2018)
		Sparidae	*Lagodon rhomboides*	Gulf of Mexico	Stomach	449	0.96	Peters et al. (2017)
		Sphyrnidae	*Sphyraena forsteri*	South Pacific	GIT	12	0.25	Markic et al. (2018)
		Synodontidae	*Saurida tumbil*	Persian Gulf	WO	4	13.50	Abbasi et al. (2018)
		Trichiuridae	*Trichiurus lepturus*	Brazil	GIT	5	0.40	Pegado et al. (2018)
		Uranoscopidae	*Uranoscopus scaber*	Italy	GIT	7	3.20	Avio et al. (2017a)
	Chondrichthyes	Squalidae	*Squalus acanthias*	Mediterranean	GIT	9	1.25	Avio et al. (2015b)
	Elasmobranchii	Carcharhinidae	*Prionace glauca*	Mediterranean	Stomach	139	0.20	Bernardini et al. (2018)
	Mammalia	Phocoenidae	*Neophocaena phocaenoides*	China	GIT	7	19.14	Xiong et al. (2018)
4.44	Mammalia	Kogiidae	*Kogia breviceps*	UK	GIT	1	4.00	Nelms et al. (2019a)
4.47	Mammalia	Delphinidae	*Stenella coeruleoalba*	UK	GIT	1	7.00	Nelms et al. (2019a)
4.48	Mammalia	Phocoenidae	*Phocoena phocoena*	UK	GIT	21	5.24	Nelms et al. (2019a)
4.49	Mammalia	Phocidae	*Halichoerus grypus*	UK	Scat	31	0.84	Nelms et al. (2018)
				UK	GIT	3	6.00	Nelms et al. (2019a)
4.5	Actinopterygii	Belonidae	*Strongylura timucu*	Brazil	GIT	2	0	Vendel et al. (2017)
		Carangidae	*Scomberoides tala*	Thailand	Stomach	3	0.70	Azad et al. (2018)
		Clupeidae	*Opisthonema oglinum*	Brazil	GIT	56	0.23	Vendel et al. (2017)
		Ephippidae	*Chaetodipterus faber*	Brazil	GIT	6	0.17	Vendel et al. (2017)
				Brazil	GIT	5	0	Pegado et al. (2018)
				Gulf of Mexico	Stomach	103	1.38	Peters et al. (2017)

	Lophiidae	*Lophius piscatorius*	Portugal	Stomach	2	0.50	Neves et al. (2015)
	Ophichthidae	*Ophichthus ophis*	Brazil	GIT	1	0	Pegado et al. (2018)
	Pomatomidae	*Pomatomus saltatrix*	Brazil	GIT	2	0	Pegado et al. (2018)
	Scombridae	*Scomberomorus commerson*	Thailand	Stomach	4	4.30	Azad et al. (2018)
		Thunnus thynnus	Mediterranean	Stomach	36	0.44	Romeo et al. (2015)
	Serranidae	*Cephalopholis argus*	Red Sea	GIT	4	0	Baalkhuyur et al. (2018)
	Stromateidae	*Peprilus paru*	Brazil	GIT	2	0	Pegado et al. (2018)
	Synodontidae	*Saurida undosquamis*	Turkey	GIT	99	1.22	Guven et al. (2017)
	Trichiuridae	*Aphanopus carbo*	Scotland	GIT	5	0	Murphy et al. (2017)
	Xiphiidae	*Xiphias gladius*	Mediterranean	Stomach	56	0.16	Romeo et al. (2015)
	Zeidae	*Zeus faber*	Portugal	Stomach	1	1.00	Neves et al. (2015)
			UK	GIT	42	2.70	Lusher et al. (2013)
Mammalia	Delphinidae	*Lagenorhynchus acutus*	UK	GIT	1	8.00	Nelms et al. (2019a)
		Tursiops truncatus	UK	GIT	1	6.00	Nelms et al. (2019a)
		Delphinus delphis	Spain	Stomach	35	11.74	Hernandez-Gonzalez et al. (2018)
			UK	GIT	16	5.69	Nelms et al. (2019a)
		Sousa chinensis	China	GIT	3	25.67	Zhu et al. (2019a)
	Otariidae	*Arctocephalus australis*	Chile	Scat	51	24.86	Perez-Venegas et al. (2018)
		Callorhinus ursinus	USA	Scat	44	13.27	Donohue et al. (2019)
	Phocidae	*Phoca vitulina*	UK	GIT	4	4.25	Nelms et al. (2019a)
	Ziphiidae	*Mesoplodon mirus*	Ireland	GIT	3	29.33	Lusher et al. (2015)

Data has been grouped into trophic levels based on the well-established FishBase (Froese and Pauly, 2010) and SeaLifeBase (Palomares and Pauly, 2010) databases and organised by class and family, with sample sizes (n) given. WO: Whole organism. [a]Wild caught bivalves only. **Plectropomus spp.* refers to *P. leopardus* (trophic level = 4.4) and *P. maculatus* (trophic level = 4.11) examined by Kroon et al. (2018a); Trophic level given is an average of the two species (trophic level = 4.25). Common names given in original manuscript (doi: 10.1371/journal.pone.0240792).

(b) Calculations for standardisation of MP contamination data

Trophic Level	Class	Family	*Species*	Average Reported (MPs g^{-1})	Soft Tissue Weight (g $individual^{-1}$)	Calculated Abundance (MPs $individual^{-1}$)	Reference
2	Bivalvia	Arcidae	*Scapharca subcrenata*	10.50	4.43	46.52	Li et al. (2015)
			Tegillarca granosa	4.00	1.29	5.16	Li et al. (2015)
		Mytilidae	*Mytilus edulis*	2.20	1.79	3.94	Li et al. (2016b)
				0.13	5.74	0.73	Vandermeersch et al. (2015)
		Ostreidae	*Alectryonella plicatula*	5.50	1.84	10.12	Li et al. (2015)
			Crassostrea gigas	0.08	2.25	0.18	Bonello et al. (2018)
		Pectinidae	*Mizuhopecten yessoensis*	2.30	24.80	57.04	Li et al. (2015)
		Pharidae	*Sinonovacula constricta*	2.00	7.53	15.06	Li et al. (2015)
		Veneridae	*Cyclina sinensis*	4.00	1.19	4.76	Li et al. (2015)
			Meretrix lusoria	4.20	2.33	9.79	Li et al. (2015)
			Ruditapes philippinarum	0.90	10.00	9.00	Davidson and Dudas (2016)
				2.50	2.21	5.53	Li et al. (2015)
2.69	Bivalvia	Mytilidae	*Mytilus galloprovincialis*	2.50	1.79	4.48	Li et al. (2015)
				0.15	5.15	0.76	Vandermeersch et al. (2015)

Data presented here is included in A1.3a table (above).

Table A9.4 Bioaccumulation of chemical additives associated with microplastic (MP) intake for marine organisms collected in situ. *Chemical additive concentrations per individual (i.e., body burden) were estimates for each species, with concentrations representative of MPs per number of total organisms in the sample size for a particular species, rather than taken from only the number of organisms that exhibited MP contamination.*

Trophic Level	Class	Family	*Species*	Location	Area Investigated	n	MP Ind^{-1}	Chemical Additives	Chemical Concentration Reported (ng g^{-1})	Reference
2	Bivalvia	Cardiidae	*Cerastoderma edule*	France	Soft tissue	50	5.72	∑PAH	0.06	Hermabessiere et al. (2019)
								∑PBDE	0.70	Hermabessiere et al. (2019)
								∑PCB	0	Hermabessiere et al. (2019)
								∑Phthalates	52.36	Hermabessiere et al. (2019)

		Mytilidae	*Mytilus edulis*	France	Soft tissue	50	5.88	∑PAH	5.48	Hermabessiere et al. (2019)
								∑PBDE	0.07	Hermabessiere et al. (2019)
								∑PCB	1	Hermabessiere et al. (2019)
								∑Phthalates	26.36	Hermabessiere et al. (2019)
2.2	Ascidiacea	Pyuridae	*Microcosmus exasperatus*	Israel	Soft tissue	15	1.78	DBP	1,643 – 2,224	Vered et al. (2019)
								DEHP	4,851 – 4,988	Vered et al. (2019)
								DnOP	0	Vered et al. (2019)

Sampling locations and sizes (n) have been included. Data has been grouped into trophic levels based on the well-established SeaLifeBase database (Palomares and Pauly, 2010). PAH = polycyclic aromatic hydrocarbons; PBDE = polybrominated diphenyl ethers; PCB = polychlorinated biphenyls; DBP = dibutyl phthalate; DEHP = bis(2-ethylhexyl) phthalate; DnOP = di-n-octylphthalate.

Table A9.5 Experimental details of microplastic (MP) laboratory exposures conducted with marine organisms. Contamination and retention, following specific exposures to MPs under controlled laboratory conditions, are presented for each species, and organised by trophic levels.

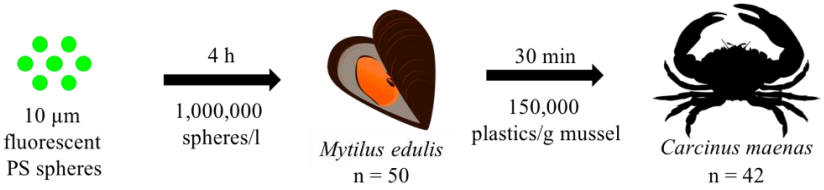
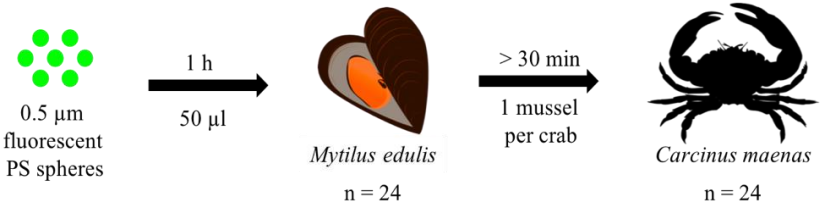
			Exposure Details								
TL	*Species*	n	Size (µm), Colour, Polymer, Shape	Time	Dose	Conc. (MPs ml^{-1})	Depuration Period	Area	MPs Ind^{-1}	Retention	Reference
2	*Mytilus edulis*	16	23 x 3000, NM, PP, fibres	1 h	Once	0.1 [1]	-	ST	0.5	-	Porter et al. (2018)
		16	23 x 3000, NM, PP, fibres	1 h	Once	0.1 [2]	-	ST	6.5	-	Porter et al. (2018)
		9	7-30, NM, PS, beads	1 h	Once	50 [3]	-	ST	340	-	Porter et al. (2018)
		9	7-30, NM, PS, beads	1 h	Once	50 [2]	-	ST	105,000	-	Porter et al. (2018)
		9	9-11, NM, PE, beads	1 h	Once	50 [2]	-	ST	130,000	-	Porter et al. (2018)
		9	7-30, NM, PS, beads	1 h	Once	50 [1]	-	ST	150,000	-	Porter et al. (2018)
		9	9-11, NM, PE, beads	1 h	Once	50 [1]	-	ST	200,000	-	Porter et al. (2018)
		5	100-3500, Black, Mix, fibres	5 d	Daily	100	-	ST	5.25	-	Qu et al. (2018)
		5	100, Black, Mix, fibres	5 d	Daily	1,000	-	ST	11.2	-	Qu et al. (2018)
		5	20-500, Pink, PVC, fragments	5 d	Daily	100	-	ST	1.5	-	Qu et al. (2018)
		5	20-500, Pink, PVC, fragments	5 d	Daily	1,000	-	ST	3	-	Qu et al. (2018)
		5	10, Green, PS, beads	5 d	Daily	100	-	ST	2.8	-	Qu et al. (2018)
		5	10, Green, PS, beads	5 d	Daily	1,000	-	ST	12	-	Qu et al. (2018)

	Evadne nordmanni	7 - 10	10, Flu, PS, beads	3 h	Once	2,000	-	GIT	0	-	Setala et al. (2014)
	Tripneustes gratilla	25	10-45, Flu, PE, beads	5 d	Once	300	420 h	STO	1.8	< 2 d	Kaposi et al. (2014)
	Brachionus koreanus	50	0.05-6, Flu, PS, beads	24 h	Once	0.00001 [a]	-	GIT	0	-	Jeong et al. (2016)
	Acartia longiremis	9	30, Flu, PS, fragments	24 h	Once	0.028	-	GIT	0	-	Vroom et al. (2017)
	Calanus helgolandicus	50	20, Flu, PS, beads	24 h	Once	75	-	Tank	3,278	-	Cole et al. (2015)
		50	10x30, NM, PA, fibres	6 h	Once	80	-	Tank	104,100	-	Procter et al. (2019)
	Allorchestes compressa	15	11-700, PE, fragments	72 h	Once	0.1 [a]	36 h	WO	18.8	< 0.5 MPs ind^{-1} at 36 h	Chua et al. (2014)
2.2	*Atactodea striata*	NM	63-250, NM, PS, beads	10 d	Daily	1	7 d	Faeces	3.5	NM	Xu et al. (2017)
	Ennucula tenuis	6	125-500 ,NM, PE, fragments	4 w	Once	1 – 25 [c]	-	ST	0.83	-	Bour et al. (2018b)
	Limecola balthica	54	10, Flu, PS, beads	24 h	Once	5 - 250	-	GIT	8.1	-	Setala et al. (2016)
	Mytilus trossulus	54	10, Flu, PS, beads	24 h	Once	5 - 250	-	GIT	27.3	-	Setala et al. (2016)
2.5	*Idotea emarginata*	24	< 100, Flu, PS, fragments	72 h	Once	0.02 – 0.35 [b]	-	GIT	41.67	-	Hamer et al. (2014)
		24	10, Flu, PS, beads	72 h	Once	0.012 – 0.12 [b]	-	GIT	20.8	-	Hamer et al. (2014)
2.69	*Mytilus galloprovincialis*	150	3, Flu, PS, beads	24 h	Once	10,000	8 d	GIT	14.2	8 h to 8 d	Capolupo et al. (2018)
2.7	*Acanthochromis polyacanthus*	112	1-2, NM, PET, beads	7 d	2x Daily	0.05 - 0.13 [d]	-	GIT	2,102*	> 6 w	Critchell and Hoogenboom (2018)
2.71	*Palaemonetes pugio*	20	93, White, PP, Fibres	3 h	Once	50	96 h	GIT	2.3	NM	Gray and Weinstein (2017)
		20	34, White, PP, Fibres	3 h	Once	50	96 h	GIT	10	NM	Gray and Weinstein (2017)
		20	93, White, PP, fragments	3 h	Once	50	96 h	GIT	22	NM	Gray and Weinstein (2017)
		20	34, White, PP, fragments	3 h	Once	50	96 h	GIT	23	NM	Gray and Weinstein (2017)
		20	116, Flu, PE, beads	3 h	Once	50	96 h	GIT	3	27.6 ± 8.57 h	Gray and Weinstein (2017)
		20	35, Flu, PE, beads	3 h	Once	50	96 h	GIT	3.5	NM	Gray and Weinstein (2017)
		20	165, Flu, PE, beads	3 h	Once	50	96 h	GIT	5	NM	Gray and Weinstein (2017)
		20	83, Flu, PE, beads	3 h	Once	50	96 h	GIT	7.5	NM	Gray and Weinstein (2017)
		20	59, Flu, PE, beads	3 h	Once	50	96 h	GIT	8	NM	Gray and Weinstein (2017)
		20	30, OP, PS, beads	3 h	Once	50	96 h	GIT	10	60.6 ± 28.5 h	Gray and Weinstein (2017)
		20	45, OP, PS, beads	3 h	Once	50	96 h	GIT	28.8	75.9 ± 13.3 h	Gray and Weinstein (2017)
2.8	*Abra nitida*	2	125-500, NM, PE, fragments	4 w	Once	1 – 15 [c]	-	ST	1.5	-	Bour et al. (2018b)
2.87	*Minuca rapax*	7	180-250, NM, PS, fragments	2 mths	Once	1,000 [c]	-	STO	0.14	-	Brennecke et al. (2015)
		27	180-250, NM, PS, fragments	2 mths	Once	108 [c]	-	STO	0.37	-	Brennecke et al. (2015)
3	*Arenicola marina*	80	400-1300, Clear, PS, crystals	28 d	Once	0.001 – 0.1 [a]	-	GIT, Faeces	1.36	-	Besseling et al. (2013)

3.5	*Dicentrarchus labrax*	120	10-45, Flu, PE, beads	36 d	Daily	1.2 [e]	2 d	WO	1.4	< 2 d	Mazurais et al. (2015)
		120	10-45, Flu, PE, beads	36 d	Daily	1.2 [e]	2 d	WO	3.3	< 2 d	Mazurais et al. (2015)
	Seriolella violacea	33	1200, Clear, PA fragments	5 min	Once	$6.7 \cdot 10^{-5}$	10 w	Video	0.5	4.4 ± 0.9 d	Ory et al. (2018b)
		33	1200, Yellow, PA fragments	5 min	Once	$6.7 \cdot 10^{-5}$	10 w	Video	0.6	4.4 ± 0.9 d	Ory et al. (2018b)
		33	1200, Blue, PA fragments	5 min	Once	$6.7 \cdot 10^{-5}$	10 w	Video	0.8	4.4 ± 0.9 d	Ory et al. (2018b)
		33	1200, Black, PA fragments	5 min	Once	$6.7 \cdot 10^{-5}$	10 w	Video	1.1	4.4 ± 0.9 d	Ory et al. (2018b)
3.7	*Sparus aurata*	15	75.6, OP, PVC, fragments	45 d	Daily	3,330 [c]	30 d	INT	0.07	> 30 d	Jovanovic et al. (2018)
		15	75.6, OP, PVC, fragments	45 d	Daily	3,330 [c]	30 d	STO	0	-	Jovanovic et al. (2018)
		15	23.4, OP, HDPE, fragments	45 d	Daily	3,330 [c]	30 d	INT	1.67	> 30 d	Jovanovic et al. (2018)
		15	23.4, OP, HDPE, fragments	45 d	Daily	3,330 [c]	30 d	STO	1.8	> 30 d	Jovanovic et al. (2018)
		15	51, OP, PS, fragments	45 d	Daily	3,330 [c]	30 d	INT	1.8	> 30 d	Jovanovic et al. (2018)
		15	51, OP, PS, fragments	45 d	Daily	3,330 [c]	30 d	STO	2.07	> 30 d	Jovanovic et al. (2018)
		15	11.7, OP, PA, fragments	45 d	Daily	3,330 [c]	30 d	STO	2.13	> 30 d	Jovanovic et al. (2018)
		15	54.5, OP, MDPE, fragments	45 d	Daily	3,330 [c]	30 d	STO	2.47	> 30 d	Jovanovic et al. (2018)
		15	87.6, OP, LDPVC, fragments	45 d	Daily	3,330 [c]	30 d	STO	5.4	> 30 d	Jovanovic et al. (2018)
		15	87.6, OP, LDPVC, fragments	45 d	Daily	3,330 [c]	30 d	INT	9.27	> 30 d	Jovanovic et al. (2018)
		15	54.6, OP, MDPE, fragments	45 d	Daily	3,330 [c]	30 d	INT	15.73	> 30 d	Jovanovic et al. (2018)
		15	11.7, OP, PA, fragments	45 d	Daily	3,330 [c]	30 d	INT	34.27	> 30 d	Jovanovic et al. (2018)

Sample sizes (n) have been included for convenience. Immediate ingestion (sampling at end of exposure duration) is reported in MP individual^{-1}. Exposure and retention times are reported in minutes (min), hours (h), days (d), weeks (w), or months (mths). 'Mix' is representative of PES, PA and PP. Trophic levels have been verified using the well-established FishBase and SeaLifeBase databases. Values varying for (Porter et al., 2018) result from dosing either [1]alongside marine snow, [2]incorporated in marine snow, or [3]alone. A dash (-) indicates that this information was not a component of the study; when it was but not reported, NM is used. TL= Trophic Level; WO= Whole organism; Flu= Fluorescent; OP= Opaque; ST= soft tissue; STO= stomach; INT= intestine; GIT= gastrointestinal tract; PP = polypropylene; PS = polystyrene; PE = polyethylene; PVC = polyvinyl chloride; PA = polyamide, nylon; HDPE = high-density PE; MDPE = mid-density PE; LDPVC = low-density PVC. [a]g ml^{-1}; [b]MPs g weight^{-1}; [c]mg kg^{-1}; [d]mg l^{-1}; [e]mg. *Values are the upper limit of ingestion rates reported. Full ingestion details for each treatment (Critchell and Hoogenboom, 2018) were not reported.

Table A9.6 Trophic transfer of microplastics (MPs) for marine organisms. Information on bioaccumulation and retention, following specific exposures to MPs under controlled laboratory conditions, are presented for each species and across two different trophic levels.

Trophic Levels	Experimental Design	Post-Feed Sampling	*C. maenas* Ingestion Reported	Retention	Reference
2 → 3.5		24 h	100% ingested MPs	14 days	(Watts et al., 2014)
2 → 3.5		1 h	1,025 ± 556 MPs ml^{-1}	< 21 days	(Farrell and Nelson, 2013)

Sample sizes (n) have been included in the experimental design. Trophic levels have been verified using the well-established SeaLifeBase database. Mussel and crab images acquired from: clipart-library.com

Table A9.7 Experimental details of chemical additive laboratory exposure conducted with marine organisms. Bioaccumulation, following specific exposures to chemical additives under controlled laboratory conditions, are presented for each species, and across trophic levels.

				Exposure Details								
TL	Class	*Species*	n	Chemicals	Conc. (ng g^{-1})	Dispersal Method	Duration	Dose	Sampling Frequency	Conc. Reported (ng g^{-1})	Depuration Period	Reference
2	Bivalvia	*Mytilus edulis*	8	PAH: Fluoranthene	100 [i]	Alone	96 h	Once	-	80,460	-	Magara et al. (2018)
						Alongside PE	96 h	Once	-	41,500	-	Magara et al. (2018)
						On PE	96 h	Once	-	2,710	-	Magara et al. (2018)
		Mytilus spp.	24	PAH: Fluoranthene	30 [i]	Alone	7 d	Once	-	117,100	7 d	Paul-Pont et al. (2016)

							Alongside PS	7 d	Once	-	89,200	7 d	Paul-Pont et al. (2016)
		Scrobicularia plana	170	PAH:	Benzo[a]pyrene	16870	On LDPE	14 d	Every 72 h	-	7.3	-	O'Donovan et al. (2018)
	Malacostraca	*Allorchestes compressa*	5	PBDE:	∑PBDEs	5 [ii]	Alongside MPs	72 h	Once	-	50 - 275	48 h	Chua et al. (2014)
							Alone	72 h	Once	-	2,000 – 2,250	48 h	Chua et al. (2014)
							On MPs	72 h	Once	-	2 - 13	48 h	Chua et al. (2014)
						50 [ii]	Alongside MPs	72 h	Once	-	175 – 1,275	48 h	Chua et al. (2014)
							Alone	72 h	Once	-	3,500 – 7,900	48 h	Chua et al. (2014)
							On MPs	72 h	Once	-	0 - 70	48 h	Chua et al. (2014)
2.69	Bivalvia	*Mytilus galloprovincialis*	180	PAH:	Benzo[a]pyrene	15000	On LDPE	28 d	Daily	7, 14, 28 d	30	-	Pittura et al. (2018)
						0.15 [i]	Alone	28 d	Daily	7, 14, 28 d	35	-	Pittura et al. (2018)
						15000	On LDPE	28 d	Daily	7, 14, 28 d	12	-	Pittura et al. (2018)
						0.15 [i]	Alone	28 d	Daily	7, 14, 28 d	17	-	Pittura et al. (2018)
			150	PAH:	Pyrene	200 - 260	On PE & PS	7 d	Daily	-	470	-	Avio et al. (2015a)
3.67	Malacostraca	*Nephrops norvegicus*	7	PCB:	∑PCBs	1350 [ii]	On PE	3 w	M, W, F*	-	214	-	Devriese et al. (2017)
			6	PCB:	∑PCBs	1350 [ii]	On PS	3 w	M, W, F*	-	154	-	Devriese et al. (2017)
			8	PCB:	∑PCBs	1350 [ii]	On PS	3 w	M, W, F*	-	181	-	Devriese et al. (2017)
			23	PCB:	∑PCBs	1350 [ii]	Alone	3 w	M, W, F*	-	229 – 2,940	-	Devriese et al. (2017)

Species have been organised by trophic level based on the well-established FishBase and SeaLifeBase databases. Exposure and retention times are reported in hours (h), days (d), weeks (w), or months (mths). Sample sizes (n) have been given as well. Concentration reported is representative of contamination at end of exposure period and prior to the depuration period (if applicable). Retention of chemicals not given for any study.

MP = microplastic; PE = polyethylene; PS = polystyrene; PAH = polycyclic aromatic hydrocarbons; PBDE = polybrominated diphenyl ethers; PCB = polychlorinated biphenyls; NM: Not Mentioned; [i]$\mu g\ l^{-1}$; [ii]ng; *Frequency of exposure to freshly dosed chemicals occurred on Monday, Wednesday and Friday of each week for the duration of the 3-week exposure (Devriese et al., 2017).

Table A9.8 Number of field and laboratory studies investigating microplastic (MP) intake in marine organisms for each trophic level. Both total number of studies and number of studies utilising chemical confirmation of MPs (i.e., FTIR, Raman, polarised light microscopy) are presented for each trophic level from in situ *studies. Laboratory exposure studies are also reported. This information was used when creating Figure 2.4 and 2.5 in the Chapter 2. Please note these numbers will equate to a value larger than the reported number of studies included in this study due to studies investigating a multitude of trophic levels.*

Trophic Level	Number of *In situ* Studies	Percent of *In situ* Studies with Chemical Confirmation (n)	Number of Lab Exposure Studies
1	1	0% (0)	0
2	26	62% (16)	12
2.1	2	50% (1)	0
2.2	3	67% (2)	0
2.3	7	57% (4)	0
2.4	2	50% (1)	0
2.5	6	83% (5)	1
2.6	5	60% (3)	1
2.7	4	50% (2)	2
2.8	5	60% (3)	2
2.9	4	25% (1)	0
3	8	50% (4)	1
3.1	23	65% (15)	0
3.2	12	75% (9)	0
3.3	19	74% (14)	0
3.4	16	75% (12)	0
3.5	18	56% (10)	2
3.6	16	81% (13)	0
3.7	16	75% (12)	1
3.8	10	70% (7)	0
3.9	8	75% (6)	0
4	17	65% (11)	0
4.1	12	83% (10)	0
4.2	11	73% (8)	0
4.3	11	73% (8)	0
4.4	24	71% (17)	0
4.5	17	59% (10)	0

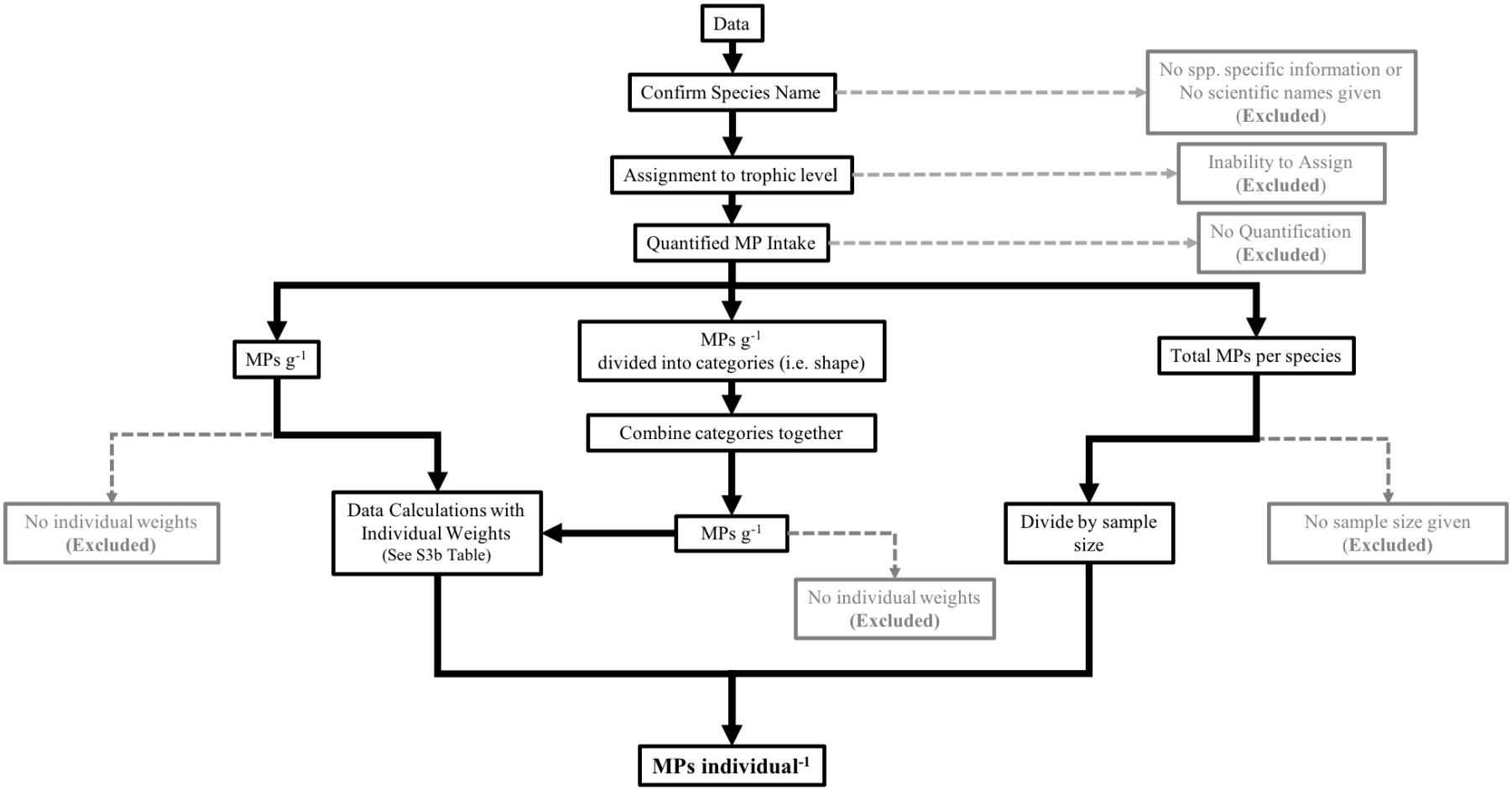


Figure A1.1 Flow diagram outlining the standardisation of microplastic (MP) contamination data for marine organisms. Data on MPs were standardised to number of MPs per individual organism (MPs individual^{-1}) (i.e., body burden).

Appendix 2: Chapter 3

*Table A2.1 Organism culturing parameters for copepods (*Parvocalanus crassirostris*) and mysid shrimp (*Mysida sp.*). Copepods were fed a mixture of* Chaetoceros muelleri *and* Tisochrysis lutea *(*T-iso*); mysid shrimp were fed frozen* P. crassirostris *copepods.*

Organism	Water Temperature (ºC)	pH	Salinity (ppt)	DO (mg L^{-1})	Tank Size	Flow Rate	Daily Feeding Rate	Culture Population Size
Copepods	25.5 ± 0.4	8.2 ± 0.1	35.5 ± 0.3	7.8 ± 0.2	200 L	8 L min^{-1}	200 ml *T-iso* & *C. muelleri* mixture	40 – 80 adults ml^{-1}
Mysid shrimp					100 L	50 L min^{-1}	50,000 copepods	350 ± 70 adults

Table A2.2 Airborne microfibre contamination isolated from airborne contamination control (AC_Ctrl) vials (n=9) placed throughout the experimental room.

Item ID	Shape	Colour	Size (mm)
AC_Ctrl_001	Fibre	Black	1.658
AC_Ctrl_002	Fibre	Black	0.630
AC_Ctrl_003	Fibre	Black	1.301
AC_Ctrl_004	Fibre	Blue	0.790
AC_Ctrl_005	Fibre	Pink	1.735
AC_Ctrl_006	Fibre	Blue	4.451
AC_Ctrl_007	Fibre	Blue	6.488
AC_Ctrl_008	Fibre	Black	0.693
AC_Ctrl_009	Fibre	Blue	3.429
AC_Ctrl_010	Fibre	Clear	1.211
AC_Ctrl_011	Fibre	Blue	3.204
AC_Ctrl_012	Fibre	Blue	1.264
AC_Ctrl_013	Fibre	Black	1.300
AC_Ctrl_014	Fibre	Blue	0.881
AC_Ctrl_015	Fibre	Blue	0.968
AC_Ctrl_016	Fibre	Blue	1.417
AC_Ctrl_017	Fibre	Black	1.129
AC_Ctrl_018	Fibre	Clear	1.238
AC_Ctrl_019	Fibre	Clear	3.896
AC_Ctrl_020	Fibre	Black	4.333
AC_Ctrl_021	Fibre	Blue	0.403
AC_Ctrl_022	Fibre	Blue	0.813
AC_Ctrl_023	Fibre	Blue	2.303
AC_Ctrl_024	Fibre	Blue	1.679
AC_Ctrl_025	Fibre	Pink	2.994
AC_Ctrl_026	Fibre	White	1.454
AC_Ctrl_027	Fibre	Black	0.454

*Table A2.3 Mean percent polyester (% PEST) ingestion and retention across time, for organisms exposed to PEST alone, PEST pre-adsorbed with bis(2-ethylhexyl) phthalate (DEHP) (i.e., PEST:DEHP), and PEST added in tandem with DEHP (i.e., PEST+DEHP). Treatments were dosed at a concentration of 10 PEST microfibres per individual; DEHP dosed at a concentration of 1 mg L^{-1}. Data presented as a percent of exposed PEST concentration for copepods (*Parvocalanus crassirostris*, n= 250 per replicate), mysid shrimp (*Mysida sp.*; n= 6 per replicate) and moon wrasse (*Thalassoma lunare*; n = 1 per replicate). Each time point contained three replicates per treatment; however, T_{48} was not measured for copepods or mysid shrimp and T_1 was not measured for fish. T_0 is representative of initial ingestion.*

Organism	Retention Time (h)	Number of PEST		
		PEST	PEST:DEHP	PEST+DEHP
Copepod	0	0.32 ± 0.07	0.35 ± 0.21	0.70 ± 0.07
	1	0.23 ± 0.09	0.28 ± 0.17	0.43 ± 0.15
	3	0.08 ± 0.05	0.12 ± 0.08	0.11 ± 0.10
	6	0.00	0.00	0.00
	12	0.00	0.00	0.00
	24	0.00	0.00	0.00
Mysid shrimp	0	35.56 ± 26.56	15.19 ± 7.84	31.11 ± 10.14
	1	22.59 ± 13.10	14.81 ± 9.88	30.74 ± 11.82
	3	13.70 ± 8.57	7.78 ± 11.06	24.07 ± 12.89
	6	12.59 ± 16.81	5.19 ± 8.18	12.59 ± 16.14
	12	6.30 ± 5.64	2.59 ± 5.72	4.07 ± 6.62
	24	5.56 ± 7.26	1.48 ± 3.38	1.11 ± 2.36
Fish	0	83.33 ± 15.28	66.67 ± 11.15	56.67 ± 23.09
	3	56.67 ± 5.77	56.67 ± 11.15	43.33 ± 5.77
	6	36.67 ± 11.55	36.67 ± 11.55	30.00 ± 10.00
	12	26.67 ± 11.55	13.33 ± 5.77	10.00 ± 10.00
	24	20.00 ± 10.00	16.67 ± 11.55	6.67 ± 5.77
	48	3.33 ± 5.77	3.33 ± 5.77	3.33 ± 5.77

*Table A2.4 Differences in initial ingestion (T_0) of polyester microfibres (PEST) by copepods, mysid shrimp, and fish between PEST treatments, including PEST alone (intercept), PEST pre-adsorbed with bis(2-ethylhexyl) phthalate, DEHP (i.e., PEST:DEHP), and PEST added in tandem with DEHP (i.e., PEST+DEHP), following a single exposure in a controlled laboratory experiment. A general linear model using a gaussian distribution with tank replicate as a nested variable (formula: glm(PEST ~ treatment + (1|tank)) was used for the post-hoc pairs tests (see Chapter 3, Table 3.2). Significant codes: * 0.05*

A) Formula: glm(PEST ~ treatment + (1|tank))

Organism	Treatment	Estimate	Std. Error	Statistic	CI Low	CI High	p-value
Copepod	Intercept	3229.23	0.31	26.43	1773.73	5879.11	< 0.001 *
	PEST:DEHP	4.14	0.51	2.78	1.52	11.25	0.006 *
	PEST+DEHP	11928.27	0.43	21.71	5111.90	27833.83	< 0.001 *
Mysid shrimp	Intercept	1.84×10^{9}	3.41	6.26	2.32×10^{6}	1.45×10^{12}	< 0.001 *
	PEST:DEHP	0.00	4.82	-2.54	0.00	0.06	0.018 *
	PEST+DEHP	0.07	4.82	-0.55	0.00	873.43	0.585
Fish	Intercept	4160.26	1.00	8.33	586.03	29534.00	< 0.001 *
	PEST:DEHP	0.19	1.41	-1.18	0.01	3.02	0.283
	PEST+DEHP	0.07	1.41	-1.89	0.00	1.11	0.108

B) Post-hoc analyses

Organism	Contrast	estimate	SE	df	t.ratio	p.value
Copepod	PEST – PEST+DEHP	-9.39	0.43	189	-21.71	< 0.001 *
	PEST – PEST:DEHP	-1.42	0.51	189	-2.78	0.016 *
	PEST+DEHP – PEST:DEHP	7.97	0.51	189	15.61	< 0.001 *
Mysid	PEST – PEST+DEHP	2.67	4.82	24.00	0.55	0.846
	PEST – PEST:DEHP	12.22	4.82	24.00	2.54	0.046 *
	PEST+DEHP – PEST:DEHP	9.56	4.82	24.00	1.98	0.138
Fish	PEST – PEST+DEHP	2.67	1.41	6.00	1.89	0.223
	PEST – PEST:DEHP	1.67	1.41	6.00	1.18	0.507
	PEST+DEHP – PEST:DEHP	-1.00	1.41	6.00	-0.71	0.768

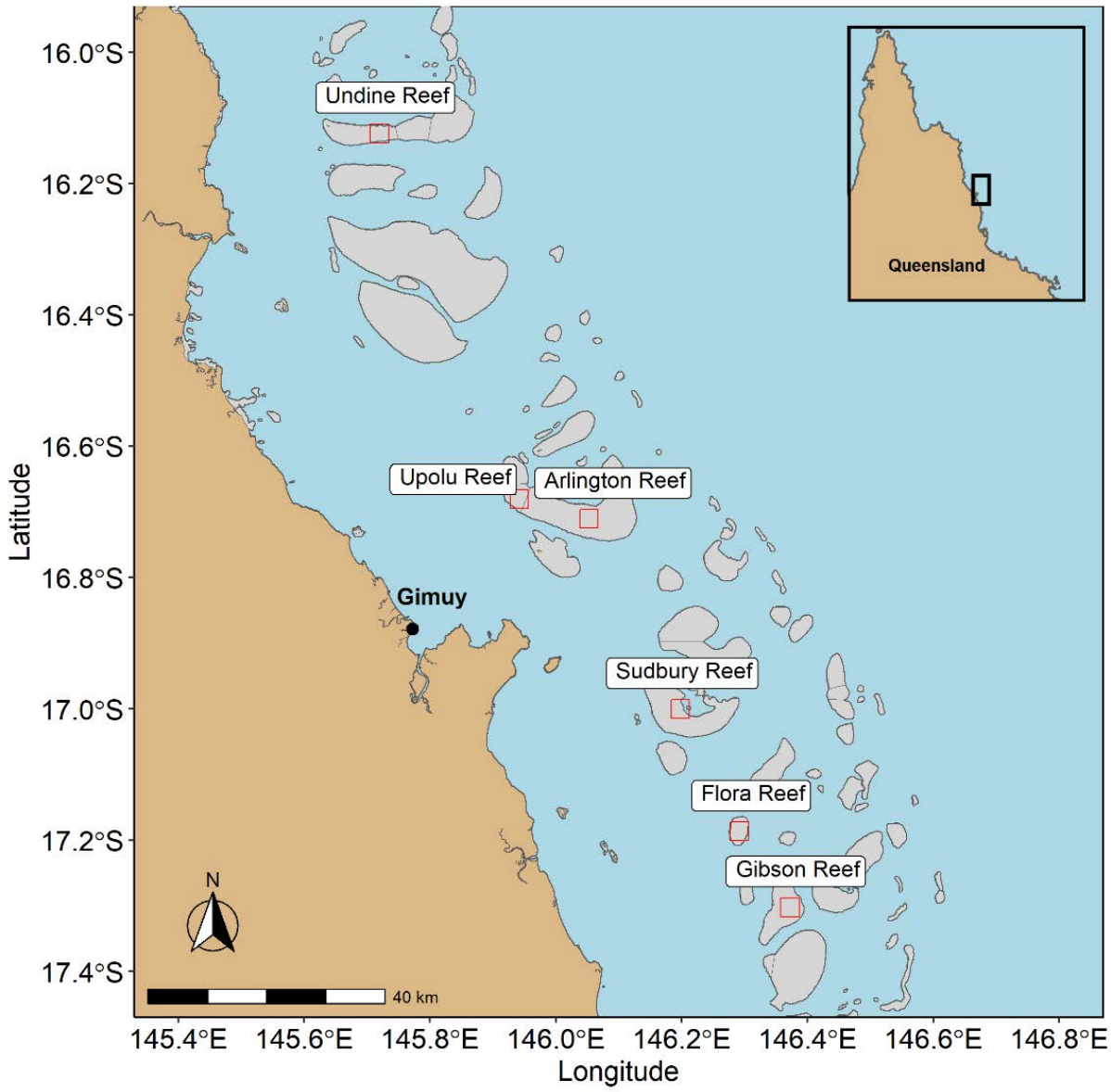


*Figure A2.1 Map of moon wrasse (*Thalassoma lunare*) collections (red boxes) along the Great Barrier Reef World Heritage Area. Collections were carried out by Cairns Marine and subsequently transferred to aquaria facilities (National Sea Simulator) at the Australian Institute of Marine Science (AIMS) for microplastic and chemical additive exposure experiments. Inset is representative of collection locations relative to Gimuy (Cairns), Queensland, Australia.*

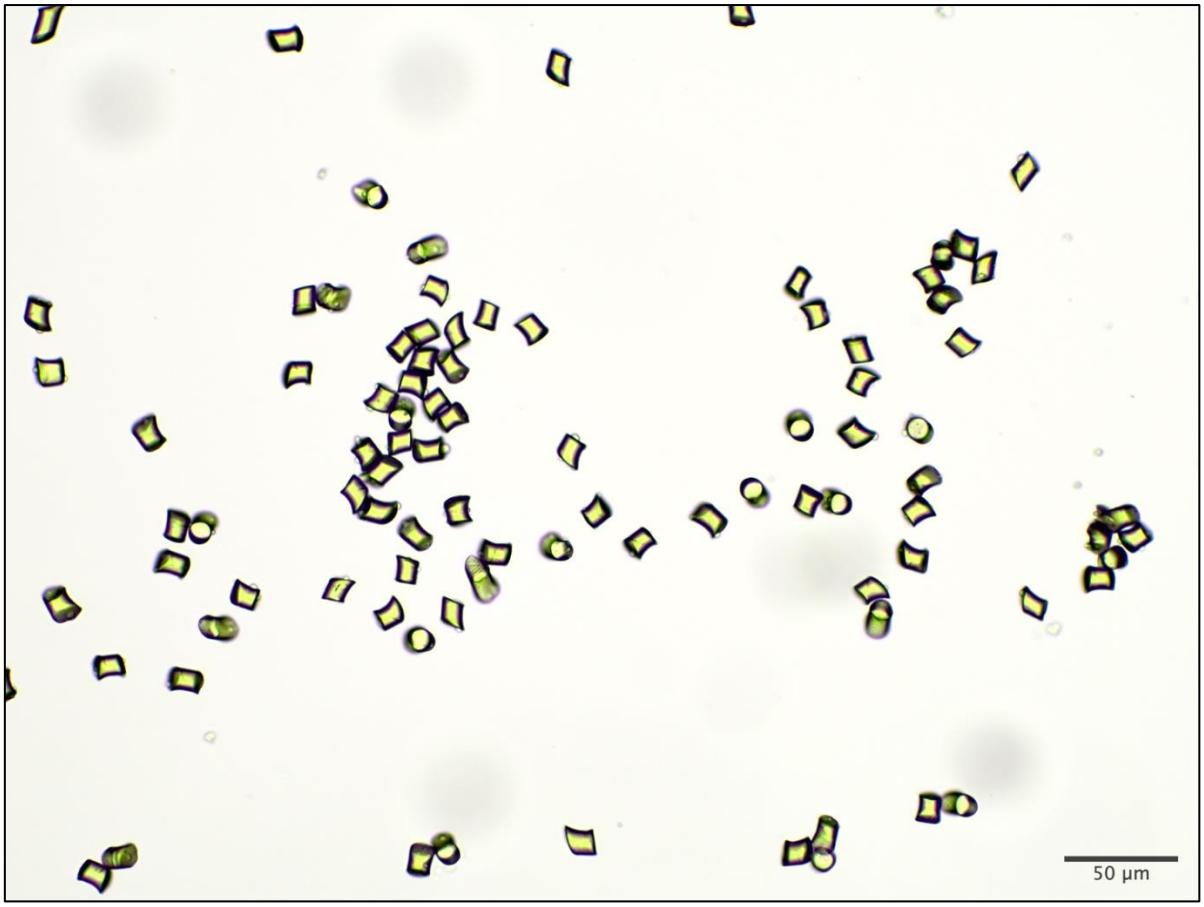


Figure A2.2 A subsample of 10 ± 2 µm fluorescent polyester microfibres following cryostat cutting procedure.

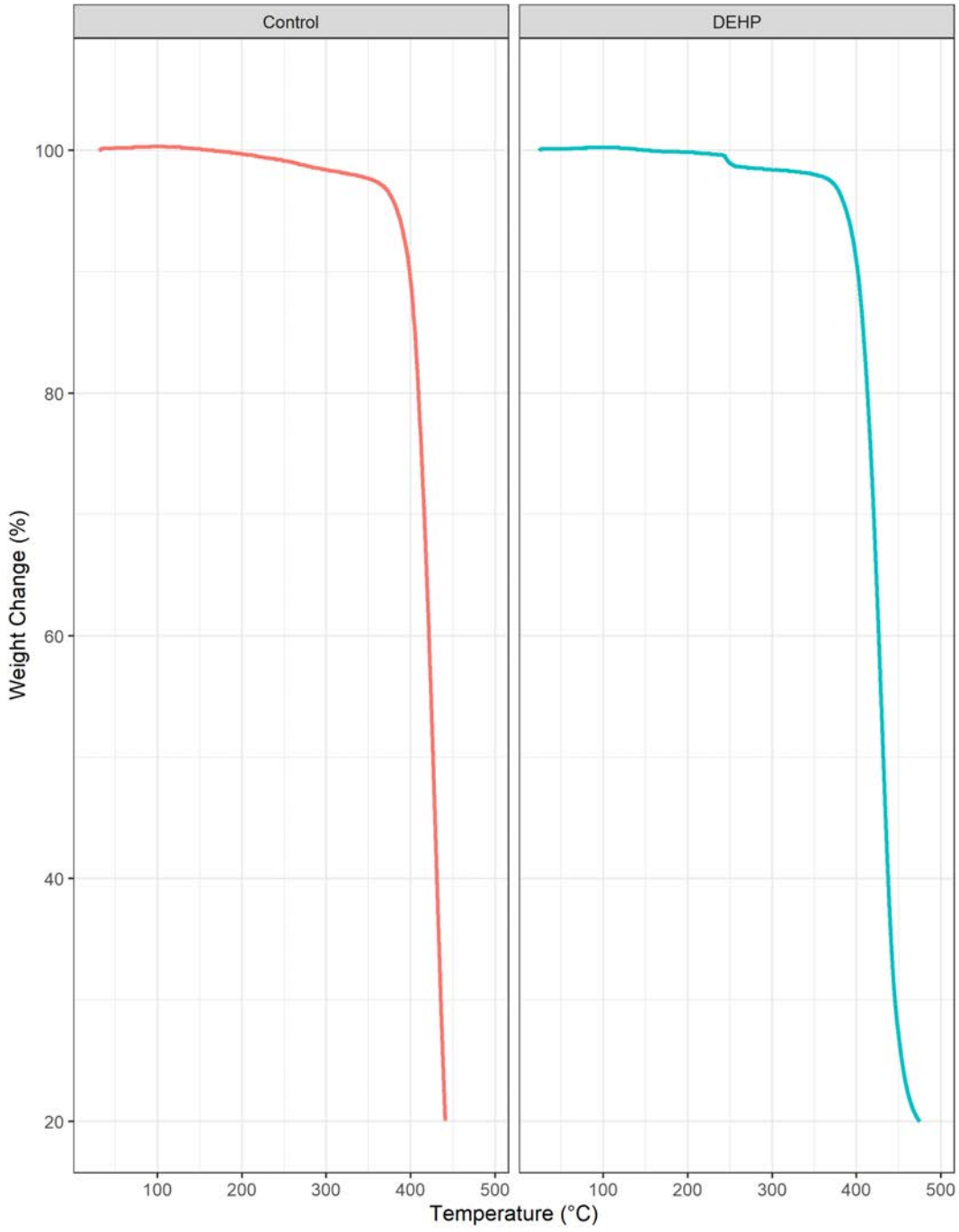


Figure A2.3 Thermogravimetric Analysis and Differential Scanning Calorimetry (TGA-DSC) results, indicating the weight change (i.e., mass loss) of control and bis(2-ethylhexyl) phthalate (DEHP)-treated polyester microfibres.

Text A2. Pilot study confirming DEHP adsorption to PEST

To test the premise that bis(2-ethylhexyl) phthalate (DEHP) absorbed to polyester microfibres (PEST:DEHP) impacts PEST ingestion by marine organisms, a pilot study was first conducted to confirm PEST:DEHP could be consistently prepared with a known amount of DEHP.

DEHP stock solution

A DEHP stock solution was prepared by adding 250 mg of DEHP (Sigma Aldrich, CAS Number: 117-81-7) to 250 ml of hexane (1 mg ml^{-1} final concentration, Fisher Scientific, CAS Number: 110-54-3).

Preparation of PEST microfibres for validation

For the pilot study, threads of PEST were cut using a scalpel blade to obtain 500 ± 50 µm fibres. Replicates (n=5) of at least 2 mg of PEST microfibres (approx. 4,000 fibres) were disentwined (i.e., unravelled into monofilaments) and placed into 20 ml scintillation vials. DEHP (1 ml of 1mg ml^{-1} DEHP in hexane stock solution) was added to each vial, the vial was covered with aluminium foil-lined lids and then placed on a mechanical shaker (Baxter Multi-Tube Vortexer, Baxter Diagnostics) for 1 h. Following, vials were left to evaporate under a constant stream of nitrogen and stored in the dark at 4°C for analysis. Controls were prepared with PEST microfibres not treated with DEHP. All preparations were analysed using Thermogravimetric Analysis and Differential Scanning Calorimetry (TGA-DSC, details below) to confirm DEHP adsorption to PEST microfibres.

TGA-DSC and Results

Microfibres were weighed (av. 2.07 ± 0.31 mg) to obtain a starting weight and then subjected to TGA, performed on a TA SDT 650 instrument at a heating rate of 10°C min^{-1} up to 500°C under a constant flow of nitrogen (50 ml min^{-1}). Results are shown below in Figure A2.3. Control PEST microfibres showed a single decomposition point at 380ºC which is indicative of PEST; the microfibre is > 95% PEST with minimal additives present (Tsanaktsis et al., 2015). The weight decrease observed in these microfibres is most likely due to the loss of moisture. PEST microfibres treated with DEHP show two thermal decomposition points (250ºC and 380ºC). The weight change at 250ºC indicates successful incorporation of DEHP into the PEST microfibres, with 0.85 ± 0.48 mg DEHP present. Based on this pilot study, this method was used to prepare PEST:DEHP for the main experiment.

Appendix 3: Chapter 4

Table A3.1 Full-text articles included and excluded from the current study. Literature search conducted in June 2022 with keywords including 'plastics', 'microplastics', and 'trophic transfer'. Databases included Google Scholar, Web of Science™, and references of relevant review papers.

Reference	Included (Reason for Exclusion)
(da Costa Araujo and Malafaia, 2021)	No (Terrestrial species)
(da Costa Araújo et al., 2020)	Yes
(Athey et al., 2020)	Yes
(Au et al., 2017)	No (Commentary article)
(Batel et al., 2016)	Yes
(Carbery et al., 2018)	No (Review article)
(Chae and An, 2020)	No (Nanoplastics)
(Chae et al., 2018)	No (Nanoplastics)
(Chagnon et al., 2018)	No (Field study – only speculated)
(Costa et al., 2020)	Yes
(Elizalde-Velázquez et al., 2020)	Yes
(Farrell and Nelson, 2013)	Yes
(Gouin, 2020)	No (Review article)
(Hanslik et al., 2020)	Yes
(Hasegawa and Nakaoka, 2021)	Yes
(Huang et al., 2021b)	No (Review article)
(Latchere et al., 2021)	No (Review article)
(Monikh et al., 2021)	No (Nanoplastics)
(Moore et al., 2022)	No (Field study – only speculated)
(Nelms et al., 2018)	No (Field study – only speculated)
(Provencher et al., 2019)	No (Review article)
(Renzi et al., 2018a)	No (Field study – only speculated)
(Setala et al., 2014)	Yes
(Stienbarger et al., 2021)	Yes
(Tangaa et al., 2016)	No (Review article)
(Tosetto et al., 2017)	Yes
(Uy and Johnson, 2022)	Yes
(Welden et al., 2018)	No (Field study – only speculated)

*Table A3.2 Statistical output from the general linear model (GLM) for polyester (PEST) ingestion at each trophic level following exposure to pre-exposed prey items as part of a trophic transfer experiment. Exposure of copepods, mysid shrimp and fish to PEST was done under varying phthalate (Bis(2-ethylhexyl) phthalate (DEHP) treatments including PEST dosed alone (intercept), PEST pre-adsorbed with DEHP (PEST:DEHP) and PEST added in tandem with DEHP (PEST+DEHP). To account for a possible tank effect, treatment replicate (i.e., tank number) was added as a random effect. SE = Standard Error. CI = Confidence Interval (95%). Significant codes: * 0.05.*

Formula: glm.nb(PEST ~ organism + treatment + (1|tank))

	Estimate	SE	Statistic	p.value	CI Low	CI High
(Intercept)	0.98	0.21	4.70	2.59×10^{-6} *	0.57	1.40
Treatment PEST:DEHP	0.02	0.20	0.10	0.918	-0.37	0.41
Treatment PEST+DEHP	0.20	0.20	1.00	0.317	-0.19	0.59
Organism Mysid shrimp	2.35	0.22	10.79	3.76×10^{-27} *	1.93	2.78
Organism Fish	3.94	0.22	18.29	1.00×10^{-74} *	3.52	4.36

Table A3.3 Airborne microfibre contamination isolated from airborne contamination (AC) controls (n=9) placed throughout the experimental room.

Item ID	Shape	Colour	Length (mm)
AC_Cnt_001	Fibre	Green	4.293
AC_Ctrl_002	Fibre	Black	6.394
AC_Ctrl_003	Fibre	Blue	0.198
AC_Ctrl_004	Fibre	Blue	3.491
AC_Ctrl_005	Fibre	Blue	1.299
AC_Ctrl_006	Fibre	Black	2.405
AC_Ctrl_007	Fibre	Green	0.394
AC_Ctrl_008	Fibre	Green	0.258
AC_Ctrl_009	Fibre	Green	0.506
AC_Ctrl_010	Fibre	Black	0.819
AC_Ctrl_011	Fibre	Blue	3.023
AC_Ctrl_012	Fibre	Blue	1.305
AC_Ctrl_013	Fibre	Blue	6.394
AC_Ctrl_014	Fibre	Black	1.204
AC_Ctrl_015	Fibre	Black	0.533
AC_Ctrl_016	Fibre	Black	0.867

*Table A3.4 Pairwise comparisons of polyester (PEST) ingestion for copepods, mysid shrimp and fish from a trophic transfer laboratory exposure under varying Bis(2-ethylhexyl) phthalate (DEHP) treatments including PEST dosed alone, PEST pre-adsorbed with DEHP (PEST:DEHP) and PEST added in tandem with DEHP (PEST+DEHP). Organisms were exposed to prey contaminated with treatment; therefore, all ingestion is a direct result of trophic transfer. SE = Standard Error. CO= Copepods; MS= Mysid shrimp; FI= Fish. Significant codes: * 0.05.*

Contrast	Estimate	SE	z.ratio	p.value
CO PEST - MS PEST	-2.35	0.22	-10.79	8.53×10^{-14} *
CO PEST - FI PEST	-3.94	0.22	-18.29	0 *
CO PEST - (CO PEST:DEHP)	-0.02	0.20	-0.10	1.000
CO PEST - (MS PEST:DEHP)	-2.37	0.30	-8.04	1.49×10^{-13} *
CO PEST - (FI PEST:DEHP)	-3.96	0.29	-13.50	0 *
CO PEST - (CO PEST+DEHP)	-0.20	0.20	-1.00	0.986
CO PEST - (MS PEST+DEHP)	-2.55	0.30	-8.62	7.92×10^{-14} *
CO PEST - (FI PEST+DEHP)	-4.14	0.29	-14.06	0 *
MS PEST - FI PEST	-1.59	0.18	-8.70	7.42×10^{-14} *
MS PEST - (CO PEST:DEHP)	2.33	0.29	7.91	2.21×10^{-13} *
MS PEST - (MS PEST:DEHP)	-0.02	0.20	-0.10	1.000
MS PEST - (FI PEST:DEHP)	-1.61	0.27	-5.96	9.00×10^{-8} *
MS PEST - (CO PEST+DEHP)	2.15	0.29	7.37	6.32×10^{-12} *
MS PEST - (MS PEST+DEHP)	-0.20	0.20	-1.00	0.986
MS PEST - (FI PEST+DEHP)	-1.79	0.27	-6.63	1.18×10^{-9} *
FI PEST - (CO PEST:DEHP)	3.92	0.29	13.38	0 *
FI PEST - (MS PEST:DEHP)	1.57	0.27	5.81	2.24×10^{-7} *
FI PEST - (FI PEST:DEHP)	-0.02	0.20	-0.10	1.000
FI PEST - (CO PEST+DEHP)	3.74	0.29	12.89	0 *
FI PEST - (MS PEST+DEHP)	1.39	0.27	5.17	8.10×10^{-6} *
FI PEST - (FI PEST+DEHP)	-0.20	0.20	-1.00	0.986
(CO PEST:DEHP) - (MS PEST:DEHP)	-2.35	0.22	-10.79	8.53×10^{-14} *
(CO PEST:DEHP) - (FI PEST:DEHP)	-3.94	0.22	-18.29	0 *
(CO PEST:DEHP) - (CO PEST+DEHP)	-0.18	0.20	-0.90	0.993
(CO PEST:DEHP) - (MS PEST+DEHP)	-2.53	0.30	-8.56	8.23×10^{-14} *
(CO PEST:DEHP) - (FI PEST+DEHP)	-4.12	0.29	-14.01	0 *
(MS PEST:DEHP) - (FI PEST:DEHP)	-1.59	0.18	-8.70	7.42×10^{-14} *
(MS PEST:DEHP) - (CO PEST+DEHP)	2.17	0.29	7.44	3.81×10^{-12} *
(MS PEST:DEHP) - (MS PEST+DEHP)	-0.18	0.20	-0.90	0.993
(MS PEST:DEHP) - (FI PEST+DEHP)	-1.76	0.27	-6.56	1.93×10^{-9} *
(FI PEST:DEHP) - (CO PEST+DEHP)	3.76	0.29	12.96	0 *
(Fish PEST:DEHP) - (MS PEST+DEHP)	1.41	0.27	5.25	5.33×10^{-6} *
(Fish PEST:DEHP) - (Fish PEST+DEHP)	-0.18	0.20	-0.90	0.993
(CO PEST+DEHP) - (MS PEST+DEHP)	-2.35	0.22	-10.79	8.53×10^{-14} *
(CO PEST+DEHP) - (Fish PEST+DEHP)	-3.94	0.22	-18.29	0 *
(MS PEST+DEHP) - (Fish PEST+DEHP)	-1.59	0.18	-8.70	7.42×10^{-14} *

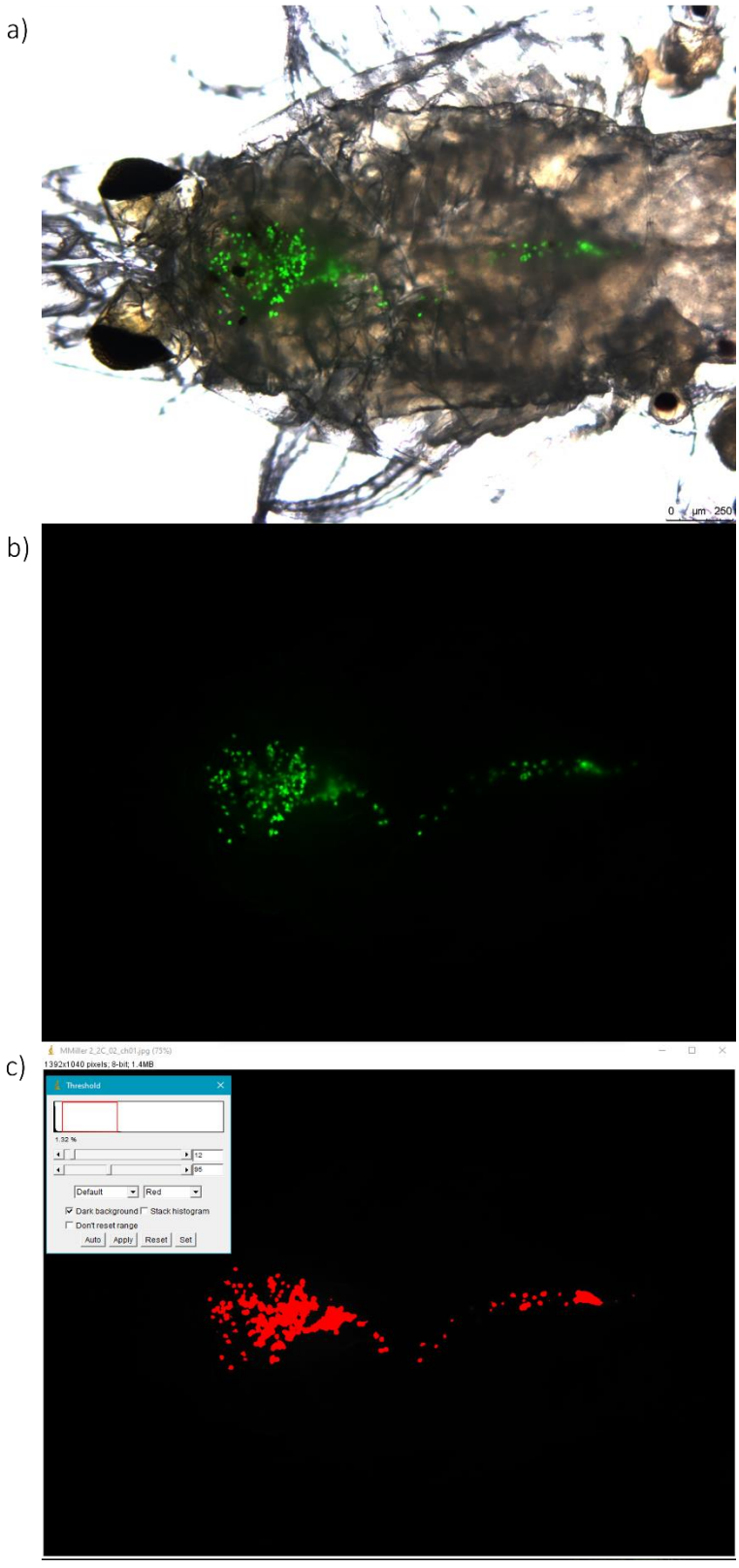


Figure A3.1 Microscopy of the gut of mysid shrimp exposed to live copepod prey contaminated with fluorescent polyester (PEST) microfibres (10 µm): (a) overlayed image with brightfield photo and I3 fluorescent filter, (b) I3 fluorescent filter photo only, and (c) application of colour threshold on 8-bit image to allow the use of the 'analyse particles' function in ImageJ.

Appendix 4: Chapter 5

*Table A4.1 Sample collection details including (a) environmental information and (b) classifications, sample sizes and details of copepods, crustaceans, and fish (*Thalassoma lunare*) collected from Backnumbers Reef and Davies Reef. Crustaceans belonging to the same larger taxa (i.e., copepods, crabs, and shrimps) were combined for each site replicate due to their size; therefore, average weights are mean values for site replicates and not individual organisms. Both the whole body and the gastrointestinal tract (GIT) weights are given for the fish. Average weight is per replicate, not per individual.*

(a)

Date	Reef	Research Vessel	Latitude Longitude	Wind Speed (Knots)	Water Temp (°C)	Depth (m)	Samples Collected
18 Oct 2019	Davies	Apollo	18° 49' 59.67" S 147° 37' 57.68" E	5 – 10	25.8	7.7	Sediment (n=3), Crustaceans (n=3), Water Column (n=3)
5 Nov 2019	Backnumbers	Apollo	18° 31' 12.7" S 147° 07' 56.2" E	5 – 10	27.2	8.4	Sediment (n=3), Crustaceans (n=3), Water Column (n=3)
9 Nov 2019	Davies	Cape Ferguson	18° 49' 59.67" S 147° 37' 57.68" E	~ 10	27.3	6 – 8	Fish (n=20)
9–11 Dec 2019	Backnumbers	Cape Ferguson	18° 31' 12.7" S 147° 07' 56.2" E	5 – 10	27.5	6 – 9	Fish (n=20)

(b)

Classification (common name)	Backnumbers Reef	Davies Reef	Total n	Size Range (mm)	Average Weight (g w.w.)
Zooplankton					
Order Calanoida (Copepod)	128	132	260	0.253 – 0.947	0.21 ± 0.07
Order Harpacticoida (Copepod)	39	41	80	214 µm – 2.48	
Benthic Crustaceans					
Family Cirolanidae (Isopod)	3	1	4	2.19 – 3.83	0.89 ± 1.41
Family Galatheidae (Squat Lobster)	25	51	76	2.07 – 12.46	0.65 ± 0.36
Family Penaeidae (Penaeid Shrimp)	3	9	12	2.05 – 20.98	0.41 ± 0.29
Infraorder Caridea (Caridean Shrimp)	23	3	26	3.74 – 8.21	
Family Xanthidae (Rubble Crab)	6	20	26	2.94 – 14.36	
Family Majidae (Spider Crab)	3	-	3	5.40 – 15.15	0.84 ± 0.97
Family Portunidae (Swimmer Crab)	2	-	2	9.40 – 9.69	
Order Amphipoda (Amphipod)	27	27	54	1.90 – 9.57	0.14 ± 0.11
Fish					
Thalassoma lunare (Moon Wrasse)	20	20	40	6.3 – 15.5	24.02 ± 19.42

Table A4.2 In-house contamination library used to check for potential contamination of samples during collection, processing, and analyses. The library includes items from field collection and laboratory processing equipment and materials, as well as items found within laboratory airborne contamination and procedural blanks. PEST = Polyester, PVC = polyvinyl chloride, PP = polypropylene, PE = polyethylene, LDPE = low density polyethylene, PAN = Polyacrylonitrile, PTFE = Polytetrafluoroethylene, HDPE = high density polyethylene.

Filename	Shape	Colour	Polymer
Lab_Blank_001	Fibre	black	PEST
Lab_Blank_002	Fibre	blue	Cotton:PEST
Lab_Blank_003	Fibre	transparent	Rayon:PEST
Lab_Blank_004	Fibre	blue	Cotton:Rayon
Lab_Blank_005	Fibre	green	Cotton
Lab_Blank_006	Fibre	transparent	Cotton:Rayon
Lab_Blank_007	Fibre	yellow	Cotton
Lab_Blank_008	Fibre	yellow	Cotton
Lab_Blank_009	Fibre	transparent	Cotton
Lab_Blank_010	Fibre	purple	Cotton
Lab_Blank_011	Fibre	yellow	Cotton
Lab_Blank_012	Fibre	blue	Cotton
Lab_Blank_013	Fibre	green	Cotton
Lab_Blank_014	Fibre	black	PEST
Lab_Blank_015	Fibre	blue	PEST
Lab_Blank_016	Fibre	black	Cotton
Lab_Blank_017	Fibre	black	Cotton
Lab_Blank_018	Fibre	black	Cotton
Lab_Blank_019	Fibre	black	Cotton
Lab_Blank_020	Fibre	black	Cotton
Lab_Blank_021	Fibre	blue	Cotton:PEST
Lab_Blank_022	Fibre	black	Cotton
Lab_Blank_023	Fibre	blue	Cotton
Lab_Blank_024	Fibre	transparent	Cotton
Lab_Blank_025	Fibre	black	PEST
Lab_Blank_026	Fibre	transparent	Acyrlic
Lab_Blank_027	Fibre	yellow	Rayon
Lab_Blank_028	Fibre	blue	Cotton:PEST
Lab_Blank_029	Fibre	transparent	PEST
Lab_Blank_030	Fibre	yellow	Cotton
Procedural_Blank_001	Fibre	blue	Cotton
Procedural_Blank_002	Fibre	yellow	Cotton
Procedural_Blank_003	Fibre	blue	Cotton
Procedural_Blank_004	Fibre	black	Rayon:Nylon
Field_Blank_001	Fibre	blue	Cotton:Rayon
40 um Plankton Net	Fibre	transparent	Nylon
350 um Plankton Net	Fibre	transparent	PEST
Apollo Davit Rope Clear	Fibre	transparent	PP
Apollo Grip Paint 24.09.20	Fragment	grey	Acrylic
Apollo Blue Rope 01	Fibre	blue	Cotton:PEST
Apollo Clear Rope 01	Fibre	transparent	PEST
Apollo Clear Rope 02	Fibre	transparent	LDPE
Apollo Green Rope 01	Fibre	green	PP:PE
Apollo White Rope 01	Fibre	white	PEST
Apollo Onboard Hose	Fragment	green	PVC
Blue Nitrile Gloves	Fragment	blue	PAN
Crustacean Foam Seal Rubble Box	Fragment	black	PE
Crustacean Rubble Box Bungee Rope	Fibre	black	PEST

Fish Collection Bag Yellow	Fibre	yellow	PEST
Fish Hand Net Pink	Fibre	pink	Nylon
Green Cotton Lab Coat	Fibre	green	Cotton
Lab Blue O-ring	Fragment	blue	Silicone
Fish Barrier Net	Fibre	transparent	PEST
Old Squeeze Bottle Lid	Fragment	transparent	PP
Parafilm	Fragment	transparent	Paraffin Wax
Pee Jar Bottom	Fragment	transparent	PP
Pee Jar Lid	Fragment	yellow	PE
Plankton Net Canvas	Fibre	white	PP
Plankton Net Nylon	Fibre	transparent	Nylon
PTFE Solvent Filter	Fibre	white	PTFE
Purple Actifibre Cloth	Fibre	purple	PEST:Rayon
Duct Tape	Fibre	grey	PE
Seawater Blue Flexible Pump Hose	Fragment	blue	PVC
Seawater White Pump Pipe	Fragment	white	PVC
Sediment Plastic Bag	Fragment	transparent	LDPE
Sediment PVC Quadrat	Fragment	white	PVC
Sieve PVC Pipe	Fragment	blue	PVC
Teflon Squeeze Bottle	Fragment	transparent	PTFE

Table A4.3 Microplastic (MP) shape categories and concentrations presented in MPs individual^{-1} for zooplankton, crustaceans and fish collected from Backnumbers Reef and Davies Reef.

	Matrix	n	Fragments	Fibres	MP Concentration (MP ind^{-1})
Backnumbers Reef	**Zooplankton**				
	Copepods	167	-	4	0.02 ± 0.01
	Benthic Crustaceans				
	Isopods	3	-	-	-
	Squat Lobsters	25	-	-	-
	Shrimps	26	-	1	0.03 ± 0.05
	Crabs	11	1	1	0.38 ± 0.54
	Amphipods	27	-	2	0.04 ± 0.07
	Fish				
	Thalassoma lunare	20	6	24	1.50 ± 1.61
Davies Reef	**Zooplankton**				
	Copepods	173	-	2	0.01 ± 0.01
	Benthic Crustaceans				
	Isopods	1	-	1	1
	Squat Lobsters	51	-	8	0.33 ± 0.44
	Shrimps	11	-	5	0.7 ± 1.13
	Crabs	20	2	14	0.74 ± 0.25
	Amphipods	27	-	-	-
	Fish				
	Thalassoma lunare	20	7	32	1.95 ± 1.46

Table A4.4 Microplastics concentrations for environmental (sediment and water column), and biological (zooplankton, benthic crustaceans, and moon wrasse fish, Thalassoma lunare*) collected at two mid-shelf coral reefs in the Great Barrier Reef, Australia. Concentrations are in converted units to allow bioconcentration, bioaccumulation, and biomagnification values to be calculated (See Chapter 3, Section 3.6)*

Matrix (units)	Backnumbers Reef	Davies Reef
Sediment (MPs kg^{-1})	0.44 ± 0.39	6.000 ± 2.40
Water Column (MPs L^{-1})	0.007 ± 0.005	0.002 ± 0.001
Zooplankton (MPs kg^{-1})	4806.33 ± 1902.87	2640.523 ± 2287.07
Benthic Crustaceans (MPs kg^{-1})	2324.03 ± 4743.59	4994.93 ± 5474.58
Fish (MPs kg^{-1})	94.27 ± 147.03	216.28 ± 264.58

*Table A4.5 Statistical output from (a) the general linear model (GLM) with a negative binomial distribution and (b) post-hoc tests for microplastic (MP) sizes (i.e., length) within each matrix examined: copepods, benthic crustaceans, moon wrasse fish (*Thalassoma lunare*), sediment and the water column at both Backnumbers Reef and Davies Reef. To account for a possible reef location effect, site number was included as a random effect. CI = Confidence Interval (95%). Significant codes: *0.05*

(a)

Formula: glm.nb(Length ~ Matrix + (1|site))						
	Estimate	Std. Error	Statistic	P Value	Low CI	High CI
(Intercept)	6.44	0.38	17.14	7.95E-66 *	5.78	7.28
Benthic Crustaceans	0.62	0.41	1.51	0.130	-0.27	1.35
Fish	0.25	0.39	0.64	0.524	-0.61	0.95
Sediment	0.61	0.41	1.48	0.140	-0.28	1.36
Water Column	0.15	0.40	0.37	0.710	-0.72	0.85

(b)

Contrast	Estimate	SE	df	z.ratio	p.value
Copepods – Benthic Crustaceans	-0.62	0.41	Inf	-1.51	0.554
Copepods - Fish	-0.25	0.39	Inf	-0.64	0.969
Copepods - Sediment	-0.61	0.41	Inf	-1.48	0.577
Copepods - Water Column	-0.15	0.40	Inf	-0.37	0.996
Benthic Crustaceans - Fish	0.37	0.19	Inf	1.91	0.312
Benthic Crustaceans - Sediment	0.01	0.23	Inf	0.02	1.000
Benthic Crustaceans - Water Column	0.47	0.20	Inf	2.35	0.129
Fish - Sediment	-0.36	0.20	Inf	-1.76	0.395
Fish - Water Column	0.10	0.17	Inf	0.62	0.973
Sediment - Water Column	0.46	0.21	Inf	2.19	0.183

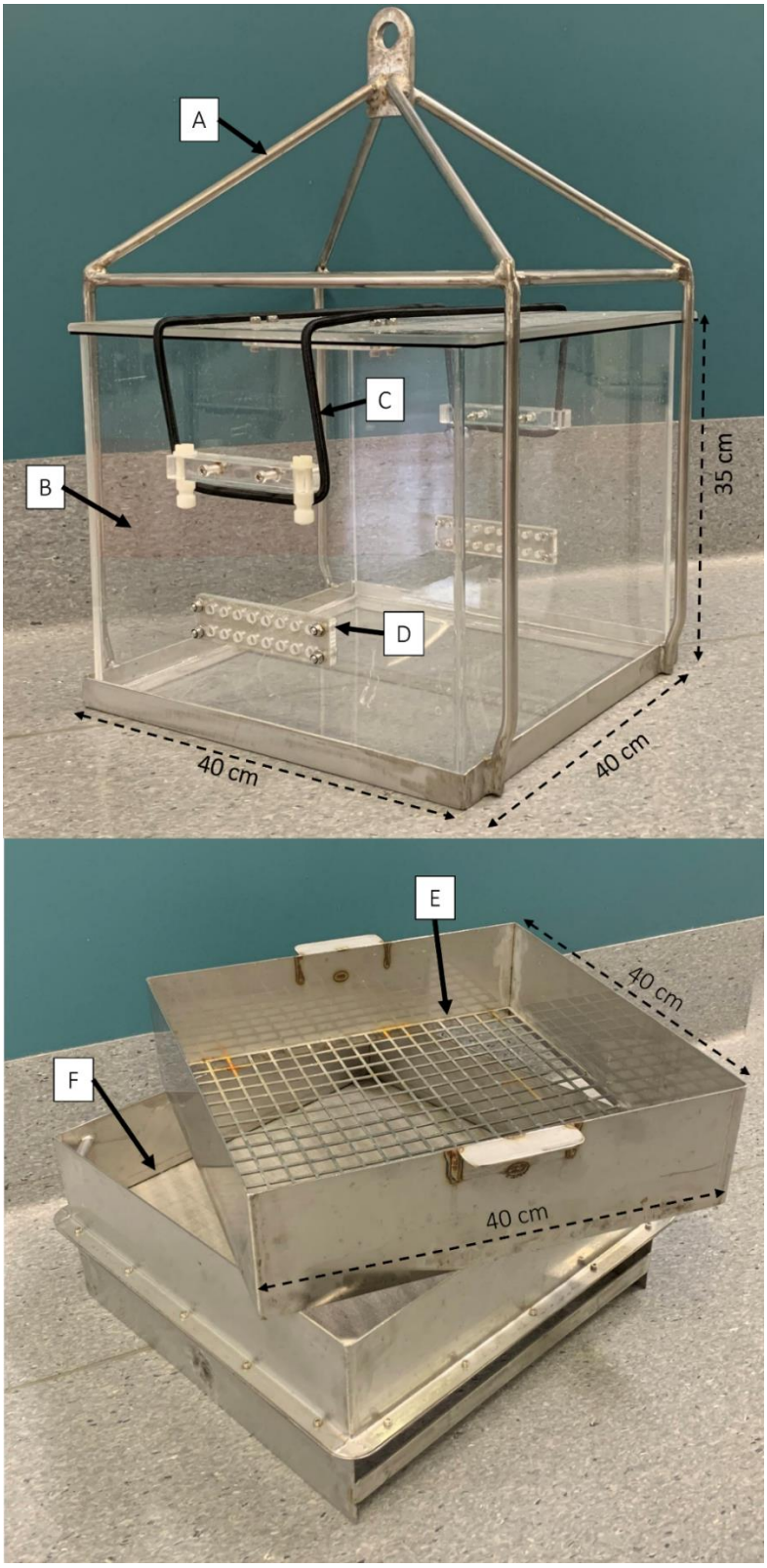


Figure A4.1 Acrylic collection boxes and stainless-steel sieve designed for sample collection of coral rubble environment to capture benthic crustaceans. (A) Stainless steel frame, (B) transparent acrylic box with removeable lid, (C) bungee cord to secure lid, (D) drain containing 315 µm stainless steel mesh, (E) 2cm stainless steel sieve, and (F) 315 µm stainless steel sieve. B, E and F are designed to stack on top of one another following collection.

Text A4. MP Contamination Units

Reporting MP contamination as MPs individual^{-1} for *in situ* organisms is standard practice in MP research (Chapter 2) as plastics are in essence heterogenous particulate matter varying in shape, size and polymer type (Andrady, 2011). Measuring these physicochemical properties is important to understand the associated ecological and physiological risks MPs pose (Lambert et al., 2017; Rochman et al., 2019). Traditionally, body burden of a pollutant is reported as the weight of pollutant per gram weight of the analysed tissue (Thornton et al., 2002); however, quantifying the weight of MPs ingested is not routinely done, due primarily to difficulties associated with handling heterogenous particulate matter less than 5 mm (Rivers et al., 2019). Therefore, the literature has adapted the classical definition of body burden to be representative of the number of MPs per gram weight of organism (Jensen et al., 2019; Kroon et al., 2018b; Santana et al., 2021) to account for the potential difference in impacts particulate MPs may have on smaller compared to larger organisms. By transforming MP contamination per individual to per gram weight of the compartment analysed (i.e., water, sediment, organism), comparisons can be made between organisms and their environment, an important component in (eco)toxicology. Here, MP contamination is reported in both units (i.e., per gram weight and per individual; see Table 5.1 and A4.2 Table) for organisms, to allow appropriate conclusions to be made with the data, as well as facilitate comparisons with previous studies (Cowger et al., 2020).

Appendix 5: Chapter 6

Table A5.1 In-house contamination library used to check for potential contamination of water samples during collection, processing, and analyses. The library includes items from field-collection and laboratory processing equipment and materials, as well as items found within laboratory airborne contamination blanks. PEST = Polyester, PVC = polyvinyl chloride, PP = polypropylene, PET = polyethylene terephthalate, LDPE = low density polyethylene, PAN = Polyacrylonitrile, PTFE = Polytetrafluoroethylene, PE = polyethylene, HDPE = high density polyethylene.

File Name	Colour	Shape	Polymer
Blank_Apr_18_Putative_01	blue	Fibre	Rayon
Blank_Apr_18_Putative_02	clear	Fibre	Cotton:PEST
Blank_Apr_18_Putative_03	clear	Fibre	Cotton
Blank_Apr_18_Putative_04	clear	Fibre	Rayon
Blank_Apr_18_Putative_05	pink	Fibre	Cotton
Blank_Aug_17_Putative_01	orange	Fibre	Cellulose
Blank_Aug_17_Putative_02	blue	Fibre	Cellulose
Blank_Aug_17_Putative_03	clear	Fibre	Cotton
Blank_Dec_17_Putative_01	black	Fibre	Cellulose
Blank_Dec_17_Putative_02	yellow	Fibre	Wool
Blank_Feb_17_Putative_01	clear	Fibre	Cotton
Blank_Feb_17_Putative_02	orange	Fibre	Cellulose
Blank_Feb_17_Putative_03	pink	Fibre	Cotton
Blank_Feb_17_Putative_04	clear	Fibre	Cotton
Blank_Feb_17_Putative_05	red	Fibre	Cotton
Blank_Feb_17_Putative_06	clear	Fibre	Cotton
Blank_Feb_17_Putative_07	white	Fibre	Cotton
Blank_Feb_17_Putative_08	clear	Fibre	Cellulose
Blank_Feb_17_Putative_09	blue	Fibre	Cotton
Blank_Feb_17_Putative_10	blue	Fibre	Cellulose
Blank_Feb_17_Putative_11	blue	Fibre	Cellulose
Blank_Feb_17_Putative_12	clear	Fibre	Cellulose
Blank_Feb_17_Putative_13	blue	Fibre	Cotton:Rayon
Blank_Feb_17_Putative_14	green	Fibre	Cotton
Blank_Feb_17_Putative_15	blue	Fibre	Cotton
Blank_Feb_17_Putative_16	blue	Fibre	Cotton
Blank_Feb_17_Putative_17	white	Fibre	Cotton
Blank_Feb_17_Putative_18	clear	Fragment	Silicate
Blank_Feb_17_Putative_19	white	Fragment	Silicate
Blank_Feb_17_Putative_20	blue	Fragment	Cellulose
Blank_Feb_19_Putative_01	clear	Fibre	Cotton
Blank_Feb_19_Putative_02	blue	Fibre	Cotton
Blank_Feb_19_Putative_03	red	Fibre	Cotton
Blank_Feb_19_Putative_04	black	Fibre	PEST
Blank_Feb_19_Putative_05	red	Fibre	Cotton
Blank_Feb_19_Putative_06	red	Fibre	Cotton
Blank_Feb_19_Putative_07	black	Fibre	Cotton
Blank_Feb_19_Putative_08	black	Fibre	Cotton
Blank_Jan_17_Putative_01	white	Fibre	Cotton
Blank_Jan_17_Putative_02	white	Fibre	Cotton:Rayon
Blank_Jan_17_Putative_03	blue	Fibre	Cellulose
Blank_Jan_17_Putative_04	clear	Fibre	Rayon
Blank_Jan_17_Putative_05	clear	Fibre	Cotton
Blank_Jan_17_Putative_06	blue	Fibre	Cotton
Blank_Jan_17_Putative_07	blue	Fibre	Cotton
Blank_Jan_17_Putative_08	clear	Fibre	Cotton
Blank_Jan_17_Putative_09	pink	Fibre	Cotton
Blank_Jan_18_Putative_01	clear	Fibre	Cotton
Blank_Jan_18_Putative_02	clear	Fibre	Cotton:Rayon
Blank_Jul_18_Putative_01	black	Fibre	Cotton
Blank_Jun_17_Putative_01	clear	Fibre	Cotton
Blank_Jun_17_Putative_02	clear	Fibre	Cotton
Blank_Jun_17_Putative_03	clear	Fibre	Cotton
Blank_Jun_17_Putative_04	blue	Fibre	Cotton
Blank_Jun_17_Putative_05	clear	Fibre	Cotton
Blank_Jun_17_Putative_06	blue	Fibre	Cotton
Blank_Jun_17_Putative_07	clear	Fibre	Cotton
Blank_Jun_17_Putative_08	clear	Fibre	Cotton
Blank_Jun_17_Putative_09	orange	Fibre	Cotton:Rayon
Blank_Jun_17_Putative_10	pink	Fibre	Cotton
Blank_Jun_17_Putative_11	blue	Fibre	Cotton
Blank_Jun_17_Putative_12	clear	Fibre	Cellulose
Blank_Jun_17_Putative_13	red	Fibre	Cotton
Blank_Jun_17_Putative_14	white	Fragment	Silicate
Blank_Jun_18_Putative_01	clear	Fibre	Cotton
Blank_Lab_Putative_01	clear	Fibre	Cotton
Blank_Lab_Putative_02	clear	Fibre	Cotton:Rayon
Blank_Lab_Putative_03	blue	Fibre	Cotton
Blank_Lab_Putative_04	blue	Fibre	Cotton
Blank_Lab_Putative_05	clear	Fibre	Cotton
Blank_Lab_Putative_06	yellow	Fibre	Cotton
Blank_Lab_Putative_07	black	Fibre	PEST
Blank_Lab_Putative_08	blue	Fibre	Cotton
Blank_Lab_Putative_09	black	Fibre	Cotton
Blank_Lab_Putative_10	clear	Fibre	Cotton
Blank_Lab_Putative_11	blue	Fibre	Cotton:Rayon
Blank_Lab_Putative_12	red	Fibre	PEST
Blank_Lab_Putative_13	green	Fibre	Cotton
Blank_Lab_Putative_14	blue	Fibre	Cotton
Blank_Lab_Putative_15	clear	Fibre	Cotton
Blank_Lab_Putative_16	green	Fibre	Cotton
Blank_Lab_Putative_17	pink	Fibre	Cotton
Blank_Lab_Putative_18	red	Fibre	Cotton
Blank_Lab_Putative_19	blue	Fibre	Cotton:Rayon
Blank_Lab_Putative_20	green	Fibre	Cotton
Blank_Lab_Putative_21	clear	Fibre	Cotton
Blank_Lab_Putative_22	green	Fibre	Cotton
Blank_Lab_Putative_23	green	Fibre	Cotton
Blank_Mar_17_Putative_01	black	Fibre	Cotton
Blank_Mar_17_Putative_02	black	Fibre	Cotton
Blank_Mar_19_Putative_01	clear	Fibre	Cotton:Rayon

Blank_Mar_19_Putative_02	black	Fibre	Cotton
Blank_Mar_19_Putative_03	red	Fibre	PEST
Blank_Mar_19_Putative_04	blue	Fibre	Cotton
Blank_May_17_Putative_01	black	Fibre	Cotton
Blank_May_17_Putative_02	blue	Fibre	Rayon:Wool
Blank_May_18_Putative_01	clear	Fibre	Cotton
Blank_May_18_Putative_02	clear	Fibre	Cotton
Blank_May_18_Putative_03	clear	Fibre	Cotton
Blank_May_18_Putative_04	clear	Fibre	Cotton
Blank_May_19_Putative_01	green	Fibre	Cotton
Blank_Oct_16_Putative_01	red	Fibre	Cotton
Blank_Oct_16_Putative_02	clear	Fibre	Cotton
Blank_Oct_16_Putative_03	red	Fibre	Cellulose
Blank_Oct_16_Putative_04	clear	Fibre	Cotton:Rayon
Blank_Oct_16_Putative_05	clear	Fibre	Cellulose
Blank_Oct_17_Putative_01	red	Fibre	Cotton
Blank_Oct_17_Putative_02	black	Fibre	Cellulose
Blank_Sep_17_Putative_01	black	Fibre	Cotton
Blank_Sep_17_Putative_02	black	Fibre	Cotton:Elastane
Blank_Sep_18_Putative_01	black	Fibre	Cotton:Rayon
Blank_Sep_18_Putative_02	blue	Fibre	Cotton
Field_40 um Sieve Mesh	clear	Fibre	Nylon
Field_40 um Sieve PVC	blue	Fragment	PVC
Field_Cod End Bottom	clear	Fragment	PP
Field_Plankton Net 350 um	clear	Fibre	PET
Field_Plankton Net Canvas	clear	Fibre	PP
Field_Plankton Net Nylon	clear	Fibre	Nylon

Field_RV_Apollo_Hose	green	Fragment	PVC
Field_RV_Apollo_Paint	white	Fragment	Acrylic
Field_RV_Apollo_Rope_01	clear	Fibre	PP
Field_RV_Apollo_Rope_02	clear	Fibre	LDPE
Field_RV_Apollo_Rope_03	green	Fibre	PP
Field_RV_Apollo_Rope_04	clear	Fibre	LDPE
Field_RV_Apollo_Rope_05	white	Fibre	PEST
Field_RV_Apollo_Rope_06	blue	Fibre	PEST
Field_RV_Ferguson_Paint_01	blue	Fragment	Isostearyl
Field_RV_Ferguson_Paint_02	blue	Fragment	Acrylic
Field_RV_Ferguson_Rope_01	green	Fibre	PP
Field_RV_Ferguson_Rope_02	green	Fibre	PP
Field_RV_Ferguson_Rope_03	clear	Fibre	PP
Field_RV_Ferguson_Rust_01	brown	Fragment	Isostearyl
Lab_Clear 50ml Vial Bottom	clear	Fragment	PP
Lab_Cotton Lab Coat	green	Fibre	Cotton
Lab_Nitrile Glove	blue	Fragment	PAN
Lab_Paper Towel	white	Fibre	Cotton
Lab_Parafilm	clear	Fragment	Paraffin Wax
Lab_PTFE Solvent Filter	white	Fibre	PTFE
Lab_PTFE Squeeze Bottle	clear	Fragment	PTFE
Lab_Purple Cloth	purple	Fibre	PEST:Viscose
Lab_Silicone O-ring	blue	Fragment	Polysiloxane
Lab_Squeeze Bottle	white	Fragment	PE
Lab_White Lab Coat	white	Fibre	Cotton:PEST
Lab_Yellow 50ml Vial Lid	yellow	Fragment	HDPE

*Table A5.2 Statistical output from the general linear model (GLM) for the plastic contamination collected at the SS Yongala National Research Station, compared to (a) physicochemical parameters including wind, current, salinity and temperature, and (b) Burdekin River discharge volume. Plastic counts, wind speed (knots), current speed (knots), salinity (ppt), temperature (ºC) and discharge volume (mL) were kept as raw data, with an offset to account for the tow volume (m^3). Month number (i.e., 1 to 12) was considered a nested variable, tow replicate a random effect. Output had 65 degrees of freedom, with a null deviation of 92.78. CI = confidence interval. Significant codes: * 0.05*

(a)

Formula: glm(Plastics ~ wind + current + salinity + temperature + offset(log(volume)) + (1|month/replicate), family = quasipoisson(link='log'))

	Estimate	Std. error	Statistic	P value	Low CI	High CI
(Intercept)	30.1	13.2	2.28	0.026 *	3.60	55.6
Wind Speed	-0.05	0.02	-2.74	0.008 *	-0.09	-0.02
Current Speed	-0.30	0.63	-0.48	0.630	-1.60	0.89
Salinity	-0.91	0.36	-2.52	0.014 *	-1.60	-0.18
Temperature	0.02	0.05	0.44	0.663	-0.08	0.13

(b)

Formula: glm(Plastics ~ average_discharge + offset(log(volume)) + (1|month/replicate))

	Estimate	Std. error	Statistic	P value	Low CI	High CI
(Intercept)	3.44	0.852	4.035	< 0.001 *	1.77	5.105
Average Discharge	1.07×10^{-5}	5.17×10^{-6}	2.068	0.042 *	5.61×10^{-7}	2.08×10^{-5}

*Table A5.3 Statistical output from the general linear model (GLM) for the plastic contamination collected at the SS Yongala National Research Station, compared to (a) physicochemical parameters including wind, current, salinity and temperature, and (b) Burdekin River discharge volume. Outliers (i.e., April 2017 and February 2019) were removed. Plastic counts, wind speed (knots), current speed (knots), salinity (ppt), temperature (ºC) and discharge volume (mL) were kept as raw data, with an offset to account for the tow volume (m^3). Month number (i.e., 1 to 12) was considered a nested variable, tow replicate a random effect. Output had 65 degrees of freedom, with a null deviation of 92.78. CI = confidence interval. Significant codes: * 0.05*

(a)

Formula: glm(Plastics ~ wind + current + salinity + temperature + offset(log(volume)) + (1|month/replicate), family = quasipoisson(link='log'))

	Estimate	Std. error	Statistic	P value	Low CI	High CI
(Intercept)	0.00	16.14	-1.62	0.112	0.00	60.96
Wind Speed	0.97	0.02	-1.97	0.053*	0.93	1.00
Current Speed	0.86	0.50	-0.31	0.758	0.31	2.21
Salinity	1.92	0.45	1.46	0.151	0.83	4.87
Temperature	1.04	0.04	1.07	0.287	0.96	1.13

(b)

Formula: glm(Plastics ~ average_discharge + offset(log(volume)) + (1|month/replicate))

	Estimate	Std. error	Statistic	P value	Low CI	High CI
(Intercept)	1.12	0.01	7.87	< 0.001*	1.09	1.15
Average Discharge	1.00	0.00	-0.75	0.457	1.00	1.00

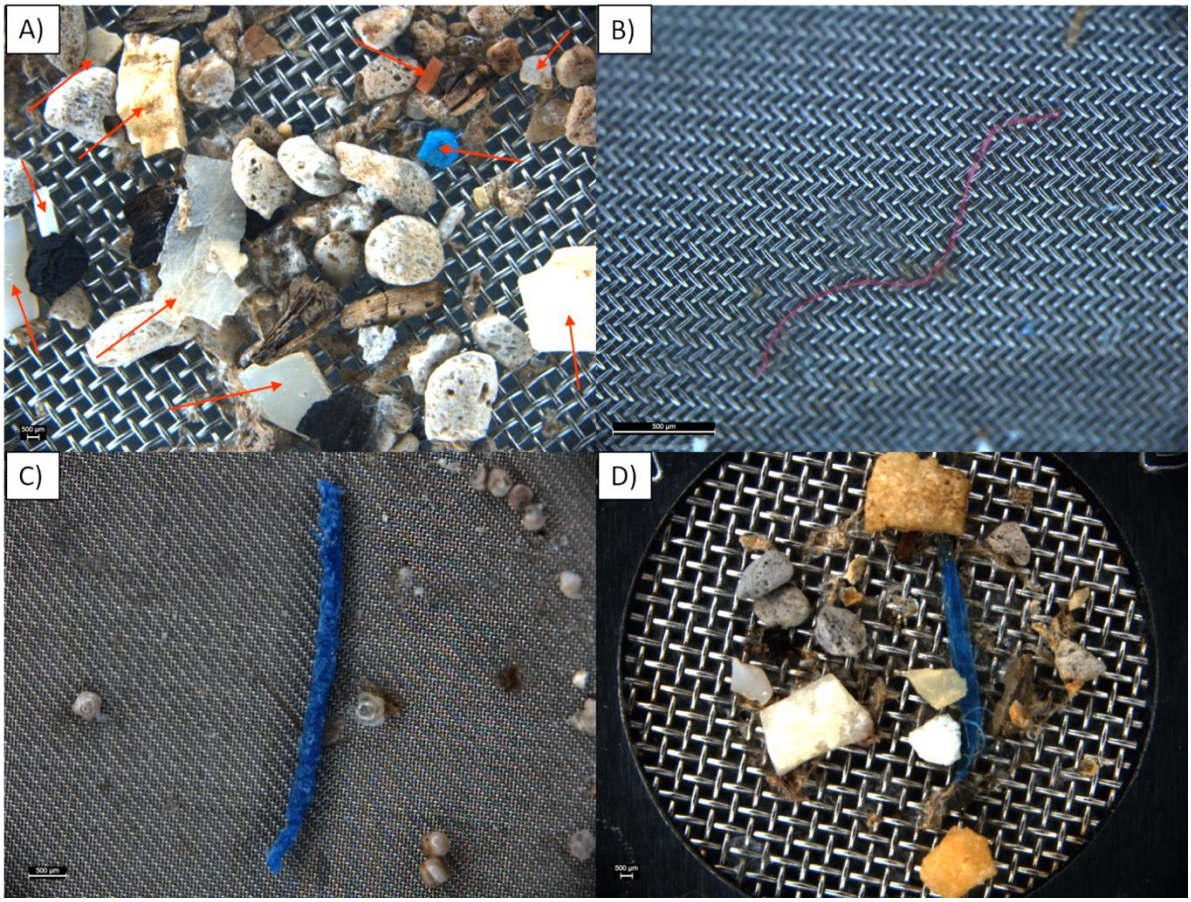


Figure A5.1 Plastic items isolated from surface seawater tows collected at the SS Yongala NRS in (a) April 2017, (b) June 2017, (c) May 2018, and (d) February 2019. Scale bars are representative of 500 µm.

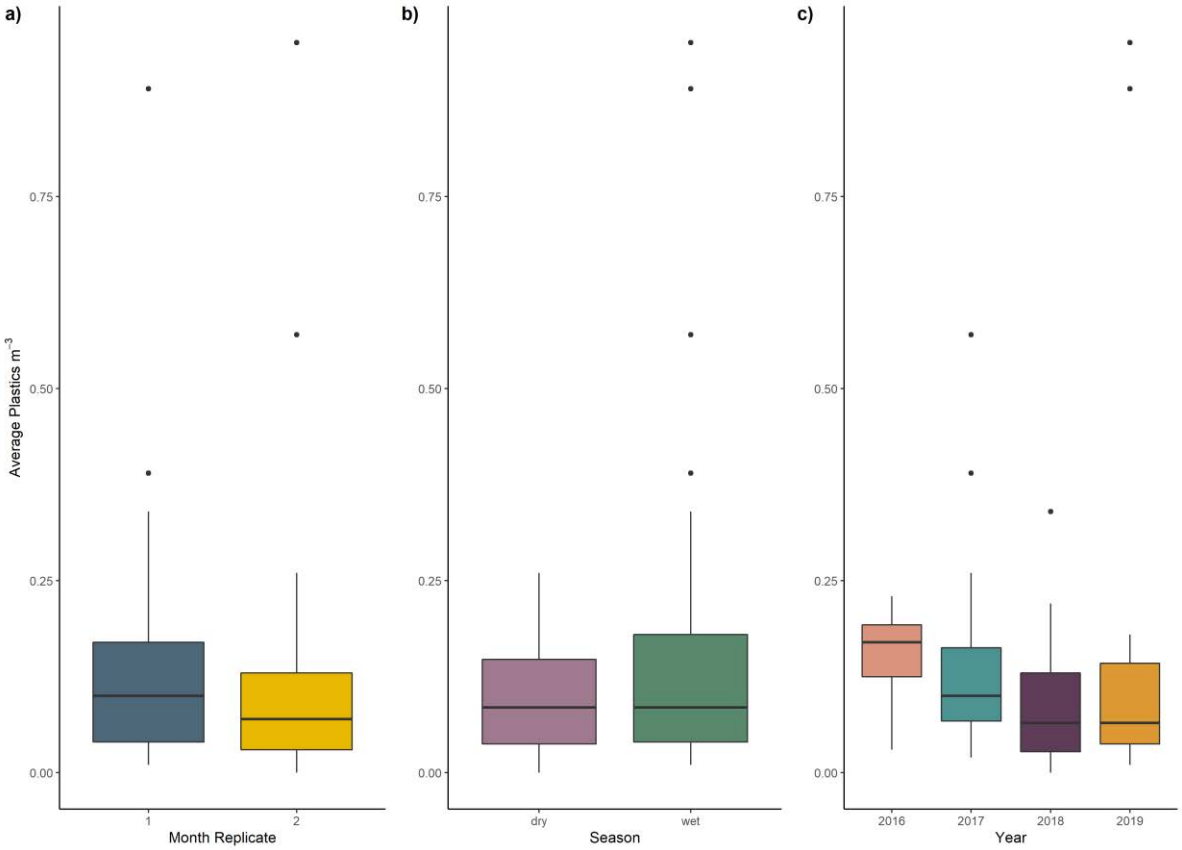


Figure A5.2 Plastic concentrations (per m^3) for tows collected at the SS Yongala National Reference Station (19°18'18.0" S, 147°37'19.2" E) between September 2016 and September 2019 (n=66) presented by (a) replicate tows (b) wet vs. dry season, and (c) each year. The median (solid black line), interquartile range (black box), minimum and maximum values (whiskers) and outliers (black points) are represented. Collections were not conducted in December 2016, January 2019, April 2019, and June 2019 due to inclement weather.