Does microplastic pollution pose a risk to marine life and food security?

by

Christopher David Walkinshaw

A thesis submitted in partial fulfilment of the degree of Doctor of Philosophy

University of East Anglia, School of Environmental Sciences

August 2022

In collaboration with

Plymouth Marine Laboratory

Abstract

Microplastics are a persistent and pervasive pollutant, ubiquitous in marine environments worldwide. Owing to their size and prevalence, microplastics have been demonstrated to be ingested by marine organisms throughout the food chain. However, the risks that microplastics pose to commercially exploited marine organisms are poorly elucidated. There is a lack of information pertaining to the inputs of microplastic into farmed marine species, the effect of environmentally relevant concentrations of microplastics on commercially important marine organisms, and ultimately whether microplastics may pose a risk to food security. In this thesis, I combine literature analysis with novel quantitative data through a suite of microplastic identification techniques and exposure experiments to explore these gaps in our current knowledge. My data demonstrates that while commercially-important organisms throughout the food chain ingest microplastics, lower trophic level organisms contain the highest body burdens of microplastics and there is little evidence that microplastics biomagnify. I identify that prolonged exposure to environmentally realistic concentrations of microplastic fibres results in lower growth rates in the commercially-exploited marine bivalve *Mytilus edulis*. I highlight a novel pathway for the contamination of farmed fish with microplastics via contaminated aquaculture feed. Finally, I performed a methods comparison of commonly used microplastic identification techniques to reveal micro-FTIR and py-GCMS are the most effective means for characterizing microplastics in complex samples, but resultant data are not readily comparable. My thesis draws attention to the prevalence of semi-synthetic and cotton microfibres in marine samples, and how their environmental risk is often ignored. This research contributes to our knowledge of how microplastics and other anthropogenic particles can contaminate aquaculture feed and adversely affect lower trophic level organisms, posing a risk to marine food security, and guides researchers as to the best techniques to use when analysing complex organic samples.

Acknowledgements

This thesis is the product of many colleagues, institutions, family and friends, without whom this would not have been possible. Firstly, I would like to extend a huge thank you to my supervisory team, Matthew Cole, Pennie Lindeque, Trevor Tolhurst, and Richard Thompson; I cannot thank you enough for your support and encouragement throughout my PhD, and for all the training and opportunities you have offered over the past four years. Special thanks to Matt, who in addition to all of the above, patiently guided me through the pitfalls of a PhD and offered unparalleled support through a global pandemic. Without your advice and insight this thesis would not have been possible. My thanks to Dr Alexandra ter Halle and Magali Albignac at Université Paul Sabatier, who kindly welcomed me into their laboratory in Toulouse and mentored me in the use of py-GCMS. I would also like to extend my thanks to the captain and crew of the RRS James Clark Ross, and all of those involved in the ChAOS research cruise JR18006, with whom I spent a fantastic 5 weeks in the Barents Sea.

To the microplastics group at PML: Matt, Rachel, Pennie and Zara, you have made my time at PML hugely enjoyable, and have been fantastic lab mates. Thank you for your support, for the many troubleshooting sessions, and for keeping me sane during pandemic lab experiments. Thank you to the wonderful PML student community who were present throughout with wise words, encouragement and friendship through difficult circumstances, even if it was from a distance of 2 meters at times.

I would not be where I am without a number of close family and friends who have offered their support and encouragement along the way. To my parents, Helen and Steven, and my brother Andrew, thank you for your love and support over not only the PhD, but all my endeavours over the last 31 years. And finally, thank you to the love of my life, Anna, who not only helped me through my PhD with boundless love and support, but also became my wife along the way.

My research was supported by the Natural Environment Research Council (NERC) through the EnvEast Doctoral Training Partnership (grant number: NE/L002582/1).

Author’s Declaration

My thesis is presented as one literature review (published) and three research papers (two submitted for publication, one being prepared for submission). All work has been the product of my planning and implementation and I am the lead author on all the papers presented within; the contributions from my supervisors and co-authors are described at the beginning of each chapter. Papers have been reformatted to provide a unified writing and referencing style throughout, with figures and tables embedded within the text. References are compiled into a single bibliography at the end of the thesis.

Contents

List of Figures and Tables

List of Abbreviations and Definitions

Anthropogenic particle	All particles of anthropogenic origin, including microplastics, semi-synthetic and cellulosic particles
FSW	Filtered seawater
FTIR	Fourier Transform Infrared spectroscopy
Macoplastic	Plastic particles > 5mm
Microplastic	Plastic particles 0.1 – 5 mm
MP	Microplastic
Nanoplastic	Plastic particles <0.1 mm
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyls
PE	Polyester
PET	Polyethylene terephthalate
pH_20	MilliQ purified H_20
PMMA	Poly(methyl methacrylate)
PML	Plymouth Marine Laboratory
PS	Polystyrene
PVC	Polyvinyl chloride
Py-GCMS	Pyrolysis gas chromatography mass spectrometry
Py-GCMS/MS	Pyrolysis gas chromatography mass spectrometry/mass spectrometry
S	Salinity
Semi-synthetic	Polymers manufactured from heavily modified natural materials e.g. rayon from regenerated cellulose
SMI	Sediment-microplastic Isolation unit
µmol	Micromoles
$ZnCl_2$	Zinc Chloride

Chapter 1: General Introduction

On 1st October 2018, when I started this PhD, the global population reached approximately 7.9 billion (Baillie and Zhang, 2018). This is an increase of nearly two billion people from the turn of the century, and more than four billion over the last 50 years (Goldewijk, 2005). Though population growth has slowed to 1.22% per annum, it is expected that 8.9 billion people will inhabit the earth by 2050 (Cohen, 2003). It is a huge challenge to feed such a large population, and farming must operate on a global scale to do so. In recognition of this challenge, shortly after the second world war the Food and Agriculture Organization of the United Nations (FAO) was founded. The FAO has outlined several priorities in the fight against hunger, including making agriculture and fisheries more productive and sustainable, increasing resilience of food systems to crises, and helping eliminate hunger and malnutrition. Through progressing these aims, the concept of food security has been widely discussed. Food security is defined by the FAO as "A situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life (FAO *et al.*, 2017). A large proportion of the global population live in coastal areas, with most of the world's megacities within the coastal zone and a projected coastal population of 879-949 million people (10.9-11.3% global population) by 2030 (Brown *et al.*, 2013; Neumann *et al.*, 2015). It is of no surprise, therefore, that fisheries and aquaculture play a huge part in the provision of food for humans. In 2018, 156 million tonnes of food for human consumption was produced by fisheries and aquaculture, with aquaculture accounting for 52% of this (FAO, 2020). Fishery and aquaculture production for human consumption has increased by 58 million tonnes since the turn of the century, with global fish consumption increasing by an annual growth rate of 3.1% from 1961 – 2017, higher than that

of other animal protein foods which increased by 2.1% per year (FAO, 2020). Marine and coastal fisheries and aquaculture are an important part of this, representing 84.4 and 30.8 million tonnes of food, respectively. Aquaculture is one of the fastest growing food production areas and is predicted to play an increasingly important role in food production in the future (Willett *et al.*, 2019; Gephart *et al.*, 2021), despite being beset by several issues, including increased risk of disease, local environmental pollution, habitat destruction, and escaped fish becoming invasive (Cole *et al.*, 2009). The 'State of food security and nutrition in the world' report (FAO, IFAD, UNICEF, 2021) identifies several major drivers challenging food security, namely conflict, climate variability and extremes (linked to climate change), economic slowdowns and downturns, and the unaffordability of healthy diets. However, it is also acknowledged that pollution is a risk to food security (Ehrlich, Ehrlich and Daily, 1993; Chakraborty and Newton, 2011), and the same report concedes that "There can be serious health consequences from different forms of environmental contamination – including from heavy metal contamination, fertilizers, pesticides, air pollution and smog, GHG emissions and microplastic pollution."

Plastics are an incredibly diverse range of materials, used in all aspects of our everyday life, from food packaging and preparation, to transportation, healthcare, and electronics. Synthetic polymers are manufactured through the polymerization of oil and gas-derived monomers, with the addition of chemical additives to alter their properties to best suit their application (Thompson *et al.*, 2009; Hale *et al.*, 2020). Plastics are suited to such a wide array of applications because they are inexpensive, lightweight, strong, are good insulators, and resist degradation. As a result, plastic production has increased from 0.35 tonnes per annum in 1950 to 348 million tonnes in 2017 (Verla *et al.*, 2019). Plastics are now so commonplace they are even theorized to form a branch of the carbon cycle, as plastics are essentially fossilized carbon manufactured into polymers (Zhu, 2021). Unsurprisingly, due to its ubiquity in our lives and widespread use in

single-use items, plastic pollution is a major problem for the global community, with ecological, social and economic impacts (Beaumont *et al.*, 2019; MacLeod *et al.*, 2021). Plastic is described as a poorly reversible pollutant as emissions cannot currently be lessened and it can persist in the environment for centuries, with estimated half-lives of high density polyethylene ranging from 58-1200 years in the marine environment (Shah *et al.*, 2008; Barnes *et al.*, 2009; Chamas *et al.*, 2020; MacLeod *et al.*, 2021). It has been estimated that in 2010, 4.8-12.7 million tonnes of plastic entered the marine environment, and without waste management improvements, this is expected to increase by an order of magnitude by 2025 (Jambeck *et al.*, 2015). Once in the environment, plastics break down into smaller and smaller fragments, forming micro- and nanoplastics, followed eventually by mineralization into inorganic molecules including carbon dioxide, water and ammonia (Andrady, 1998, 2011; Thompson *et al.*, 2004; Barnes *et al.*, 2009). Since their presence in our environment was first highlighted (Thompson *et al.*, 2004), the scientific community have sought to define and understand them, in order to evaluate their risk and trace their sources and sinks. In 2008, at an international workshop hosted by the NOAA, a working definition that microplastics are all plastic particles <5mm in diameter was adopted by the field at large (Arthur, Baker and Bamford, 2009), though this has since been adapted by most researchers to allow for a distinction with nanoplastics, which make up the nano-size fraction <100 nm (Koelmans, Besseling and Shim, 2015); in this thesis, I therefore define microplastics as plastic particles with a maximum Feret diameter in the range of 0.1 µm – 5 mm. Microplastics can be defined as primary or secondary in origin, with primary microplastics manufactured in this size range for their purpose, e,g, microbeads in cosmetics, pre-production pellets (often called nurdles), bio-beads used in sewage treatment processes etc., and secondary microplastics which are formed from the breakdown of macroplastics within the environment through UV degradation, abrasion and wave action (Andrady, 2011). The washing of textile items results in the release of microfibres, which enter marine environments through water

treatment plants (Napper and Thompson, 2016; De Falco *et al.*, 2020), and synthetic tyre particles are produced through road friction, where they are subsequently washed into waterways and are transported into the marine environment (Kole *et al.*, 2017). Once in the marine environment, microplastics can travel vast distances in ocean currents (van Sebille, England and Froyland, 2012; Horton and Dixon, 2018), and several microplastic sinks have been identified, including ocean gyres, sediments, shorelines, the deep sea, sea ice, and biota (Law *et al.*, 2010; Browne *et al.*, 2011; van Sebille, England and Froyland, 2012; Van Cauwenberghe *et al.*, 2013, 2015; Woodall *et al.*, 2014; Peeken *et al.*, 2018; Kanhai *et al.*, 2019). The size of microplastics makes them highly bioavailable to many marine species, and ingestion has been observed in organisms throughout the food chain in their natural environment, e.g. zooplankton (Desforges, Galbraith and Ross, 2015), bivalves (Van Cauwenberghe and Janssen, 2014), fish (Lusher, McHugh and Thompson, 2013), sea mammals (Besseling *et al.*, 2015; Nelms *et al.*, 2019) and seabirds (Amélineau *et al.*, 2016), including in marine organisms consumed by humans (Rummel *et al.*, 2016; Cho *et al.*, 2019; Karbalaei *et al.*, 2019). While there has been a focus on the study of microplastics in the environment leading to a huge amount of information regarding these pollutants, there is a lack of studies investigating pollution by other anthropogenic particles such as semi-synthetic and cotton microfibres. These particles are released through many of the same processes as microplastics, e.g. washing of textiles and abrasion from general use (Napper and Thompson, 2016; Zambrano *et al.*, 2019), and they are similar in size, colour, and morphology to microplastics, suggesting they are also bioavailable to the same marine organisms. Though these particles are generally cellulosic-based microfibres, they are heavily modified through chemical treatment, and can contain zinc salts and flame retardants, among other potentially hazardous chemicals (Barker, 1975), and may therefore pose an environmental risk in a similar way as microplastics (Chen *et al.*, 2007; Moriam *et al.*, 2021). Due to the similarity of these particles to microplastics, I use the term ‘anthropogenic particles’ to refer to both sets of particles throughout this thesis.

The FAO has identified microplastics as a potential challenge to food security (FAO, IFAD, UNICEF, 2021). The risks posed by microplastics are poorly understood, and there is a disparity between current knowledge about the effects of microplastics on marine organisms at environmentally-relevant levels and the perception of the risk of microplastics to food security. Studies have demonstrated effects from microplastics on marine organisms such as increased mortality (Lee *et al.*, 2013; Gray and Weinstein, 2017), decreased reproduction and fecundity (Lee *et al.*, 2013; Cole *et al.*, 2015), and disturbed larval development (Sussarellu *et al.*, 2016). However, these studies were often performed at concentrations many orders of magnitude greater than those currently observed in the marine environment, due to a lack of data accurately reporting environmental concentrations of microplastics. Nonetheless, this research has led to the perception that marine organisms may be less healthy to consume due to microplastic contamination (SAPEA, 2019). Although the disparity between theorized environmental concentrations and laboratory exposure concentrations has decreased, this is an issue that persists to the present day, especially as current environmental microplastic concentrations are disputed (Lindeque *et al.*, 2020). It is therefore difficult to determine what the current and future risks from microplastics really are, and we cannot accurately describe risks to food security using current information.

In this thesis, "***Does microplastic pollution pose a risk to marine life and food security?***", I explore the risks that microplastics pose to food security, considering their capacity to bioaccumulate and cause harm, and their prevalence in aquaculture feed, through both analysis of the current literature and laboratory-based experiments. I also assess the ability of current microplastic identification methods to identify microplastics and other anthropogenic particles within complex organic samples, with the aim of harmonizing methods for the creation of comparable results.

In Chapter 2, "***Microplastics and seafood: lower trophic organisms at highest risk of contamination***", I explore the current knowledge

base pertaining to the contamination of commercially important fished and farmed marine organisms with microplastics. Through a semi-systematic review of the available literature, I surmise that microplastics are not likely to biomagnify within the food chain and that organisms at lower trophic levels have the highest body burdens of microplastics (mass g^{-1}). I explore the factors that influence the consumption and retention of microplastics by marine organisms, highlight risks to fisheries and aquaculture, and identify knowledge gaps in the field. Discussion of anthropogenic particles other than microplastics highlights the lack of knowledge about the effects from other microparticle pollutants such as cellulosic microfibres.

In Chapter 3, "***Impact of polyester and cotton microfibers on growth and sublethal biomarkers in juvenile mussels***", I investigate the effects of anthropogenic particles on the Blue mussel (*Mytilus edulis*), a lower trophic level, commercially important organism. Juvenile mussels were exposed to environmentally-relevant concentrations of polyester and cotton microfibres over a three-month timescale. Clearance rate, respiration rate and growth rate were recorded throughout the exposure period, and I subsequently discuss the impact of a significantly reduced growth rate on juvenile mussels and marine food security.

In Chapter 4, "***Detection and characterization of microplastics and microfibres in fishmeal and soybean meal***", I consider whether aquaculture feeds may be increasing exposure of farmed finfish to microplastics. As fishmeal is typically manufactured from low trophic level organisms, I hypothesised that fishmeal will present a novel route for contamination of farmed fish with anthropogenic particles, while plant based meals would not contain anthropogenic particle contamination. However, the presence of anthropogenic particles within both soybean meal and fishmeal led me to the conclusion that anthropogenic particles in feeds primarily stem from post-harvest contamination. The results were used to estimate the anthropogenic particle burden fed to Atlantic salmon over their commercial lifespan, and I surmise the effects of this on nutrition and food security.

In Chapter 5, "***Identification of anthropogenic particles within complex organic samples"***, I use the experience gained through this PhD in handling complex organic samples such as fishmeal and soybean meal to perform a methods comparison between commonly used microplastic identification techniques. I analysed fishmeal and soybean meal samples using four commonly used analytical techniques and compared their efficacy in the identification of both microplastics and other anthropogenic particles within these complex organic samples. I provide a pragmatic comparison of these methods in the hope of guiding researchers in the best techniques to use when approaching future research studies and evaluate the inter-comparability of data stemming from FT-IR and py-GCMS analysis.

My thesis contributes to the knowledge base of researchers seeking to understand which marine organisms may be most at risk from anthropogenic particles, how environmentally-relevant concentrations of anthropogenic particles may affect marine organisms, and the effect that this may have on aquaculture. The work within this thesis informs researchers and policymakers of the current and predicted future effects of microplastic pollution on marine food security and provides a guide to the best techniques for researchers to use when analysing the complex sample matrices that are often investigated in environmental research.

Chapter 2: Microplastics and seafood: lower trophic organisms at highest risk of contamination

This chapter is a reformatted version of my publication:
Walkinshaw, C., Lindeque, PK., Thompson, RT., Tolhurst, T. and Cole, M. (2020): Microplastics and seafood: lower trophic organisms at highest risk of contamination, Ecotoxicology and Environmental Safety 190(1), 110066, https://doi.org/10.1016/j.ecoenv.2019.110066
CW conducted all literary research, processed the data and was lead author of the review, MC guided the development of the review, and all authors provided comments and edits that helped shape the final manuscript.

Microplastic debris is a prevalent global pollutant that poses a risk to marine organisms and ecological processes. It is also suspected to pose a risk to marine food security; however, these risks are currently poorly understood. In this review, we seek to understand the current knowledge pertaining to the contamination of commercially important fished and farmed marine organisms with microplastics, with the aim of answering the question “Does microplastic pollution pose a risk to marine food security?”. A semi-systematic review of studies investigating the number of microplastics found in commercially important organisms of different trophic levels suggests that microplastics do not biomagnify, and that organisms at lower trophic levels are more likely to contaminated by microplastic pollution than apex predators. We address the factors that influence microplastic consumption and retention by organisms. This research has implications for food safety and highlights the risks of microplastics to fisheries and aquaculture, and identifies current knowledge gaps within this research field.

2.1 Introduction

Microplastics are a ubiquitous global contaminant, identified throughout the marine environment, including seawater, sediment and biota (Cole *et al.*, 2011; Law and Thompson, 2014). Microplastics describe tiny plastic particulates, although a coherent definition remains under debate, especially in terms of their size (Frias and Nash, 2019; Hartmann *et al.*, 2019). For the purposes of this review, we refer to microplastics and nanoplastics as synthetic solid particles or polymer matrices, with at least one dimension ranging 0.1 µm–5 mm. The literature describes microplastic shapes in a myriad of different ways, from spheres, beads and fragments, to films, filaments and fibres; for consistency, we here opt for using the terms “bead” (any spherical plastic), “fibre” (plastic threads such as those used in clothing), or “fragment” (irregularly shaped particulates). Microplastics can be further classified based on their origin: primary microplastics are manufactured in the micro size range, and include cosmetic microbeads, pre-production pellets and industrial scrubbers; secondary microplastics are formed by the breakdown of macroplastics within the environment (Andrady, 2017). Microplastic fibres have been identified as a particular concern for the environment, owing to their abundance and bioavailability, with research suggesting that microplastic fibres can contribute up to 91% of all plastics collected in global seawater samples (Barrows, Cathey and Petersen, 2018).

Plastic production has increased rapidly since its inception, with an estimated 8.3 billion metric tonnes of virgin plastic produced to date. Approximately 4.6 billion metric tonnes of this (55%) has been produced since 2000 (Geyer, Jambeck and Law, 2017). Microplastics enter the marine ecosystem through many different pathways, including riverine transport, sewage and wastewater effluent, direct release (e.g. from shipping and ports) and atmospheric deposition (Boucher and Friot, 2017). Plastics are incredibly durable, and rather than undergoing a straightforward process of mineralization in the

marine environment, plastics first degrade into smaller and smaller fragments, eventually forming micro- and nanoplastics (Andrady, 1998, 2011). Microplastic debris can travel vast distances via oceanic currents and winds, impinging on remote habitats including mid-oceanic islands and the polar ice caps (Barnes *et al.*, 2009; Peeken *et al.*, 2018). Sinks of microplastics include the ocean gyres, sediments, shorelines, polar sea ice, and biota, including animals destined for human consumption (Hardesty *et al.*, 2017; Peeken *et al.*, 2018). Whilst there are efforts to remove microplastics from the marine environment, it is widely accepted that once released, it neither practically nor economically feasible to recapture marine microplastics for recycling or responsible disposal.

Microplastics pose a risk to marine life and ecological processes (Galloway, Cole and Lewis, 2017a), and it has been suggested they may further impact on food security (Barboza *et al.*, 2018a), socio-economic wellbeing (Beaumont *et al.*, 2019) and human health (Galloway, 2015). The perceived risks, pathways, effects, and consequences arising from microplastic pollution on food security and ecosystem health in the marine environment are displayed in Fig. 2.1.

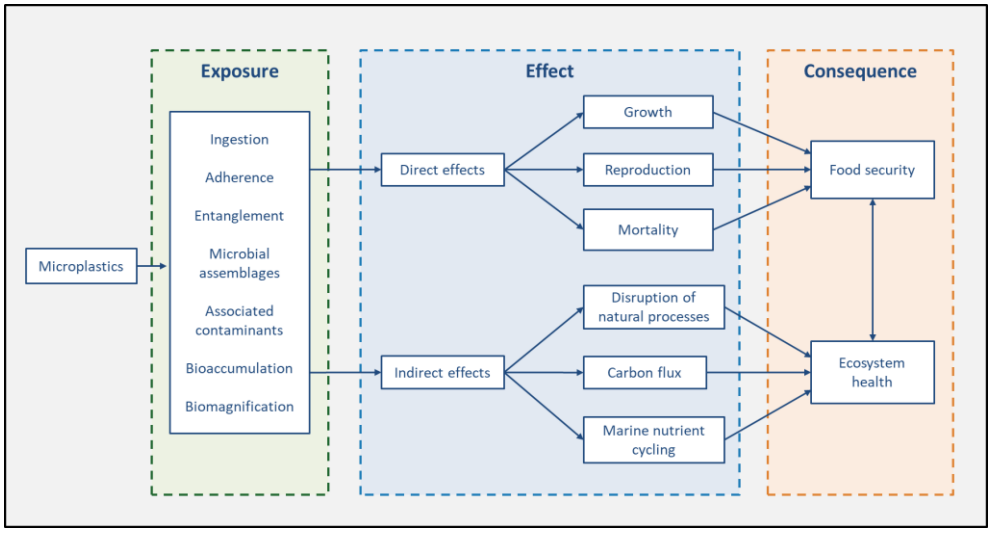


Fig. 2.1. Perceived impact pathways of microplastics on food security and ecosystem health.

2.1.1 Marine food security

Fisheries and aquaculture provide a critical proportion of the world's food supply, providing over 4.5 billion people with at least 15% of their average per capita intake of animal protein (Béné *et al.*, 2015), and production is predicted to grow in the future, from 171 million tonnes in 2016 to approximately 201 million tonnes in 2030, an increase of 17.5% (FAO, 2018). Global fish exports in 2017 were valued at 152 billion USD (FAO, 2018). Total capture from fisheries has remained fairly constant since the 1990s and is not expected to increase considerably, with growth instead expected from aquaculture, predominantly in Asia, which as a continent accounts for almost two thirds of global fish consumption (Béné *et al.*, 2015). The FAO predicts that aquaculture production will reach 109 million tonnes in 2030 (FAO, 2018).

Food security is defined by the Food and Agriculture Organisation as "a situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life" (FAO *et al.*, 2017). Current identified risks to food security include climate variability due to both short-term events and climate change, eutrophication, ocean acidification, oxygen depletion, conflict, economic recession, pathogens, and pollution (Chakraborty and Newton, 2011; Wollenberg *et al.*, 2016). Larger plastic debris, particularly derelict fishing gear (i.e., abandoned or lost nets, lines, pots), has been shown to pose a substantial risk to food security. For example, in Chesapeake Bay the removal of 34,408 derelict fishing pots over the time period 2008-2014 led to the harvest of an additional 13,504 metric tonnes in blue crab (*Callinectes sapidus*) over the same period, valued at 21.3 million USD (Scheld, Bilkovic and Havens, 2016). However, whilst there has been considerable research into the effects of microplastics on marine organisms, evidence is lacking on the effect of microplastics on food security and food safety. We hypothesise that in marine ecosystems already affected by a multitude

of environmental stressors, microplastics may represent an additional risk to food security.

In this review, we critically assess microplastics research with relevance to fishing and aquaculture, the health of commercially exploited organisms, and food security, to understand the current state of microplastics research and evaluate whether microplastics pose a risk to food security. Several marine pollutants are known to biomagnify, causing heightened risk to higher trophic organisms, however, very little research is available to show whether this may occur with microplastics, with current research giving opposing viewpoints (GESAMP, 2016; Akhbarizadeh, Moore and Keshavarzi, 2019; Hantoro *et al.*, 2019). We evaluate currently available data regarding microplastic content within organisms of different trophic levels to assess whether biomagnification is likely to be a risk with microplastic contamination. Current research gaps will also be discussed to highlight areas where unknown risks may threaten marine food security and human health.

2.2 Methods

2.2.1 Sourcing reference material

In order to investigate the prevalence of microplastics in commercially exploited marine organisms, including fish, shellfish, crustaceans and macroalgae, we undertook a semi-systematic review of the scientific literature, performed by using a specific set of search terms separated by Boolean operators (Table 2.1), utilising the academic literature search engines *Web of Science*, *ScienceDirect*, *PubMed* and *PLOS ONE*. This search method was supplemented by use of a snowballing method, where further literature was identified in the references of the articles reviewed to encompass the broadest set of literature. Only articles published up to the end of 2018 were included in the data analysis in this review. See Table 2.2 for a summary of the number of

articles found from each search engine. These articles were considered for relevant information and subjected to a quality control step (see below); literature that passed this stage was utilised in this review.

Table 2.1. Search terms and Boolean operators used in the identification of scientific literature.

Search term	**Boolean operator**	**Search term**
Microplastic Microplastic pollution Marine microplastic	AND OR	Food security Food Marine Health Fish (including individual species searches) Effect Shellfish (including individual species searches) Bivalve (including individual species searches) Organism

Table 2.2. Relevant literature identified through searches of different academic literature search engines

Academic search engine	**Results retrieved**
Web of Science	955
ScienceDirect	1516
PubMed	668
PLOS ONE	46

2.2.2 Quality control

The primary literature from which data was extracted for analysis had been peer-reviewed prior to publication, providing a base level of quality assurance. We additionally conducted a quality assessment to verify that: (1) experimental replication was performed for statistical analysis; and (2) suitable controls were implemented in the study

protocol (e.g., negative controls in toxicity testing, procedural blanks, and contamination controls in environmental analyses). If any of these quality control parameters was not met, the literature was not included in this review. After these steps, the identified literature was cross-referenced with available data showing organisms of global importance to aquaculture and fisheries. Following further narrowing of studies to select those that analysed organisms of commercial importance, 32 pieces of literature were selected to ascertain the data presented in this review.

2.2.3 Data analysis

In the literature data is typically presented as the number of microplastics per individual (MP/individual) for fish, or microplastics per gram (wet weight, w. w.) (MP/gram) for shellfish. For assessing whether microplastics biomagnify within lower trophic level organisms it was necessary to convert MP/individual values by ascertaining mean wet weights for individual species, drawn from primary and grey literature. MP/gram w. w. values were subsequently estimated by dividing average microplastics per organism by the average mass of that organism as reported in the literature (see Supplementary information for details).

2.3 Results

2.3.1 Risks to food security

Prevalence of microplastics in commercially exploited species

Microplastics can be ingested by a wide range of marine life, and the presence of microplastics in marine organisms destined for human consumption has been widely reported. Tables 2.3 and 2.4 below show the 10 most caught marine species and 10 most farmed aquaculture species in 2016 (FAO, 2018), alongside evidence of their capacity to ingest microplastic debris. 60% of the most farmed aquaculture species have been investigated for the presence of

microplastics, and 80% of the most caught marine species have been investigated. The organisms that are not mentioned in any microplastic ingestion studies up to the end of 2018 represented a total of approximately 22.5 million tonnes of food in 2016.

Table 2.3. 10 most cultured aquaculture species in 2016 (data from FAO, 2018). NIF = no information found.

Common name	**Species name**	**Production (thousand tonnes, 2016)**	**Habitat**	**Feeding strategy**	**Microplastic ingestion reference**
Grass carp	*Ctenopharyng-odon idellus*	6 068	Freshwater	Herbivorous	NIF
Silver carp	*Hypophthalmic-hthys molitrix*	5 301	Freshwater	Planktivorous	Jabeen *et al.*, 2017
Cupped oysters NEI	*Crassostrea spp.*	4 864	Estuarine	Filter feeder	Van Cauwenberghe and Janssen, 2014; Rochman *et al.*, 2015; Phuong *et al.*, 2018; Waite, Donnelly and Walters, 2018
Common carp	*Cyprinus carpio*	4 557	Freshwater	Omnivorous	Jabeen *et al.*, 2017
Japanese carpet shell	*Ruditapes philippinarum*	4 229	Seawater and estuarine	Filter feeder	Li *et al.*, 2015
Nile tilapia	*Oreochromis niloticus*	4 200	Freshwater	Omnivorous	Rochman *et al.*, 2015; Biginagwa *et al.*, 2016
Whiteleg shrimp	*Penaeus vannamei*	4 156	Seawater	Planktivorous (plus more: detritus, worms, bivalves and crustaceans)	NIF

Common name	Species name	Production (thousand tonnes, 2016)	Habitat	Feeding strategy	Microplastic ingestion reference
Bighead carp	*Hypophthalmic-hthys nobilis*	3 527	Freshwater	Planktivorous	NIF
Crucian carps	*Carassius spp.*	3 006	Freshwater	Omnivorous	Jabeen *et al.*, 2017; Yuan *et al.*, 2019
Catla	*Catla catla*	2 961	Freshwater	Planktivorous	NIF

Table 2.4. 10 most caught marine species in 2016 (data from FAO, 2018). NIF = no information found.

Common name	Species name	Production (thousand tonnes, 2016)	Habitat	Feeding strategy	Microplastic ingestion reference
Alaska pollock	*Theragra chalcogramma*	3 476	Demersal	Fish and invertebrates	NIF
Peruvian anchovy	*Engraulis ringens*	3 192	Pelagic	Planktivorous	Ory *et al.*, 2018
Skipjack tuna	*Katsuwonus pelamis*	2 830	Pelagic	Fish, crustaceans, molluscs	Rochman *et al.*, 2015; Choy and Drazen, 2013; Markic *et al.*, 2018
Sardinellas NEI	*Sardinella spp.*	2 290	Pelagic	Planktivorous	NIF
Jack and horse mackerels NEI	*Trachurus spp.*	1 744	Pelagic/ demersal	Fish and plankton	Neves *et al.*, 2015; Foekema *et al.*, 2013; Lusher, McHugh and Thompson, 2013; Murphy *et al.*, 2017; Markic *et al.*, 2018; Güven *et al.*, 2017
Atlantic herring	*Clupea harengus*	1 640	Pelagic	Planktivorous	Ogonowski *et al.*, 2017;

Common name	Species name	Production (thousand tonnes, 2016)	Habitat	Feeding strategy	Microplastic ingestion reference
					Foekema *et al.*, 2013; Rummel *et al.*, 2016; Hermsen *et al.*, 2017
Pacific chub mackerel	*Scomber japonicus*	1 599	Pelagic	Fish and plankton	Neves *et al.*, 2015; Rochman *et al.*, 2015; Güven *et al.*, 2017; Ory *et al.*, 2018
Yellowfin tuna	*Thunnus albacares*	1 463	Pelagic	Fish, crustaceans, molluscs	Choy and Drazen, 2013; Markic *et al.*, 2018
Atlantic cod	*Gadus morhua*	1 329	Demersal	Fish and crustaceans	Foekema *et al.*, 2013; Bråte *et al.*, 2016; Liboiron *et al.*, 2016; Rummel *et al.*, 2016
Japanese anchovy	*Engraulis japonicus*	1 304	Pelagic	Planktivorous	Tanaka and Takada, 2016

Fish

Many species of edible demersal, pelagic and reef fish, sampled from across the globe, have been found to ingest microplastics (Lusher, McHugh and Thompson, 2013; Neves *et al.*, 2015; Rochman *et al.*, 2015; Bellas *et al.*, 2016; Bråte *et al.*, 2016; Rummel *et al.*, 2016; Tanaka and Takada, 2016; Critchell and Hoogenboom, 2018; N. C. Ory *et al.*, 2018; N. Ory *et al.*, 2018). Of the seven most farmed aquaculture species which are fish (Table 2.3), all are freshwater species, and their feeding strategies are mostly planktivorous or omnivorous, with the exception of the grass carp which is herbivorous and feeds mostly on aquatic weeds. These fish may be likely to consume microplastics due to their prey being within a similar size

range. However, microplastic ingestion investigations have only been performed on Common carp, Crucian carps, Nile tilapa and Silver carp, and no data is available for the other three species, even though they represent a combined 12.5 million tonnes of farmed fish (as of 2016). These studies gave a combined average amount of microplastics per organism of 2.5 ± 1.3 MP/individual (Common carp), 1.9 ± 1.0 MP/individual (Crucian carps), and 3.8 ± 2.0 MP/individual (Silver carp). Nile tilapia data was presented by the authors as the number of individuals which had consumed microplastics, which was an average of 16% (Rochman *et al.*, 2015; Biginagwa *et al.*, 2016). Where it is possible to view the morphology of plastic particles ingested, fibres are the most common microplastic shape seen and make up 57.6-86.5% of the plastic shapes observed.

Of the ten most caught species (Table 2.4), all are marine fish; the majority are pelagic species that consume mostly plankton and small fish, with three exceptions (pollock, tuna and cod). The microplastic content of these fish are much more studied than common aquaculture species, with 80% of the top ten most fished species included in at least one microplastic study. Collating all available literature on these organisms gives the following percentages of each species that were seen with microplastics in their gastrointestinal tract (GIT): 0.9% Peruvian anchovy; 9.4% Skipjack tuna; 24.5% Jack and Horse mackerels; 8.8% Atlantic herring; 23.3% Pacific chub mackerel; 23.4% Yellowfin tuna; 2.8% Atlantic cod, and 76.6% Japanese anchovy (Neves *et al.*, 2015; Foekema *et al.*, 2013; Lusher, McHugh and Thompson, 2013; Murphy *et al.*, 2017; Güven *et al.*, 2017; Ogonowski *et al.*, 2017; Rummel *et al.*, 2016; Hermsen *et al.*, 2017; Rochman *et al.*, 2015; Ory *et al.*, 2018; Choy and Drazen, 2013; Markic *et al.*, 2018; Bråte *et al.*, 2016; Liboiron *et al.*, 2016; Tanaka and Takada, 2016). Other species of commercial importance that have been included in several pieces of literature (plus percentages seen with microplastics in their GIT) include Scads (*Decapterus spp*, 46%.), European pilchards (*Sardina pilchardus*, 26%), Blue whiting (*Micromesistius poutassou*, 29.8%), and Atlantic mackerel (*Scomber scombrus*,

23.2%). As with aquacultured species, fibres are the most common microplastic shape seen, forming 30-87.6% of the plastic shapes observed. Unfortunately it is not possible to view in detail the most common size of microplastics observed in each species due to how the data is reported, however this information may not be reliable due to constraints in minimum observable size in the methodology used (e.g. choice of filters, sensitivity of analytical techniques, Lusher *et al.*, 2017). Notable by its absence in the literature is the Alaska Pollock (*Theragra chalcogramma*) and members of *Sardinella spp.*, neither of which were found to have been analysed to investigate microplastic ingestion in the literature. Both species are an extremely important food source, with more than 3.47 million tonnes of Pollock and 2.29 million tonnes *Sardinella spp.* fished in 2016.

Shellfish

Cupped oysters (*Crassostrea spp.*) and Japanese carpet shell (*Ruditapes philippinarum*) are among the most prevalently aquacultured shellfish species worldwide. Microplastic ingestion in shellfish is generally reported as the number of microplastics per gram of wet tissue. In Cupped oysters, the average result reported ranged from 0.18 to 3.84 microplastics gram^{-1} w. w., and in the Japanese carpet shell, the average reported result ranged from 0.9 to 2.5 microplastics gram^{-1} w. w.

By far the most studied shellfish are mussels of the family *Mytilidae*. 9 pieces of literature were identified that studied the amount of microplastics found in sea mussels in their natural environments, with ingestion ranges varying from 0.2-5.36 microplastics g^{-1} w. w. (Bråte et al., 2018; Catarino *et al.*, 2018; De Witte *et al.*, 2014; Li *et al*., 2015, 2016; Phuong *et al.*, 2018; Qu *et al.*, 2018; Van Cauwenberghe and Janssen, 2014; Van Cauwenberghe *et al.*, 2015). Whilst ingestion values look different when analysing the number of microplastics ingested per individual, when normalised for soft tissue weight, the values for all three species overlap, seemingly showing that microplastic ingestion in shellfish is not species-specific. Though

shellfish can show selective feeding, rejecting particles based on size (Newell and Jordan, 1983; Defossez and Hawkins, 1997), they are found to ingest microplastics. Whilst these species all ingest similar amounts of microplastics, it is possible that they selectively ingest different size microplastics due to organism size, with for example oysters being able to ingest larger particles than mussels. Data from the analysis of mussels and oysters taken from the French Atlantic coast (Phuong *et al.*, 2018) suggests this, as both organisms ingested a majority of microplastics in the 50-100 µm size range, but mussels ingested a higher proportion of 20-50 µm particles than oysters (37% and 15 %, respectively), and oysters ingested a higher proportion of > 100 µm particles than mussels (32% and 11%, respectively).

Crustaceans

Crustaceans form a very large and diverse group of organisms including many that are important for worldwide food security, such as crabs, lobsters, crayfish and prawns. Many edible species of crustaceans have been shown to ingest microplastics (Devriese *et al.*, 2015a; Welden and Cowie, 2016a; Abbasi *et al.*, 2018). Organisms such as copepods and krill are also critically important as a food for organisms which are consumed by humans, and have been reported to ingest microplastics (Botterell *et al.*, 2019). No studies have been performed to investigate microplastic ingestion in the Whiteleg shrimp, one of the top ten most farmed aquatic species with 4.2M tonnes farmed in 2016 (Table 2.3), however, investigations have taken place with other commercially important species. Brown shrimp, *Crangon crangon,* a commercially important crustacean fished in the eastern Atlantic and Mediterranean Sea, were found with an average of 0.68 ± 0.55 microplastics gram^{-1} w. w. and 63% of the 165 shrimp analysed containing microplastics (Devriese *et al.*, 2015a). Green tiger prawn, *Penaeus semisulcatus*, an organism of commercial importance in East Africa and Asia, was found to have ingested an average of 7.8 particles per individual (1.5 particles gram^{-1}, n=12) in the Musa estuary, Persian Gulf (Abbasi *et al.*, 2018). Nylon fibres were observed in the stomachs of 5.93% *Plesionika narval* (narwhal shrimp), an important fishery in

the Aegean Sea, although it is hypothesised by the authors that these fibres may result from the fishing method (Bordbar *et al.*, 2018). Other commercially important species that have been observed to contain microplastics include *Eriocheir sinensis* (Wójcik-Fudalewska, Normant-Saremba and Anastácio, 2016), *Carcinus maenas* (Watts *et al.*, 2014, 2015), and *Nephrops norvegicus* (Murray and Cowie, 2011; Welden and Cowie, 2016b).

Macroalgae

Seaweeds have been consumed as a traditional food around the globe; however, consumption of seaweed has been increasing in recent years with much of this increase from farming of seaweed rather than from harvesting wild crops. Statistics from the Food and Agriculture Organisation of the United Nations state that aquatic plant production grew from 13.5 million tonnes to over 30 million tonnes from 1995 to 2016, with 96.5% of the 31.2 million tonnes produced in 2016 from aquaculture (FAO, 2018). Seaweeds for consumption are generally classified into three groups: red algae (Rhodophyta) such as Dulse and Nori, brown algae (Phaeophyceae) such as kelp and green algae (Chlorophyta, Charophyta, Mesostigmatophyceae, Chlorokybophyceae and Spirotaenia) such as sea lettuce. *Fucus vesiculosus* is a common seaweed in the British Isles and Atlantic coastlines, in the class of brown algae, and is often consumed as a health supplement. Recent studies have shown the ability for 20 µm polystyrene microparticles to sorb to *F. vesiculosus* (Sundbæk *et al.*, 2018). Trophic transfer via this macroalgae has also been observed; Gutow *et al.* (2016) demonstrated the ability for the common periwinkle *Littorina littorea* to ingest microplastics via *Fucus vesiculosus*. Algal pieces were exposed to polystyrene microbeads (10 µm), fragments (1-100 µm), and polyacrylic fibres (90 to 2200 µm), followed by a washing step. Feeding assays with the three types of microplastic-contaminated algal pieces showed that *Littorina* littorea did not show a feeding preference between contaminated and non-contaminated algal pieces, and microplastics were found in the stomach content, gut

and faecal pellets, with 89% of *L. littorea* faecal pellets containing microplastics.

2.3.2 Factors influencing microplastic consumption

Feeding strategy

Broadly speaking, there are two main ways for marine organisms to ingest microplastics: direct ingestion from the natural environment; or indirect ingestion, including trophic transfer from prey and consumption of contaminated aquaculture feedstock. Furthermore, there is some indication that microplastics can be taken up via the gills (Watts *et al.*, 2014). Dietary strategy may be a defining characteristic influencing microplastic ingestion in fish, with planktivores more likely to consume microplastics direct from the natural environment, while piscivores (e.g., tuna) would be expected to consume microplastics mainly through trophic transfer via prey or accidental ingestion while feeding.

Direct ingestion of microplastics is often a consequence of feeding strategy. Indiscriminate feeders show no selection in the matter that they ingest, ingesting prey in proportion to their availability in the environment, whilst discriminate feeders select based on preferential feeding factors (colour, size etc.). Filter feeders such as some bivalves can be considered as indiscriminate feeders as they feed by filtering water through their gills, capturing particulate matter such as plankton and microalgae. This is generally in a non-selective manner; however some of the filtered matter can be rejected. This has been shown recently by Ward *et al.* (2019), who demonstrated that the bivalves *Crassostrea virginica* and *Mytilus edulis* selectively ingested microplastics preferentially, based on the physical characteristics of the plastic. In this way, microplastics are ingested if they resemble the properties of the organic matter these organisms feed on, such as in size and shape. Discriminate feeders may directly ingest microplastics either when they resemble prey items, or incidentally whilst feeding, e.g., in contaminated feedstock; this feeding strategy is generally

utilised by higher trophic-level organisms. Discriminate feeders such as fish may therefore ingest microplastics that resemble their prey. Amberstripe scad (Decapterus muroadsi) appear to ingest blue microplastics preferentially as they resemble their copepod prey in both colour and size (Ory *et al.*, 2017). Evidence of selective feeding on the blue copepods *Pontella sinica* and *Sapphirina spp.* was seen, as was selectivity for blue microplastics.

Indirect ingestion, or "trophic transfer" occurs when organisms consume prey that have already consumed microplastics. Trophic transfer from blue mussels *Mytilus edulis* to the shore crab *Carcinus maenas* has been observed in laboratory conditions (Farrell and Nelson, 2013; Watts *et al.*, 2014). Farrell and Nelson (2013) fed 0.5 µm fluorescent polystyrene microspheres to *M. edulis*, with *C. maenas* subsequently being fed one mussel per crab. Microspheres were subsequently detected in the stomach, hepatopancreas, ovary, gills and haemolymph of the crabs. Results from Nelms *et al.* (2018) suggest the ability for microplastics to be ingested by grey seals (Halichoerus grypus) through trophic transfer from Atlantic mackerel (Scomber scombrus). Detritivores may also be prone to indirectly consuming microplastics present in faeces of contaminated organisms; for example coprophagous copepods can ingest microplastics present in other copepods' egests (Cole *et al.*, 2016). Feedstock contaminated with microplastics may be a risk to aquaculture, as fishmeal is a commonly used fish feed manufactured from whole fish, therefore any microplastics within the fish may pass into the processed fishmeal (Karbalaei *et al.*, 2019).

Trophic level

The percentage of planktivorous and piscivorous fish populations contaminated with microplastics might suggest that trophic level and feeding strategy alone are not indicative of microplastic ingestion, however, this may be due to a difference in how microplastics data are usually presented (Table 2.5). For example, Markic *et al.* (2018), saw no significant difference in their study on plastic ingestion rate in 23

species of fish in the South pacific based on their trophic level, with the only significant difference in ingestion rates seen between benthic predators and omnivores. However, while similar proportions of the total population of marine organisms with different dietary strategies contained microplastics, the number of microplastics per gram of tissue may be very different. For example, data presented in this review shows a similar percentage of *S. japonicus* (23.3%) and *T. albacares* (23.4%) contained microplastics, but the average weight of *T. albacares* caught by Markic *et al.* (2018) is 5228.7 g, whereas the average caught weight for *S. japonicus* by Güven *et al.* (2017) was 28.86 g. Using these weights, the average amount of plastic particles per gram (wet weight) for S*comber japonicus* from Güven *et al.* (2017) is estimated as 0.33 particles $gram^{-1}$ and the maximum number of microplastics found per gram in *Thunnus albacares* from Markic *et al.* (2018) is estimated at $5.9x10^{-4}$ particles $gram^{-1}$, a 1000-fold difference.

In order to investigate this further, 11 commercially exploited taxa, including bivalves, crustaceans and fish, were selected for analysis from a variety of trophic levels. Taxa were selected that had either a wide range of literature available for analysis (e.g. *Mytilus spp.*, *Scomber japonicus*), or were at a trophic level not covered by other data (e.g. *Thunnus* albacares, *Katsuwonus* pelamis). The data were normalized to give the number of microplastics ingested per gram wet weight of these organisms. Table 2.5 lists the fish, crustaceans and bivalves in which the number of microplastics per gram wet weight of tissue has been calculated.

Table 2.5. Number of microplastics per gram wet weight marine organisms.

Species	**Common name**	**Family**	**Diet**	**Microplastics per gram wet weight**	**Raw data references**
Katsuwonus pelamis	Skipjack tuna	Fish	Largely piscivorous	0.000249	Markic *et al.,* 2018
Thunnus albacares	Yellowfin tuna	Fish	Largely piscivorous	0.00059	Markic *et al.*, 2018
Clupea harengus	Atlantic herring	Fish	Planktivorous	0.01	Foekema *et al.*, 2013
Engraulis ringens	Peruvian anchovy	Fish	Planktivorous	0.057	Ory *et al.*, 2018
Trachurus spp.	Jack and horse mackerels NEI	Fish	Planktivorous	0.000126-0.14	Foekema *et al.*, 2013; Neves *et al.*, 2015; Güven *et al.*, 2017; Markic *et al.*, 2018
Scomber japonicus	Pacific chub mackerel	Fish	Planktivorous	0.0025-0.33	Neves *et al.*, 2015; Güven *et al.*, 2017; Ory *et al.*, 2018
Crangon crangon	Brown shrimp	Crustacean	Planktivorous/ herbivorous	0.13-1.23	Devriese *et al.*, 2015
Penaeus semisulcatus	Green tiger prawn	Crustacean	Planktivorous/ herbivorous	1.5	Abbasi *et al.*, 2018
Ruditapes philippinarium	Japanese carpet shell	Shellfish	Filter feeder	0.9-2.52	Li *et al.*, 2015; Davidson and Dudas, 2016
Crassostrea spp.	Cupped oysters	Shellfish	Filter feeder	0.18-3.84	Foekema *et al.*, 2013; Van Cauwenberghe and Janssen, 2014; Phuong *et al.*, 2018; Waite, Donnelly and Walters, 2018
Mytilus spp.	Sea mussels	Shellfish	Filter feeder	0.2-5.36	De Witte *et al.*, 2014; Van Cauwenberghe and Janssen, 2014; Li *et al.*, 2015, 2016; Van Cauwenberghe *et al.*, 2015; Bråte *et al.*, 2018; Catarino *et al.*, 2018; Phuong *et al.*, 2018; Qu *et al.*, 2018

There is up to four magnitudes of difference between microplastics per gram present in shellfish compared to higher trophic level fish. The data presented above therefore suggests that trophic level and feeding strategy may play a key role in the level of microplastic contamination within marine organisms; though similar percentages of the total population of organisms at different trophic levels contain microplastics within their body tissues, lower trophic level organisms have a higher proportion of microplastic comparatively with body weight, which may be more indicative of risks from microplastics. Fig. 2.2 displays a comparison of microplastics per gram wet weight of the organisms in Table 2.5 with the amount of mercury in tissues of similar organisms reported by Plessi, Bertelli and Monzani (2001; *Mytilus spp.*) and the FDA (FDA, 2017; all other species). Mercury is well known to biomagnify, and values are inversely proportional with the microplastic data presented here, which shows a decrease in microplastic concentration with increasing trophic level. Based on this data, we conclude that unlike other contaminants such as organochlorines (Borgå, Gabrielsen and Skaare, 2001) or mercury (Lavoie *et al.*, 2013), microplastics do not biomagnify. This is likely because the evidence currently suggests that microplastics do not, in most cases, translocate from the digestive system into tissues or circulatory fluid, therefore it is a more transitory contaminant with a limited residence time within organisms.

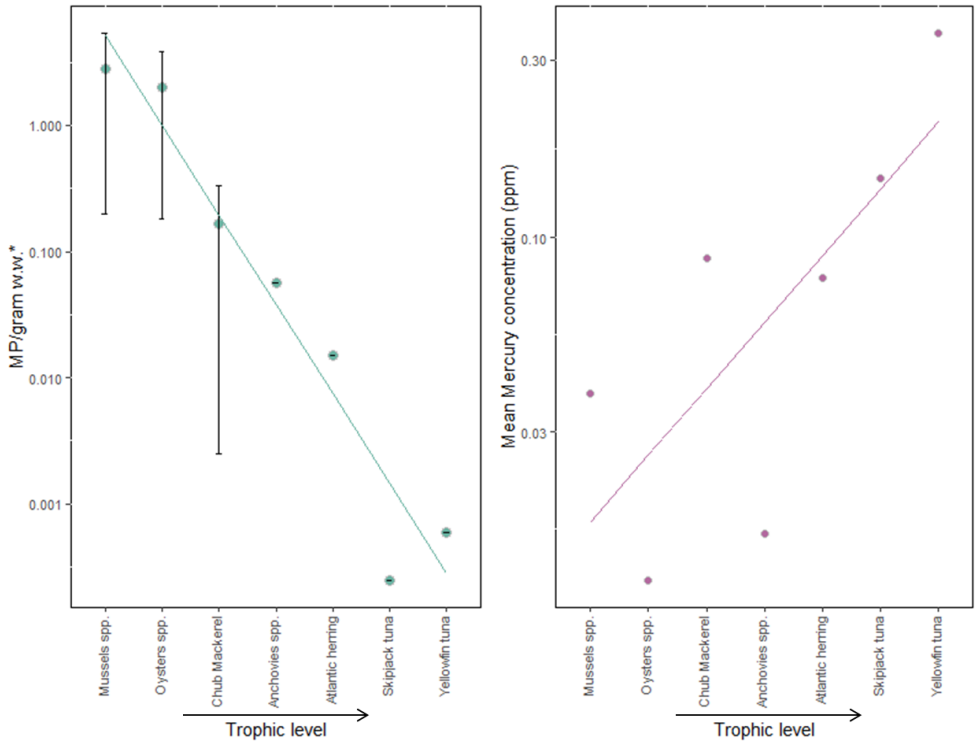


Fig. 2.2. A comparison of the number of microplastics (MP) per gram wet weight of organisms of different trophic levels to the amount of Mercury (ppm) reported in the tissues of similar organisms as listed by the FDA (FDA, 2017) and Plessi, Bertelli and Monzani (2001). Trophic level shows general increase with direction of arrow. Line of best fit added to show trend in data. *Value is average value of ranges shown in Table 2.5 and Table S2.1, with error bars displaying the range of the results.

Environmental concentrations

It is possible that another variable such as habitat may have a pronounced effect on the amount of microplastic ingestion. Markic *et al*. (2018) saw a significant difference in the vertical habitat of a species and their plastic ingestion rates. Although they did not see a significant difference with respect to horizontal distribution (Neritic/Neritic-oceanic/Oceanic), it may be expected that for example fish caught in an oceanic gyre or other area of high microplastic load may have a higher incidence of ingestion than those caught in other areas. In fact, this is observed in the study in question; significantly higher ingestion of microplastic debris was observed in a sampling area within the South Pacific ‘garbage patch’ than in fish from other

locations. This was seen with for example Thunnus albacares, where ingestion was seen in 70% of individuals within the garbage patch, and 24% and 15% at two locations outside of this area. In juvenile fish, there was an increased incidence of microplastic ingestion and increasing concentrations of microplastic in seawater with proximity to the coast, with higher encounter rates where microplastic concentrations exceeded those of fish larvae (Steer *et al.*, 2017).

Environmental concentrations may be a particularly important variable for microplastic ingestion in crustaceans and molluscs (Li *et al.*, 2019). As bivalves are filter feeders, any differences in microplastic ingestion are likely due to microplastic distribution in their habitat. Li *et al.* (2016) investigated microplastic abundance in mussels in 22 sites along the coast of China, and significant differences in microplastic ingestion were seen at different sites. Wild mussels contained on average 2.7 items/g (4.6 items/individual) and farmed mussels contained on average 1.6 items/g (3.3 items/individual). In heavily contaminated areas, mussels contained an average of 3.3 items/g (5.3 items/individual), whereas in less contaminated areas, microplastic abundance in mussels was significantly lower (1.6 items/g or 3.3 items/individual). Gut content of individuals of the crustacean *Nephrops norvegicus* collected from three sites in North and West Scotland had significantly different microplastic ingestion; 84.1%, 43% and 28.7% of *N. norvegicus* individuals ingested microplastic in the Clyde Sea Area, North Minch and North Sea, respectively (Welden and Cowie, 2016a), suggesting crustaceans may also ingest microplastics relative to environmental availability.

2.3.3 Risks of microplastics to marine organisms

Retention in the digestive system (gut blockages)

Following ingestion, microplastics may be rejected by the organism through pseudofaeces or post-ingestion rejection, egested through faeces, transferred across the GIT epithelium, or be retained in the GIT. Microplastic retention in the digestive system may adversely affect organism health through physical perforation of the gut or by

giving the organism a feeling of false satiety, decreasing feeding activity and nutrient intake.

Shore crabs fed with 10 µm polystyrene microspheres had plastic detected in the foregut 5 days after exposure to microplastic-containing mussels (Watts et al., 2014). In this feeding experiment, crabs were fed with mussels that had been exposed to microplastics and subsequently sampled over a 21-day period, and n=6 crabs were analysed for microplastics in the foregut at each time point post-ingestion. Polystyrene microspheres were detected in all six crabs after 24hrs; decreasing to 50-66% of the crabs from days 2-5. Microplastics were then not detected in the crab faecal pellets after 7 and 22 days post-exposure (but were on day 14).

Blue mussels (*Mytilus edulis*) were shown to ingest 9% of all available microplastic fibres (approx. 450 µm length) in an ingestion study where microplastic fibres were ingested alongside the microalgae *Rhodomonas salina* (Woods et al., 2018). Mussel filtration rate decreased when exposed to microplastic fibres in addition to *R. salina*, and though most fibres (71%) were rejected as pseudofaeces, 9% were ingested, and < 1% were excreted in faeces. Microplastics were identified in the gills, digestive gland and other soft tissues at all time points over a 72 hr exposure period. In another experimental study, 2 of 31 Palm Ruff (*Seriolella violacea*) fish were shown to retain microplastics after a 49-day exposure period (Ory et al., 2018). The transitory nature of microplastics within the digestive system of organisms may explain why microplastics do not appear to biomagnify. If microplastics pass through the GIT of organisms and are not retained within the GIT or tissues, it is much less likely that organisms at higher trophic levels will ingest significant amounts of microplastics through a carnivorous diet.

Research by Welden and Cowie (2016) suggests whilst Norway lobster (*Nephrops norvegicus*) are seen to retain microplastics within their foregut for extended periods of time, the main route by which they are removed is by ecdysis, whereby the individual moults and sheds

its gut lining. This gut lining was found to contain microplastics which were removed from the individual during moulting.

Are growth rate, reproduction or function affected?

Any changes to growth rate, reproduction, mortality or behaviour due to external factors may significantly alter population dynamics. In the case of commercially important organisms, this may significantly affect the efficiency and profitability of fishing and aquaculture. Lower growth rates may mean that fewer organisms can be harvested in a season, or lower reproduction rates may cause population decreases in following seasons, both of which would have a negative effect on food security. A similar concept is discussed by Galloway, Cole and Lewis (2017), who propose that, though chronic exposure to microplastic is not usually lethal, it is associated with reductions in energy, growth, fecundity and reproductive output. These individual and population-level effects can as a consequence cause ecosystem level effects, such as community shifts and changes to ecosystem function, which would result in risks to food security.

Several articles have shown reduction of growth rates and reproductive function (Cole et al., 2015; Sussarellu et al., 2016), and behavioural changes (Cole et al., 2015; Sussarellu et al., 2016; Ribeiro et al., 2017; Woods et al., 2018) in marine organisms as a result of exposure to microplastics. Significant effects from microplastic exposure were observed in laboratory exposure studies with the Pacific oyster (*Crassostrea gigas*) (Sussarellu et al., 2016). Significantly higher algal consumption was observed for oysters exposed to microplastics, possibly in an attempt for the oyster to compensate for lower nutrient intake. Significant reproductive effects were observed; exposed female oysters had fewer, smaller oocytes and a reduction in D-larval yield; exposed male oysters had lower sperm velocity. *C. gigas* larval growth was significantly slower, with a reduction in mean size of 18.6% at 17 days post-fertilization and a 6-day lag time to metamorphosis.

Behavioural changes are observed in clams; 20 µm polystyrene microplastics also induced effects on antioxidant capacity, DNA damage, neurotoxicity and oxidative damage in *Scrobicularia plana* (Ribeiro et al., 2017), and reduced clearance rate in *Atactodea striata* (Xu et al., 2017). Behaviour may also be affected in the presence of nanoplastics. For example, Wegner et al (2012) observed no pseudofaeces production in *Mytilus edulis* exposed to microalgae alone, but found heightened pseudofaeces production in *Mytilus edulis* exposed to microalgae (Pavlova lutheri) and 30 nm polystyrene, along with a decrease in filtering activity.

Risk of disease

Once in the marine environment, microplastics are quickly colonised by a variety of organisms termed the plastisphere (Zettler, Mincer and Amaral-Zettler, 2013). The plastisphere is a risk to the marine environment, aquaculture and food security as it has the potential to support pathogenic microorganisms, and allow them to become more bioavailable to the organisms consuming microplastics. Recent research has identified hazardous microorganisms present on microplastics, along with microorganisms usually found in sewage and gut-associated pathogens (Oberbeckmann, Löder and Labrenz, 2015). The microbial biofilms discussed here affect the physical characteristics of the plastic, including size and buoyancy, which could in turn affect the vertical distribution of microplastics within the water column, transporting microplastics to the benthos (Kaiser, Kowalski and Waniek, 2017; Kooi et al., 2017). This, in addition to the horizontal transport of microplastics via ocean currents and wind therefore means that microplastics have the capacity to transport microorganisms to new environments over vast distances, suggesting the potential for microplastics to act as a vector for the transfer of invasive pathogens to new environments.

High concentrations of microplastic debris in the North pacific subtropical gyre have resulted in an increase in the pelagic insect *Halobates sericeus* and in *H. sericeus* egg densities (Goldstein, Rosenberg and Cheng, 2012). Jiang et al. (2018) profiled bacterial

communities attached to microplastic samples taken from intertidal locations around the Yangtze estuary in China, and found a wide range of bacterial taxa, including some that are associated with human and animal pathogens: *Vibrio* (0.4% of taxonomic abundance, found at Xiangshan bay); *Leptolyngbya* (1.6% abundance, found at Chongming island), and *Pseudomonas spp.* (<0.01% abundance, all plastics).

Harmful pathogens travelling large distances could have severe implications for food security. One potential example of this would be the colonisation of marine plastics by HAB (harmful algal bloom) species. When floating plastic debris collected along the North-west Mediterranean were analysed, several potentially harmful dinoflagellates were identified, including *Ostreopsis spp.*, *Coolia spp.* and *Alexandrium taylori* (Masó et al., 2003), all of which can cause HABs. *Alexandrium spp.* can cause paralytic shellfish poisoning (PSP), which is hazardous to both marine organisms and humans. *Alexandrium catanella* has caused significant economic losses to the salmon industry in Chile, for example in 2009 when a large bloom was associated with a loss of over $10 million to the Chilean Salmon industry (Mardones et al., 2015). *Alexandrium taylori* has also been shown to produce paralytic shellfish toxins and has recently been identified for the first time in Malaysian waters (Lim et al., 2005). Invasive HAB species, potentially transported by microplastics, could therefore be incredibly damaging to global fishery and aquaculture industries.

Marine plastic debris collected from multiple locations in the North Atlantic was analysed and bacterial assemblage sequenced to characterize the plastisphere community (Zettler, Mincer and Amaral-Zettler, 2013). In this diverse community, the bacteria genus *Vibrio* and dinoflagellate genus *Alexandrium* were identified. Both of these genii contain species that are pathogenic to both humans and animals. Several strains of *Vibrio spp.* including potentially pathogenic *Vibrio parahaemolyticus* were also detected on microplastics and in seawater from the North and Baltic sea by Kirstein et al. (2016). Microplastics samples from a transect taken along the Slovenian coast

of the North Adriatic Sea were subjected to DNA extraction, amplification and phylogenetic analysis, and the bacterial pathogen *Aeromonas salmonicida* was identified on the particles (Viršek et al., 2017). This species is pathogenic to several commercially important species, such as salmonids.

Chemical additives and adhered contaminants

Microplastics contain chemicals added during plastic manufacture to enhance certain properties, and have also been shown to adsorb and concentrate contaminants from the environment such as PCBs, PAHs, and metals (Teuten et al., 2007; Brennecke et al., 2016). Many of these contaminants can be toxic to marine organisms. Several researchers have therefore investigated whether microplastics can act as a vector for contaminant transfer to marine organisms, and whether this is a significant pathway compared to other methods of contaminant ingestion.

Chemical additives

Chemical additives in plastics enhance the different properties that make plastics so useful; some act as fire retardants, while others may act as stabilisers, foaming agents or strength enhancers. When plastic pollution occurs, these additives slowly leach from plastics into their surrounding media, for example seawater. This has led to concerns that they may enter biological systems and affect the health of exposed organisms, however, there is also a growing set of evidence that the overall exposure of organisms to these chemicals from plastics is negligible compared to other sources.

The potential for leaching of nonylphenol (NP) and bisphenol A (BPA) in the GIT of *Arenicola marina* (lugworm) and *Gadus morhua* (Atlantic cod), and a comparison of exposure to these two substances by microplastics alone and total environmental exposure, was investigated utilising a biodynamic model by Koelmans, Besseling and Foekema (2014). They suggest that for cod, ingestion of microplastic is highly unlikely to lead to negative effects from NP and BPA and is negligible compared to uptake from water and prey. For lugworms,

though ingestion of microplastic was hypothesised to be a substantial exposure pathway in certain conditions, the low concentrations of NP and BPA involved would not cause a risk to the lugworm.

Adhered contaminants

In addition to leaching chemical additives, plastic particles can sorb contaminants from the environment, giving a possible route for the concentration of these chemicals, potentially increasing their toxicity if they are released into a marine organism. Teuten et al., (2007) investigated the uptake and release of the hydrophobic organic contaminant phenanthrene by three virgin plastic polymers: polyethylene, polypropylene, and polyvinyl chloride. All three sorbed phenanthrene with varying efficiency, however all three plastics greatly exceeded the sorption of phenanthrene onto two natural sediments.

Ašmonaitė et al. (2018) investigated the effect of ingestion of large (100-400 µm) polystyrene microplastics (PS-MPs) on the rainbow trout (*Oncorhynchus mykiss*). Trout were exposed to virgin microplastics as well as microplastics exposed to either sewage effluent or environmental water in a harbour. All three sets of PS-MPs contained chemical contaminants including PAHs, plasticizers and surfactants, however, a wider variety of compounds were detected after exposure to sewage and harbour water, confirming the ability for PS-MPs to sorb contaminants from the aquatic environment. Rainbow trout were experimentally exposed to these microplastics following a dietary-exposure protocol, however no significant changes in hepatic biomarker responses were observed, suggesting that PS-MPs did not induce adverse hepatic stress in rainbow trout; however, Ašmonaitė et al. (2018) theorize that this may be due to the size of the PS-MPs used, as oxidative stress effects have been observed for smaller polystyrene particles (Jeong et al., 2016; Lei et al., 2018). Ašmonaitė, Sundh, et al. (2018) also show that PS-MPs did not affect intestinal health in the same species.

A review and reinterpretation of the available literature by Koelmans et al. (2016) and a modelling study by Bakir et al. (2016), both

investigating the relative importance of microplastics as a pathway for the transfer of adhered contaminants from microplastics to biota, suggest that this is not a significant route for exposure to adhered contaminants when compared to bioaccumulation from natural prey and water.

Metals

Heavy metal pollution within the marine environment is increasingly becoming a serious threat to ecosystems (Naser, 2013) and may therefore become a risk to food security in the near future. Brennecke et al. (2016) examined the adsorption of two heavy metals, copper and zinc, leached from antifouling paint, to virgin polystyrene beads and aged polyvinylchloride fragments in seawater. Both heavy metals adsorbed onto the two microplastic types, with concentrations of Cu and Zn increasing significantly on PVC and PS over the 14-day experiment. Significantly greater adsorption of Cu onto PVC fragments was observed, with the authors theorizing this was due to the higher surface area and polarity of PVC.

The effect of exposure to microplastic (0.26 and 0.69 mg/L), mercury (0.010 and 0.016 mg/L) and mixtures of the two substances (same concentrations) on the gills and liver of juvenile European bass (Dicentrarchus labrax) over a 96-hour period showed that, while both alone caused oxidative stress in the gills and liver, the concentration of mercury in both gills and liver was significantly higher in the presence of microplastics than their absence (Barboza et al., 2018b). This result is therefore indicative of a synergistic effect of microplastics on the accumulation of mercury within fish tissue. Heavy metals are proven environmental contaminants, and their interaction with microplastic debris therefore has potential to significantly alter the toxicity of microplastics within the marine environment.

Transfer across biological membranes

Microplastic ingestion may not be indicative of negative effects, as microplastics may be egested again quickly either by post-ingestion rejection or through faeces. However, if microplastics or nanoplastics

are able to transfer into the tissues or circulatory system, for example by transfer across the gut lining or gill structures, this may lead to greater accumulation and negative effects as the organism may not be able to remove them. Transfer to tissues, organs and the circulatory system has been seen in laboratory studies in crabs (Farrell and Nelson, 2013; Watts et al., 2014; Brennecke et al., 2015), bivalves (Browne et al., 2008; Von Moos, Burkhardt-Holm and Köhler, 2012; Al-Sid-Cheikh et al., 2018) and fish (Avio, Gorbi and Regoli, 2015; Lu et al., 2016).

Uptake of microplastics into the tissues of the blue mussel *Mytilus edulis* can cause changes on the cellular and tissue level (Von Moos, Burkhardt-Holm and Köhler, 2012). *M. edulis* were exposed to High-density polyethylene (HDPE) with irregularly shaped particles from >0-80 µm in size at a concentration of 2.5 g/L for up to 96 hours. Microplastic particles were found on the gills and in the digestive system, lysosomal system, connective tissue and digestive gland. Effects of microplastic exposure included granulocytoma formation after 3 hrs, and lysosomal membrane destabilization after 6 hrs; both effects are associated with the toxicological response of organisms to pollutants (Moore, 1985; Moore et al., 2008).

Zebrafish *Danio rerio* exposed to polystyrene microplastic beads (5, and 20 µm) at 20 mg/L for up to 7 days showed microplastic accumulation in the fish gills and gut (5 and 20 µm particles), and in the liver by 5 µm particles only (Lu et al., 2016). Toxicity testing, exposing *D. rerio* to 5 and 70 µm particles at 20, 200 and 2000 µg/L for 3 weeks showed that at 2000 µg/L both particle sizes caused inflammation and lipid accumulation in the liver. Particle size did not cause any observable histopathological differences in fish tissues.

Smaller plastic particles are more likely to transfer across biological membranes than particles at the larger end of the micro-scale, for example through the villi or M-cells of the peyer's patches within the intestine (Galloway, 2015). However, biologically-facilitated fragmentation of microplastics to nanometre-sized fragments has been reported to occur through microplastic ingestion by Antarctic krill

(*Euphausia superba*, Dawson et al., 2018). Here, 31.5 µm polyethylene beads (average size, ±7.6 standard deviation, S.D) were ingested by krill, and microplastic fragments identified in krill tissues and faecal pellets were decreased by an average of 78% (7.1 µm ± 6.2 S.D) and 81% (6.0 µm ± 5.0 S.D). This is the first time that fragmentation of microplastics to nanoplastics has been reported in planktonic crustaceans, and could be indicative of a mechanism for microplastic translocation to tissues in crustaceans where initially they may have been too large.

2.4 Discussion

2.4.1 What does the data show?

All of the commercially important organisms studied here, where data was available, were shown to contain microplastics. The population of animals shown to ingest microplastics varied widely by species, and when normalized for weight, the number of microplastics ingested per gram wet weight decreased with increasing trophic level. We conclude that commercially important organisms towards the base of the food chain (bivalves, crustaceans and small planktivorous fishes) are more likely to be contaminated with higher concentrations of microplastics, potentially posing a greater risk to their health and having implications for perceived or actual food safety.

The number of journal articles on the topic of microplastics has increased significantly over recent years: a search for ‘microplastics’ in Web of Science shows 473 papers published in 2018, up from 71 published in 2014. However, there are still gaps in our knowledge, particularly pertaining to commercially important organisms. It is critically important that more targeted research is done to assess the risk of microplastics to commercially important seafood species; several species, such as Alaska pollock, Grass carp and Whiteleg shrimp have had no research published on their ingestion of

microplastics within the natural environment. As similar species have shown microplastic ingestion we can surmise that they will most likely be ingesting plastics, but we have no idea of the scale of this or effects on these populations. As these three organisms had a combined production of 13.7 million tonnes of food in 2016, this is a huge gap in this research field and potentially an important risk to consider for worldwide food security.

The data presented in Fig. 2.2 and Table 2.5 suggests that microplastics do not biomagnify. Comparing microplastic concentrations within the GIT of different marine organisms to Hg concentrations within similar organisms (Fig. 2.2), normalizing by organism weight, shows contrasting trendlines; Hg presence in organism tissues (ppm) biomagnifies with increasing trophic level whereas the number of microplastics g^{-1} w. w. decreases with increasing trophic level. Whilst the data presented here suggests that microplastics within marine organisms do not biomagnify, this may not be the case for nanoplastics. These particles are small enough to possibly pass through the gut lining and into the tissues of organisms (Al-Sid-Cheikh *et al.*, 2018), therefore they may be more likely to bioaccumulate in animal tissues and may potentially biomagnify through the food chain, although there is no data as of yet to support this hypothesis.

2.4.2 What factors influence microplastic consumption?

Feeding strategy and environmental prevalence are primary drivers for microplastic consumption. Generally, lower trophic level organisms appear to ingest more microplastics due to feeding strategy, as observed by our biomagnification data (Fig. 2.2 and Table 2.5). However, there can be huge variations, for example although they occupy the same ecological niche, 76.6% Japanese anchovy were found with microplastics within their GIT (Tanaka and Takada, 2016), but only 0.9% Peruvian anchovy (Ory *et al.*, 2018). This is most likely due to the location where the fish were caught and the sample digestion methodology utilised. The Japanese anchovy were caught in Tokyo bay, which is in extremely close proximity to a very large level

of anthropogenic activity, with a drainage basin population of 29 million people, whereas the Peruvian anchovy were caught in further offshore locations in proximity to smaller population centres, therefore less microplastic pollution may be expected. Tanaka and Takada (2016) also removed and digested the entire GIT, whereas Ory *et al*. (2018) instead removed and digested only the gut contents; such differences in methodology may lead to differing identification efficacies. These differences in sampling site and methodology may have resulted in the large difference in the number of anchovy caught containing microplastics, and care should always be taken when comparing ingestion studies to identify any sampling bias such as identified here.

Though trophic transfer does not appear to be an important factor in microplastic consumption, it is possible that organisms at aquaculture facilities may be exposed to dietary microplastic through contaminated fishmeal. In 2014, 15.8 million tonnes of fish were reduced to fishmeal (Green, 2016), for use as a feedstock in the agriculture sector. Miles and Chapman (2006) estimate that in 2010, 56% of fishmeal was used in the aquaculture sector, 20% in pig feed and 12% in chicken feed. This therefore represents a novel way for microplastics to be introduced into human food, with potential risks to many different agriculture industries. Fishmeal is advertised as a nutritious and protein-rich feedstock (Miles and Chapman, 2006), therefore microplastic contamination through the processing of contaminated organisms or contamination during fishmeal processing may affect this nutritional value and have knock-on effects on global agriculture.

2.4.3 What are the issues with current studies?

Problems with laboratory analysis of microplastics remain, with several papers likely underestimating the amount of microplastics found in organic material due to worries about contamination and the use of filters with pore sizes too large to catch smaller microplastics. Microplastic fibres are commonly removed from analysis due to concerns about contamination (Rochman *et al.*, 2015; Rummel *et al.*, 2016; Ory *et al.*, 2018). Fibres are one of the most common types of microplastic debris worldwide (Lusher *et al.*, 2014; Barrows, Cathey

and Petersen, 2018), therefore it is critical that research should utilise methodology to reduce contamination (laminar flow cabinets, non-synthetic laboratory consumables and clothing etc.), to allow for more robust and realistic analyses of environmental microplastic concentrations, as concentrations are very likely to be under-represented without the inclusion of microplastic fibres in results. Smaller microplastics are often missed from analysis due to equipment constraints, both in collection and analysis. Foekema *et al.*, (2013) and Rummel *et al*. (2016) only analysed particles larger than 0.2 and 0.5 mm respectively, due to the diameter of the sieve mesh used. Both Güven *et al.* (2017) and Foekema *et al.* (2013) investigated microplastic in the GIT of *Trachurus spp.;* Güven *et al.* filtered digested *Trachurus mediterraneus* stomach and intestine content through a 26 µm mesh, with the resulting percentage of *Trachurus* shown to ingest microplastics as 68% of the population; Foekema *et al.* filtered digested *Trachurus trachurus* samples through a 0.2 mm seive and found microplastics in 1% of the population. Güven *et al.* also included microplastic fibres in their results, while Foekema *et al.* did not. Mean microplastic size identified by Güven *et al.* was 656.18 µm ± 803.31 SD, median particle size observed by Foekema *et al*. was 800 µm. Extrapolation of observed environmental concentrations of microplastics compared to their size shows that as mesh size or bead diameter decreases, the number of microplastics found per litre seawater increases by several orders of magnitude (Lenz, Enders and Nielsen, 2016). This shows a clear bias of microplastics identified due to methodology, and without standardization it is very difficult to accurately compare microplastic studies in a rigorous manner.

Methodological differences are also clear in the preparation of samples for microplastic analysis. When preparing fish digestive tracts for microplastic analysis, some researchers inspect the entire GIT, while others opt to inspect only the stomach contents. Both of these methods involve manually inspecting GIT contents for microplastics once scraped from their respective lining, while another method more commonly in use in newer studies is to digest the entire GIT, filtering

this solution to remove most of the organic matter and make microplastics more visible and easier to quantify. Common solvents used to digest the organic material are H_2O_2, KOH, HNO_3 and $HClO_4$ (Foekema *et al.*, 2013; Li *et al.*, 2015, 2016; Van Cauwenberghe *et al.*, 2015; Davidson and Dudas, 2016; Jabeen *et al.*, 2017; Phuong *et al.*, 2018; Qu *et al.*, 2018; Waite, Donnelly and Walters, 2018), with combinations of these solvents sometimes used to increase digestion efficacy (De Witte *et al.*, 2014; Devriese *et al.*, 2015a). Some of these treatments have been shown to have a destructive effect on microplastic particles (Cole *et al.*, 2014; Lusher *et al.*, 2017) therefore care should be taken to ensure microplastics are not damaged or eliminated due to the digestion protocol utilised. One option is to use digestive enzymes; for example Cole *et al*. (2014) and Courtene-Jones *et al.* (2017) have utilised enzymatic digestion with proteinase K and trypsin, respectively, with no observed impacts on microplastics. However, the methods utilized to effectively measure microplastics whilst avoiding microplastic alteration or destruction must be balanced against the cost, speed and effort required.

2.4.4 What are the risks of microplastics to fisheries and aquaculture?

Measuring the cost of microplastic pollution to ecosystem services, such as food provisioning through fisheries and aquaculture, is very challenging, and research into this is still in its infancy. Measuring the economic cost of marine litter is complex due to the wide range of impacts on the environment, social and economic sectors (Newman *et al.*, 2015), and it can be expected to be even more challenging to look at the cost of only microplastics as a proportion of this. The close relationship between ecosystem services and the marine environment means that adverse environmental effects from microplastic pollution will have impacts on food provisioning, which could add risk to global food security. Research has been done to attempt to put a cost to large marine debris. A survey of Scottish fish vessels reported that 86% of vessels reported reduced catch and 95% reported snagging on their nets on seafloor debris, with an estimated cost of €11.7-13 million per

year; the equivalent of 5% of the total revenue of affected fisheries (Mouat, Lozano and Bateson, 2010). Estimated values such as this are not available to look at the cost of microplastic pollution, however the risks of microplastics identified in this review may all add a cost to fisheries and aquaculture that we cannot currently quantify. Microplastics carrying pathogenic microbes or invasive species may decimate native populations of commercially important organisms such as shellfish and crustaceans. Increasing concentrations of microplastic within the marine environment may put a stress on the energetic burden of marine organisms; if organisms have to spend more energy to consume nutritionally valuable food this will decrease the energy available for growth and reproduction, and could decrease mean population size and reproductive output. This would mean that commercially exploited organisms could take longer to reach a harvestable size, leading to decreased profits in the fisheries and aquaculture sector, and smaller organism size would lower the nutritional value of seafood.

Currently, there is no evidence that significant amounts of microplastics can translocate to the tissues of fish from e.g. the digestive tract or gills, and as most fish are consumed gutted or as processed pieces (e.g. fillets), there is little evidence that larger fish will transfer microplastics to humans through diet. However, in the case of smaller fish such as anchovies, as well as shellfish and edible seaweeds, where the whole organism is often consumed, there is a greater risk of humans consuming microplastics, with implications for food safety and food security. Studies have suggested that European consumers may consume 11,000 microplastics per year (Van Cauwenberghe and Janssen, 2014) or 4620 microplastics per year (Catarino *et al.*, 2018) through seafood. Although it has been a concern that microplastics may leach additives or adsorbed chemical contaminants into humans upon ingestion, the estimated chemical exposure to humans of persistent organic pollutants and plastic additives following consumption of seafood is expected to be negligible, at <0.1% of total dietary exposure (FAO, 2017). Although

risks from seafood ingestion are not currently clear, it is possible that studies such as these will affect the perception of consumers, leading to a change in consumer habits and diet, before robust studies can be performed to give a clear picture of the effects of plastic pollution (Koelmans *et al.*, 2017) on food safety and food security. The results of a survey by the German Environment Agency found that 62% of the population studied felt that they were strongly (39%) or moderately (23%) contaminated by plastic particles in food and drinking water (SAPEA, 2019); microplastics research that is reported whilst failing to address human health and food security concerns may heavily alter public perceptions in similar ways. This may cause a lowering of seafood value and reduced profits in the seafood and aquaculture sector, potentially impacting public health in areas which rely heavily on seafood diets. In addition to researching the prevalence and effect of microplastics that are ingested by organisms in the marine environment, significant numbers of microplastics may be added to seafood during processing stages and packaging; such concerns should be researched through analysing microplastic content throughout the production process, to eliminate any potential areas of contamination that may occur.

Microplastics are present in commonly consumed aquatic species sourced from both aquaculture and the marine environment. Processing steps may remove some microplastics, e.g. by removing the GIT of fish, or washing shellfish and molluscs, however microplastics have been identified in processed aquatic biota that is being sold for consumption (Karami, Golieskardi, Ho, *et al.*, 2017; Karami *et al.*, 2018). The effect pathways of microplastics on the health of commercially important marine organisms, and possible risks to human health from consuming these organisms, must therefore be researched more thoroughly, to evaluate the potential effect of microplastic pollution to food security.

2.5 Conclusion

This review examined the presence of microplastics within commercially important marine organisms, and the risks they may have on organism health. All commercially important organisms analysed in this review were shown to contain microplastics. Investigation of microplastic concentrations at different trophic levels suggests that microplastics do not biomagnify, and organisms at lower trophic levels are at greater risk of microplastic contamination. While organisms higher up the food chain may not contain as many microplastics per gram body weight, risks are still present from contaminant transfer and chronic effects, potentially including increased feeding pressure as a result of the higher risk to lower trophic level organisms. This review highlights that some marine organisms that are important to global food security are omitted from current microplastics research, and that microplastics are a risk to the health of marine organisms worldwide. As fisheries and aquaculture are critical for global food security, this has implications for food security and food safety. Microplastics present an added risk to an already stressed environment, and further research on the effects of microplastic pollution is required to be able to perform comprehensive risk assessments on the effect of microplastics on food security.

2.6 Acknowledgements

This work was supported by the Natural Environment Research Council through the EnvEast Doctoral Training Partnership (grant number NE/L002582/1). MC and PL are funded by the Waitrose Plastic Plan Fund (Mussel Power).

2.7 Supplementary information

Table SI2.1. Mean number of microplastics per gram wet weight of organisms, calculated where required from data provided. MP = microplastic.

Species	Mean weight (g)	Mean no. MP found	Mean no. MP per gram wet weight	References
Katsuwonus pelamis	6018.5	1.5	0.000249	Markic *et al.*, 2018
Thunnus albacares	5228.7	3.1	0.00059	Markic *et al.*, 2018
Clupea harengus	198	2	0.01	Foekema *et al.*, 2013
Engraulis ringens	17.4	1	0.057	Ory *et al.*, 2018
Trachurus spp.	18.71-238	0.07-2.58	0.000126-0.14	Foekema *et al.*, 2013; Neves *et al.*, 2015; Güven *et al.*, 2017; Markic *et al.*, 2018
Scomber japonicus	28.86-228	0.57-9.4	0.0025-0.33	Neves *et al.*, 2015; Güven *et al.*, 2017; Ory *et al.*, 2018
Crangon crangon	N/A - calculated by author	N/A - calculated by author	0.13-1.23	Devriese *et al.*, 2015b
Penaeus semisulcatus	N/A - calculated by author	N/A - calculated by author	1.5	Abbasi *et al.*, 2018
Ruditapes philippinarium	N/A - calculated by authors	N/A - calculated by authors	0.9-2.52	Li *et al.*, 2015; Davidson and Dudas, 2016
Crassostrea spp.	N/A - calculated by authors	N/A - calculated by authors	0.18-3.84	Van Cauwenberghe and Janssen, 2014; Phuong *et al.*, 2018; Waite, Donnelly and Walters, 2018
Mytilus spp.	N/A - calculated by authors	N/A - calculated by authors	0.2-5.36	De Witte *et al.*, 2014; Van Cauwenberghe and Janssen, 2014; Li *et al.*, 2015, 2016; Van Cauwenberghe *et al.*, 2015; Bråte *et al.*, 2018; Catarino *et al.*, 2018; Phuong *et al.*, 2018; Qu *et al.*, 2018

Where required, the average weight of the organism was calculated from data provided. If any of the required data were not available, the corresponding author was contacted. The mean number of microplastics (MP) per gram wet weight of the organism was then calculated using the following formula:

$$MP\ per\ gram\ wet\ weight = \frac{Mean\ no.MP\ in\ organism}{Mean\ organism\ weight\ (g)}$$

Where multiple references are available for a single marine organism, the mean number of microplastics per gram wet weight were calculated individually and then the minimum and maximum values were used in the table above and in Table 2.5 and Fig. 2.2.

Chapter 3: Impact of polyester and cotton microfibers on growth and sublethal biomarkers in juvenile mussels

This chapter is a reformatted version of my publication currently under review:

Walkinshaw, C., Lindeque, PK., Thompson, RT., Tolhurst, T. and Cole, M.

CW, MC, TT, PKL and RT designed the experiments. CW carried out the experiments, data collection, conducted statistical analysis and wrote the manuscript. All authors contributed to editing and improving the final manuscript.

Anthropogenic microfibres are a prevalent, persistent and globally distributed form of marine debris. Evidence of microfibre ingestion has been demonstrated in a range of organisms, including *Mytilus spp.* (mussels), but the extent of any impacts on these organisms are poorly understood. This study investigates, for the first time, the effect of exposing juvenile mussels to polyester and cotton microfibres at environmentally relevant concentrations (both current and predicted future scenarios) over a chronic timescale (94 days). Sublethal biomarkers included growth rate, respiration rate and clearance rate. Mussels were exposed to polyester (median length 149 µm) and cotton (median length 132 µm) microfibres in three treatments: polyester (~8 fibres L^{-1}), polyester (~80 fibres L^{-1}) and cotton (~80 fibres L^{-1}). Mussels exposed to 80 polyester or cotton microfibres L^{-1} exhibited a decrease in growth rate of 35.6% (polyester) and 18.7% (cotton), with mussels exposed to ~80 polyester microfibres L^{-1} having a significantly lower growth rate than the control population ($P < 0.05$). This study demonstrates that polyester microfibres have the potential to adversely impact upon mussel growth rates in realistic future scenarios, which may have compounding effects throughout the marine ecosystem and implications for commercial viability.

3.1 Introduction

Microplastics are a persistent and pervasive contaminant that have been identified in freshwater, terrestrial and marine ecosystems worldwide (Thompson *et al.*, 2004; Andrady, 2011; Horton *et al.*, 2017; Karbalaei *et al.*, 2018; Li, Liu and Paul Chen, 2018). Studies suggest that as much as 4.8 to 12.7 million metric tons of plastic enters the world's oceans every year (Jambeck *et al.*, 2015) and this is expected to rise as plastic manufacturing rates are forecast to increase (PlasticsEurope, 2020). Microplastics are found in the environment in a myriad of different shapes, commonly categorized as fragments, fibres, films, or beads. Fibres are one of the most common morphologies of microplastic identified in environmental studies (Browne *et al.*, 2011; Desforges *et al.*, 2014; Ivar Do Sul and Costa, 2014; Kooi and Koelmans, 2019; Lindeque *et al.*, 2020; Rebelein *et al.*, 2021), accounting for up to 91% of the total identified microplastics in some studies (Barrows, Cathey and Petersen, 2018). These small anthropogenic fibres, termed microfibres, are typically composed of polyester, polypropylene or nylon, however numerous studies also report the presence of naturally derived and semi-synthetic cellulosic microfibres (e.g. cotton, rayon) in environmental samples (Bessa *et al.*, 2018; Yu *et al.*, 2018; Avio *et al.*, 2020; Suaria *et al.*, 2020). Despite their prevalence in environmental samples, semi-synthetic microfibres, which have been manufactured from heavily modified natural materials such as regenerated cellulose, have received relatively little attention compared to their plastic counterparts (Suaria *et al.*, 2020). Microfibres are predominantly generated from the breakdown of textiles, stemming from the day-to-day use and washing of clothes, and from the decay of marine infrastructure such as netting and rope (Napper and Thompson, 2016; Carr, 2017; Hernandez, Nowack and Mitrano, 2017; Zambrano *et al.*, 2019; Mishra *et al.*, 2020; Xu *et al.*, 2020; Xue *et al.*, 2020). For an informative review on the sources, sinks and exposure pathways of microfibres, see Suaria *et al.* (2020).

Bivalve shellfish, including mussels, oysters and clams, are highly cultivated marine species critical for global marine food security (FAO, 2020; Azra *et al.*, 2021). Bivalve shellfish have been demonstrated to readily consume microplastics (e.g. Van Cauwenberghe *et al.*, 2015; Li *et al.*, 2016, 2019; Digka *et al.*, 2018; Woods *et al.*, 2018), and in a recent review, shellfish were shown to typically have far higher body burdens of microplastics (microplastics gram^{-1} wet weight) than pelagic and demersal fish (Walkinshaw *et al.*, 2020). The environmental concentration of microplastics with a size range that is bioavailable to mussels is approximately 0.01 - 10 microplastics L^{-1} (Lenz et al. (2016). However, such values are likely underestimated owing to the complexities of sampling and identifying <100 µm microplastics (Lindeque *et al.*, 2020; Rebelein *et al.*, 2021); indeed, localised studies have shown waterborne microplastic concentrations of 88 items L^{-1} (Gray *et al.*, 2018). There are concerns microplastics might pose a risk to these ecologically and economically important organisms, for example, by reducing growth rates or survival, or increasing risk of disease, thereby posing a risk to shellfish commercial viability. As a result of microplastic exposure, marine mussels (e.g. *Mytilus edulis, Mytilus galloprovincialis*) can display inflammatory responses (Von Moos, Burkhardt-Holm and Köhler, 2012), increased antioxidant enzyme levels (Revel *et al.*, 2019; Cole *et al.*, 2020; Li *et al.*, 2022), increased hemocyte mortality (Paul-Pont *et al.*, 2016) and a reduction in the number of byssal threads produced and attachment strength (Green *et al.*, 2019). However, most studies investigating the effect of microplastic ingestion by marine organisms use plastic concentrations many orders of magnitude larger than those currently seen in the environment (Phuong *et al.*, 2016; Lim, 2021); Lenz, Enders and Nielsen (2016) note this discrepancy may result in inaccurate predictions and perceptions of the effect of microplastics upon the marine ecosystem. Furthermore, the majority of toxicity studies have not investigated the effects of microplastic fibres, which are the most common morphology of plastic identified in environmental samples (Christoforou *et al.*, 2020; Zhang *et al.*, 2020; Rebelein *et al.*, 2021). Other research gaps or critiques we have identified include:

studies are typically performed over relatively short periods of time, precluding the monitoring of chronic health effects; a lack of non-plastic controls; and, a focus upon the adult life stage, with little data emerging on the risks to juvenile or larval stages, which are generally much less resilient to environmental pollutants that affect metabolic rates (Stevens and Gobler, 2018). Two studies which did consider effects on juvenile life stages of bivalve shellfish are Capolupo *et al.* (2018), who investigated the effect of the ingestion of 3 µm polystyrene microplastics on *Mytilus galloprovincialis* larvae, observing RNA transcriptional changes as a result of microplastic ingestion at 50,000 and 500,000 microplastics L^{-1} (upregulation of shell biogenesis and immunomodulation genes and inhibition of genes coding for lysosomal enzymes); and Thomas *et al.* (2020), who observed increased mortality and a decrease in lysosomal membrane stability and condition index (a measure of the physiological state of the animal) in juvenile oysters exposed to 10^6 polystyrene beads L^{-1} (6 µm diameter) over 80 days. In both these studies, however, microplastic concentrations were far greater than what is currently reported in the environment; calculations by Lenz, Enders and Nielsen (2016) estimate particles of this size may be present at 27 – 170 microplastics L^{-1}.

This study seeks to address these research gaps by undertaking a chronic exposure study in which juvenile mussels are exposed to current environmentally relevant and feasible future-scenario concentrations of microfibres over a three-month period. Microfibres comprised both polyester and cotton to enable a direct comparison of the toxicity of microplastic fibres and a cellulose-based semi-synthetic microfibre control. We seek to test the hypothesis that sublethal health markers, such as growth rate, respiration rate and clearance rate, are affected in mussels by the presence of anthropogenic microfibres. This study provides evidence of the risk that microfibres pose to the growth and energetics of *Mytilus spp.*, a keystone genus important for global food security (Larsson *et al.*, 2017).

3.2 Methods

3.2.1 Contamination control

Care was taken at all stages to prevent the contamination and cross-contamination of microfibre exposure treatments. Wherever possible, glass apparatus and consumables were used to prevent the introduction of microfibres. When this was not possible, sterile, clean plastic consumables were used. All apparatus and consumables were rinsed thoroughly with Milli-Q prior to use. Samples and equipment were covered with aluminium foil wherever possible to minimize exposure to airborne contamination. Microfibre manufacture, mussel preparation, and microscopy were performed within the Plymouth Marine Laboratory ultraclean microplastics facility, which minimizes microplastic contamination via a HEPA filtered positive pressure airflow system (which removes 99.95% of airborne particles with a diameter of 0.3 µm), in addition to controlled personnel entry, cotton lab coats (of a different colour to the cotton microfibres used in the study) and tack mats to remove footwear contamination. Water samples from all mussel header tanks were checked for contamination concurrently with experimental microfibre quantification; no other microplastics were observed within these samples.

3.2.2 The model organism

Juvenile *Mytilus spp*. (~1 cm anterior-posterior length; n=200) were gathered on 15th June 2020 from Trebarwith Beach, Cornwall (50.643794N, -4.763651W). Mussels were harvested from intertidal rocks and placed into buckets filled with seawater from the same location; buckets were placed into cool boxes surrounded by ice packs and transported to a 15°C controlled temperature laboratory. Subsequently, mussels were housed in two 5 L beakers filled with filtered seawater. Filtered seawater (FSW) was prepared using natural seawater sampled from station L4 (www.westernchannelobservatory.org) in the western English

Channel, diluted with ultrapure water to Salinity = 35, filtered through two 5 µm filter cartridges in parallel (RS Pro House S cartridge) and UV sterilized (EHEIM reeflexUV 350). Mussels were fed by pipetting 1 mL microalgae feed, comprising *Isochrysis*, *Pavlova*, *Tetraselmis*, *Thalassiosira weissflogii*, and *Thalassiosira pseudonana*, into each beaker (Instant Algae Shellfish Diet 1800, Reed Mariculture). Mussels were visually inspected every 24 hours for one week, removing any deceased organisms; seawater was changed and mussels were fed every 48 hours.

3.2.3 Manufacture of microfibres

Small sections of 100% cotton or 100% polyester fabric (verified using a PerkinElmer Spotlight 400 FT-IR microscope) were cut from new textiles, and microfibres were manufactured from these garments through cryogrinding. The textiles chosen were brightly coloured (yellow cotton; red polyester) to allow for easy identification of fibres and to distinguish from any contamination. Textile sections were frozen using liquid nitrogen, and ground using a mortar and pestle. Resultant fibres were then rinsed several times with Milli-Q water and size-fractionated by vacuum filtration over two filters (10 and 500 µm nylon mesh discs) to retain microfibres approximately 10-500 µm in length. The two microfibre stocks were stored in Milli-Q water at ~2°C to limit microbial growth, and 50 fibres from each stock were measured using an Olympus SZX16 microscope (Magnification: 8.06x) with CellSens software (Olympus). Polyester microfibres had a median length of 148.95 µm (mean 293.5 µm, SE ± 47) and a median diameter of 10.21 µm (mean 12.03 µm ± 0.72 SE). Cotton microfibres had a median length of 132.33 µm (mean 171.5 µm, SE ± 22.0) and a median diameter of 19.23 µm (mean 20.47 µm ± 0.69 SE).

3.2.4 Experimental set up

All experimental work was conducted within a controlled temperature laboratory (15°C, 16:8h light/dark cycle). Header tanks (80 L clear polypropylene crates with lids; n=4) were filled with 40 L FSW (salinity 35 ± 1), with the addition of microalgae (Shellfish Diet 1800) to a final

concentration of 0.74 µg L^{-1} (concentration based upon initial experiments, see supplementary information). Water temperature and salinity were monitored throughout the experiment, and salinity adjusted by adding Milli-Q water where required. One header tank was used for each treatment, with the addition of either cotton or polyester microfibres to achieve nominal concentrations of either 8 or 80 microfibres L^{-1}, with 8 microfibres L^{-1} being representative of microplastic concentrations observed in the natural environment (Desforges *et al.*, 2014; Lenz, Enders and Nielsen, 2016; Barrows *et al.*, 2017; Karlsson *et al.*, 2017; Cai *et al.*, 2018), and 80 microfibres L^{-1} representing a feasible future-scenario environmental concentration given continued growth in global plastic production rates, and representative of current microplastic concentrations in heavily polluted water bodies close to anthropogenic input such as estuaries (Naidoo, Glassom and Smit, 2015; Gray *et al.*, 2018). Actual concentrations were verified throughout the exposure period by filtering 1 L subsamples through a GFF filter (Whatman) and enumerating microfibres under an Olympus SZX16 microscope. Recirculation pumps in each tank ensured tank homogeneity, and a peristaltic pump (Watson Marlow 323S) was used to dispense treatments to mussel aquaria (1 L glass beakers with lids, n=5 per treatment) at a flow rate of 76 mL/h, thereby ensuring mussels had a continuous supply of food and microfibres and that aquarium volume was maintained at 1 L volume which was replaced every 13 hours. All filters were removed from pumps to prevent microfibre retention within pumps. Header tanks were swapped with clean tanks weekly, and old tanks cleaned thoroughly with a surface active cleaning agent (Decon 90) and MilliQ water; header tanks were placed randomly (Excel random number generator) each week to remove any positional effect. The 20 mussel aquaria were housed in large spill trays so that displaced water could be continuously removed. To ensure mussels remained within the water column, for each replicate five juvenile mussels were secured onto a wooden spatula (spaced 5-10 mm apart), using non-toxic, aquarium-safe silicone, and then spatulas fully submerged into individual aquaria (see supplementary figure SI3.1).

Each replicate aquaria therefore housed five mussels, for a total of 25 mussels per treatment. Aquaria housing the mussels were cleaned weekly, with the mussels carefully removed and the 20 aquaria emptied and cleaned, before being rinsed and refilled with 1 L of the corresponding treatment solution from the header tank, ensuring consistent microfibre exposure concentrations throughout the study. The experiment was performed over a 94-day period. An experimental flowchart and labelled picture of the experimental set-up can be found in the supplementary data (SI3.2 and SI3.3, respectively).

3.2.5 Evidence of microfibre ingestion

To verify that the mussels had consumed the experimental fibres, at the end of the exposure period mussels were digested to isolate experimental microfibres. Mussels were euthanized via freezing, then soft tissues excised and rinsed in Milli-Q water to remove any microfibres present within the cavity around the soft tissues. Soft tissues were placed into pre-weighed individual glass vials and dried in a dehydrator overnight, then weighed again (Oxford A2205D) to ascertain mussel dry weight (mg). Tissues were digested using 10 mL 10% KOH with addition of 0.01% Tween20 surfactant for 48 hours at 50°C, in a rotational incubation chamber (Stuart Scientific SI50; 125 rpm). Both polyester and cotton are resistant to KOH digestion in the conditions utilized in this experiment (Treilles *et al.*, 2020). Digested samples were filtered onto polycarbonate filter discs (10 µm pore size, 47 mm diameter) in a laminar flow hood, and rinsed with copious amounts of Milli-Q. Filters were covered and dried in a dehydrator, then visually inspected under an Olympus SZX16 microscope to quantify experimental microfibres; experimental microfibres were first identified by their physical characteristics (i.e. distinctive shape, size and colour), then a subset were verified using a PerkinElmer Spotlight 400 FT-IR microscope and comparing to the spectra from the pre-exposure microfibres (see supplementary figure SI3.5).

3.2.6 Measurement of biological endpoints

Three variables were measured throughout the experiment: mussel shell length, clearance rate, and respiration rate. Clearance and respiration rate measurements were taken on alternate weeks to reduce handling stress. Where mussel mortality occurred, the data for the affected mussel was removed from the whole time series, to remove any bias from pre-mortality effects on mussel growth, clearance rate or respiration.

As mussels were kept alive throughout the experimental period, soft tissue weight could not be used to assess mussel growth. Instead, mussel anterior-posterior shell length was measured (McKinney, Glatt and Williams, 2004) for the assessment of mussel growth on days 0, 11, 21, 32, 44, 59, 72, 86 and 93. Mussels on spatulas were removed from aquaria and gently placed onto the stage of an Olympus SZX16 stereomicroscope where images of the mussels were captured using a DP74 camera and CellSens software (Olympus, v2.1), and promptly returned to their aquaria. Images were analysed using Image J version 1.52a (Schneider, Rasband and Eliceiri, 2012); shell lengths (µm) for individual mussels were measured in triplicate for each timepoint.

Mussel clearance rates (L h^{-1}) were measured on days 30, 44, 58, 72, 86 and 93 to track shifts in feeding activity. A stock of 10 L FSW containing 1 mL of Shellfish Diet 1800 was prepared, and 500 mL aliquots dispensed into 1 L beakers (n=20). Spatulas housing the mussels were removed from aquaria and carefully placed into these beakers for 2 hours, before being placed back in their aquaria. 10 mL samples from each beaker were transferred into labelled 15 mL Falcon tubes at T=0, T=1h and T=2h. At each timepoint, the water in the beaker was gently mixed before the solution was removed to ensure sample homogeneity. Samples were filtered onto clean GF/F filters (0.1 µm pore size); filters were placed into 15 mL Falcon tubes with 10 mL 90% acetone solution and left overnight in a -20°C freezer, to extract chlorophyll. Chlorophyll *a* concentrations were measured in triplicate using a fluorometer (Turner Designs Trilogy, model #7200-

000). The fluorometer was calibrated using a serial dilution of chlorophyll stock solution of known concentration (1057.9 µg L^{-1}) to create a standard curve; the linear regression equation of this line of best fit ($R^2 > 0.99$) was used to convert sample fluorescence (RFU) to chlorophyll-*a* concentration (µg L^{-1}) in the clearance rate samples. Subsequently, clearance rates were calculated using Chlorophyll *a* values following the method set out in Widdows and Staff (2006) and the equation in Coughlan (1969) (see supplementary information).

Oxygen consumption rates of mussels in each replicate were measured on days 22, 37, 51, 65, 79 and 93 and were used to calculate respiration rates (µmol L^{-1} O_2). Glass respiration vials (40 mL) were fitted with an oxygen sensitive spot (503090, World Precision Instruments) and filled to the brim with aerated FSW. Spatulas housing the mussels were removed from each beaker and carefully placed into the respiration vial; the vial was then sealed with a rubber bung, and the mussels left to acclimate for 5 minutes. Oxygen concentrations in the vials were ascertained via a fibre optic oxygen meter (Oxy mini, World Precision Instruments) and oxygen saturation (%) and phase shift was recorded every 15 minutes for an hour (T = 0, 15, 30, 45, and 60 minutes after measurement start time). This timeframe ensured that oxygen concentrations never decreased by more than 50% during the monitoring period. Mussels were then promptly returned to their aquaria. Data were calibrated using both a 0% oxygen solution (Hanna Instruments HI7040) and a 100% oxygen saturated seawater solution. The average phase shift results of these two standards were used to create a calibration curve, through which the recorded phase shift values were converted to a temperature and salinity corrected $\%O_2$ saturation value. Finally, the $\%O_2$ saturation data was converted into µmol L^{-1} O_2 as per Talbot *et al.* (2019) (see Supplementary information).

3.2.7 Statistical analysis

Statistics were conducted using R statistical analysis software (version 3.6.0). Data were tested for normality (Shapiro-Wilk) and homogeneity

of variance (Levene's test). Where data passed assumptions of normality, parametric ANOVA tests with Tukey's post-hoc testing were used. In data where results violated *a-priori* assumptions of normality, non-parametric Kruskal-Wallis tests were performed, followed by Dunn's post-hoc pairwise testing to observe whether individual experimental groups differ significantly. Statistical significance is assigned where $P < 0.05$ (95% confidence interval).

3.3 Results

3.3.1 Microfibre concentrations

Microfibre concentrations were monitored throughout the exposure period (Table 3.1), with average waterborne concentrations of: 7.88 fibres L^{-1} ± 0.74 SE (nominal: 8 polyester fibres L^{-1}); 78.8 fibres L^{-1} ± 5.8 SE (nominal: 80 polyester fibres L^{-1}); and 81.7 fibres L^{-1} ± 7.5 SE (nominal: 80 cotton fibres L^{-1}). Conditions will be referred to herein by their nominal concentrations (8 or 80 fibres L^{-1}) for clarity.

3.3.2 Digestion of mussels and identification of microfibres

Digestion and microscopic analysis of mussels revealed experimental microfibres within the soft tissues, which were verified via FT-IR spectral analysis (Table 3.1, supplementary figure SI3.4 and SI3.5). In the polyester treatments, an average of 8.65 ± 1.6 SE and 18.8 ± 3.4 SE fibres were identified within mussels exposed to 8 L^{-1} microfibres and 80 L^{-1} microfibres, respectively. In the cotton treatment, an average of 34.4 fibres ± 3.9 SE were identified (Table 3.1). In all treatments, we observed an average of 4.25 ± 0.87 SE non-experimental fibres per individual, likely owing to airborne contamination in the controlled temperature laboratory; this background contamination was removed from the results.

Table 3.1. Nominal and measured microfibre concentrations in aquaria for each condition and mean experimental microfibres identified in digested mussel soft tissues following exposure experiment. SE = standard error.

Microfibre polymer	Nominal concentration (microfibres L^{-1})	Measured concentration (microfibres L^{-1})	Experimental microfibres in mussel tissues (microfibres $mussel^{-1}$)
Control (no microfibres added)	0	N/A	0
Polyester	8	7.88 ± 0.74 SE	8.65 ± 1.6 SE
Polyester	80	78.8 ± 5.8 SE	18.8 ± 3.4 SE
Cotton	80	81.7 ± 7.5 SE	34.4 ± 3.9 SE

3.3.3 Mortality

The average survival rate across treatments was 72.5%, with the majority of individuals lost in the first few weeks of the exposure (79% of the total mortality was recorded in the first month). Treatment had no significant effect on mortality (Kruskal-Wallis, P = 0.77). Where >50% mussel mortality was observed within an aquarium (i.e. the loss of 3 out of 5 individuals), this replicate was removed from further analysis; this occurred in one control aquaria, two 80 L^{-1} cotton exposure aquaria, and one 80 L^{-1} polyester exposure condition, leaving a minimum of n=3 replicates for each condition.

3.3.4 Effects of microfibers on mussel growth

Mussel growth was observed in all treatments. The largest increase in mean shell length over the three-month period was in the control group (938.5 µm ± 230.0 SE). Mean growth was comparatively lower in all treatment groups (Fig. 3.1A): 906.3 µm ± 89.0 SE (8 polyester L^{-1}),

644.1 µm ± 88.0 SE (80 polyester L^{-1}) and 778.4 µm ± 130.0 SE (80 cotton L^{-1}). Significant reductions in growth were observed in the 80 polyester microfibres L^{-1} treatment group from Day 32 onwards (Kruskal-Wallis, $P<0.05$), and on Day 59 only in the 80 cotton microfibres L^{-1} treatment (Kruskal-Wallis, $P<0.05$).

Mean growth rate (Fig. 3.1B) in the control was 9.75 µm day^{-1} $mussel^{-1}$. Mussels exposed to 80 polyester microfibres L^{-1} had significantly reduced growth rates (6.28 µm day^{-1} $mussel^{-1}$; 35.6% decrease; Kruskal-Wallis, $P = 0.004$) as compared with the control. The mean growth rates of the 8 polyester L^{-1} exposure group (9.45 µm day^{-1} $mussel^{-1}$; 3.1% decrease; Kruskal-Wallis, $P = 0.40$) and the 80 cotton L^{-1} exposure group (7.93 µm day^{-1} $mussel^{-1}$; 18.7% decrease; Kruskal-Wallis, $P = 0.09$) appeared lower than the control, but this decrease was not statistically significant. In comparing polyester and cotton (at 80 microfibres L^{-1}), no significant difference in growth rate was observed (Kruskal-Wallis, $P = 0.12$).

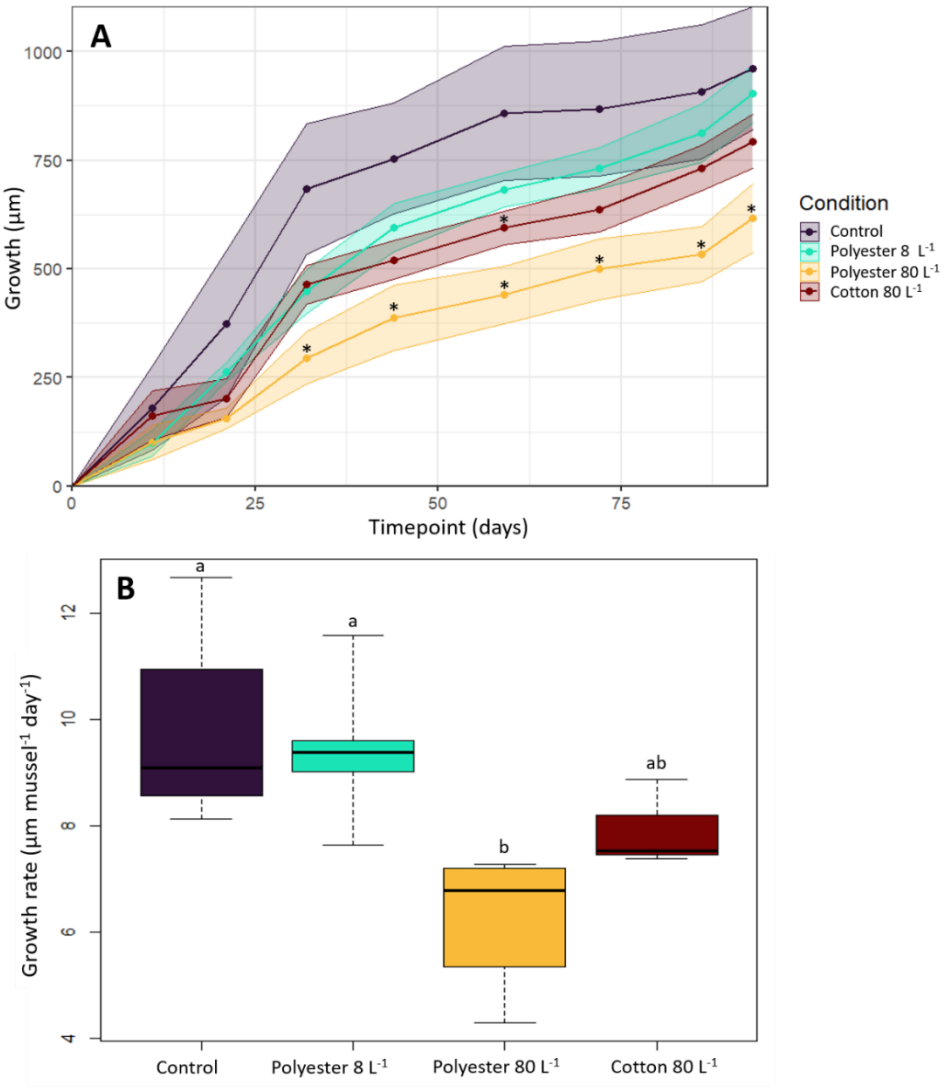


Figure 3.1. (A) Plot displaying mean increase in mussel shell length over time (µm) for each experimental condition. Coloured shading shows SE around the mean at each timepoint. * denotes results which are significantly lower than the control at each timepoint, P<0.05 (Kruskal-Wallis with Dunn's, post-hoc test). (B) Box and whisker plot showing median mussel growth rate (µm day^{-1} mussel^{-1}) for each experimental exposure condition. Box displays interquartile range, whiskers display full range. Letters denote statistical significance between treatments, P <0.05 (Kruskal-Wallis test with Dunn's post-hoc test). For a colour version of this figure, the reader is directed to the online version.

3.3.5 Effect of microfibres on mussel clearance rate

Mussel clearance rate decreased over the experimental period for all experimental treatments (Fig. 3.2A), although this decrease was not uniform between conditions. Statistical difference in clearance rates differed by timepoint; for example, in the 8 polyester L^{-1} exposure

mussels had a significantly lower clearance rate than the control group at day 44 (Kruskal-Wallis, P = 0.004), however at day 58, mussels in both the 8 polyester L^{-1} and 80 polyester L^{-1} exposure conditions exhibited significantly higher clearance rates than the control group (Kruskal-Wallis, P = 0.030 and 0.011, respectively). The 80 cotton L^{-1} condition had a statistically higher clearance rate compared to the control (Kruskal-Wallis, P = 0.024) on day 30. After day 58, no significant difference in clearance rate between experimental groups was observed. Clearance rate in each experimental exposure condition varied more and had a greater range than the control group; control clearance rate varied by a maximum of 67.1% from the first timepoint (day 30), whereas the three experimental groups varied by a maximum of 85.4%, 77.6%, and 88.9% (8 polyester L^{-1}, 80 polyester L^{-1} and 80 cotton L^{-1} respectively). The 80 polyester L^{-1} and 80 cotton L^{-1} clearance rate results did not differ significantly at any timepoint (P > 0.05, all timepoints).

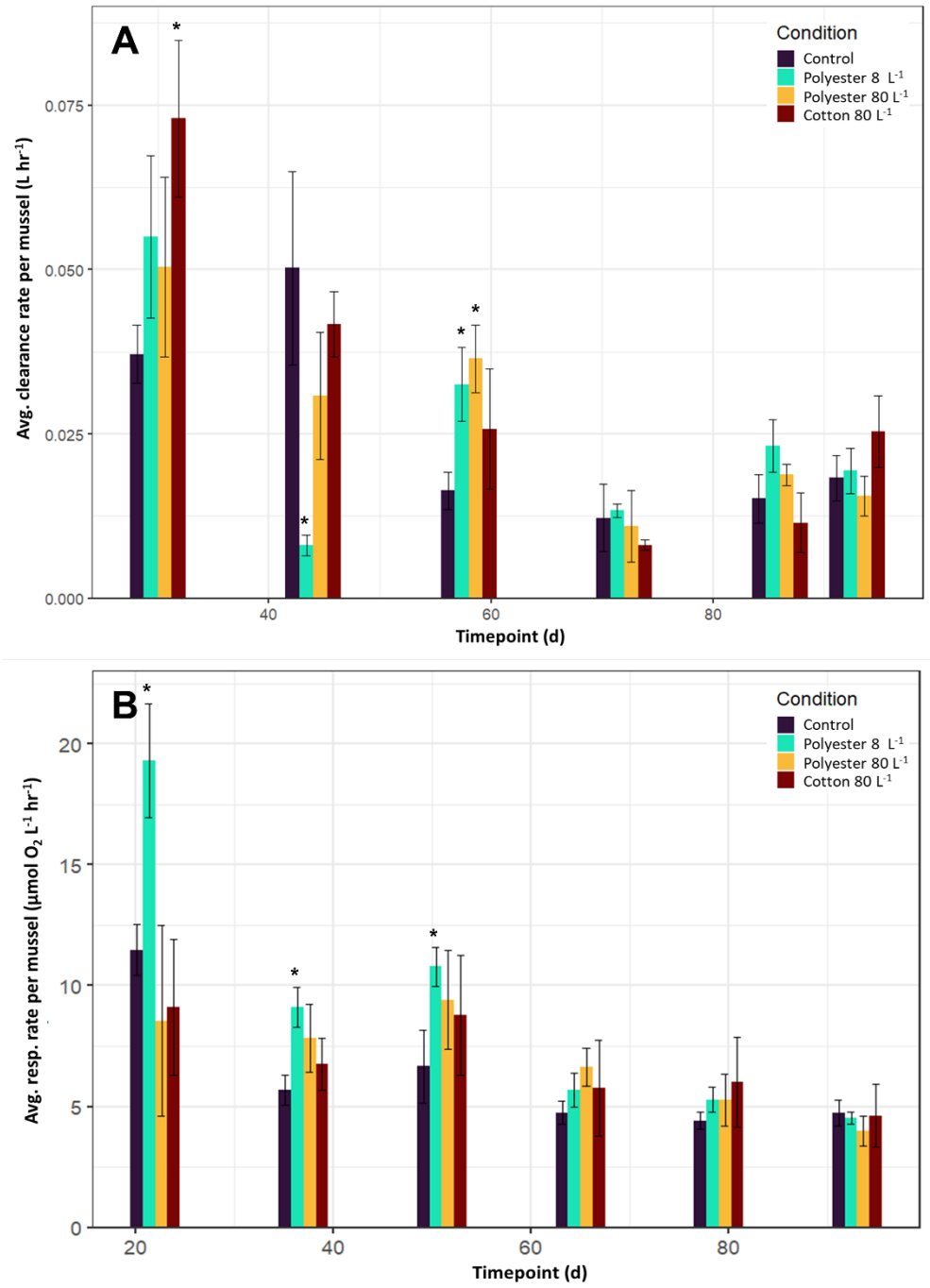


Figure 3.2. (A) Average clearance rate per mussel (L h^{-1} $mussel^{-1}$) for each experimental exposure condition. x-axis denotes days after experiment start. (B) Average respiration rate per mussel (µmol O_2 L^{-1} h^{-1}) for each experimental exposure condition. x-axis denotes days after experiment start. * denotes statistical significance when compared to the control group at each timepoint, P <0.05 (Kruskal-Wallis test with Dunn's post-hoc test).

3.3.6 Effect of microfibres on mussel respiration rate

Mussel respiration rate decreased over the experimental period for all conditions (Fig. 3.2B). In the control group, respiration rates stabilized

more quickly than in all three microfibre exposure conditions. Compared to the control group, respiration rate was significantly higher in the 8 L^{-1} polyester exposure group in the first three experimental timepoints (Kruskal-Wallis: d22, P = 0.044; d37, P = 0.014; d51, P = 0.013). Throughout the experiment there was no significant difference in respiration rate between the control group and the 80 L^{-1} cotton or polyester microfibre exposure groups. As with the clearance rate results, there was no significant difference between the 80 L^{-1} polyester or cotton microfibre exposures throughout the experiment (P > 0.05 at every timepoint).

3.4 Discussion

This is the first chronic exposure experiment assessing the effect of anthropogenic microfibres on bivalves. Juvenile mussel growth rates were significantly reduced when exposed to 80 polyester microfibres L^{-1}, with significant differences in growth evident after 32 days of exposure. Microfibre treatments had no significant impact on respiration rates or clearance rates.

3.4.1 Microfibre ingestion

The uptake of cotton and polyester microfibres by juvenile mussels was confirmed by isolating experimental microfibres from the soft tissues. When exposed to a higher concentration of polyester microfibres, a higher number of polyester microfibres were identified in mussel tissues, suggesting that mussels will ingest microfibres in loose correlation with environmental availability (Scott *et al.*, 2019). Almost twice as many cotton microfibres were found in soft tissues as compared with polyester microfibres (when provided at equal concentration); this may be due to the mussels having a greater capacity to deal with cellulosic fibres such as cotton, as *Mytilus spp.* are known to possess a high level of cellulase activity (Fernández-Reiriz et al., 2001; Labarta et al., 2002), or may possibly reflect subtle

differences in fibre morphology (cotton microfibres had a greater diameter than polyester microfibres) or variance in the egestion time of the different microfibres. The two microfibres were different in colour, however there is no evidence that mussels can selectively ingest particles based on their colour. While we can conclude microfibres were ingested by the mussels, owing to the small size of the juvenile mussels utilized here, we were unable to excise and digest specific tissues, and cannot confirm which organs they might affect or for how long the microfibres may be retained. Previous studies using adult specimens have identified microplastics within the digestive gland, gills, digestive tract, mantle and circulatory system (Browne *et al.*, 2008; Kolandhasamy *et al.*, 2018; Woods *et al.*, 2018; Cole *et al.*, 2020; S. Wang *et al.*, 2021).

3.4.2 Biological endpoints

Exposure to anthropogenic microfibres were demonstrated to reduce growth rates of juvenile mussels. Significant reductions in growth were observed in mussels following 32 days exposure to 80 polyester microfibres L^{-1}. This reflects probable future scenarios for microplastics in coastal waters as global plastic production rates rise, and is representative of microplastic concentrations already observed in heavily polluted marine and estuarine sites, which have seen microplastic concentrations of 88-247 particles L^{-1} (Naidoo, Glassom and Smit, 2015; Song *et al.*, 2015; Gray *et al.*, 2018). Our results highlight the importance of conducting chronic exposure studies when considering microplastic toxicity. While the impact of microplastics on lower levels of biological hierarchy (i.e. molecular endpoints) can become evident over short timescales (Galloway, Cole and Lewis, 2017b), the impact of environmentally relevant concentrations of microplastics on apical endpoints (e.g. growth, reproduction, survival) that have the greatest relevance to populations and communities, require far longer observation periods. Other aquatic organisms have also exhibited lower growth rates when exposed to microplastics, including fish (Naidoo and Glassom, 2019), zooplankton (Ziajahromi *et al.*, 2017), crustaceans (Welden and Cowie, 2016c), and corals

(Chapron *et al.*, 2018). Clearance and respiration rates decreased over the first 6 weeks of exposure, and then remained stable for the remainder of the experiment. We suspect that initially higher respiration and clearance rates stemmed from handling stress and change of conditions, which were alleviated as the mussels acclimated to experimental conditions. Previous studies have indicated that mussel acclimation to laboratory conditions takes around 14-21 days (Widdows, 1973, 1976; Moyen, Somero and Denny, 2020). However, the clearance and respiration rate measurements observed here suggest that juvenile mussels may take even longer to acclimate. In the control group, mussel respiration rates normalized to ~4.5 µmol O_2 L^{-1} hr^{-1} between Days 22-37, and clearance rates normalized to ~0.02 L hr^{-1} between Days 44-58. The clearance and respiration rates of mussels exposed to microfibres appeared to take longer to stabilize (Respiration: Days 51-65; Clearance: Days 58-72).

We found no clear evidence of microfibre impact upon feeding. In the literature, mussels exposed to 3000-30,000 polyester microfibres L^{-1} (length 459 µm) for 72 hours exhibited significant reductions in their filtration rates (Woods et al., 2018); however, these concentrations are approximately 340x higher than used in this experiment and are not reflective of environmental concentrations. Respiration data showed greater consistency across time. While higher microfibre concentrations showed no significant effect on respiration, exposure to 8 polyester micofibres L^{-1} resulted in higher respiration rates on Day 22, 37 and 51. Given the stochastic nature of the respiration and clearance rate data, these biological parameters are not recommended as useful toxicological endpoints in future microplastic exposure studies. Similarly, exposure to 1 and 10 µm polystyrene beads for 8 days had no significant effect on Pacific oyster (*Crassostrea gigas*) larvae feeding or growth at ≤100 microplastics mL^{-1} (Cole and Galloway, 2015). Furthermore, European flat oysters (*Ostrea edulis*) exposed to HDPE and PLA at concentrations of 0.8 and 80 µg L^{-1} for 2 months showed no alteration in respiration, clearance rates or growth rate compared to control organisms (D. S.

Green, 2016). We postulate that observed reductions in growth rate stem from a reduction or shift in the energetic budget of individual mussels. Energetic shortfalls, resulting from reducing feeding or assimilation rates, could play an instrumental role in curtailing growth. Such effects could stem from a 'false satiation' effect, as indigestible anthropogenic particles replace volumetric mass of digestible matter, (Science Advice for Policy by European Academies (SAPEA), 2019; De Ruijter *et al.*, 2020; Koelmans *et al.*, 2020), or alternatively shifts in feeding behaviors to avoid consuming microplastics. For example, the copepod *Calanus helgolandicus* has been observed to shift to foraging smaller microalgae to avoid consuming larger microplastics, which resulted in reduced energetic uptake and consequently the production of smaller eggs with reduced hatching success (Cole *et al.*, 2015). Previous studies have shown adult mussels can increase pseudofaeces production to reduce uptake of 75-1075 µm nylon microfibres when 34-495 microfibres are offered to actively feeding organisms (Ward *et al.*, 2019). However, we observed no consistent decrease in mussel clearance rates, indicating that the presence of microfibres did not reduce energetic intake. Reduced growth rate could also stem from energy being diverted away from growth into processing ingested microfibres in the gastrointestinal tract (Wright *et al.*, 2013; Sussarellu *et al.*, 2016), or repairing damage caused by microplastics. A number of toxicity studies provide evidence that microplastics can cause adverse sub-lethal health effects at the molecular and cellular level of biological hierarchy in adult *Mytilus edulis* and *Mytilus galloprovincialis*. For example: exposure to <100 µm polyethylene and polystyrene powders for 7 days can alter granulocyte/hyalinocyte ratios indicative of an increased immune response (Avio *et al.*, 2015; Cole *et al.*, 2020); exposure to >0–80 µm high-density polyethylene grains for up to 96 hours can result in decreased lysosomal membrane stability and increased granulocytoma formation indicative of cellular damage (Von Moos, Burkhardt-Holm and Köhler, 2012); exposure to a mixture of <400 µm polyethylene and polypropylene powder for 10 days increased superoxide dismutase (SOD) activity in the digestive glands and gills,

indicative of oxidative damage and haemocytic DNA damage (Revel *et al.*, 2019). A heightened immune response, DNA, cellular and tissue repair, and upregulation of antioxidative pathways will all require energetic expenditure, thereby shifting energy away from growth and reproduction (Trestrail, Nugegoda and Shimeta, 2020). Indeed, dynamic energy budget modelling of Adult Pacific oysters (*Crassostrea gigas*) exposed to 2 and 6 µm polystyrene microspheres reveals homeostatic changes in stress and immune responses resulting in energy flow disruption, redirecting energy away from reproduction towards organism maintenance and growth (Sussarellu *et al.*, 2016).

3.4.3 Comparing polyester and cotton microfibres

While plastic microfibres such as polyester are widely investigated in the literature, this study is among the first to consider the effect of anthropogenic cellulosic fibres such as cotton. Growth rates of mussels exposed to 80 cotton or polyester microfibres L^{-1} were not significantly different, however when compared to the control condition, only polyester microfibres caused a consistent significant decrease in growth rate. Given the comparable morphologies of the cotton and polyester microfibres in this study, the difference in their effect on mussel growth rate may be due to their chemical characteristics, which were not investigated in this study. Future research should therefore consider the mechanisms by which different anthropogenic polymers (whether natural, semi-synthetic or fully synthetic, with and without additives and dyes) may affect organisms. Though the perceived decrease in growth rate of 19% in mussels exposed to 80 cotton microfibres L^{-1} was not significantly lower than the control group in this study, given the large numbers of semi-synthetic microfibres used in textiles and found in marine samples (Suaria *et al.*, 2020), we recommend investigations into the effects of manmade cellulosic microfibres on marine biota are made a research priority. Greater use of positive controls in microplastic studies, such as the use of cotton microfibres as a natural fibre control, are

recommended to help explain the mechanisms by which anthropogenic particulates cause toxicity in marine organisms.

3.4.4 Environmental relevance

Mussels provide a range of ecosystem services, including biofiltration, food provision and carbon sequestration (van der Schatte Olivier *et al.*, 2020). A reduction in growth rate will result in smaller mussels with comparatively lower clearance rates, reducing their capacity to function as effective biofilters and remove carbon through consumption and egestion of phytoplankton. While mussels with reduced growth rates may reach the same size as the control population over a longer period of time, this may pose a risk to bioenergetics within marine food webs, as in natural populations, smaller mussels will be less energetically valuable to predators (e.g. gastropods, echinoderms, seabirds and mammals). While in commercial populations, a reduction in growth rate will increase the time taken for mussels to reach harvestable size, with smaller mussels having lower commercial value. Molluscs (mostly bivalves) were the second-most farmed group of aquatic animals after finfish in 2018 (FAO, 2020), with 17.7 million tonnes produced by global aquaculture; as such, negative impacts on the growth of bivalve shellfish has the potential to impact upon their commercial viability.

Data presented here explores the effect of microfibres on mussel health in a controlled environment. However, in the marine environment, mussels are exposed to multiple stressors, from pollutants to climate change (e.g. warmer temperatures, acidification). Effects observed here may therefore be compounded through multi-stress impacts on mussels which may leave populations less resilient and lead to decreased population health (Negri *et al.*, 2013; Horton and Barnes, 2020). As bivalve populations take longer to grow they may be more prone to disease, or susceptible to morbidity or mortality following extreme events (e.g. heat stress, oxygen depletion) (LeBlanc *et al.*, 2005; Anestis *et al.*, 2007; Seuront *et al.*, 2019). Global bivalve populations are already declining, with many bivalve species listed as

endangered and several classified as extinct (Zhang *et al.*, 2020); it is possible that increasing concentrations of microplastics and microfibres may exacerbate this rate of decline, though further research is required to confirm this hypothesis.

3.5 Conclusion

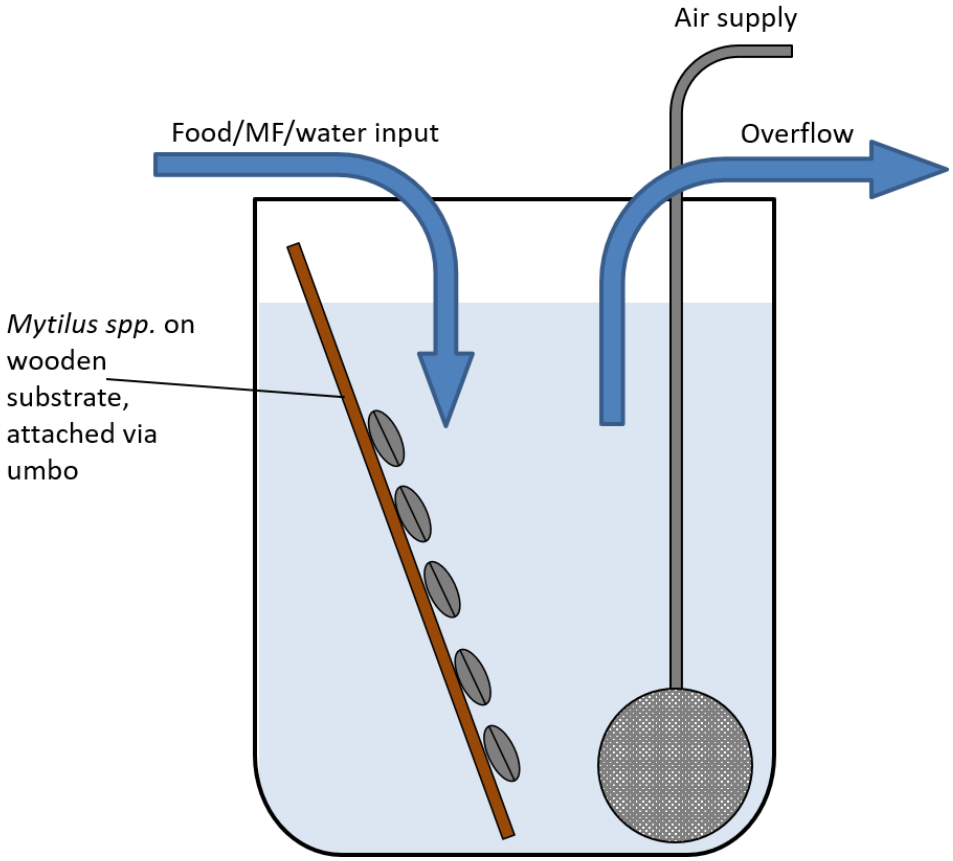
The growth rate of juvenile mussels was significantly decreased when exposed to 80 polyester microfibres L^{-1}. Furthermore, the mean growth rate of mussels exposed to 80 cotton microfibres L^{-1} was reduced by 19%; whilst not significant in this study, this result highlights the importance of investigating other anthropogenic particles released alongside microplastics, such as cotton and semi-synthetic microfibres. The reduction in growth rate observed from polyester microfibre exposure may increase the time taken for bivalves to grow to a harvestable size, increasing the time and investment required for the growing of bivalves for human consumption. This may potentially present a risk to commercial viability and food security. The effects detailed here could also have broadscale effects on marine ecosystems. As bivalves are a prey item for many species, sublethal health effects such as reduced growth rates will cause additional stress in an ecosystem already suffering from multiple stressors through the effects of climate change, overfishing, and anthropogenic pollution.

3.6 Acknowledgements

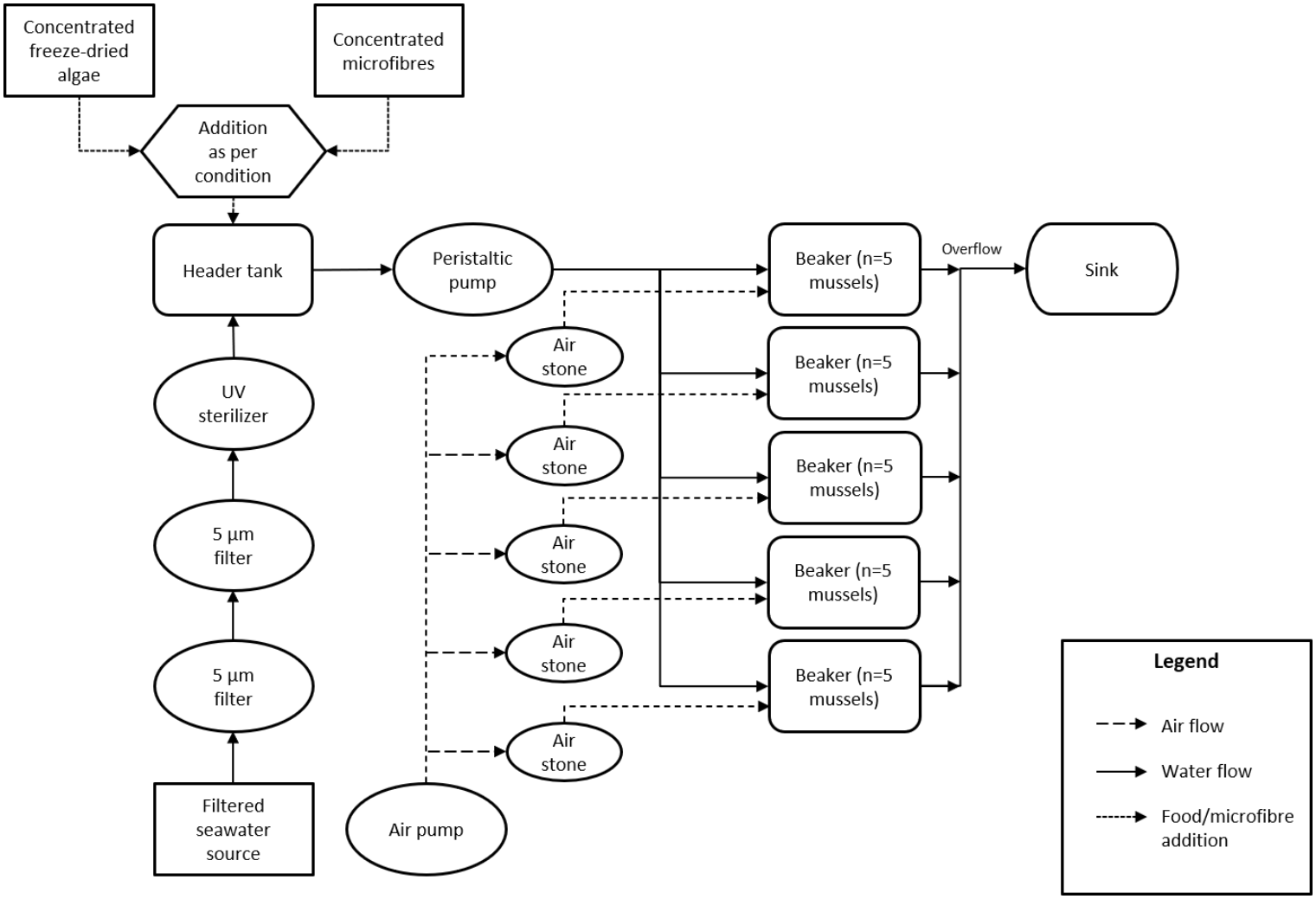
This work was supported by the Natural Environment Research Council through the EnvEast Doctoral Training Partnership (grant number NE/L002582/1). MC and PL were funded by the March Marine Initiative. We would like to thank Ana Queirós (PML) for providing training in respiration measurements and Zara Botterell (PML) for their assistance in field sampling.

3.7 Supplementary information

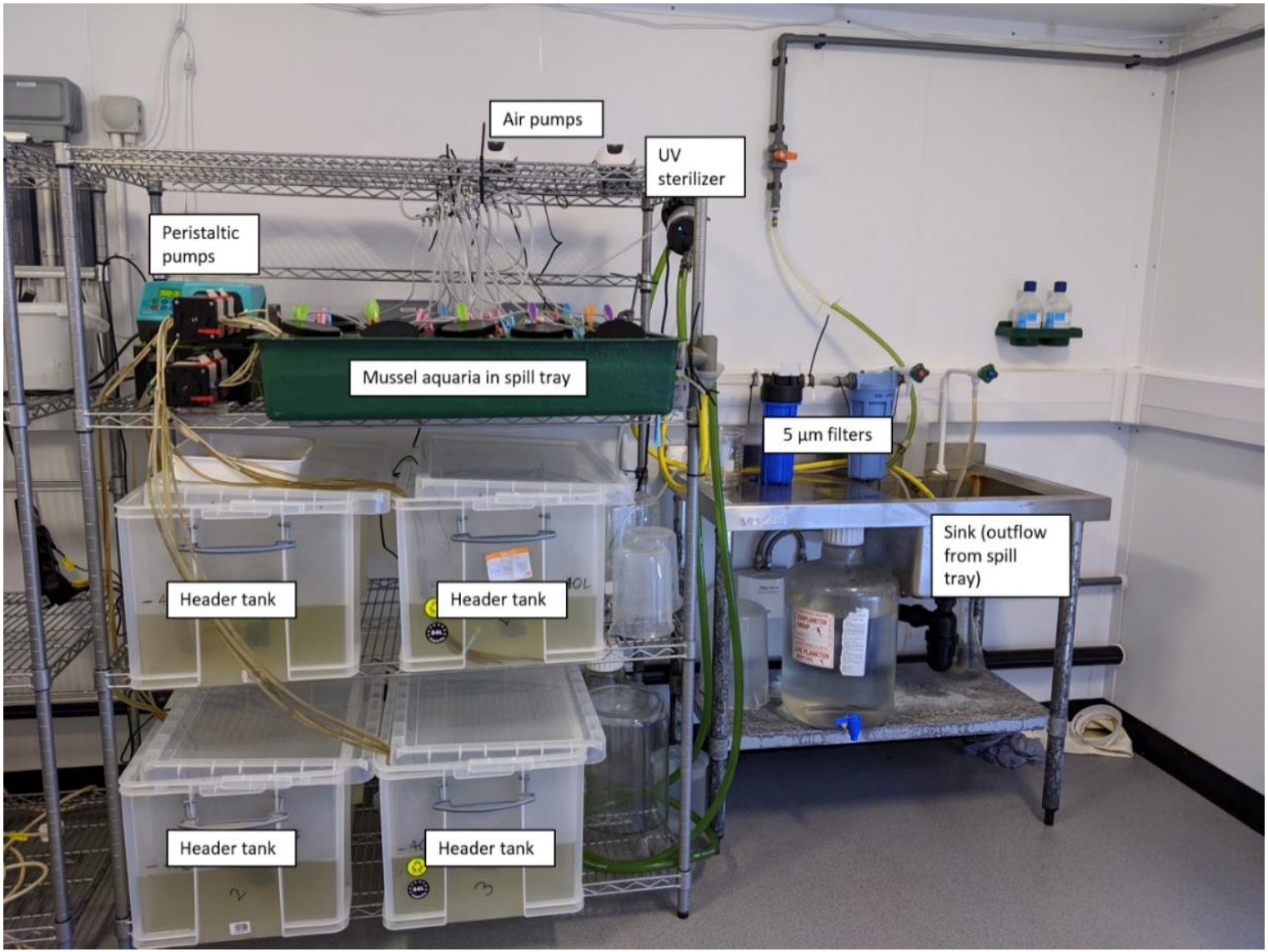
Supplementary Methods



Supplementary figure SI3.1. Diagram of mussel aquaria, with mussels attached to wooden spatula via mussel umbo. Mussels were kept in this way to keep them suspended in the water column to allow for better exposure to food and microfibres, to facilitate easy handling reducing handling stress, and allowing faeces to travel to the bottom of the beaker (keeping the area around the mussels cleaner). MF = microfibre.



Supplementary figure SI3.2. Mussel experimental setup flow chart for each experimental condition.



Supplementary figure SI3.3. Mussel experimental setup

Calculation of header tank algal concentration

The microalgal concentration used was based on initial experiments to ascertain the clearance rate and food uptake of juvenile mussels in the size range tested. 9 mussels (shell length approx. 1cm) were placed into 3 1 L beakers (3 mussels per beaker, n=3), filled with 1 L filtered seawater and left to acclimate for 1 hour. 0.349 mL microalgae solution (Instant Algae Shellfish Diet 1800, Reed Mariculture) was added to each beaker, mixing gently until homogenous. 10 mL solution (n=3) was removed from each beaker at t = 0, 15, 30, 45, 60 mins (gently mixing solution each time before removing sample). Each sample was filtered onto Whatman GF/F filter papers, which were placed into falcon tubes with 10 mL 90% acetone solution and left at -20 °C overnight. All solutions were read using a fluorometer and chlorophyll-a concentration was calculated using a standard curve. The average concentration for each sample was calculated (µg/L) and mussel clearance rate was subsequently calculated using the methods in Widdows and Staff (2006) and the formula from Coughlan (1969):

$$Clearance\ Rate = \frac{V}{nt} . (log_e conc_0 - log_e\ conc_t)$$

V = Volume (L)

n = number of mussels

t = time of measurement (hours)

$conc_0$ = algal concentration at time 0

$conc_t$ = algal concentration at time t

Mussel clearance rate was calculated as 0.113 L hr^{-1} $mussel^{-1}$. Using an environmentally relevant algal concentration of 10 µg/m^3 (unpublished data), and the dispense speed of algal solution into the header tanks, it was calculated that the header tanks should be

maintained at an algal concentration of 0.74 µg L^{-1} to provide mussels with a constant concentration of algae to maintain maximum clearance rate.

Calculation of respiration rate

To calculate mussel respiration rates, the corrected %O_2 saturation data was converted into µmol L^{-1} O_2 following the equation below:

$$[O2](\mu\text{mol L}^{-1}) = \left[\frac{P_{atm} - P_W(T)}{P_N} . \frac{\%\ O_2\ saturation}{100} . 0.2095 . \alpha(T) . 40 . \frac{M(O_2)}{V_M}\right] . 31.25$$

Where:

Patm is the atmospheric pressure (mbar)

P_W(T) is the vapour pressure of water at measurement temperature (K)

P_N is standard pressure (1013 mbar)

0.2095 is the volume content of O2 in air

α(T) is the Bunsen absorption coefficient at measurement temperature (K)

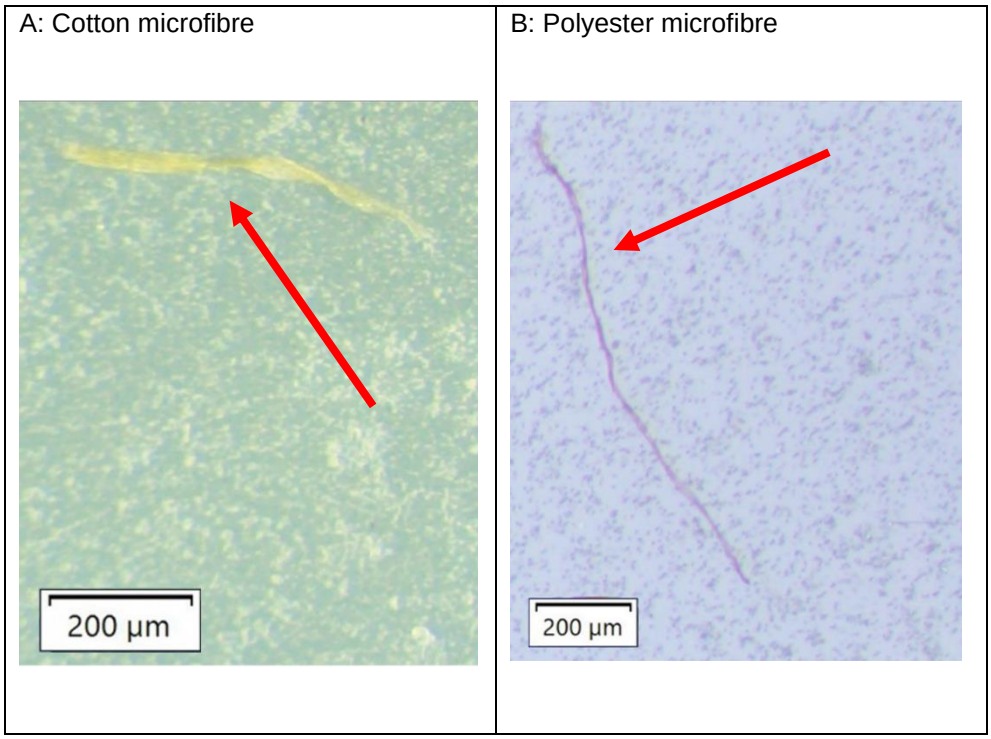
40 is the volume of the respiration chamber in mL

M(O_2) is the molecular mass of oxygen (32 g mol^{-1})

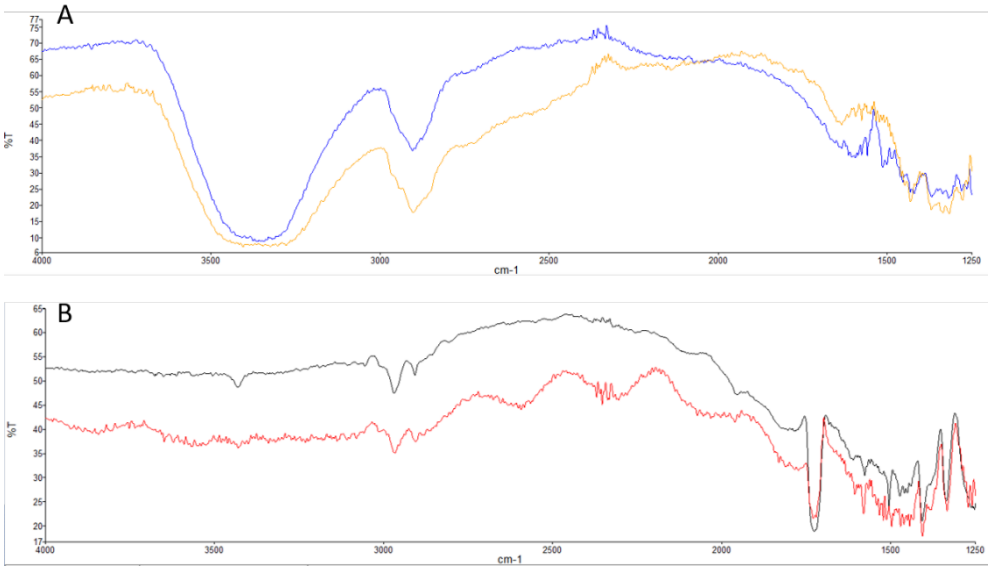
V_M is the molar volume (22.414 L mol^{-1})

31.25 is the conversion factor from mg L^{-1} to µmol L^{-1}

Supplementary Results



Supplementary figure SI3.4. Experimental microfibres identified within mussel soft tissue digests. A = cotton fibre, B = polyester fibre.



Supplementary figure SI3.5. Example experimental microfibre FT-IR spectra pre-exposure and post-exposure (identified within mussel digests). A = cotton microfibres (yellow = pre-exposure, spectral match = 0.892; blue = post-exposure, spectral match = 0.782), B = Polyester microfibres (black = pre-exposure, spectral match = 0.897, red = post-exposure, spectral match = 0.673).

Appendix A – Scope for growth of juvenile mussels exposed to polyester and cotton microfibres

This study is intended as supplementary content not included in the manuscript owing to limitations in the calculation of scope for growth. It is included here to address the energetic budget theory alluded to in the main text, but was not included in the manuscript submitted for publication.

Introduction

The biological measurements recorded in this chapter can be used to investigate the energy reserves for the exposed mussels over the experimental time period. There are two general types of energy budget models that can be used to do this: dynamic energy budget (DEB) models or the scope for growth (SFG) model. These methods are compared by Filgueira, Rosland and Grant (2011), who surmise that both methods perform similarly when modelling *Mytilus edulis* growth; both models can therefore be used to calculate the excess energy available to mussels from energy intake that can be used for growth and reproduction. As scope for growth has proven itself as a sensitive method for assessing the effect of pollutants on physiological rates of the organism (Widdows *et al.*, 2002; Halldórsson, Svavarsson and Granmo, 2005; Albentosa *et al.*, 2012), we use this model to create a simplified energy budget for juvenile mussels exposed to polyester or cotton microfibres over a chronic timescale.

It is important to note that there are limitations to the application of this method using the data generated in this study. Normally scope for growth would be calculated using respiration rate, clearance rate and absorption efficiency measured sequentially on the same day, however due to the experimental setup, laboratory limitations, and in

efforts to minimize stress, I could not take these measurements together. Clearance and respiration rate measurements were taken one week apart, and absorbance efficiency was calculated at the end of the experiment. The SFG results reported here are therefore an estimation of energy reserves, and while they can be used to compare between experimental treatments they are not directly comparable with other studies.

Methods

The clearance and respiration rates calculated throughout this study were used to calculate the average scope for growth (SFG) of mussels in each experimental condition by following the method set out in Widdows and Staff (2006). In brief, the SFG method seeks to provide an estimate of the energy budget available for growth and reproduction, by converting physiological measurements into their energy equivalents (J h^{-1}). Calculating this energy budget requires the user to ascertain the clearance rate, respiration rate, food absorption efficiency, and energy lost through excretion. Food absorption efficiency is calculated following the method set out in Conover, (1966), where the absorption efficiency is calculated as a ratio of the ash-free dry weight:dry weight ratio of the food and the ash-free dry weight:dry weight ratio of the faeces. Food absorption efficiency was calculated at the end of the experimental period. While normally food absorbance efficiency would be calculated alongside clearance rate measurements, this could not be done due to the experimental set up. The calculation of food absorption efficiency requires that faeces is collected after the clearance rate measurements, with faeces collected after the first 5-6 hours discarded and faecal pellets collected after this period (Widdows and Staff, 2006). This would result in the mussels being kept away from the constant algal and microfibre exposure in the experiment for 24 hours every two weeks; consequently, this may compromise the other results and therefore food absorption efficiency was only measured at the end of the experimental period. Other

studies have successfully estimated SFG without absorption efficiency calculations, therefore we considered this an acceptable limitation to the method (Halldórsson, Svavarsson and Granmo, 2005). Given that energy lost via excretion accounts for less than 5% of the total energy budget, this value can be removed and Widdows and Staff (2006) suggest a simplified energy equation can be used:

Where:

Scope for growth (SFG) = (I × A) – R

Energy ingested (I) = (maximum clearance rate L g^{-1} h^{-1}) × (mg organic matter L^{-1}) × (23 J mg^{-1} organic matter)

Where the energy content of the algal food is 23 J mg^{-1} (Widdows and Staff, 2006)

Respiratory energy expenditure (R) = (µmoles O_2 g^{-1} h^{-1}) × 0.456

Where 0.456 J $\mu mole^{-1}$ O_2 is the heat equivalent of oxygen uptake (Widdows and Staff, 2006)

A = absorbed food energy (energy ingested x food absorption efficiency)

SFG results are presented at three timepoints: after one, two and three months of the experimental period (3 months = end of experimental period). Clearance rate measurements for these three timepoints were collected on d30, d58 and d93, and respiration rate measurements were collected on d22, d65 and d93.

Results

SFG results (Figure A1; Table A1), calculated at the start, midpoint and end of the experiment, show that SFG is significantly greater than the control at the first timepoint in both the 80 L^{-1} polyester (Kruskal-Wallis, P = 0.027) and 80 L^{-1} cotton (Kruskal-Wallis, P = 0.005) groups. SFG decreases significantly in the 80 L^{-1} polyester and cotton treatments over the experimental period, decreasing by 83.3% (80 polyester L^{-1}, Kruskal-Wallis, P = 0.003) and 71.6% (80 cotton L^{-1}, Kruskal-Wallis, P = 0.018). The decrease in SFG is most pronounced in the 80 L^{-1} cotton exposure condition between the first measurement and the midpoint, which also displayed the largest decrease in energy absorbed over the experimental period (Table A1), though this was followed by a small recovery in SFG at the last timepoint. The 80 L^{-1} polyester exposure group showed a constant decrease in SFG over the course of the experiment. In contrast, SFG remained stable in the control (Kruskal-Wallis, P = 0.384) and 8 polyester microfibres L^{-1} (Kruskal-Wallis, P = 0.389) treatments. The average amount of energy absorbed by the mussels appears to vary more than the energy consumed through respiration over the course of the experiment; thus it is food energy absorption which appears to drive the change in SFG recorded. As absorption efficiencies were similar for all conditions (0.904, 0.882, 0.901, and 0.897 for the control, 8 L^{-1} polyester, 80 L^{-1} polyester and 80 L^{-1} cotton conditions, respectively), the main driver for change in SFG appears to be energy ingested.

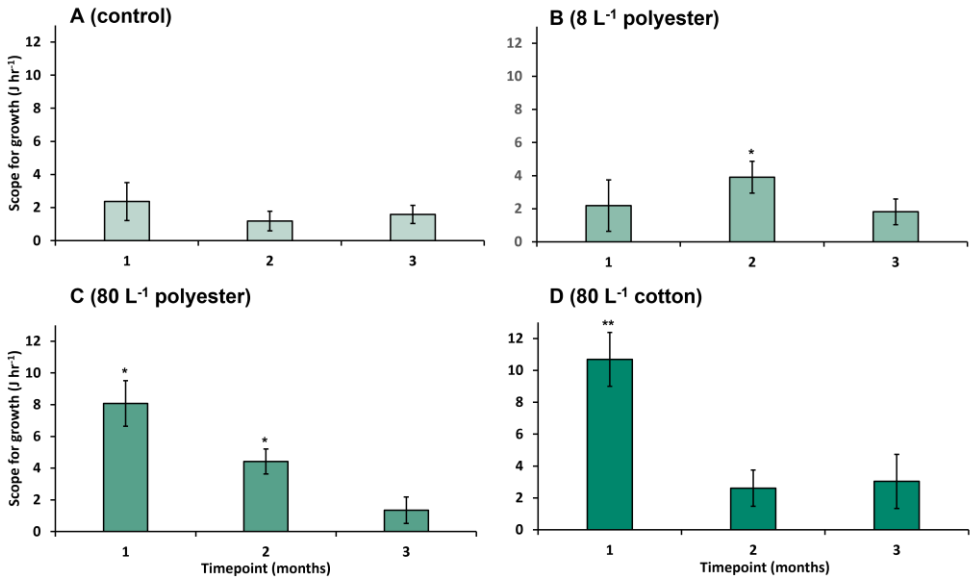


Figure A1. Scope for growth per mussel (J h^{-1}) for each experimental group after 1 month, 2 months, and 3 months exposure (3-month datapoint = experiment endpoint). A = control, B = 8 L^{-1} polyester, C = 80 L^{-1} polyester, D = 80 L^{-1} cotton. * and ** denote statistical significance when compared to the control group, $P < 0.05$ and $P < 0.01$, respectively.

Table A1. Energy absorbed, energy respired, and scope for growth per mussel (J h^{-1}) for each experimental group at the start, midpoint, and end of the exposure experiment.

Condition	**Timepoint (months after experiment start)**	**Energy absorbed, A (J h^{-1})**	**Energy respired, R (J h^{-1})**	**Scope for growth, SFG (J h^{-1})**
Control	Month One	7.60	5.23	2.37
	Month Two	3.35	2.17	1.19
	Month Three	3.75	2.16	1.59
Cotton 80 L^{-1}	Month One	14.83	4.15	10.69
	Month Two	5.24	2.63	2.61
	Month Three	5.15	2.11	3.04
Polyester 8 L^{-1}	Month One	10.99	8.80	2.19
	Month Two	6.50	2.60	3.90
	Month Three	3.88	2.06	1.82
Polyester 80 L^{-1}	Month One	11.98	3.90	8.07
	Month Two	7.44	3.02	4.42
	Month Three	3.17	1.82	1.35

Discussion

This study estimates, for the first time, SFG of mussels exposed to two different types of microfibre pollutant; synthetic polyester fibres and cellulosic cotton fibres. SFG results calculated at the start, midpoint and end of the experimental period show initially high SFG values for the 80 L^{-1} polyester and cotton exposure groups, (significantly higher than the control group), followed by a significant decrease in both groups over the experimental period. This trend was not observed for the control or 8 L^{-1} polyester exposure conditions, which had lower, more stable SFG values throughout the experiment. SFG results should be considered as a trend over the whole experimental period when comparing experimental groups, as animals naturally have variation in growth rates and physiological responses. Therefore, the SFG results are considered here over the course of the whole

experiment, rather than as a snapshot at the end of the experimental period, to give context of the baseline SFG at experiment onset. SFG results (Table A1) suggest that the amount of energy ingested is the critical variable which drives the SFG equation, with larger changes in the amount of energy absorption recorded over the experimental period than in the amount of energy respired. Results observed here are similar to those observed by Watts *et al.* (2015), who reported a significant decrease in SFG over time in the green shore crab *Carcinus maenas* when fed for four weeks with food where 0.3-1% of the food content was replaced with 500 µm blue polypropylene microfibres; Watts *et al.* also saw an initially higher SFG in their most concentrated exposure condition compared to the control. Gardon *et al.* (2018) also observed a significant decrease in SFG when the pearl oyster *Pinctada margaritifera* was exposed to a mixture of 6 and 10 µm polystyrene beads (0.25, 2.5 and 25 µg L^{-1}) for two months, however SFG was only calculated at one timepoint rather than over the course of the experiment. Thomas *et al.* (2020) observed a similar phenomenon, with an initial increase in condition index of juvenile oysters exposed to the highest concentration of microbeads before a subsequent statistically significant decrease in this value in future timepoints.

Interestingly, these SFG results contrast with the growth results; higher SFG values would usually predict more growth, but this was not the case in this study, as the control group with lower scope for growth over the experimental period exhibited the most growth. It is possible that the increased amount of substrate in the 80 L^{-1} cotton and polyester conditions due to the presence of both microalgae and microfibres stimulated a higher clearance rate in the mussels. If the mussels identified the cotton and polyester microfibres as a potential foodstuff, they may respond by increasing their clearance rate, as is observed on d30 and d58 in Fig. 3.2. As microfibres were observed within the mussel body at the end of the experiment, it is not likely that the mussels shifted their feeding strategy to completely eliminate microfibre ingestion, but instead acclimated to microfibre exposure

over time. The lowering of SFG observed here may therefore be due to a decrease in clearance rate as mussels acclimated to the presence of microfibres within the experimental exposures, without a similar change in respiration rate. As microfibres are observed to remain within organisms for extended periods of time, e.g. up to 75 h in shrimp (Gray and Weinstein, 2017) and 14 days in crabs (Watts *et al.*, 2014), the SFG method may not give reliable data for the amount of inorganic material ingested, as it relies on quick egestion of materials for detection. The lower control and 8 L^{-1} polyester exposure group results may be caused by a lower level of substrate (microalgae with/without experimental fibres) stimulating a lower clearance rate than the other two groups, subsequently meaning less acclimation is required by the mussels when fed with microalgae only, or microalgae with 8 L^{-1} polyester microfibres. Higher clearance rates in the 80 L^{-1} polyester and cotton exposure rates may not have resulted in more growth as post-ingestive selection to eliminate microfibres had an energetic cost, and ingested microfibres occupied volumetric space in the gut, potentially also leading to sublethal health effects causing further energetic cost, decreasing growth rates (Science Advice for Policy by European Academies (SAPEA), 2019; De Ruijter *et al.*, 2020; Koelmans *et al.*, 2020). These results suggest that the SFG method may be unsuitable for measuring sublethal health effects from the ingestion of microplastics.

Chapter 4: Detection and characterization of microplastics and microfibres in fishmeal and soybean meal

This chapter is a reformatted version of my publication currently under review:

Walkinshaw, C., Lindeque, PK., Thompson, RT., Tolhurst, T. and Cole, M.

CW, MC, TT, PKL and RT designed the experiments. CW carried out the experiments, data collection, conducted statistical analysis and wrote the manuscript. All authors contributed to editing and improving the final manuscript.

Aquaculture is an increasingly important source of nutrition for global food security, which is reliant on animal- and plant-based feeds. Anthropogenic particles, including microplastics and semi-synthetic cellulosic fibres, are prolific marine pollutants that are readily consumed by marine organisms, including small pelagic fish commonly used in fishmeal. Conversely, there is no indication plants can accumulate anthropogenic microparticles. We explore whether aquaculture feed presents a route of contamination for farmed fish. Commercially-sourced aquaculture feedstocks, including fishmeals and soybean meal, were processed (KOH digestion and $ZnCl_2$ density separation) and anthropogenic particles characterised using microscopy and spectroscopic methods. Both fishmeal and soybean meals contained anthropogenic particles, with concentrations ranging 1070-2000 particles kg^{-1}. The prevalence of anthropogenic particles in plant-based feeds indicates that the majority of contamination occurs post-harvest. Based on our findings, farmed Atlantic salmon may be exposed to a minimum of 1788–3013 anthropogenic particles from aquaculture feed across their commercial lifespan.

4.1 Introduction

Fisheries and aquaculture provide over 15% of the animal protein consumed by 4.5 billion people worldwide (Béné *et al.*, 2015). With a rapidly expanding global population, aquaculture is becoming an increasingly important approach for supplying seafood to market, and intrinsic to marine food security; in 2019, aquaculture provided 52% of fish production for human consumption with a value of 250 billion USD (FAO, 2020). Aquaculture can be used to grow a variety of species, including macroalgae, crustaceans and molluscs, however finfish dominates global production, contributing >54.3 million tonnes of food worth 139.7 billion USD (FAO, 2020). High value finfish species such as Atlantic Salmon and European seabass are typically maintained in open systems (e.g. sea pens), relying on aquaculture feed for sustenance and nutrition (Halwart, Soto and Arthur, 2007). Aquaculture feed typically comprises protein-rich pellets, powders or cakes, prepared from animal (e.g. fishmeal) or plant (e.g. soybean meal) material. For fishmeal, feedstock derives from targeted capture of small marine fish such as Peruvian anchoveta (*Engraulis ringens*), Pacific sardine (*Sardinops sagax*), and Atlantic herring (*Clupea harengus*), by-catch, and by-products (i.e. offal, trimmings) from the processing of larger commercial fish species (Cashion *et al.*, 2017). Fishmeal is created by cooking, pressing, drying and then grinding these tissues into a solid powder, at which point it can be pressed into a cake (Salin *et al.*, 2018). This process removes most of the water from the fish tissue, and centrifugation of the pressed material removes the fish oil, which is sold separately and can also be used in animal feed. Historically this process would have occurred outside, letting the fish dry out in the sun before processing it, whereas this process now occurs in large scale processing plants (Hertrampf and Piedad-Pascual, 2000; Windsor, 2001). Soybean meal is manufactured by cleaning, cracking and dehulling soybeans, followed by oil extraction (either by solvent or mechanical means), cooking and finally grinding into a meal (Willis, 2003). Additionally, feeds often contain a variety of additives to enhance digestibility of the feed, or

provide probiotics and immune stimulants to improve the health of the animal (Encarnação, 2016). While herbivorous fish can consume a feed that is either partially or completely comprised of plant proteins and oils (Viola, Arieli and Zohar, 1988), carnivorous fish require the addition of animal-derived proteins and oils. In 2013, approximately 16.3 million tonnes of fish were reduced to fishmeal and fish oil (FAO, 2014), of which 60% of total fishmeal and 80% of total fish oil production were used in aquaculture (Boyd, 2013). In recent years, the use of fishmeal within aquaculture feeds has been diminishing, largely owing to economic and consumer pressure stemming from overfishing of lower trophic species for feeding commercial species (Naylor *et al.*, 2009; Olsen and Hasan, 2012; Shannon and Waller, 2021); fishmeal is typically being replaced by plant-based meals, such as soybean, wheat and corn meal which is considered a cheaper and more sustainable option (Salin *et al.*, 2018).

Microplastics, describing plastic particles and fibres 1 µm – 5 mm in size, are a persistent, globally prevalent contaminant (Cole *et al.*, 2011; Hale *et al.*, 2020). These particles stem from industry (e.g. biobeads used in sewage treatment works as substrate for bacterial filtration of wastewater, pre-production pellets), highways (e.g. tyre-particles) and household effluent (e.g. microfibres released during laundry cycles, scrubbing agents) (Andrady, 2011; Napper and Thompson, 2016), or form through the degradation of macroplastic litter (Napper *et al.*, 2022). In the natural environment, microplastics degrade slowly and can persist for decades (Andrady, 2011). They are found in almost every environment worldwide, including freshwater, marine, benthic and terrestrial environments, and throughout the atmosphere resulting in their transport and deposition into remote ecosystems (Peeken *et al.*, 2018; Rochman, 2018; Bergmann *et al.*, 2019). Whilst microplastics are among the most commonly studied marine pollutants, there are other types of anthropogenic microparticles that may also pose a risk to the marine environment; these include cellulosic microfibres comprised of cotton and semi-synthetic polymers manufactured from regenerated cellulose (e.g.

rayon). Herein, we use the umbrella term ‘anthropogenic particles’ to refer to microplastics, semi-synthetic polymers and cotton particles. Cotton and semi-synthetic polymers are commonly used in textiles, such as clothing and agricultural fleece, and can enter the marine environment through household effluent, agricultural runoff and aeolian deposition (Napper and Thompson, 2016). Determining the environmental prevalence of these microfibres has been challenging, owing to the difficulties in differentiating between anthropogenic and natural cellulosic materials and issues with contamination, for example fibres shedding from operators’ lab coats or contamination from clothing or atmospheric fallout during sample collection. Nevertheless, numerous studies point to the presence of these fibres in considerable quantities alongside plastic microfibres (Remy *et al.*, 2015; Talvitie *et al.*, 2017; Halstead *et al.*, 2018; Nunes *et al.*, 2021; Savoca *et al.*, 2021).

Owing to their ubiquity in the marine environment, anthropogenic particles are inevitably taken up into living organisms, through ingestion or inhalation (Galloway, Cole and Lewis, 2017b). The presence of anthropogenic particles within commercially exploited aquatic species is well evidenced (Choy and Drazen, 2013; Foekema *et al.*, 2013; Rummel *et al.*, 2016). Chronic exposure to microplastics can have negative effects on commercially important marine organisms, with evidence of reduced growth and reproductive outputs (Cormier *et al.*, 2021); such effects could reduce the productivity and profitability of commercial aquaculture facilities (Walkinshaw *et al.*, 2020) . Recent studies have identified the presence of both plastic and semi-synthetic microfibres in farmed sea bream and common carp (Savoca *et al.*, 2021), and current evidence suggests that farmed fish typically contain more microplastic than wild-caught fish (Wootton et al., 2021). Yet, despite the importance of farmed seafood for human health and food security, the prevalence and effects of anthropogenic particles on farmed fish remain poorly elucidated. Farmed aquaculture species can be subject to anthropogenic particle exposure via their natural environment (e.g. through seawater and atmospheric

deposition), release from equipment, infrastructure and clothing, and their food and feeding supply system. Several studies have identified microplastics within fishmeals (Hanachi *et al.*, 2019; Karbalaei *et al.*, 2020; Gündoğdu *et al.*, 2021; Thiele *et al.*, 2021; Yao *et al.*, 2021; Wang *et al.*, 2022), however cellulosic microfibres were not considered in the majority of these studies. Contamination of aquaculture feed can occur where anthropogenic particles are present in source material (Hanachi *et al.*, 2019). For example, fishmeal is typically manufactured using planktivorous fish (commonly termed forage fish) which have been widely identified to contain high body burdens of anthropogenic particles (Lusher, McHugh and Thompson, 2013; Tanaka and Takada, 2016; Collard *et al.*, 2017; Welden, Abylkhani and Howarth, 2018; Walkinshaw *et al.*, 2020). While studies have identified plastic particles ≤45 µm can adsorb onto aquatic plants (Mateos-Cárdenas *et al.*, 2019; Dovidat *et al.*, 2020), there is currently no evidence that anthropogenic particles can permeate into plant material; therefore, anthropogenic particles in plant-based feeds (e.g. soybean meal) are unlikely to derive from source material. However, anthropogenic particles can also contaminate feeds during processing, transport and packaging; for example, anthropogenic particles may be released through mechanical abrasion of equipment, shedding of fibres from clothing and airborne deposition (Dris *et al.*, 2017; Roblin *et al.*, 2020). In comparing anthropogenic particle concentrations in both animal- and plant-based feedstocks, the origin of these contaminants can be elucidated.

In this study, we investigate the potential exposure of commercially exploited finfish species to anthropogenic particles via aquaculture feed. We apply optimised methods for isolating and characterising >25 µm anthropogenic particles in ten commercially-available aquaculture feeds, including a variety of fish meals and a soybean meal. We hypothesise that there are a wide range of anthropogenic particles present in aquaculture feed, including both microplastics and semi-synthetic cellulosic fibres. The analysis of both fishmeal and soybean meal will allow us to explore the hypothesis that anthropogenic particle

contamination of aquaculture feed is predominantly driven by the level of contamination in the source material. Finally, we test the hypothesis that the use of aquaculture feed in fish farming increases risk of anthropogenic particulate exposure in farmed finish as compared to wild stock by calculating the additional anthropogenic particle load that farmed salmon will incur from the consumption of aquaculture feed.

4.2 Methods

4.2.1 Contamination control and blanks

All sample processing took place within a laminar flow hood in the ultraclean microplastics laboratory in Plymouth Marine Laboratory (Plymouth, UK). The laboratory minimizes microplastic contamination through use of a HEPA filtered positive pressure airflow system (which removes 99.95% of airborne particles with a diameter of 0.3 µm), controlled personnel entry, tack mats to remove footwear contamination and cotton lab coats to supress release of polymeric clothing fibres. Wherever possible, glass apparatus and consumables were used to avoid plastic contamination. All flasks were sealed with aluminium foil and parafilm whenever taken out of the laminar flow hood and when in the orbital shaker incubator. Procedural blanks (n=3), comprised of performing all experimental steps without the initial addition of fishmeal or soybean meal, were performed and analysed in the same way as test samples to identify any background contamination. Positive controls (n=3), spiked with a known quantity of nylon fibres and polystyrene beads, were taken through the process to determine methodological efficacy.

4.2.2 Digestion and density separation

Ten commercially-available aquaculture feeds were chosen for investigation, comprising nine fishmeals of marine origin and one soybean meal (Table 4.1). Fishmeal is a complex organic matrix, comprising dehydrated flesh, bone and abiotic material. Therefore, it was necessary to employ an optimised two-step process, including

chemical digestion and density separation, to effectively isolate anthropogenic particles from this substrate. Aquaculture feed was manually mixed within its container and 10 g subsamples weighed with a mass balance and placed into a clean conical flask with 200 mL of 10% KOH + 1% Tween 20. Flasks were sealed with aluminium foil and parafilm to prevent airborne contamination, and placed into an orbital shaker incubator (Sanyo Orbisafe orbital incubator) and digested for 48 hours at 50 °C, 125 rpm. Treilles et al. (2020) show that both plastic and cotton are resistant to KOH degradation at this concentration. After digestion, undigested material was vacuum filtered sequentially onto 100 µm, 63 µm and 25 µm filter mesh discs, rinsing filtration equipment with ultrapure water to ensure no loss of material; filter discs were dried overnight in a dehydrating oven set to 60°C. Samples were subsequently density separated using a sediment-microplastic isolation (SMI) unit (Coppock *et al.*, 2017) filled with $ZnCl_2$ solution (solution density 1.5 g cm^3); the solution was mixed and left to separate out for 30 minutes, and then the lower-density particulates in the supernatant were filtered back on to corresponding mesh discs to retain any anthropogenic particles <1.5 g cm^3. The mesh disc was then placed into a Petri dish and dried for 12 hours in a dehydrator at 60°C. Between repeats, the SMI unit was cleaned with ultrapure water and the $ZnCl_2$ solution was recycled by filtering through a 0.2 µm GF/F glass fibre filter. $ZnCl_2$ solution density was checked, and if this was below 1.5 g cm^3 a new solution was manufactured. The two-step protocol removed on average of 97.5% of the sample material by mass, making identification of anthropogenic particles using microscopy viable, but precluding the use of scanning technologies (e.g. Raman, FT-IR imaging) as such methods require pristine microplastics without other detrital matter.

Table 4.1. Aquaculture feed details including country of origin and main species within the feed. Samples are referred to throughout the text by the name designated in brackets.

Sample	**Country of origin**	**Main species**
LT-94 (LT94a)	Norway	Atlantic herring (*Clupea harengus*)
LT-94 (LT94b)	Norway	Atlantic herring (*Clupea harengus*)
Provimi 66 (Pv66a)	UK	White fish and salmon trimmings
Provimi 66 (Pv66b)	UK	White fish and salmon trimmings
Pre-digested fish protein (CP70)	UK	Pre-digested white fish and fish trimmings
White fish (WF)	Scotland	White fish
Sardine and anchovy (SA)	South America	Sardine and anchovy
Squid (Sq)	Unknown	Dried Whole Squid
Krill (Kr)	Antarctic Krill	Antarctic krill (*Euphausia superba*)
Soybean meal (Soy)	Unknown	Defatted heat treated soya (*Glycine max*)

4.2.3 Anthropogenic particle identification

Anthropogenic particles were identified by performing a multi-stage identification process involving microscopic screening based on visual characteristics, supplemented with polymeric verification using Fourier-transform infrared (FTIR) spectroscopy. This approach aligns with the methodologies used for analysis of environmental samples elsewhere (Jones-Williams et al., 2020). Each mesh disc was systematically checked for potential anthropogenic particles manually using an Olympus SZX16 microscope and CellSens software (Olympus, version 2.1); mesh discs were placed onto a glass slide with a 3 mm^2 grid and each square analysed to identify particles of interest. Particles of interest were identified through morphology, colour, and

texture that may allude to being anthropogenic in origin. For each of these particles, colour, shape (fibre, fragment or film), and length of longest dimension (µm) was recorded. A subset of 400 particles (48.5% total identified particles) were selected for polymeric analysis; these particles were placed on to 0.02 µm anodiscs for FTIR analysis. To reduce analytical effort, where particles showed a high degree of morphological similarity, only a subset of these particles were analysed (33% of selected particles); spectroscopic data was used to estimate the polymer composition of the other particles on the mesh disc.

Potential anthropogenic particles were verified using a Perkin Elmer Spotlight 400 imaging system comprised of a PerkinElmer Frontier FT-IR spectrometer (MCT detector, KBr window) and PerkinElmer Spotlight 400 microscope, with SpectrumIR software (PerkinElmer, 2017, version 10.6.0.893). The spectrometer was used in transmittance sampling mode, with 20 scans (range = 1250 – 4000 cm^{-1}) at a resolution of 4 cm^{-1}. Resultant spectra were compared with bespoke and publicly-available reference libraries, including the spectral library created by Primpke et al. (2018), who utilised a near identical spectral range in the creation of this reference library (1250 – 3600 cm^{-1}). Particles with spectral matches > 70% were used as confirmation of particle composition. However, as organic soiling on the surface of plastic particles can reduce spectral match accuracy, particles with spectral matches < 70% were also included in the results where physical characteristics (morphology, colour, structure) matched similar particles within the sample that were successfully characterised as being of anthropogenic origin. Following the polymer identification steps, polymers were assigned to one of three categories based on their origin: Petroleum-based plastic, semi-synthetic or cotton. Semi-synthetic polymers were defined as cellulose-based polymers manufactured synthetically from regenerated cellulose, such as rayon, cellophane, and cellulose acetate. There is added complexity in distinguishing anthropogenic cellulosic particles from natural cellulose in the samples. In order to distinguish anthropogenic cellulosic particles such as rayon, an extra step was added following

FTIR analysis. If the resultant spectra identified the particle as cellulose, rayon, cellophane, or cellulose acetate, the particle was again screened visually and was only included if the colour and morphology was indicative of being of anthropogenic origin, i.e. a non-natural uniform colour and uniform shape with no organic structures visible.

4.2.4 Analysis and statistics

Concentrations of anthropogenic particles within fishmeal samples were calculated as mean number of particles per 10 g sample, with the total of all three meshes (100, 63 and 25 µm) comprising each replicate. Data was then used to calculate the mean number of particles kg^{-1}. Exposure of farmed Atlantic salmon to anthropogenic particles (P_E) was calculated by taking the approximate weight of a salmon upon harvest and multiplying this by the feed conversion ratio (FCR, a measure of the weight of feed needed for 1 kg biomass gain in the farmed organism) to calculate the total feed consumed (F_C). This value is then multiplied by the approximate percentage inclusion of fishmeal or soybean meal in salmon feed ($\%I_{FM}$ and $\%I_{SBM}$ for fishmeal and soybean meal, respectively) to calculate the mass of each feed included in the meal. The resulting value is also multiplied by the mean number of anthropogenic particles kg^{-1} identified in each feed (AP_{FM} and AP_{SBM} for fishmeal and soybean meal, respectively) to calculate the estimated number of anthropogenic particles ingested by Atlantic salmon through fishmeal and soybean meal. This calculation is shown below:

$$(1)\ F_C = Salmon\ mass\ (kg)\ \times FCR$$

$$(2)\ P_E = (F_C\ \times \%I_{FM}\ \times\ AP_{FM}) + (F_C\ \times \%I_{SBM}\ \times\ AP_{SBM})$$

Data is presented as mean with standard errors of the mean, unless otherwise stated. Statistical analyses were performed using R (version

4.1.0). Data were tested for normality using Shapiro-Wilk tests, and normally-distributed data were tested by ANOVA with Tukey's post-hoc testing. Where data violated assumptions of normality, non-parametric Kruskal-Wallis tests with Dunn's post-hoc pairwise testing were performed to investigate whether individual experimental groupings differ significantly. The significance level for both tests was set at $\alpha = 0.05$.

4.3 Results

4.3.1 Anthropogenic particle identification

Analysis of process blank samples showed a mean of 5 particles per sample, all of which were fibres, of which 4.67 fibres filter^{-1} were semi-synthetic and 0.33 fibres filter^{-1} were identified as polyester. Mean blank results were removed from each replicate. Positive controls found mean recovery rates of 100% and 94% for nylon fibres and polystyrene beads respectively. Owing to the high recovery rates, no corrective factor was applied to the results.

Across all aquaculture feed subsamples, 865 suspected anthropogenic particles were identified via microscopy, with 64% of selected particles identified as being anthropogenic in origin using FT-IR. For all particles assessed: the most prevalent morphology was fibres (82.5%), followed by fragments (16.8%) and films (0.8%); the most common colour of anthropogenic particle was blue (70%), followed by red (11.8%) and black (6.5%); and the longest dimension of particles and fibres ranged from 24 – 11,400 µm, with a mean throughout all samples of 1218 µm (median 732 µm).

Accounting for contamination in procedural blanks, the mean number of anthropogenic particles, including semi-synthetic, cotton and petroleum-based polymers, ranged from 10.7 - 20 particles per 10 g (Table 4.2), equating to 1070-2000 particles kg^{-1}.

4.3.2 Particle characteristics

Fibres were predominant in all samples regardless of feed origin. When comparing fibre prevalence between samples, only sample LT94a and LT94b were significantly different (Kruskal-Wallis/Dunn test, $P < 0.05$). Only 1 anthropogenic particle fragment (comprised of polyamide) was identified across all soybean meal samples, compared to an average concentration of 1.0-5.3 fragments per 10 g fishmeal sample; statistical analysis revealed significantly (Kruskal-Wallis/Dunn test, $P < 0.05$) more fragments in Pv66a, Pv66b, LT94b, and Krill meal when compared with the soybean meal. When comparing LT94a/LT94b and Pv66a/Pv66b a difference in the number of anthropogenic particles can be observed, with LT94a containing considerably more fibres and less fragments than LT94b. Pv66a has a similar number of fragments but more fibres on average than Pv66b.

Table 4.2. Anthropogenic particles identified in meals of different origin. Mean results (n=3) with result range displayed in brackets.

Fishmeal	**Mean particles per 10g replicate**	**Mean fibres per 10g replicate**	**Mean fragments per 10g replicate**	**Mean films per 10g replicate**
LT94a	20.0 (8-35)	18.3 (8-33)	1.7 (0-3)	0.0
LT94b	10.7 (6-16)	5.3 (1-13)	5.3 (3-9)	0.0
Pv66a	14.3 (9-17)	11.0 (9-13)	3.3 (0-6)	0.0
Pv66b	11.0 (10-12)	7.3 (4-9)	3.3 (1-6)	0.3 (0-1)
CP70	13.0 (1-31)	11.0 (0-29)	2.0 (1-3)	0.0
WF	14.7 (7-26)	12.7 (5-23)	1.7 (1-3)	0.3 (0-1)
S&A	12.7 (7-16)	11.3 (7-14)	1.3 (0-2)	0.0
Sq	11.3 (7-15)	10.0 (5-14)	1.0 (0-2)	0.3 (0-1)
Kr	13.3 (9-19)	11.0 (7-16)	2.3 (2-3)	0.0
Soy	12.3 (8-17)	12.0 (8-17)	0.3 (0-1)	0.0

Petroleum-based polymers were identified in all feeds tested, with the number of particles and the number of different polymer types identified varying between samples (Fig. 4.1). In total, 18 different petroleum-based polymers were identified. In order to simplify results,

the most commonly identified microplastic pollutants were split out (polyamide, polyester, polyethylene, polypropylene and polystyrene), while the rest of the plastic polymers were identified as 'other'. This category included plastics such as polyvinyl chloride, polytetrafluoroethylene (PTFE), epoxy and alkyd urea resins, and copolymers (see Supplementary Tables SI4.1 and SI4.2 for full details of all polymers identified). Polyester was the most common petroleum-based polymer identified in the samples, being present in all samples except for CP70 at a concentration of 66.7 – 633.3 particles kg^{-1}. The least diversity in polymer type identified within samples is observed with the soybean meal sample, which only had particles from 4 of the polymer categories used here (polyamide, polyester, semi-synthetic and other); marine meals contained polymers from 5 – 9 categories.

Semi-synthetic polymers were found in all samples tested and ranged from 8 – 73% of all particles identified (Fig. 4.1 and 4.2). Semi-synthetics were most predominant in LT94a, Krill and soybean meal, where they represented >50% of the total number of anthropogenic particles identified. Cotton was found in all samples except for soybean meal, and was most prevalent in CP70, where it represented 53% of the total particles identified. Petroleum-based polymers were the most prevalent in all other samples. Cotton contamination ranged from 0 – 700 particles kg^{-1}, with semi-synthetic contamination varying from 133-1467 particles kg^{-1} and contamination by petroleum-based plastics ranging from 267 – 1267 particles kg^{-1}.

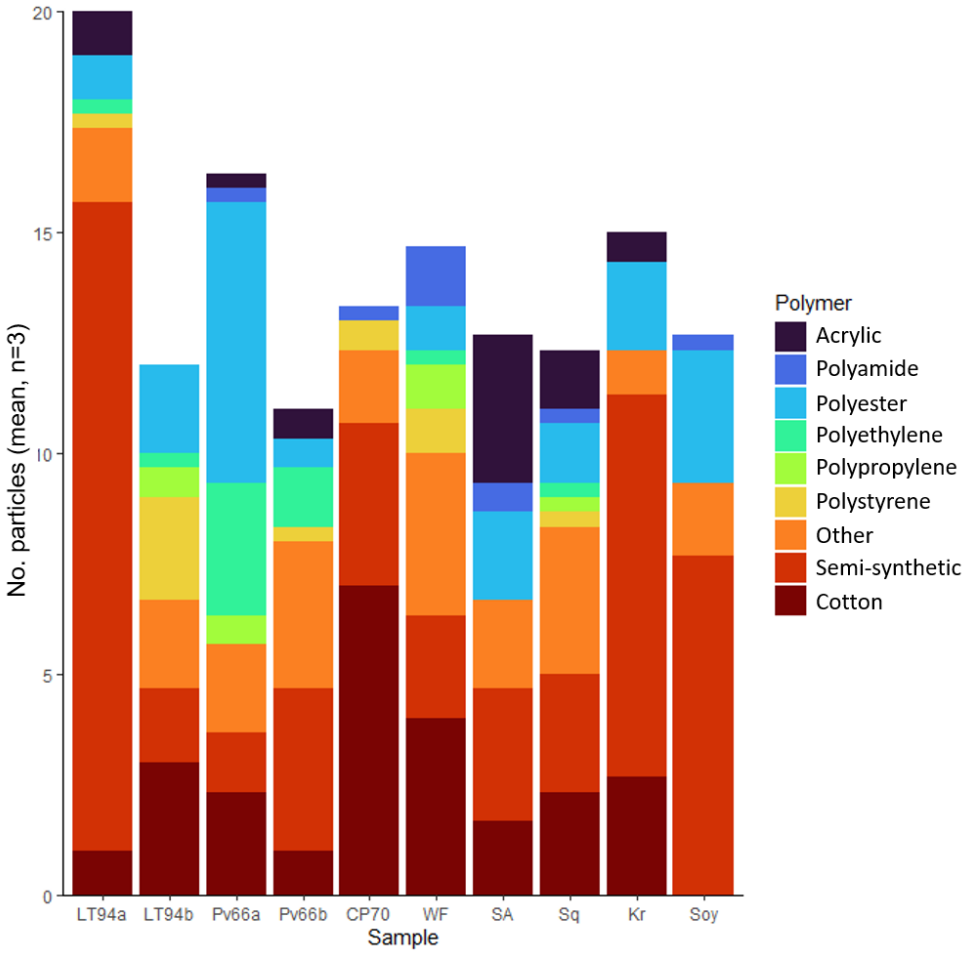


Figure 4.1. Polymer composition of identified anthropogenic particles within each sample (mean per 10g replicate, n=3).

4.3.3 Size fractionation of particles

The total number of particles captured on each mesh did not correlate with mesh size and was not consistent between samples (Fig. 4.2). This was also the case for the number of petroleum-based plastics, semi-synthetics and cotton particles identified on each mesh. The number of fragments identified correlated with mesh size in 5 out of the 10 sample types (LT94a, LT94b, Pv66a, Pv66b, CP70), with decreasing numbers of fragments identified with decreasing mesh size. The number of fibres showed no correlation to mesh size, and not enough films were identified for trends to emerge. The size of particles captured also did not correlate with mesh pore size (Fig. 4.3). The 25 µm mesh captured fibres with lengths up to 1700 µm, which had passed through both the 100 µm and 63 µm meshes. This may be because, whilst fibres are measured by their longest dimension

(length), they are very small in diameter and have the capacity to pass through larger mesh sizes lengthways (Barrows *et al.*, 2017; Covernton *et al.*, 2019). The diameter of a subset of microfibres from sample LT94a were measured and ranged from approximately 10-30 µm, many of which would pass through a 25 µm mesh if oriented appropriately. Fragments >100 µm were also identified on the 25 µm meshes in this study, despite having been passed through the 100 µm and 63 µm meshes; this phenomenon can occur because: (a) particles with a large axial ratio may permit them to pass through coarse meshes when orientated in a certain position (in a similar way to fibres); and (b) owing to inconsistency in mesh pore size across a filter that may be exacerbated by pressure from the vacuum pump pulling fragments through mesh pores during filtration.

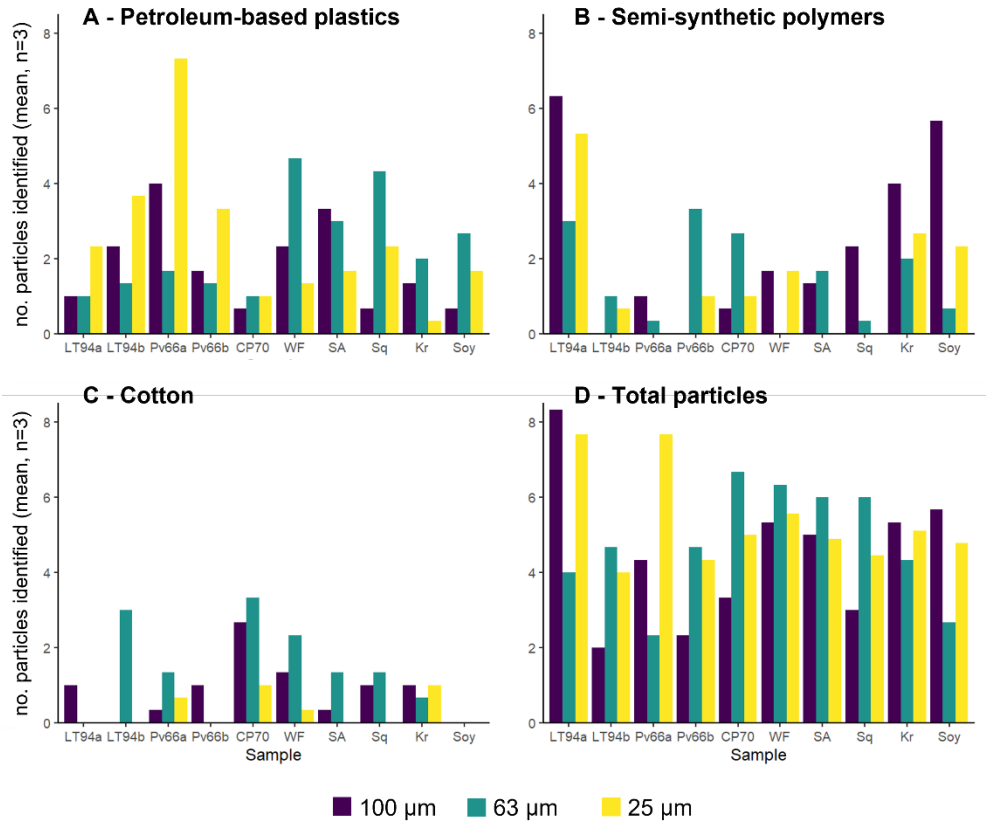


Figure 4.2. Mean number of particles identified of each category (petroleum-based plastics, semi-synthetic polymers, cotton, and total of all particles) on 100, 63, and 25 µm pore size filters for each 10 g sample (n=3).

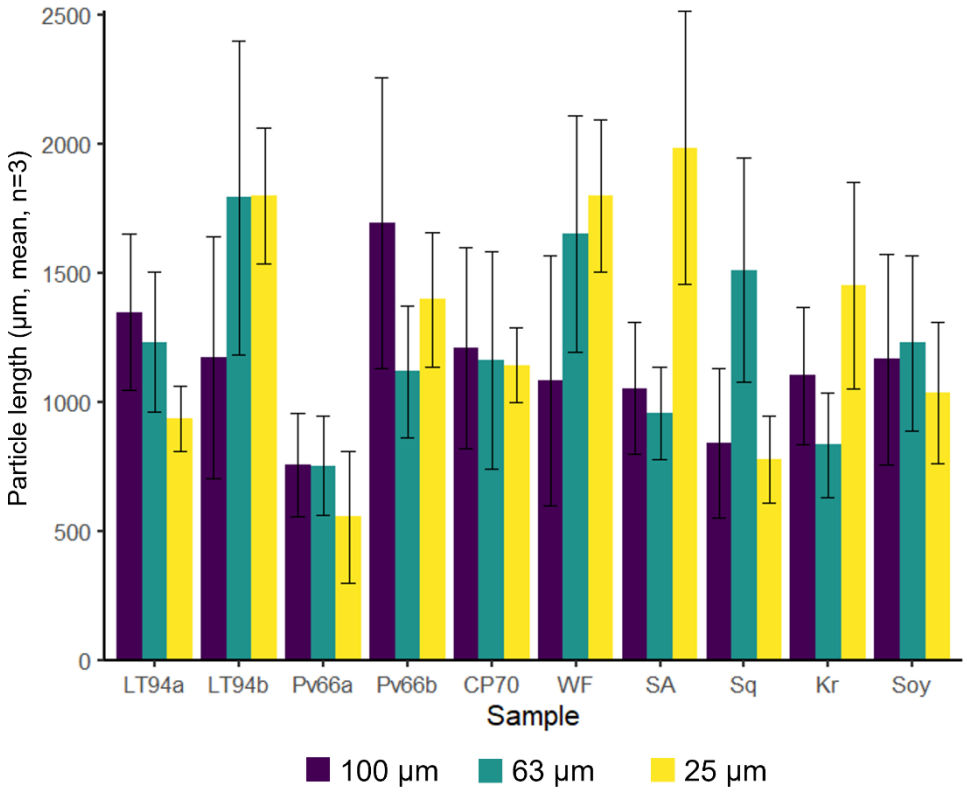


Figure 4.3. Mean length of particles (calculated as largest dimension in µm) identified on 100, 63 and 25 µm pore size filters for each 10 g sample (n=3, error bars = standard error).

4.3.4 Lifetime exposure of salmon to anthropogenic particles through aquaculture feed

Using our results, we estimated anthropogenic particle exposure via aquaculture feed for farmed Atlantic Salmon. Atlantic Salmon have a feed conversion ratio (FCR) of approximately 1.1, meaning that they require 1.1 kg feed for 1 kg biomass gain. Aquaculture feeds are variable in biomass content, with fishmeal, fish oil, plant-based meal and meal from other origins (e.g. poultry) all used in different proportions by different producers for different species. The latest figures from some producers show fishmeal making up 15% of Atlantic salmon feed (Mowi, 2021), and though soybean meal content in aquaculture feed is also highly variable, prior research has shown up to 20% soybean content within feed caused no observable difference in Atlantic salmon health (Olli, Krogdahl and Våbenø, 1995). Atlantic Salmon are frequently grown to a size of 4-5 kg before harvest (Cohen *et al.*, 2016; Davidson *et al.*, 2016). With a diet comprising 15%

fishmeal (0.66 – 0.83 kg) and 20% soybean meal (0.88-1.1 kg), we calculate Atlantic Salmon will be exposed to 1788 – 3013 anthropogenic particles throughout their commercial lifespan from aquaculture feed, with 706-1660 particles from fishmeal and 1082-1353 particles from soybean meal.

4.4 Discussion

Anthropogenic particles, including microplastics and cellulosic microfibres, were identified in all aquaculture feeds tested, with an average of 1070-2000 anthropogenic particles kg^{-1} across fishmeals and soybean meal. In other studies, mean microplastic content in fishmeal ranges from 0-10,000 particles kg^{-1} (Karbalaei *et al.*, 2020; Gündoğdu *et al.*, 2021; Thiele *et al.*, 2021; Yao *et al.*, 2021; Wang *et al.*, 2022). The orders of magnitude difference in microplastic and anthropogenic particle concentrations may stem from high variability in the source material and heterogenous particle distributions within aquaculture feeds; this is evident within our study, where anthropogenic particle concentrations from the same type of fishmeal sourced from two different suppliers (LT94a/b) contained the lowest and highest particle concentrations observed. Inter-laboratory comparisons may be further compounded by methodological differences in extracting and enumerating anthropogenic particles in complex organic substrates (Lusher *et al.*, 2017). For example, the use of larger pore size filters can preclude the capture of microfibres (Lindeque *et al.*, 2020; Athey and Erdle, 2022). This is illustrated in the difference in microfibre prevalence between our study (82.5% total particles) which utilises a minimum filter pore size of 25 µm, and that of Hanachi et al. (2019) (6% total particles), who used a filter pore size of 149 µm. Particle capture rates depend upon the shape of the particle and the shape of the filter pore (Lees, 1964b, 1964a; Lees and Sherigold, 1965). The smallest cross-sectional area is the most important determining factor, and in the case of sediment grains the longest dimension of the particle usually has little effect on whether the

particle will pass through any given hole (e.g. Fernlund, 1998; Fig. 1). This means that prolate and rod shaped particles tend to pass through the holes in a sieve according to their intermediate diameter. Our results demonstrate a similar process occurs during filtration of anthropogenic particles; fibres of over 1000 µm length and fragments with highly heterogeneous morphologies were able to pass through filters of 63-100 µm pore size. This has important implications for the extraction of different shaped microplastics from the environment, and demonstrates the importance of using small pore size filters and sequential filtration to improve microfibre capture rates. Due to these methodological limitations we surmise that the number of anthropogenic particles identified in studies such as this will almost always be conservative.

We identified microplastics in all feeds tested; conversely, Gündoğdu *et al.* (2021) identified no microplastics within fishmeal derived from Antarctic Krill, and Hanachi *et al.* (2019) identified no microplastics in soybean meal. In this study, semi-synthetic and/or cellulosic microfibres were also identified in all types of aquaculture feed, making up >50% of the anthropogenic particles in krill and soybean meals. However, cellulosic microfibres were not investigated in detail in other studies examining aquaculture feed. For example, Gündoğdu *et al.* (2021) characterised particles using Raman spectroscopy and compared results with the spectra of 13 commercially-available materials including cellulose, but did not include any cellulosic particles in their results; while Hanachi *et al.* (2019) identified low levels of rayon within salmon, sardine and kilka meal (4% total particles) but not in soybean meal. Numerous studies describe challenges in the identification of semi-synthetic particles owing to difficulties in differentiating naturally-occurring and anthropogenic cellulosic fibres using spectroscopy (Dris *et al.*, 2017) and issues of contamination (Halstead *et al.*, 2018). There is often a perception that semi-synthetic plastics may pose less of a risk to the natural environment, compared with synthetic plastic, given their comparatively faster degradation times (Ladewig, Bao and Chow,

2015; Henry, Laitala and Klepp, 2019; Zambrano *et al.*, 2019, 2020). When tested in an aqueous medium with the addition of sewage sludge to simulate natural aquatic environments, rayon yarn exhibited 62% biodegradation after 243 days, whereas polyester yarn did not degrade (Zambrano *et al.*, 2019). However, their prevalence in aquaculture feed demonstrates that semi-synthetic polymers and cellulosic microfibres (e.g. cotton) may enter marine food webs irrespective of their biodegradability. We advocate that where feasible, microplastics research should also consider the prevalence, fate and biological effects of these anthropogenic particles.

Irrespective of source material, aquaculture feeds contained similar levels of anthropogenic particles, with fishmeal containing an average of 1070-2000 anthropogenic particles kg^{-1} and soybean meals containing an average of 1230 anthropogenic particles kg^{-1}. Nanoplastics and very small microplastics ≤2 µm could potentially contaminate plant vascular systems via the apoplastic space in plant root cells, consisting of their cell walls and extracellular space (Li *et al.*, 2020; Azeem *et al.*, 2021). However, while particles could adhere to the external surfaces of plants (Mateos-Cárdenas *et al.*, 2019), there is no indication that anthropogenic particles in the size range observed in soybean meal (24 – 11,400 µm) can directly contaminate vascular plants. Therefore, we surmise that the anthropogenic particles identified in soybean meal will have stemmed from post-harvest contamination, such as from processing, packaging and transportation. Given microplastics are widely evidenced in marine organisms (Lusher, McHugh and Thompson, 2013; Tanaka and Takada, 2016; Collard *et al.*, 2017; Welden, Abylkhani and Howarth, 2018), we had anticipated fishmeals would contain higher levels of anthropogenic particles compared with soybean meals. While we demonstrated the types of anthropogenic particles differed between fishmeal and soybean meal, there was no significant difference in anthropogenic particle concentrations between feeds of different origin. It is possible that anthropogenic particles present within source material (i.e. fish tissues) were broken-down or destroyed during

manufacture, either through mechanical abrasion or combustion owing to the use of high temperatures (up to 500°C in direct air drying) during desiccation (Hertrampf and Piedad-Pascual, 2000). The melting points of plastics, including polyamides, polyethylene and polystyrene, range 170-290°C, meaning they would be subject to degradation during processing. However, some fishmeals (e.g. LT94), are cooked at temperatures of 90-100°C, and yet did not display significantly higher levels of anthropogenic particles compared with other types of fishmeal. Future studies may wish to consider the prevalence of toxic by-products, including PAHs with a higher number of aromatic rings, free radicals and toxic heavy metals, that are emitted by anthropogenic particles during combustion (Simoneit, Medeiros and Didyk, 2005; Valavanidis *et al.*, 2008). Based on our data, we conclude that post-harvest contamination is the predominant source of anthropogenic particles in aquaculture feed.

We estimate that farmed Atlantic Salmon will be exposed to 1788 – 3013 anthropogenic particles via fishmeal and soybean meal over their commercial lifespan. However, farmed finfish may also be exposed to anthropogenic particles through other feed ingredients, for example fish oil and other vegetable- and animal-based products, as well as their natural environment. Wang et al. (2022) estimated that farmed Atlantic Salmon consume 9,361 microplastic items over their commercial lifespan; differences in exposure data can be explained by Wang using a higher feed conversion ratio (1.2 compared with 1.1 used here) and assuming a higher proportion of fishmeal used in the salmon's diet (42% compared with 15% used here). In recent years, the proportion of fishmeal used in aquaculture diets has been decreasing in response to limited supply (Olsen and Hasan, 2012), concerns about ecosystem health and overfishing (Deutsch *et al.*, 2007; Brunner *et al.*, 2009), increasing costs (Tacon and Metian, 2008), and the development of alternative feeds (Hemaiswarya *et al.*, 2011; Rust *et al.*, 2011; Bandara, 2018; Lock, Biancarosa and Gasco, 2018; Ferrer Llagostera *et al.*, 2019). In 2020, up to 70% of the diet of farmed salmon may be composed of plant-based meals (Mowi, 2021);

as other plant-based materials are likely to have undergone similar processing steps as soybean meal, we hypothesise that these feeds will also contain anthropogenic particles. In addition to exposure through their feed, farmed fish are exposed to anthropogenic particles present in seawater (Auta, Emenike and Fauziah, 2017; Luo *et al.*, 2019), and stemming from airborne deposition (Roblin *et al.*, 2020; Szewc, Graca and Dołęga, 2021), workers' clothing (De Falco *et al.*, 2020) and aquaculture equipment (Floerl, LM and Bloecher, 2016; Chen, Li and Wang, 2021). The consumption of anthropogenic particles by finfish may have profound consequences for farmed populations; for example, there is growing evidence that microplastics can negatively affect growth and reproductive output (Galloway, Cole and Lewis, 2017b), which in commercially-exploited species could result in longer time-to-market and decreased commercial and nutritional value (Walkinshaw *et al.*, 2020). Following consumption, anthropogenic particles such as microplastics are often passed through the gastrointestinal tract and excreted through faeces (Ory *et al.*, 2018; Spanjer *et al.*, 2020), in which they will sink through the water column (Cole *et al.*, 2016). In open cage aquaculture facilities, this may lead to hotspots of anthropogenic particles in the benthos directly beneath aquaculture facilities, which may result in environmental perturbations for underlying benthic communities (Coppock *et al.*, 2021).

4.5 Conclusion

All aquaculture feeds tested contained microplastic and semi-synthetic particles, with 90% of the samples also containing cotton microfibres. As both animal- and plant-based feeds contained high concentrations of anthropogenic particles regardless of feed origin, we consider it likely that the majority of particles and fibres stem from post-harvest contamination. Contamination of aquaculture feed with anthropogenic particles adds an additional exposure route for farmed species with

potential consequences for fish health, and risks to nutritional value, profitability and ultimately food security.

4.6 Acknowledgements

CW was supported by the Natural Environment Research Council through the EnvEast Doctoral Training Partnership (grant number NE/L002582/1). MC and PL would like to acknowledge funding from the UKRI Global Challenges Research Fund (GCRF) funded project ‘Reducing plastic waste in the Galapagos and Eastern Pacific’.

4.7 Supplementary information

Supplementary Table SI4.1. Morphological characteristics of anthropogenic microparticles identified within feed samples (mean, n=3).

	LT94a	LT94b	Pv66a	Pv66b	CP70	WF	S&A	Sq	Kr	Soy
Total particles	20.00	10.67	14.33	11.00	13.00	14.67	12.67	11.33	13.33	12.33
Fibres	18.33	5.33	11.00	7.33	11.00	12.67	11.33	10.00	11.00	12.00
Granules	1.67	5.33	3.33	3.33	2.00	1.67	1.33	1.00	2.33	0.33
Films	0.00	0.00	0.00	0.33	0.00	0.33	0.00	0.33	0.00	0.00
Black	0.00	0.67	5.00	0.33	0.33	0.00	0.67	0.33	1.00	0.33
Blue	18.33	9.00	3.33	5.00	12.33	10.00	9.67	6.33	12.00	7.00
Clear	0.00	0.00	2.33	0.00	0.00	0.33	0.67	1.00	0.00	0.33
Clear/blue	0.00	0.00	0.00	0.00	0.00	0.33	0.00	1.00	0.00	0.00
Green	1.00	0.00	1.67	1.67	0.00	0.67	0.00	0.33	0.00	0.33
Purple	0.33	0.00	0.67	0.67	0.00	1.67	1.67	0.00	0.00	1.00
Red	0.67	2.33	0.67	1.33	1.00	1.33	1.33	2.33	1.33	3.33
Pink	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Yellow	0.00	0.00	0.33	0.00	0.00	0.33	0.00	0.00	0.00	0.00
Brown	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Supplementary Table SI4.2. Polymer identity of anthropogenic microparticles detected within feed samples (mean, n=3).

	LT94a	**LT94b**	**Pv66a**	**Pv66b**	**CP70**	**WF**	**S&A**	**Sq**	**Kr**	**Soy**
Rayon	11.33	1.00	1.00	2.33	3.33	2.33	0.33	2.67	8.67	7.67
Cellulose	3.00	0.00	0.00	1.33	0.33	0.00	2.67	0.00	0.00	0.00
Cotton	1.00	3.00	2.33	1.00	7.00	4.00	1.67	2.33	2.67	0.00
Cellophane	0.33	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Azlon	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Acrylic	1.00	0.00	0.33	0.67	0.00	0.00	3.33	1.33	0.67	0.00
Alkyd urea resin film	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00
Bakelite	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Celanese/acetate	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Epoxy resin	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ethylene/maleic acid anhydride copolymer	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33
Polyamide	0.00	0.00	0.33	0.00	0.33	1.33	0.67	0.33	0.00	0.33
Phenol resin	0.00	1.00	0.00	1.67	1.00	2.00	0.33	1.33	0.67	1.33
Poly(butadiene:acrylonitrile) film	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00
Poly(phenylene disulfide)	0.00	0.00	0.00	0.00	0.00	1.33	0.33	0.00	0.00	0.00
Polyacrylamide	0.00	0.67	0.00	0.00	0.00	0.33	0.67	2.00	0.00	0.00
Polyester	1.00	2.00	6.33	0.67	0.00	1.00	2.00	1.33	2.00	3.00
Polyethylene	0.33	0.33	3.00	1.33	0.00	0.33	0.00	0.33	0.00	0.00
Polypropylene	0.00	0.67	0.67	0.00	0.00	1.00	0.00	0.33	0.00	0.00
Polystyrene	0.33	2.33	0.00	0.33	0.67	1.00	0.00	0.33	0.00	0.00
Polyvinyl chloride	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00

	LT94a	LT94b	Pv66a	Pv66b	CP70	WF	S&A	Sq	Kr	Soy
Styrene/isoprene copolymer	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00
Olefin copolymer	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00
PTFE	1.33	0.00	0.33	0.33	0.67	0.00	0.67	0.00	0.00	0.00
Petroleum-based plastics	4.33	7.33	12.67	6.33	2.67	8.33	8.00	7.33	3.67	5.00
Semi-synthetics	14.67	1.33	1.33	3.67	3.67	2.33	3.00	2.67	8.67	7.67

Appendix B – Sample digestion and density separation method development

1 Introduction

Identifying microplastics within complex, organically-rich matrices such as fishmeal is hugely challenging and requires the application of suitable protocols in order to remove as much extraneous material as possible. Two processes are commonly performed to remove organic and inorganic material that occlude microplastics, generally termed: digestion and density separation. Digestion involves the use of chemicals or enzymes (e.g. Proteinase K, Trypsin) to break down and remove organic materials from a sample. Chemicals commonly used for digestion include acids (e.g. HCl and HNO_3), bases (e.g. KOH and NaOH) and reagent mixtures (e.g. Fenton's reagent). There are advantages and disadvantages for each chemical (Cole *et al.*, 2014; Lusher *et al.*, 2017; Hurley *et al.*, 2018), but typically the choice of chemical is about making a trade-off between effectively destroying organics without damaging or destroying microplastics within the sample. KOH is one of the most commonly applied chemicals for removing animal tissues to isolate microplastics (Foekema *et al.*, 2013; Rochman *et al.*, 2015). Density separation involves the use of a high-density solution that enables plastic particles to float, while inorganic matter such as sediments sink (silicon dioxide, the major constituent of sand, has a density of 2.648 g cm^{-3}). Common solutions include NaCl, NaI and $ZnCl_2$ dissolved in H_2O, usually to 1.33 g cm^{-3}, (NaCl) or 1.5 g cm^{-3} (NaI and $ZnCl_2$) (Coppock *et al.*, 2017). The density of common plastics can be seen in Table B1; from this table it can be seen that the density of a saturated NaCl solution is not high enough to separate some plastics from sediments (e.g. PVC, PE), and a solution with a density of ≥ 1.5 g cm^{-3} is preferred.

Table B1. The densities of common plastic polymers

Plastic	**Density (g cm^{-3})**
Polyvinyl chloride (PVC)	1.35-1.45
Polyester (PE)	1.38-1.39
Polypropylene (PP)	0.905
Polystyrene (PS)	0.96-1.05
Low density polyethylene (LDPE)	0.91-0.93
High density polyethylene (HDPE)	0.94-0.97

Following sample pre-treatment, the identification of the polymer makeup of suspected anthropogenic particles within fishmeal and soybean meal requires the use of spectroscopic methods. Fourier transform infrared (FTIR) spectroscopy is often used to identify microplastics in environmental and biotic samples (Käppler *et al.*, 2016). There are various FTIR techniques, including ATR-FTIR (attenuated total reflectance), µATR-FTIR, and transmittance or reflectance micro-FTIR. ATR-FTIR cannot be used to identify microplastics, precluding its use in this method. µATR-FTIR can be used to identify microplastics, however as this method relies on contact between the ATR crystal and the particle, it risks particles being lost through temporary attachment to the crystal, and the crystal may become contaminated if thorough cleaning is not maintained, confounding particle identification. Micro-FTIR, the technique utilized in this study, whereby IR radiation is focussed on a small area of a microplastic via a microscope, is non-contact, and can identify particles down to approximately 10 µm (Chen *et al.*, 2020).

In this Appendix, I report the development of an optimised method for isolating and characterising ≥25 µm microplastics present in fishmeal. All methods were trialled using LT-94a fishmeal (see Chapter 4). I compared a variety of digestion and density-separation protocols, incorporating size fractionation to improve visualisation of smaller microplastics. To identify polymer type, I compared the use of transmission and reflectance micro-FTIR using three common

microplastics: polyester microfibres (50-500 µm); nylon microfibres (100 µm); and polystyrene beads (20 µm). The optimised protocols were successfully applied to a range of fishmeals and soybean meal, as reported in Chapter 4.

2 Isolating Microplastics

For all tests, dry fishmeal was pre-weighed and the mass loss (%) calculated by filtering digested fishmeal onto pre-weighed meshes and drying for 12 hours at 60°C.

2.1. Fenton's Reagent

Fenton's reagent was trialled as a digestion medium. Fishmeal was incubated with 30% H_2O_2 solution and an Iron (II) sulphate catalyst solution (usually ferrous sulphate, $FeSO_4 \cdot 7H_2O$), with a final concentration of 20g Iron catalyst in 1L pH_20 (MilliQ purified H_20), adjusted to pH 3.0-5.0 (Tagg *et al.*, 2017; Hurley *et al.*, 2018). Reagent mixtures were added to either 5 g or 10 g fishmeal, with reagents added in ratios from 1:1 to 3:1 H_20_2:Fenton's reagent, to a total reagent volume of 60-100 mL. This solution was left for 24-48 hours, and the resulting digestate filtered on to 100 µm filters for analysis. We observed that Fenton's reagent was ineffective in the digestion of fishmeal material, with only 8-59% fishmeal digested by mass. This reagent also caused a considerable amount of foaming which may result in the loss of microplastics from the digestion solution. Upon filtration of the digestate, it was apparent that the low percentage digestion precluded the identification of anthropogenic particles, therefore further testing was not performed.

2.2 KOH

Several different ratios of fishmeal:KOH were incubated for 24 h at temperatures of 50-60°C to determine optimal conditions for digestion. Tests used 2.5-25g fishmeal and 50-500 mL of 10% KOH. This initial testing showed that using 10 g fishmeal with a 1:20 ratio of fishmeal:KOH yielded the most effective digestion, removing 90.2%

total sample by mass. Initial testing also showed that temperatures of 50-60°C did not have an effect on digestion efficacy, therefore 50°C was selected as a suitable incubation temperature. Further testing was performed using both 10% and 20% KOH, which showed that doubling the KOH concentration did not result in a higher amount of fishmeal material digestion, with 90.2% (10% KOH) and 90.4% (20% KOH) material digested by mass. Lastly, increasing the digestion period from 24 to 48 hours increased digestion efficacy to 91.0%.The optimum KOH digestion condition was found to be incubation of 10g fishmeal with 200mL KOH for 48 hours at 50°C (91.0% total material digested).

2.3 KOH additives

Following initial testing, several protocol adaptations were trialled (n=3) to optimise the KOH digestion of fishmeal. The addition of surfactants (Tween® 20), detergents (Decon® 90) and a solvent (70% Ethanol spray) was trialled, along with the addition of a KOH neutralization step using HCl (Thiele, Hudson and Russell, 2019). Addition of 1% Tween 20 surfactant, which can help disaggregate agglomerations of organic material and breakdown lipids, increased digestion efficacy by 3.9%. Addition of small volumes of 70% Ethanol via wash bottle was observed to prevent foaming and improve filtration. However, the addition of detergents and a neutralization step did not increase digestion efficacy.

2.4 Density separation

Density separation was trialled on KOH + 1% Tween 20 digested samples. To prepare a high-density solution, $ZnCl_2$ was dissolved in ultrapure H_2O to a density of approx. 1.5 g cm^{-3}. Samples were removed from meshes and added to a Sediment-Microplastic Isolation (SMI) containing $ZnCl_2$ and mixed via a magnetic flea for 5 minutes, before allowing the solution to settle for 30 minutes, or until a clear solution with no particles in suspension was observed. The supernatant containing floating particles was isolated and filtered back on to the retained mesh via vacuum filtration, and the mesh was dried

for 1 hour at 60°C on a plate dryer. The addition of a density separation step increased the mass reduction of the fishmeal sample to 98.3%.

2.5 Size fractionation

Lastly, a size fractionation step was added whereby the digestate is filtered onto 100 µm, 63 µm and 25 µm pore size meshes, in order of decreasing pore size. Size fractionation increased the efficacy of the digestion protocol to 99.1% total material removal by mass, whilst retaining microplastics and anthropogenic particles for digestion (positive controls found mean recovery rates of 100% and 94% for nylon fibres and polystyrene beads, respectively, see Chapter 4 for details). It also allowed for faster vacuum filtration and diluted the sample onto multiple meshes to simplify particle identification by microscopy.

2.6 Optimised method

Often digestion or density separation are performed individually, however here we found greater efficacy in the removal of extraneous material when both processes were performed sequentially. Inorganic materials are often not affected by the chemicals which are used to digest the organic material, but will be removed during density separation, therefore both processes may be required where there is a mixed sample matrix, for example in seawater samples where both organic material (e.g. plankton) and inorganic material (e.g. sediments) are present, or in complex sample matrices such as sewage sludge or the fishmeal sample analysed here. This combined digestion, size fractionation and density separation protocol gave the best performance and was therefore selected for use in this study. A summary of the protocol is shown in Table B2. When the optimized protocol was utilized in the full study, digestion efficacies in fishmeals and soybean meal ranged from 92.7% (soybean meal) to 99.7% (LT94a fishmeal), see Table B4 for details.

Table B2. An optimized protocol for the removal of extraneous materials within fishmeal.

Step	Process
1	10 g fishmeal added to a conical flask with 200 mL KOH + 1% Tween 20
2	Solution left for 48 hours in a shaker incubator at 50°C, 125 rpm
3	Digestate filtered sequentially on to 100 µm, 63 µm and 25 µm meshes via vacuum filtration, rinsing with pH_20 between filtration steps
4	Filters dried at 60°C overnight
5	SMI unit primed with 1.5 g cm^{-3} $ZnCl_2$ solution, stirred with a magnetic flea. Mesh filter added to solution and manually shaken to remove materials. Once filter is clean, filter removed and retained. Solution left to mix for one minute before stirring stopped.
6	Solution left to separate for 30 minutes or until $ZnCl_2$ solution is clear.
7	Supernatant with floating debris isolated and vacuum filtered back on to retained filters
8	Filter dried at 60°C over night and stored until analysis

3 FTIR Analysis

A selection of microplastics were prepared for comparing micro-FTIR analysis: 50-500 µm polyester microfibres (manufactured in Chapter 3), 100 µm nylon microfibres (Goodfellow Cambridge Ltd., prepared following method in Cole (2016)), and 20 µm polystyrene microbeads (Spherotech, Illinois, USA), all suspended in ultrapure H_20. Microplastics stocks were mixed and 15 µL transferred via pipette to each substrate for analysis using reflectance or transmittance micro-FTIR.

3.1 Reflectance micro-FTIR

A flat piece of aluminium foil was attached to a microscope slide using electrical tape, and several small boxes were marked onto the foil surface using forceps. Potential microplastics were identified and moved onto a droplet of water placed within one of the squares. The slide was loosely covered with a glass petri dish lid and dried on a plate dryer at 60°C for 30 minutes. The slide was then moved carefully

onto the FTIR stage and particles located using the µFTIR microscope camera. Particles were scanned using reflectance microscopy with an accumulation of 10 scans and a wavelength range of 800-4000 cm^{-1}.

3.2 Transmittance micro-FTIR

Several small circles were drawn onto an anodisc filter using a black marker pen (fine tip), and the anodisc filter placed into a glass petri dish. Potential microplastics were identified and moved onto a droplet of water placed within one of the circles. The filter was covered loosely with a glass petri dish lid and dried on a plate dryer at 60°C for 30 minutes. The anodisc filter was then placed carefully into position on a steel filter holder and a 1 mm BaF_2 window was placed carefully on top of the filter to prevent particle loss and press microfibres into one focal plane for improved micro-FTIR signal. Particles were scanned using transmittance microscopy, with an accumulation of 10 scans and a wavelength range of 1250-4000 cm^{-1}.

3.3 Comparison

Higher search scores were achieved with the BaF2 window/anodisc transmission method with all microplastic types (Figure B1, Table B3). Reflectance micro-FTIR could not successfully identify the polystyrene beads, with a polystyrene search score range of 0.38-0.41. Transmittance FTIR gave search score range of 0.46-0.64 for polystyrene microbeads, which is lower than the desired cutoff (0.7), but greater than the reflectance method. The poor search score results obtained here are likely due to the small size (20 µm) and challenging morphology of the polystyrene microbeads. One notable advantage of the transmittance method used here is that the BaF_2 window acts to cover and confine the particles so they may be lost less easily, although once the window is removed, the particles may stick to it through static adding a risk of particle loss following µFTIR scanning. Although the anodisc transmittance method must be used with a smaller wavelength range, missing out on the 800-1250 cm^{-1} range, this does not appear to affect the accuracy of the results as observed in Table B3.

For the successful use of transmittance microscopy, particles must be sufficiently thin (<100 µm) to avoid total absorption of IR radiation and lack of signal for polymer identification (Käppler *et al.*, 2016). This will be considered in the full study, and any sufficiently large particles will be identified using ATR-FTIR, which can be used with larger plastic particles with minimum dimensions greater than 100 µm.

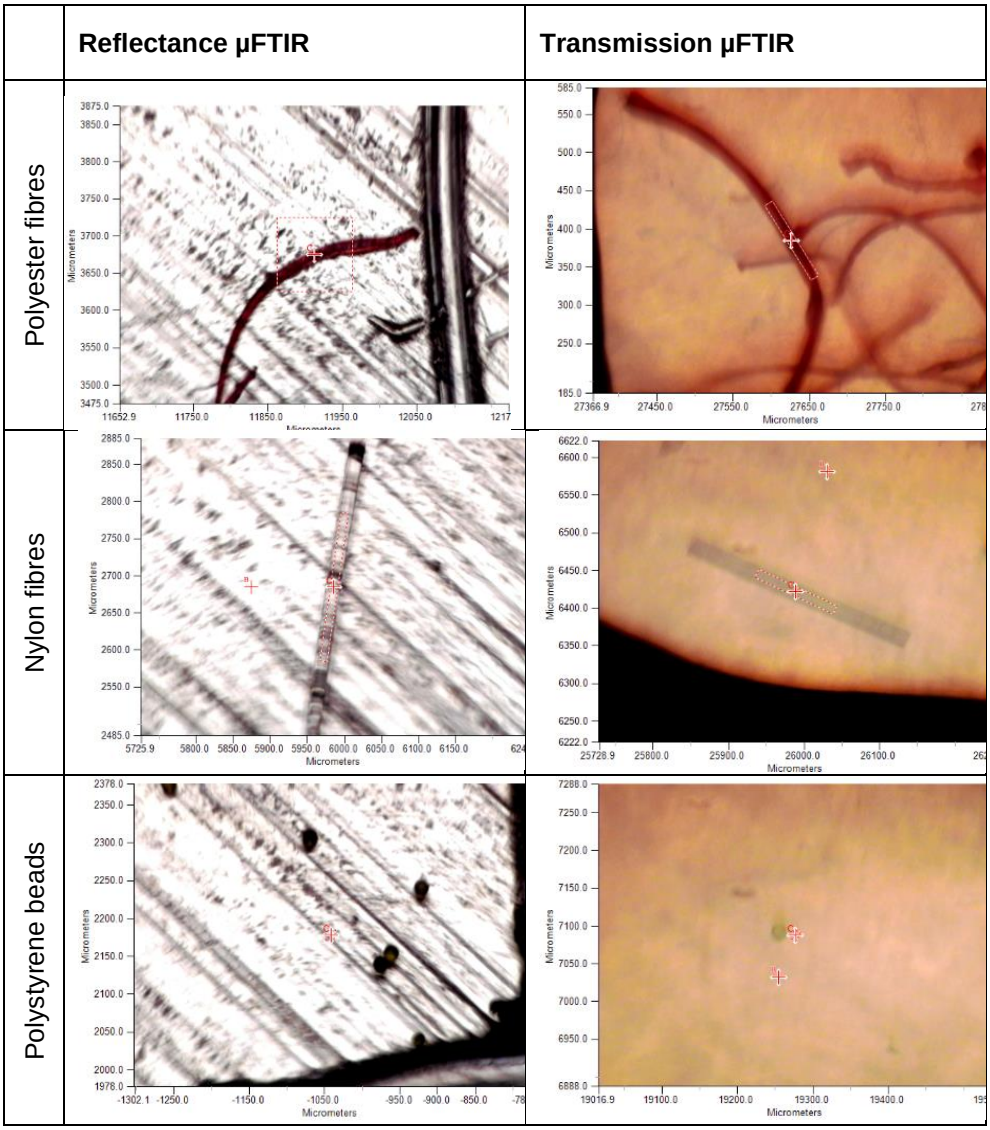


Figure B1. Images of each polymer taken from µFTIR microscope for particles identified using reflectance or transmittance µFTIR.

Table B3. Search score from spectral libraries for particles of each polymer type.

Fibre	Maximum reflectance ID score	Maximum transmittance ID score
Polyester fibre	0.844	0.879
Nylon fibre	0.887	0.905
Polystyrene beads	0.406	0.638

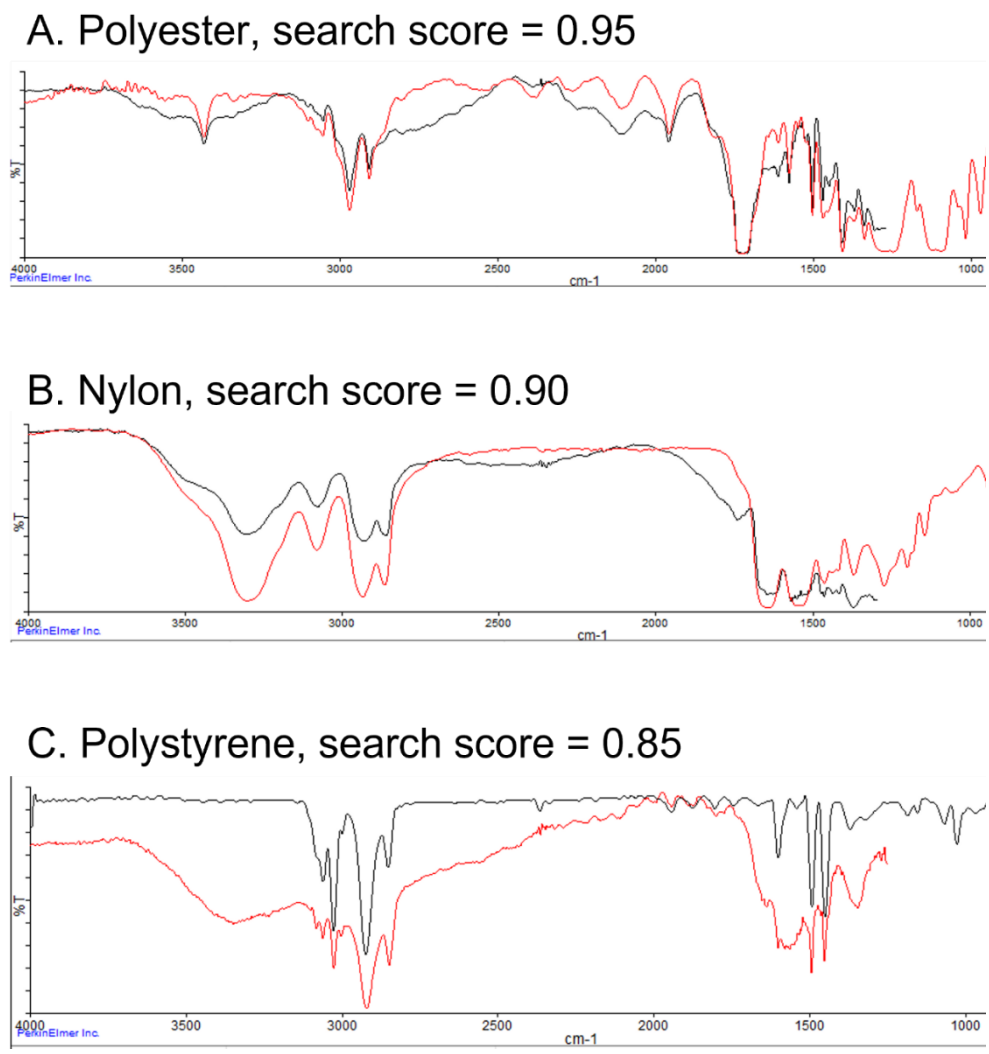


Figure B2. FTIR transmittance spectra of polyester, nylon and polystyrene found within fishmeal samples by transmission micro-FTIR.

Table B4. Final digestion protocol efficacy with all fishmeal and soybean meal samples (n=3).

Sample ID	Replicate	Mesh pore size (µm)	Mass before processing (g)	Mass after processing (g)	Mass difference (g)	Total difference (g)	% digestion	Mean % digestion (n=3)
LT94a	1	100	7.709	7.731	0.022	0.04	99.6	99.67
		63	7.67	7.678	0.008			
		25	7.707	7.717	0.01			
	2	100	7.72	7.738	0.018	0.027	99.7	
		63	7.67	7.675	0.005			
		25	7.696	7.7	0.004			
	3	100	7.716	7.741	0.025	0.031	99.7	
		63	7.674	7.677	0.003			
		25	7.702	7.705	0.003			
LT94b	1	100	7.721	7.744	0.023	0.162	98.4	98.84
		63	7.683	7.743	0.06			
		25	7.703	7.782	0.079			
	2	100	7.716	7.755	0.039	0.074	99.3	
		63	7.677	7.706	0.029			
		25	7.706	7.712	0.006			
	3	100	7.713	7.748	0.035	0.112	98.9	
		63	7.67	7.68	0.01			
		25	7.709	7.776	0.067			
Pv66a	1	100	7.722	7.769	0.047	0.414	95.9	95.69
		63	7.681	7.723	0.042			
		25	7.704	8.029	0.325			
	2	100	5.925	5.988	0.063	0.436	95.7	
		63	5.891	5.915	0.024			
		25	5.93	6.279	0.349			
	3	100	5.925	6.174	0.249	0.449	95.5	
		63	5.897	6.015	0.118			
		25	5.927	6.009	0.082			
Pv66b	1	100	7.716	7.784	0.068	0.122	98.8	98.37
		63	7.669	7.699	0.03			

Sample ID	Replicate	Mesh pore size (µm)	Mass before processing (g)	Mass after processing (g)	Mass difference (g)	Total difference (g)	% digestion	Mean % digestion (n=3)
		25	7.699	7.723	0.024			
		100	7.715	7.802	0.087			
	2	63	7.678	7.724	0.046	0.16	98.4	
		25	7.699	7.726	0.027			
		100	7.722	7.857	0.135			
	3	63	7.669	7.726	0.057	0.209	97.9	
		25	7.699	7.716	0.017			
		100	7.716	7.725	0.009			
	1	63	7.677	7.677	0.000	0.016	99.8	
		25	7.699	7.706	0.007			
		100	7.725	7.731	0.006			
CP70	2	63	7.683	7.7	0.017	0.028	99.7	99.44
		25	7.702	7.707	0.005			
		100	7.713	7.792	0.079			
	3	63	7.672	7.692	0.02	0.124	98.8	
		25	7.701	7.726	0.025			
		100	7.716	7.758	0.042			
	1	63	7.673	7.687	0.014	0.087	99.1	
		25	7.707	7.738	0.031			
		100	7.719	7.75	0.031			
WF	2	63	7.669	7.695	0.026	0.086	99.1	98.89
		25	7.696	7.725	0.029			
		100	7.709	7.802	0.093			
	3	63	7.676	7.723	0.047	0.161	98.4	
		25	7.705	7.726	0.021			
		100	7.712	7.808	0.096			
	1	63	7.674	7.717	0.043	0.203	98.0	
		25	7.704	7.768	0.064			
SA		100	7.719	7.794	0.075			98.25
	2	63	7.67	7.678	0.008	0.108	98.9	
		25	7.707	7.732	0.025			

Sample ID	Replicate	Mesh pore size (µm)	Mass before processing (g)	Mass after processing (g)	Mass difference (g)	Total difference (g)	% digestion	Mean % digestion (n=3)
	3	100	7.711	7.875	0.164	0.218	97.8	
		63	7.673	7.705	0.032			
		25	7.703	7.725	0.022			
Sq	1	100	7.712	7.773	0.061	0.069	99.3	99.38
		63	7.671	7.675	0.004			
		25	7.701	7.705	0.004			
	2	100	7.716	7.757	0.041	0.049	99.5	
		63	7.671	7.675	0.004			
		25	7.696	7.7	0.004			
	3	100	7.705	7.748	0.043	0.07	99.3	
		63	7.669	7.693	0.024			
		25	7.713	7.716	0.003			
Soybean	1	100	7.722	7.998	0.276	0.553	94.5	92.71
		63	7.667	7.937	0.27			
		25	7.696	7.703	0.007			
	2	100	7.716	8.044	0.328	0.763	92.4	
		63	7.671	7.888	0.217			
		25	7.698	7.916	0.218			
	3	100	7.719	8.227	0.508	0.872	91.3	
		63	7.669	7.907	0.238			
		25	7.698	7.824	0.126			
						total average digestion (%)		**97.52**

Chapter 5: Identification of anthropogenic particles within complex organic samples

This chapter is being prepared for publication:
Walkinshaw, C., Lindeque, PK., Thompson, RT., Tolhurst, T., ter Halle A., Albignac M., and Cole, M.
CW, MC, TT, PKL and RT designed the experiments. CW carried out the experiments, data collection, conducted statistical analysis and wrote the manuscript. ATH and MA trained CW in the use of py-GCMS and assisted with py-GCMS data collection. All authors will contribute to editing and improving the final manuscript.

The analysis of microplastics and other anthropogenic particles such as rayon and cotton in field and laboratory studies often involves the analysis of complex sample matrices, which contain a variety of organic and inorganic materials precluding the identification of microplastics and other anthropogenic particles. In this study, we perform a methods comparison to observe whether four commonly used microplastic identification techniques, Nile red staining, micro-FTIR, FTIR imaging, and py-GCMS, can identify microplastics within real-world, complex samples, namely fishmeal and soybean meal. We compare their efficacy in identifying not only microplastics, but other anthropogenic particles such as semi-synthetic (e.g. rayon) and cotton microfibres. While Nile red staining and FTIR imaging could not reliably identify microplastics or other anthropogenic particles within these samples, applying microscopy and micro-FTIR resulted in the identification of an array of microplastics within fishmeal and soybean meal samples in addition to semi-synthetic and cotton particles. Py-GCMS identified plastic polymers within samples but was limited by the number of polymer markers available, and could not be used to identify other anthropogenic particles. As FTIR and py-GCMS have different data outputs, we investigated the potential to estimate

polymer mass from FTIR data and compared this to py-GCMS polymer masses to test the comparability of these two techniques. Both techniques identified similar masses of polystyrene within fishmeal and soybean meal samples, however py-GCMS identified 130x higher mass of polyester within fishmeal samples. While FTIR and py-GCMS can both identify microplastics within complex samples, and offer advantages and disadvantages based on experimental requirements, data output in this study was not comparable, with further research needed to bring these two methods into alignment.

5.1 Introduction

Microplastics are a pervasive pollutant that have been identified in a wide range of environmental compartments, including freshwater, seawater, sediments, soil, ice, snow, air, and biota (Thompson *et al.*, 2004; Barnes *et al.*, 2009; Van Cauwenberghe *et al.*, 2013; Horton *et al.*, 2017; Peeken *et al.*, 2018; Bergmann *et al.*, 2019; Corradini *et al.*, 2019; Klein and Fischer, 2019). Alongside microplastics, other types of anthropogenic particles including cotton and semi-synthetic microfibres comprised of regenerated cellulose (e.g. rayon), have been routinely identified within environmental matrices (Halstead *et al.*, 2018; Nunes *et al.*, 2021; Remy *et al.*, 2015; Savoca *et al.*, 2021; Talvitie *et al.*, 2017). Microplastics, nanoplastics and semi-synthetic microfibres are either directly manufactured (e.g. pre-production pellets and scrubbing agents) or derive from fragmentation of larger objects (e.g. textiles, macroplastic debris, rope) through physical, chemical and biotic processes (Andrady, 2011). These persistent anthropogenic particles can enter the natural environment via a number of pathways, including agricultural runoff, wastewater and atmospheric deposition (Napper and Thompson, 2016). In complex samples such as seawater, animal tissues, wastewater effluent, sewage, organic materials, soils and sediments (Löder and Gerdts, 2015; Stock *et al.*, 2019; Zarfl, 2019; Möller, Löder and Laforsch, 2020;

Zhang *et al.*, 2022) organic and inorganic materials can occlude anthropogenic particles. Over the past decade, many methodologies have been developed to isolate anthropogenic particles from complex samples (Lusher *et al.*, 2017). Commonly applied techniques include chemical or enzymatic digestion to remove organic materials (e.g. small organisms, biological materials) (Courtene-Jones *et al.*, 2017; Lusher *et al.*, 2017), and density separation which utilizes high-density solutions (1.3-1.6 g cm^{-3}) to separate dense particles (e.g. sediments, sand) from plastic polymers which have relatively lower densities (commonly 1-1.5 g cm^{-3}) (Coppock *et al.*, 2017; Hanvey *et al.*, 2017). These methods can result in a >95% reduction in sample mass (Karami *et al.*, 2017; Thiele *et al.*, 2019, Chapter 4 methods), however the resulting filtrate will often still contain undigested materials that make microplastic identification and characterization challenging. Microscopy is commonly used by researchers to visually identify and characterize anthropogenic particles by size, shape and colour; however, this method is subject to selective bias and microscopy alone cannot be used to confirm the chemical composition of particles. Several protocols have championed the use of fluorescent Nile red dye as a means to identify microplastics objectively (Fischer *et al.*, 2016; Tamminga, Hengstmann and Fischer, 2018). However, there is evidence that relying on Nile red staining alone can result in the misidentification of certain organic materials as microplastics, confounding data analysis (Nalbone *et al.*, 2021). Limitations in visual identification have led to the increased use of vibrational spectroscopic methods in microplastics research, such as Raman, and reflectance, absorbance or Attenuated Total Reflectance (ATR) Fourier-transform infrared (FTIR) spectroscopy, to ascertain the chemical composition of particles. These techniques use electromagnetic radiation (infrared light in the case of FTIR, visible light in the case of Raman) which interacts with the sample through absorption or scattering; the resultant vibration of specific molecules within the sample provides information about its chemical structure which can be matched with spectral libraries (Käppler *et al.*, 2016; Xu *et al.*, 2019). In microplastics research, microscopy coupled with FTIR (termed micro-FTIR) is

increasingly used, where the IR light is focused on a small area of a particle identified via microscope, allowing smaller particles (≥10 μm) to be identified (Chen *et al.*, 2020). Visual and spectroscopic methods are often combined, with suspected anthropogenic particles identified and characterized using microscopy, and the chemical composition of these particles determined using FTIR or Raman spectroscopy (Käppler *et al.*, 2016; Xu *et al.*, 2019; Veerasingam *et al.*, 2021). More recently, FTIR spectral imaging techniques have been developed to create spectroscopic maps of entire filters, with each image pixel having a corresponding spectra that can be matched to specific polymers (Löder *et al.*, 2015; Rummel *et al.*, 2016; Mintenig *et al.*, 2017; Primpke *et al.*, 2017). FTIR imaging is automated, removing the subjective bias of users when visually selecting for suspected anthropogenic particles. The technique has been successfully utilized in the analysis of sand and Arctic zooplankton (Primpke *et al.*, 2017; Botterell *et al.*, 2022). However, in order for this spectroscopic mapping to work successfully, the protocol requires pristine samples with minimal organic or inorganic material masking the particles; therefore, analysis of complex samples will require substantial pre-treatment steps to remove all extraneous materials e.g. chemical or enzymatic digestion, or density separation, with such protocols potentially resulting in particle damage or loss (Mintenig *et al.*, 2017; Botterell *et al.*, 2022). An alternative method for determining the chemical composition of microplastics is pyrolysis gas chromatography/mass spectrometry (py-GCMS). Samples processed using py-GCMS are thermally degraded and the decomposition products identified through gas chromatography and mass spectrometry; specific molecular signatures can be used as a marker of a given polymer, and standard curves for each polymer marker enable quantification and estimation of polymer mass (Yakovenko, Carvalho and ter Halle, 2020). These techniques have been successfully employed by the research community to identify nanoplastics and microplastics in an array of environmental matrices (Neves *et al.*, 2015; Erni-Cassola *et al.*, 2017; Ter Halle *et al.*, 2017; Tagg *et al.*, 2020; Botterell *et al.*, 2022), however comparisons

between py-GCMS and FTIR are not widely available. Each method displays advantages and disadvantages (e.g. bias, cost, complexity), leading to debate over which methods are most appropriate in a given situation (Käppler *et al.*, 2018; Zarfl, 2019; Gomiero *et al.*, 2020; Primpke, Fischer, *et al.*, 2020). FTIR is widely seen as more user-friendly, with large publicly-available polymer identification libraries available, but experiences difficulties with samples containing large amounts of extraneous materials and cannot identify very small microplastics and nanoplastics. In contrast, py-GCMS can identify very small microplastics and nanoplsatics within complex samples containing large amounts of extraneous materials, but is less user friendly and spectral libraries have not yet been developed to identify as many different polymers as FTIR libraries. A major obstacle to data harmonization is that each analytical approach produces different types of data, with optical and spectroscopic methods providing particle count and py-GCMS providing particle mass; it is currently unclear whether these parameters are comparable, or indeed which metric is more appropriate when considering the toxicity of microplastics (Senathirajah *et al.*, 2021; Thornton Hampton *et al.*, 2022). Another limitation is the challenge of accurately identifying semi-synthetics and cotton microfibres present in environmental samples; even when we ignore the issue of airborne contamination (Halstead et al., 2018) it is unclear how effective spectrosopic and thermoanalytical techniques can be in differentiating between natural and anthropogenic cellulosic particles (Dris *et al.*, 2017).

In this study we compare four methods to identify anthropogenic particles, including microplastics and anthropogenic cellulosic particles (e.g. rayon, cotton), within complex organic samples. This study takes a pragmatic approach to comparing methods. Typically, method comparisons use spiked samples with known quantities of plastics; however, these microplastics are typically limited in shape, size, colour, morphology and polymer, and are added at unrealistic concentrations, thereby enabling easier identification. Instead, we used aquaculture feeds of unknown anthropogenic particle content as

“real world” examples, allowing us to more accurately represent the challenges of identifying anthropogenic particles within complex environmental matrices. Aquaculture feeds comprised of fishmeal are made up of whole wild-caught fish, bycatch and fish by-products, while soybean meal contains the by-products of soybean oil extraction. These samples are rich in processed animal and plant tissues, including proteins, lipids, and difficult to process materials such as plant cell walls and bone fragments. Prior studies, including Chapter 4 of this thesis, have shown aquaculture feeds contain a diverse and environmentally-relevant array of anthropogenic particles (Karbalaei *et al.*, 2020; Gündoğdu *et al.*, 2021; Thiele *et al.*, 2021; Yao *et al.*, 2021; Wang *et al.*, 2022). Fishmeal and soybean meal samples were processed by chemical digestion, size fractionation and density separation to isolate anthropogenic particles, and these samples were subsequently analyzed using: (1) Nile red staining followed by microscopy and micro-FTIR polymer verification, (2) microscopy followed by µ-FTIR polymer verification, (3) µ-FTIR spectral imaging and (4) py-GCMS analysis. The results of this study allow us to compare the suitability of different analytical methods for identifying and characterizing both microplastics and other anthropogenic particles in complex organic samples, and determine the comparability of resultant data. We further consider the advantages and disadvantages of the methods with regard to cost, training requirements, operator bias, and processing time, and evaluate whether particle count and mass can be used interchangeably.

5.2 Methods

5.2.1 Sample processing

Fishmeal (n=3) and soybean meal (n=3) samples were processed using the method described in Chapter 4. Briefly, the bag containing the fishmeal/soybean meal was manually mixed and 10 g samples transferred to clean conical flasks, 200 mL 10% KOH with 1% Tween®

20 (filtered to 0.2 µm) added and the flask sealed with aluminium foil. The samples were digested in an orbital shaker incubator (Sanyo Orbisafe orbital incubator) for 48 hours at 50°C, 125 rpm. Once digested, the material was size fractionated by sequential filtration through 100 µm, 63 µm and 25 µm nylon mesh filters which were dried overnight at 60°C in a dehydrator (Callow TS-9688-3(A-03). Once dry, samples were density separated using ~1.5 g cm^{-3} $ZnCl_2$ solution (filtered to 0.2 µm) within a Sediment Microplastic Isolation (SMI) unit (Coppock *et al.*, 2017). After 30 minutes (or until solution was visibly separated), the supernatant was isolated, filtered back on to their original meshes and dried in a dehydrator overnight at 60°C. The processing steps resulted in average sample mass being reduced by 99.7% (fishmeal, mass reduction of 9.96-9.97 g) and 92.7% (soybean meal, mass reduction of 9.13-9.45 g).

5.2.2 Methods for particle identification and characterization

Four different methods (Fig. 5.1) were utilized to identify and characterize microplastics and other anthropogenic particles within fishmeal and soybean meal: (1) Nile red staining followed by microscopy and micro-FTIR particle characterization; (2) visual identification of particles via microscopy followed by micro-FTIR characterization; (3) FTIR imaging; (4) py-GCMS. These methods were compared to evaluate their suitability for the identification of microplastics and other anthropogenic particles in a complex organic sample.

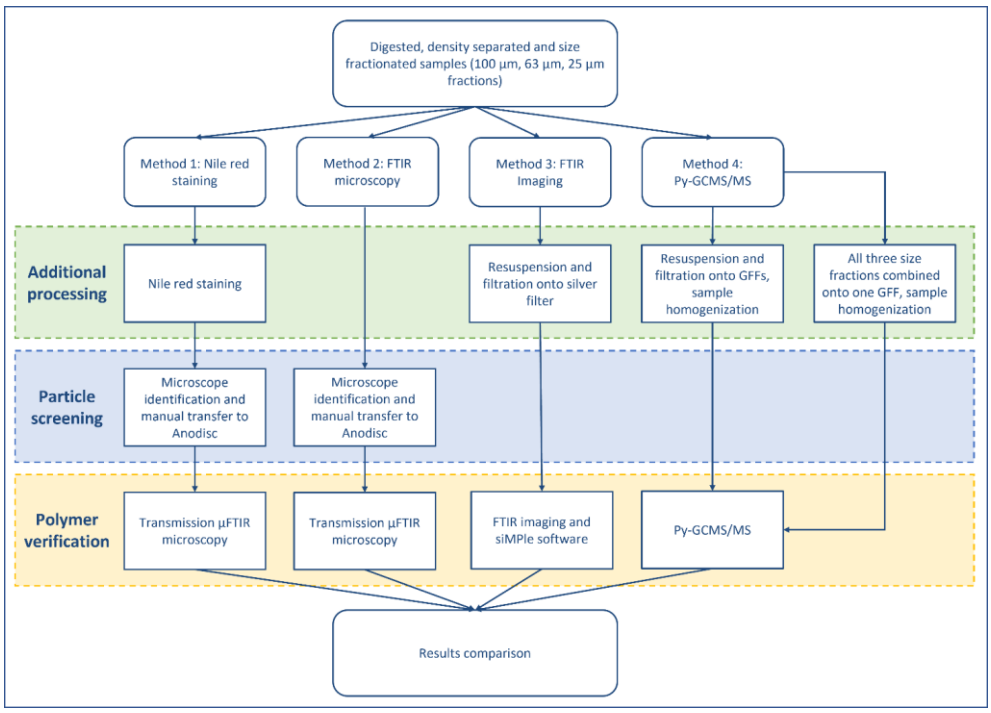


Figure 5.1. Flow chart displaying the additional sample processing, particle screening and polymer verification methods required for each of the four particle identification methods utilized. GFF = glass fibre filter, 0.7 µm pore size.

Nile red staining

Nile red has been recommended as a semi-selective fluorescent stain that can be used to identify microplastics with less subjective bias than by visual identification. Nile red stocks solutions were prepared by dissolving Nile red powder in acetone, to concentrations of 10 µg/mL and 1 mg/mL, as used elsewhere (Prata, Sequeira, *et al.*, 2021; Shruti *et al.*, 2022). Samples were housed in Petri dishes and Nile red solution added dropwise to saturate the sample and mesh for 30 minutes. The sample was rinsed with purified H_2O and dried for 1 hour at 60°C. To identify Nile red stained microplastics, the mesh was visually inspected under an Olympus SZX16 microscope with an RFP2 filterblock (exitation 540-580 nm, emission 610 nm). Owing to high levels of fluorescence, samples were rinsed with 70% ethanol to remove excess stain (Sfriso *et al.*, 2020; Prata, Godoy, *et al.*, 2021; Shruti *et al.*, 2022); samples were subsequently dried at 60°C for 1 hour, and the microscopy steps repeated.

Given a very high proportion of particles within the sample took up the Nile red stain, a selection of stained and unstained particles were selected for chemical characterization using micro-FTIR. FTIR analysis was conducted using a Perkin Elmer Spotlight 400 imaging system comprised of a PerkinElmer Frontier FT-IR spectrometer (MCT detector, KBr window) and PerkinElmer Spotlight 400 microscope with SpectrumIR software (PerkinElmer, 2017, version 10.6.0.893). Particles were transferred to aluminium oxide filters (Anodiscs) and scanned in transmittance sampling mode (20 scans, range = 1250 – 4000 cm^{-1}, resolution 4 cm^{-1}). The resulting spectra were compared with bespoke and publicly-available reference libraries (Primpke *et al.*, 2018); particles with spectral matches >70% were used as confirmation of polymer identity.

Micro-FTIR

Micro-FTIR analysis was performed per the methodology laid out in Chapter 4. Briefly, filters were checked systematically for potential anthropogenic particles via microscopy, with the size, shape and colour of potential anthropogenic particles recorded before they were transferred using forceps to a 0.02 µm Anodisc. Particles were scanned via micro-FTIR in transmittance sampling mode (20 scans, range = 1250 – 4000 cm^{-1}, resolution 4 cm^{-1}) and compared to spectral libraries as above. As cellulose is naturally present within the environment, whenever rayon, cellulose, cotton and cellophane particles were identified via micro-FTIR, their anthropogenic origin was confirmed by referring to the original photo taken of the particle when it was identified by optical microscopy. The image was inspected and only particles which were of a non-natural colour, absent of natural structures (e.g. visible cellular structure), and had a uniform shape (or uniform diameter along their length in the case of microfibres) were categorized as anthropogenic particles.

FTIR Imaging

FTIR imaging was performed using a PerkinElmer Spotlight 400 imaging system comprised of a PerkinElmer Frontier FT-IR

spectrometer (MCT detector, KBr window) and PerkinElmer Spotlight 400 microscope, with SpectrumIR software (PerkinElmer, 2017, version 10.6.0.893). FTIR imaging was performed in reflectance mode, with spectral imaging performed using a resolution of 16 cm^{-1} and 4 accumulations at a resolution of 6.25 µm (wavelength range = 750 – 4000 cm^{-1}) and an interferometer speed of 1 cm s^{-1}. Samples were refiltered onto a 5 µm silver filter (Sterlitech) and dried on a plate dryer (COSORI C0194-CW) set at 60°C for 1 hour before scanning, which was sufficient in to evaporate any water which may preclude polymer identification. All spectra were baseline corrected using a corresponding clean silver filter (5 µm pore size, Sterlitech) as a background sample.

Following spectral imaging, the free software program siMPle (https://simple-plastics.eu/), version 1.1) was used as a tool to identify and quantify anthropogenic particles within the spectral image by autonomously comparing all spectra within the image to a reference spectral database, following the methods of Primpke *et al.* (2020). A Pearson's correlation coefficient threshold of 0.65 was used against the first and second derivative, and the second and third thresholds were set at 0.4 and 0.3, respectively (Johnson *et al.*, 2020; Botterell *et al.*, 2022).

Py-GCMS

The py-GCMS method requires the sample to be on glass fibre filters (GFFs). Therefore, an extra processing step was performed, whereby the samples were resuspended in 70% ethanol and filtered onto GFFs (calcinated at 500°C), which were placed in glass vials with lids and dried in the dehydrator overnight at 60°C. Visual and spectroscopic techniques require samples that have low levels of extraneous material necessitating the size fractionation of complex samples. However, py-GCMS is less reliant on the removal of extraneous materials in advance of analysis, and therefore size fractionation of samples onto multiple filters may not be required, reducing processing time. To test this, additional fishmeal (n=3) and soybean meal (n=3) samples were processed, and two sets of samples were analysed (Fig.

1). The first set were processed in the same way as the micro-FTIR samples and were size fractionated into 100 µm, 63 µm and 25 µm fractions. The second set of samples were processed identically, but all three size fractions were then combined onto a single GFF filter.

Following sample digestion and density separation, additional steps were taken to prepare samples for py-GCMS. Firstly, the sample and GFF were homogenized by milling at 30-40 amplitudes per second for 1 minute over 2-3 cycles (FRITSCH Pulverisette 23 with zirconium oxide chamber and balls), within a fume cabinet which was cleaned with ethanol before use. All fishmeal samples were successfully milled following this procedure, however several of the soybean samples were too hard and contained too much material to be homogenized following this method and were instead cryoground using a SPEX Sample Prep 6775 Freezer/Mill. These samples were added to a clean cryogrinding tube and ground with the following settings: 5 minutes precool, followed by 5 cycles of a 45 second runtime followed by a 1-minute cool time, with a grinding speed of 8 cycles per second. Homogenized samples were poured onto clean aluminium foil and transferred to a clean, calcinated glass vial with a lid. 5 µL tetramethylammonium hydroxide (TMAH, 25% solution in Ethanol) was slowly added to each tube in a clean fume cabinet and the tubes were left for 30 minutes to dry. TMAH is a quaternary ammonium salt which is added to counter the loss of structural information in organic compounds by depolarizing pyrolysis fragments through methylation, giving a higher yield from biopolymers (Challinor, 1989, 2001).

2 mg sub-samples were analysed using a pyrolysis gas chromatography/mass spectrometry system comprising a CDS Pyroprobe 6150 Pyrolyser (with 48 position autosampler module) coupled to a Thermo scientific Trace 1310 gas chromatography (GC) unit and Thermo scientific TSQ 9000 triple quadrupole mass spectrometer (MS). The triple quadrupole MS used here provides high selectivity where high sensitivity is also required, such as for example in the complex organic sample matrix analysed here where high selectivity is required to isolate ions of interest from background

interferences. Py-GCMS uses high temperatures to thermally decompose samples in an inert atmosphere; large molecules break into fragments which are separated via gas chromatography and detected via mass spectrometry. Triple quadrupole mass spectrometry further confirms the presence of the polymer marker by performing another ionization step, fragmenting the marker ion and detecting these breakdown products. The ratio of these ions can then be used to confirm that the polymer marker is present within the sample. The outside surfaces of the quartz vials were cleaned with ethanol before addition to remove any residue which may cause unsuccessful release into the pyrolyser. Samples were added to the autosampler which was covered with foil to prevent contamination, and samples were pyrolyzed at 600°C. The presence of six polymers were investigated within fishmeal and soybean meal: polyethylene terephthalate (PET), polypropylene (PP), polyesters (PE), polystyrene (PS), poly(methyl methacrylate) (PMMA), and polycarbonate (PC). The polymer markers utilised to identify these polymers are detailed in Table 5.1. The resultant pyrogram, generated using Chromeleon software, is used to identify polymers within the sample via the identification of specific marker ions appearing as peaks at particular retention times; these peaks are cross-referenced with a custom database to identify the breakdown marker peaks. The area of these peaks are used to calculate the mass of the polymer present within the sample by comparison to a calibration curve, which is generated using purified polymer standards containing each polymer breakdown marker diluted to known masses. Mass (ng) polymer per 1 mg sample (M_P) is calculated by dividing the polymer mass identified by the sample weight (mg); this value is subsequently used to calculate polymer content as µg polymer per kg source material (fish or soybean meal) through the following calculation:

$$Polymer\ content\ (\mu g\ kg^{-1}) = \frac{M_P}{(\frac{Mass\ Sample}{Mass\ Filter})} \cdot (Mass\ Sample \cdot 100)$$

Process blanks (n=3) were taken through this process and used to define the LOD (limit of detection) for each sample, equal to 3x sample standard deviation. In addition, a repeatability test was performed, consisting of three aliquots of one homogenized sample, which were analysed to investigate inherent variation within py-GCMS sampling and analysis. Mean result and coefficient of variation were used as an indicator of sample homogeneity and repeatability.

Table 5.1. Markers used to identify each polymer through py-GCMS analysis.

Polymer	**Marker**
PS	Styrene trimer (5-hexene-1,3,5-triyltribenzene)
PP	Dimethyl heptane
PE	1,12 tridecadiene
PMMA	Methyl-methacrylate
PET	dimethyltherephthalate
PC	Methyl-bis-phenol A

5.2.3 Comparability of FTIR and py-GCMS data

FTIR analysis provides polymer identification of microplastic particles, along with the number of particles identified, but cannot provide the polymer mass. In contrast, py-GCMS analysis provides the polymer mass in the sample but cannot identify the number of particles which provide this total polymer mass. To directly compare these two results, a conversion is therefore required. As the particles identified by FTIR have complementary dimensions from initial microscopic evaluation, we can calculate particle volume; polymer density information subsequently allows us to calculate particle mass, and therefore total polymer mass in each sample. This method has been trialled previously by Simon, van Alst and Vollertsen (2018) and Primpke, Fischer, *et al.* (2020). Both estimated particle mass by assuming an ellipsoid shape when calculating volume, where three values for the radius were used; the Feret diameter, shortest dimension, and the third dimension which was calculated as 67-70% of the minor dimension. However, this equation assumed all particles are a similar morphology, and will not accurately estimate the mass of microfibres whose second and third dimensions, the fibre diameter, are equal and are many times smaller than their major dimension (length). As microfibres often make up the majority of the particles identified in samples (including in this study), this may lead to large inaccuracies in the estimation of polymer mass within samples. Simon, van Alst and Vollertsen (2018) overcame this by calculating the volume of microfibers separately using the equation for the volume of a cylinder (including a 40% void fraction), which will more reliably estimate the volume of microfibers. Here, we will test a method for calculating particle masses whereby particles are split into three groups with corresponding mass equations: fibres, fragments, and films. Microfibres are cylindrical in morphology, with consistent thickness through the fibres, therefore mass can be accurately estimated using the equation to calculate the volume of a cylinder [1]; fragments have no consistency in shape, however their major:minor dimension ratios

are much more similar to each other than in the case of cylinders, with estimates of 0.67-0.7 previously calculated (Simon, van Alst and Vollertsen, 2018; Primpke, Fischer, *et al.*, 2020), therefore we will calculate these using the equation for the volume of a sphere, assuming equal major and minor dimensions [2]. For films, the minor dimension is much smaller than the large major dimension, therefore we will assume that these particles are cuboidal with equal length and width, and a depth 100x smaller than the major dimension [3]:

[1]: $V = \pi r^2 l$

[2]: $V = 4/3\ \pi r^3$

[3]: $V = M^2(M/100)$

Where: V = volume, r = radius, l = length, M = length of major dimension

Initial results for fishmeal and soybean meal samples were obtained in Chapter 4, reported as number of each polymer identified per kg sample. Here, these results have been converted to mass (ng) and concentration (µg/kg). Images of each particle identified in Chapter 4 were re-analysed to measure particle dimensions. The diameter of all microfibres included in this analysis were measured in five places along their length using an Olympus SZX16 microscope with CellSens software (Olympus, version 2.1); the mean of these diameters was then used to estimate the radius of the microfibre in equation [1], above. We also calculate particle masses using the ellipsoid equation of Simon, van Alst and Vollertsen, 2018; Primpke, Fischer, *et al.,* 2020), assuming a major: minor dimension ratio of 0.7, and compare this to the masses derived using our equations.

5.2.4 Data analysis

Data were analysed using Excel and R (version 4.0.1). Data were checked for homogeneity of variance and normality tested using

Shapiro-Wilk and Levene's tests. Data which passed assumptions of normality were tested using ANOVA and Tukey's post-hoc testing; data which violated these assumptions were tested using Kruskal-Wallis tests with Dunn's post-hoc testing. Significance level was set at $\alpha = 0.05$ for all statistical tests.

5.3 Results

5.3.1 Nile Red staining

Samples were successfully stained with Nile red solution, and addition of a wash step reduced background fluorescence from the filter. However, visual examination of filters revealed that, even after a solvent wash, the majority of the material retained on the filters fluoresced under excitation as a result of Nile red staining. A number of stained and unstained particles were chemically characterised using FTIR to evaluate the selectivity of Nile Red. Notably, spectroscopic analysis identified a number of particles that either did not fluoresce or fluoresced weakly (no greater than surrounding materials) as microplastic (e.g. PET) or semi-synthetic (e.g. rayon, cotton), and particles that were clearly fluorescent as natural (e.g. bone, Fig. 5.2). Given Nile red staining was not selective for microplastics, with high background fluorescence, further spectroscopic analysis was halted.

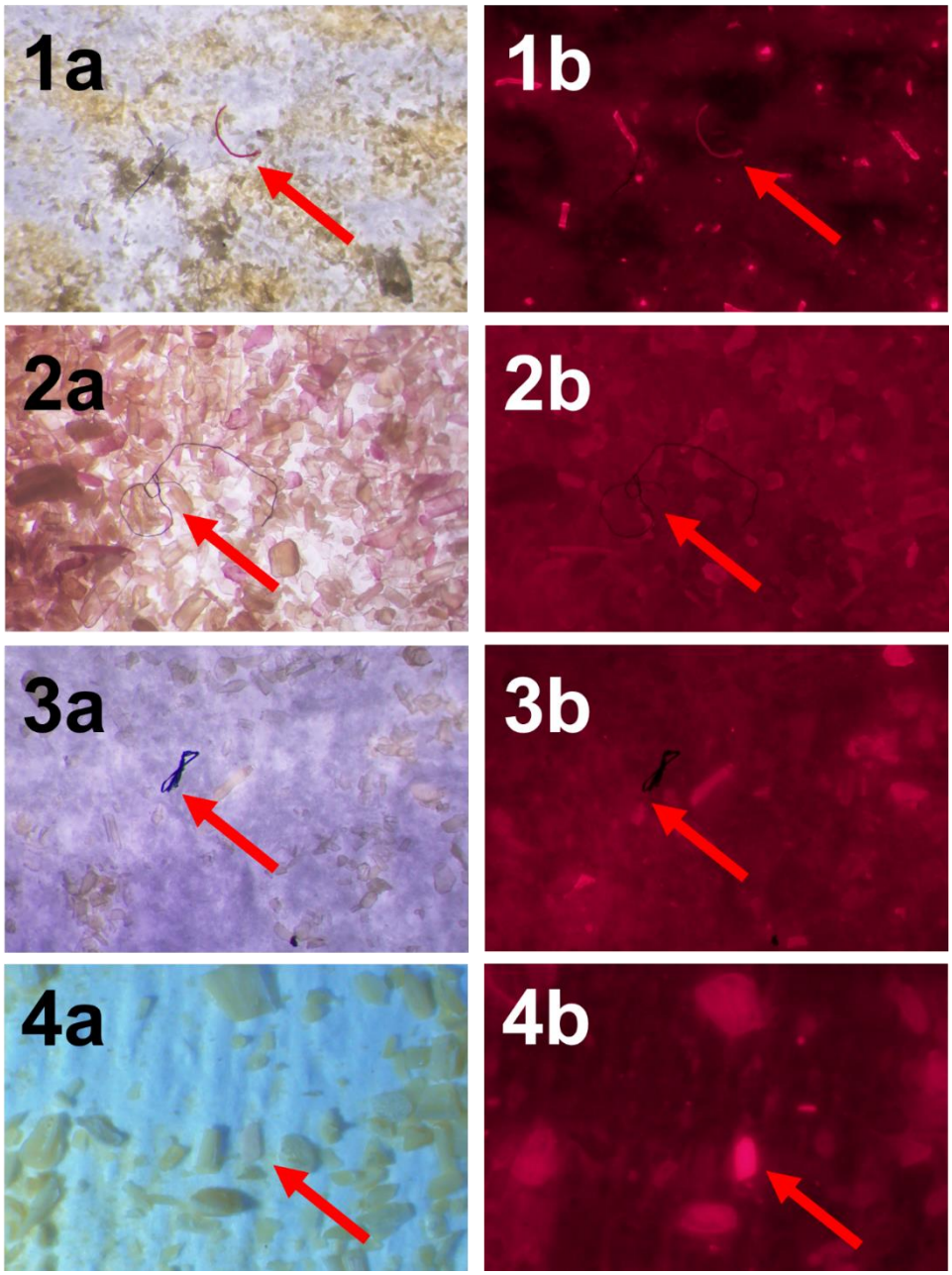


Figure 5.2. Anthropogenic particles visualised under optical microscopy (left hand column) and Nile red fluorescence (right hand column). Particle 1 = polyester (FTIR spectral match = 0.81), particle 2 = cotton (FTIR spectral match = 0.82), particle 3 = rayon (FTIR spectral match = 0.75), particle 4 = bone (FTIR spectral match = 0.83). Microscope magnification (1.98x) and optical settings identical for all pictures.

5.3.2 micro-FTIR

Optical microscopy followed by micro-FTIR verification of particles successfully identified both microplastic and other anthropogenic particles within fishmeal and soybean meal samples. Results were initially reported and discussed in Chapter 4 as number of particles per

kilogram. In brief; 2000 and 1230 anthropogenic particles kg^{-1} were identified in fishmeal and soybean meal samples, respectively; semi-synthetic particles were predominant, comprising 55% of the total anthropogenic particles in fishmeal and 73% in soybean meal. Cotton microfibres were identified in fishmeal (100 particles kg^{-1}) but were not identified in the soybean meal samples. Ten different anthropogenic particle types were found within the fishmeal samples, including six plastic polymers (acrylic, epoxy resin, polyester, polyethylene, polystyrene and PTFE) and four non plastic particles (rayon, cotton, cellophane, and 'anthropogenic cellulose' particles, which were not categorized within the other three groups). Five different types of anthropogenic particle were identified within soybean meal, four plastic polymers (polyamide, phenol resin, polyester and a copolymer (ethylene maleic anhydride)) and rayon.

5.3.3 FTIR spectral imaging

Initially, a 2000 μm^2 section of filter was imaged (Fig. 5.3a) to observe whether samples were clean enough for successful spectral imaging. Once the spectral map image was produced (Fig. 5.3b), a section of the image which contained a fibre was selected, and the resulting spectrum was compared to spectral libraries for particle identification. Multiple points on the spectral map along the length of the fibre were selected for comparison to spectral libraries, however no polymer could be identified from any of the resultant spectra (Fig. 5.3c). The spectral maps produced by the FTIR were also transferred to the open-source platform siMPle for automated spectral map processing and identification of anthropogenic particles to observe whether this process could identify the particle. The spectral map was successfully imported and initial processing created a visible particle map, however, the filters were too soiled with background materials for successful interpretation of results (Fig. 5.3d), and no particle information could be retrieved. The microfibre was subsequently transferred to an Anodisc and scanned using transmission micro-FTIR, and was identified as polyester (search score 0.76). Neither plastic polymers or other anthropogenic particles could be identified

using this method due to the increased background interference from the large amount of material still present within the sample following digestion and density separation. Following multiple processing failures, this method was abandoned in favour of other particle identification methods.

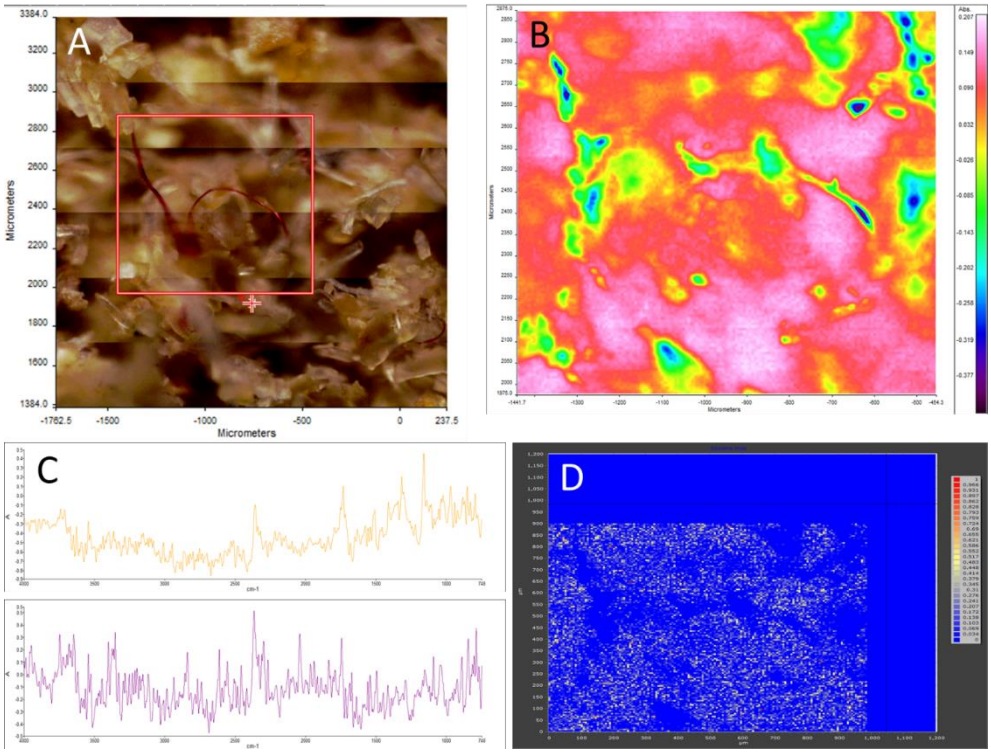


Figure 5.3. FTIR spectral imaging of fishmeal sample on 5µm silver filter. Panel A: Visible image scan. Panel B: spectral map of area within red box in A. Panel C: Example spectra of two pixels selected within area of red fibre seen in A. Spectral match of yellow spectrum = ethyl pyruvate (search score: 0.481), spectral match of purple spectrum = adenosine-5'-monophosphate disodium salt (search score: 0.235). Panel D: Imported spectral map into siMPle software, showing inability to identify single particles within this extensively soiled filter.

5.3.4 Py-GCMS

Py-GCMS analysis of the size fractionated samples identified polyester, polypropylene and polystyrene in fishmeal samples (n=3), and polyethylene terephthalate and polystyrene in soybean meal samples (n=2). Mean values per 10g replicate in fishmeal were 62052 ng polyester (15727-120935 ng), 846 ng polypropylene (0-2538 ng) and 90 ng polystyrene (0-271 ng), equivalent to 6205, 84.6 and 9.02

µg/kg respectively. Mean values per 10g replicate in soybean meal were 649 ng polyethylene terephthalate (0-1107 ng) and 129 ng polystyrene (0-338 ng), equivalent to 64.9 and 12.9 µg/kg respectively (See Table 5.2 for a summary of results). No trace of poly(methyl methacrylate) or polycarbonate were identified in either fishmeal or soybean meal. Similar polystyrene content was identified in both fishmeal and soybean meal (9 µg/kg and 12 µg/kg, respectively); no other polymers were identified in both sample types. A very high polyester content was found in the fishmeal samples (6205 µg/kg), approximately 70-700x greater than the mean concentration of the other polymers identified. Mean results from each mesh size show that larger particles dominate polymer mass in fishmeal samples; 63% polymer mass was identified in the >100 µm size fraction, with 9.8% and 26% of the total mass found in the 63-100 µm and 25-63 µm size fractions, respectively. Conversely, no polymer mass was identified in the >100 µm size fraction in the soybean samples, with polymer mass evenly split between the 63-100 µm and 25-63 µm size fractions. A repeatability test, consisting of three aliquots of one homogenized sample analysed to investigate inherent variation within sampling and analysis, was performed and good repeatability was observed, with coefficients of variation of 7.8-16.5% for polypropylene, polyester and polystyrene mass within one sample.

Table 5.2. Polymer contamination identified within fishmeal and soybean meal samples, which are either analysed as size fractionated or combined samples. - symbol signifies no polymer mass detected.

Polymer	**Fishmeal size fractionated (µg/kg, n=3)**	**Fishmeal combined sample (µg/kg, n=3)**	**Soybean meal size fractionated (µg/kg, n=2)**	**Soybean meal combined sample (µg/kg, n=3)**
Polyester (PE)	6205.2	26294.5	-	-
Polyethylene terephthalate (PET)	-	-	64.9	666.8
Polypropylene (PP)	84.6	7988.2	-	-
Polystyrene (PS)	9.02	14.4	12.9	53.9
Poly(methyl methacrylate) (PMMA)	-	-	-	-
Polycarbonate (PC)	-	-	-	-
Total polymer	6299	34297.1	77.8	720.8

In the combined samples, the same polymers were identified as were found in the size fractionated samples for both fishmeal and soybean meal. Mean polymer concentrations in the combined samples were higher than identified in the size fractionated samples, with 14.4 µg/kg polystyrene, 7990 µg/kg polypropylene and 26,300 µg/kg polyester identified in fishmeal, and 53.9 µg/kg polystyrene and 667 µg/kg polyethylene terephthalate identified in soybean meal. Mean fishmeal polypropylene results from the combined method were almost 100x

higher than the mean size-fractionated samples; similarly, mean polyethylene terephthalate content was 10x greater using this method in soybean samples. When the mean total mass of all polymers is compared, the disparity between these methods is reduced but still observable, with the combined samples having a mean total polymer mass 5.4-9.3 times greater than the size fractionated separate mesh samples (Table 5.2). Standard curves for each polymer marker compared to the quantities of each polymer marker identified in fishmeal and soybean meal samples are displayed in Fig. 5.4. Plots marked (A) on the left-hand side show the full set of results, including results of the combined mesh samples which were up to 10x greater in mass than the maximum calibration standard; plots marked (B) have the combined mesh results removed to give higher resolution to size fractionated results. PP and PE results varied much more than expected; the mass of PE and PP in the combined mesh results were 4-10x greater than the highest calibration standard, and some size fractionated PE results were up to 2.5x greater than the highest calibration standard, however the majority of the individual mesh masses were within the standard curves. PET masses identified in soybean meal samples had good distribution through the standard curve and could therefore be accurately quantified. An extended range of marker masses should be utilised to create these standard curves in the future to give greater resolution at the top and bottom of the range, as the difference in polymer mass identified in our results was a maximum of approximately 8000 ng.

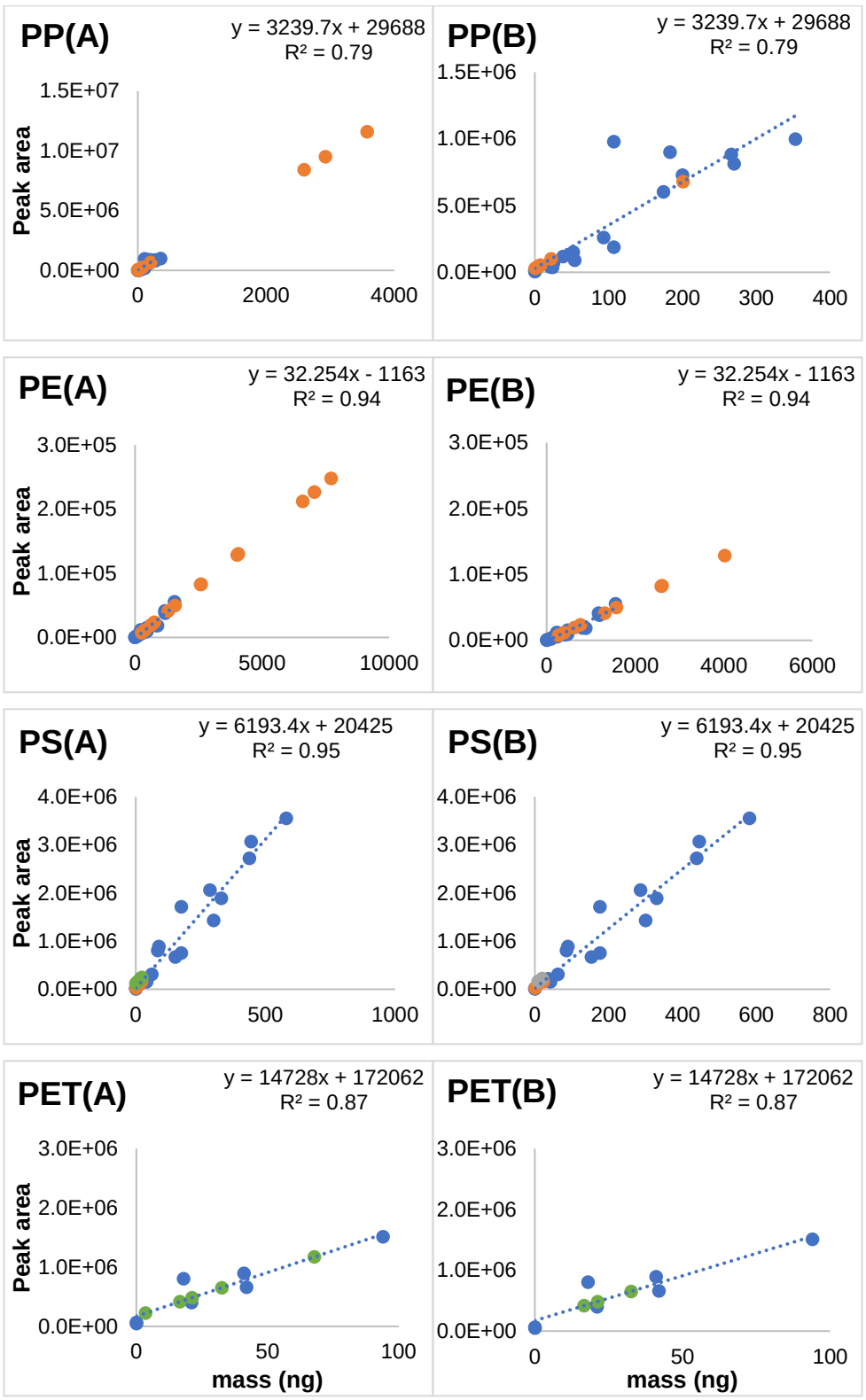


Figure 5.4. Calibration curves overlaid with fishmeal and soybean meal results. Blue dots = calibration standards, orange dots = fishmeal samples, green dots = soybean meal samples. Blue line = linear calibration curve. Plots marked (A) have all results included, plots marked (B) have combined results removed. Equation of line of best fit of calibration standard curve included above each plot.

5.3.5 Comparability of FTIR and Py-GCMS data

Following mass conversion of FTIR data, mean total anthropogenic particle mass per 10g fishmeal and soybean sample was calculated as 4202 and 1756 ng respectively (Fig. 5.6). This is equivalent to 420 µg/kg (fishmeal) and 175 µg/kg (soybean meal). Rayon, the most prevalent particle type identified within both sample types, constituted 1652 and 1274 ng within fishmeal and soybean meal, respectively (Fig. 5.5). Individual microplastic polymer masses ranged from 39 ng (polyethylene) to 628 ng (acrylic) in fishmeal and from 2 ng (copolymer) to 185 ng (polyester) in soybean meal. The method of calculating mass of Simon, van Alst and Vollertsen (2018) and Primpke, Fischer, *et al.* (2020) was trialled and the equation developed in this study gave higher particle masses by a factor of 1.2. To compare the polymer quantities identified via micro-FTIR and py-GCMS (Fig. 5.6), only the py-GCMS results from the separate mesh analysis were used to allow for a direct comparison, as this was the method used in the micro-FTIR analysis.

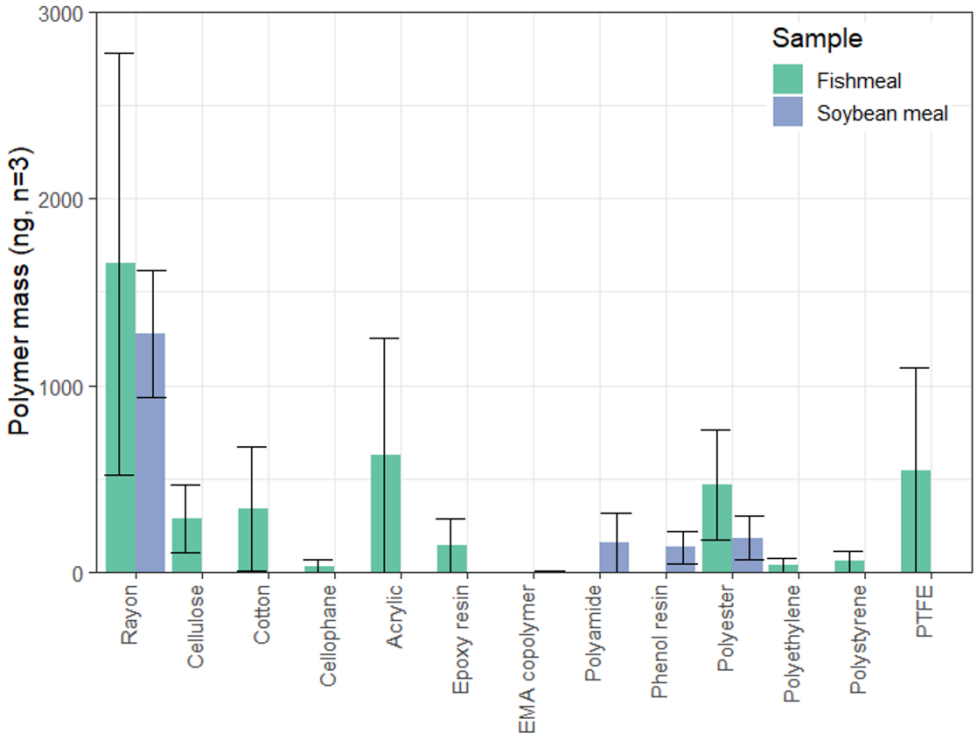


Figure 5.5. Polymer masses (ng) identified in fishmeal (green) and soybean meal (blue) by micro-FTIR, n=3. Error bars = standard error.

While the average mass of polystyrene in the fishmeal results was similar between techniques, there was significantly more polyester identified in fishmeal samples through py-GCMS than micro-FTIR (Kruskal-Wallis chi-squared = 3.8571, df = 1, p-value = 0.04953); indeed, polyester mass was 130x greater in py-GCMS samples (62052 ng) than FTIR samples (471 ng). The total mass of polymer identified in fishmeal through py-GCMS was greater than that found through micro-FTIR (Kruskal-Wallis chi-squared = 3.8571, df = 1, p-value = 0.04953), but in soybean meal, the total amount of polymer identified did not differ significantly (Kruskal-Wallis chi-squared = 1.3333, df = 1, p-value = 0.2482).

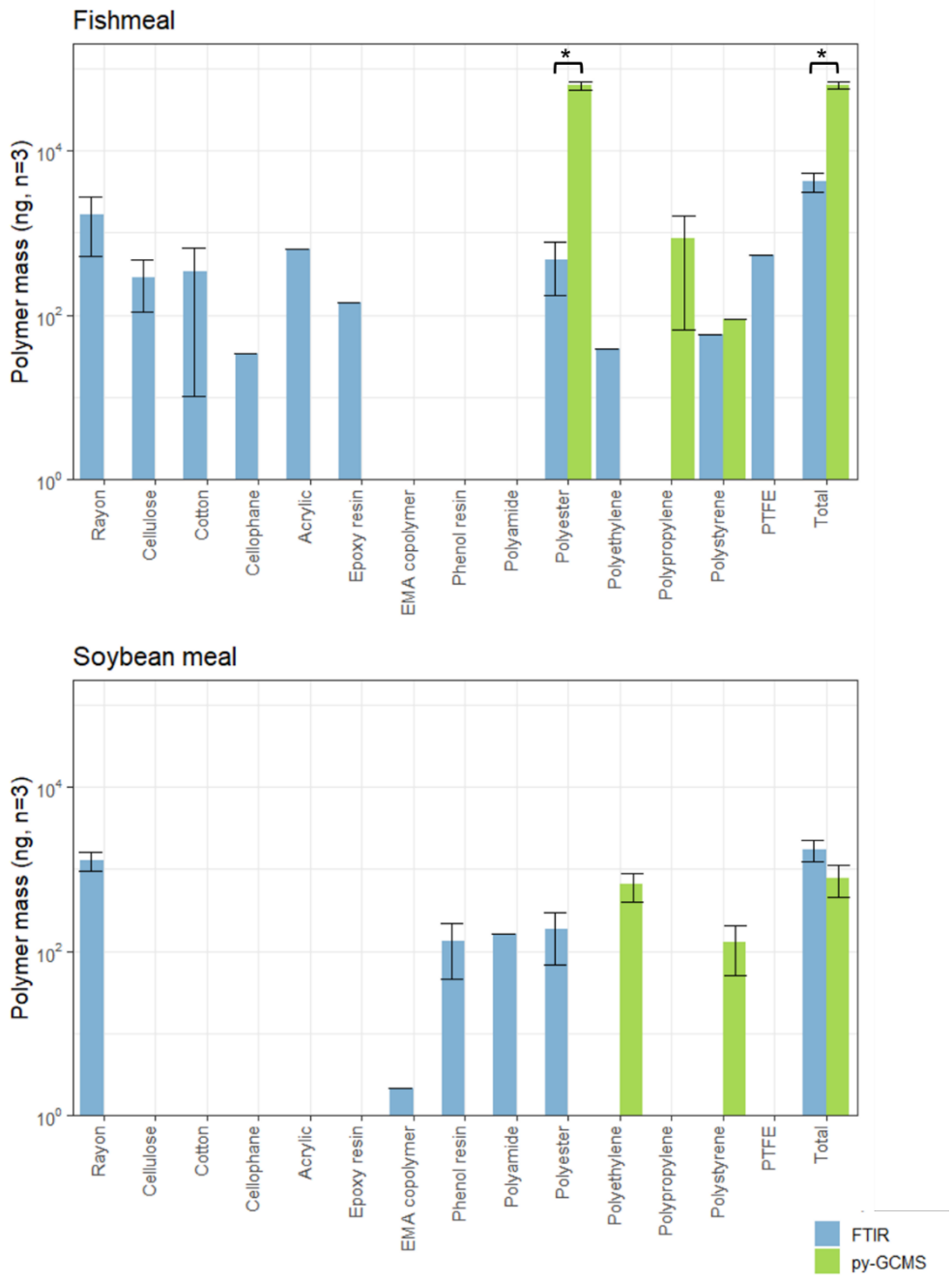


Figure 5.6. Polymer masses (ng) identified in 10 g fishmeal or soybean meal samples characterised by transmission micro-FTIR (blue) and py-GCMS (green). Note $\log_{10}$ scale y axis. Error bars = standard error.

5.4 Discussion

Py-GCMS and micro-FTIR were used to successfully identify microplastics within fishmeal and soybean meal samples. Four different microplastic polymers were identified using py-GCMS, while micro-FTIR identified a wider range of microplastics plus semi-synthetic and cotton microfibres. Micro-FTIR mass conversions suggest a discrepancy between the mass of polymers identified between py-GCMS and micro-FTIR, with much higher polyester concentrations found via py-GCMS. Nile red staining and FTIR imaging could not successfully differentiate anthropogenic particles within fishmeal and soybean meal samples.

5.4.1 Methods comparison

Nile Red staining

In this study Nile Red staining did not assist in the identification of anthropogenic particles over microscopy alone. This reflects the findings of other studies where Nile Red has been shown to readily bind to strongly hydrophobic polymers, including HDPE, LDPE, polypropylene, and polystyrene (Erni-Cassola *et al.*, 2017; Prata *et al.*, 2019; Wiggin and Holland, 2019), but there is evidence other polymers, including polyester, acrylic, polyamide, tyre rubber, PVC, polyethylene terephthalate, and nylon, do not efficiently take up Nile red stain (Shim *et al.*, 2016; Erni-Cassola *et al.*, 2017; Prata *et al.*, 2019; Stanton *et al.*, 2019; Wiggin and Holland, 2019; Wang *et al.*, 2021; Nel *et al.*, 2021). Furthermore, Nile red has been shown to not uniformly stain coloured microplastics (Stanton *et al.*, 2019), while white and transparent microplastics appear to stain efficiently which may overestimate their presence in proportion to dyed microplastics (Stanton *et al.*, 2019). Here, we observed that polyester fluoresced weakly and several brightly coloured fibres made of rayon and cotton did not fluoresce at all, resulting in false negatives. There is conflicting evidence on the ability of Nile red to stain semi-synthetic and other

anthropogenic microfibres: Stanton *et al.* (2019) observed fluorescence in cotton, wool, silk and rayon even after H_2O_2 treatment and Prata *et al.* (2019) observed fluorescence in linen, rayon and viscose; whereas, in support of our findings, Wiggin and Holland (2019) found cotton and rayon did not take up the stain. One of the major disadvantages in the use of Nile red staining for identification of anthropogenic particles is the potential staining of organic materials within samples producing false positives. We observed that organic detritus such as bone fragments fluoresced brightly; in other studies, fish egg shells, shrimp and fish muscle, and chitin have also been observed to fluoresce (Shim *et al.*, 2016; Maes *et al.*, 2017; Prata *et al.*, 2019; Prata, Sequeira, *et al.*, 2021; Sturm, Horn and Schuhen, 2021). Without further particle characterization through spectroscopy, Nile red stained organic materials may therefore cause overestimation of microplastic abundance in environmental samples (Stanton *et al.*, 2019; Nalbone *et al.*, 2021). Nile red may also interfere with Raman identification of polymers, confounding spectroscopic polymer identification (Erni-Cassola *et al.*, 2017; Hengstmann and Fischer, 2019). Recent methodological developments including optimisation of organic material removal (Prata *et al.*, 2021), co-staining with Calcofluor white or Evans blue dyes (Maxwell *et al.*, 2020; Wang *et al.*, 2021) and automated image processing (Konde *et al.*, 2020; Meyers *et al.*, 2022) may help to offset these concerns in the future. If these methods can validate the use of Nile red staining to identify anthropogenic particles within samples to a high degree of accuracy with low false-positive and false-negative rates, the low costs and low technical skill required would make this a highly cost-effective technique (Prata *et al.*, 2021; Sturm, *et al.*, 2021). However, given the selectivity issues, we consider it is not currently feasible to use Nile red staining to identify microplastics and other anthropogenic particles within complex organic samples.

Micro-FTIR

Transmittance micro-FTIR successfully identified microplastics within all samples and was the only method tested which identified semi-

synthetic and cotton particles within samples. This is partly due to the presence of easily accessible, high quality spectral libraries along with the ability to add spectral information into these libraries to increase the range of particles identifiable by FTIR. However, the ability to visually observe morphological features such as colour, shape and cellular structures to distinguish anthropogenic particles from natural cellulosic particles is critical for the identification of semi-synthetic and cotton particles. While the same spectral libraries are available for use with FTIR imaging, the absence of microscopic validation of particle morphology, colour and structural information currently precludes the ability for FTIR imaging to identify cellulosic semi-synthetic and cotton fibres.

FTIR is the most widely applied of the methods tested, and has been successfully used in complex sample matrices including sewage sludge, sediment and biota (Mahon *et al.*, 2017; Steer *et al.*, 2017; Li, Liu and Paul Chen, 2018; Corami *et al.*, 2020; Deng *et al.*, 2020; Lindeque *et al.*, 2020). However, FTIR is limited by the visual sorting step, which requires trained operators to identify potential anthropogenic particles by physical characteristics. It is possible that operators may underestimate microplastics that are difficult to distinguish from natural or background materials due to their colour (e.g. black, white, brown, and pale yellow particles), and smaller microplastics that cannot be seen by microscopy, e.g. particles <24 µm maximum dimension in this study. As a result, micro-FTIR is inevitably hampered by operator bias, and it is reported that up to 70% of the particles selected for FTIR confirmation are not confirmed as plastics by FTIR spectroscopy (Hidalgo-Ruz *et al.*, 2012). Though this value was lower in this study, with 64% of the 865 suspected anthropogenic particles identified as being anthropogenic in origin, this substantial false positive visual identification rate increases processing time by a considerable margin. Researchers may attempt to reduce the analysis time by selecting a subset of particles to analyse by FTIR, with as little as 10% particles verified spectroscopically in some studies (Mahon *et al.*, 2017), estimating the total number of microplastics in a

sample based on this subsample. However, this again leads to errors as not all particles are verified. FTIR can be used to successfully identify polymers within a wide range of samples, however researchers should be aware of these limitations and work to minimise selection and subsampling bias within analyses.

FTIR Imaging

FTIR imaging has been shown to be useful in automating microplastic identification within environmental samples that have very small amounts of extraneous materials on filters (Botterell *et al.*, 2022). However, in this study, though a mean of 97.5% of the material was removed from the original sample, too much background material was present to successfully identify anthropogenic particles. While suspected particles produced greater absorbance values on the spectral map, potential anthropogenic particles could not be sufficiently differentiated from the background. This is likely due to the large amount of material present on the filter creating too much background noise for successful particle characterization, exacerbated by the requirement for the use of a smaller diameter filter (13 mm) concentrating particles within a smaller area compared to the µ-FTIR method (47 mm diameter filter).

For the analysis of microplastic contamination within wastewater, Simon et al. (2018) performed several processing steps to purify microplastics and remove enough of the sample matrix to allow for FTIR imaging, namely sieving to remove particles >500 µm, treatment with cellulase enzymes followed by Fenton's reagent, and filtration to retain two fractions <80 µm and >80 µm. However, the authors were still required to subsample 2-6% of the final sample for successful FTIR imaging, a considerable subsampling procedure which may not be appropriate with other sample matrices such as the heavily heterogeneous fishmeal and soybean meals analysed here. While processing similar wastewater samples, Tagg *et al.* (2020) performed a 7-day 30% H_2O_2 digestion followed by treatment with Fenton's reagent which allowed filtration onto 47 mm, 5 µm filters and analysis using FPA-based reflectance micro-FTIR imaging, though the pixel

size of 25 μm^2 limited detection of small microplastics. While it is possible to split samples into several smaller samples, or to subsample from the processed final sample, diluting the material sufficiently for particle identification would also increase sample processing time and cost and may not be appropriate for all studies. Additionally, increasing the number of processing steps, or the use of more caustic digestion agents, risks increased particle loss.

py-GCMS

Py-GCMS identified plastic polymers within all samples tested, with total polymer mass identified in samples ranging from 38 – 7526 µg/kg, but could not identify non-plastic anthropogenic particles within samples due to limitations in marker availability. Size fractionated samples (size fractions: 25-63 µm, 63-100 µm, >100 µm) were compared to samples where all three fractions were combined into one sample; while both techniques identified the same polymer types within the sample, the combined samples identified higher polymer masses, being higher by a factor of 5.4-9.3. While sample heterogeneity may play a part in this disparity, there are likely other factors, e.g. polymer loss during processing, and background masking of breakdown products near the limit of detection, which may exacerbate this difference. The analysis of the entire sample in one not only increased sensitivity, but also reduced processing time, although it could reduce the amount of information retrieved from the sample, as the results would not give information about the approximate particle size that can be inferred by size-fractionating samples. In this study, polymer concentrations ranged over four orders of magnitude, highlighting that standard curves must be extended in future analysis, unless initial testing can be performed to estimate the mass range expected in samples.

5.4.2 Comparing identification techniques

Research into the presence of microplastics and other anthropogenic particles within the environment is critical to understand the sources,

sinks and effects of these particles as they pass through different environmental compartments. Researchers use a suite of methods for the identification of these particles, but they are not directly comparable, therefore consideration of the research question, sample type, and desired outputs must be considered during experimental planning. As the use of Nile red and FTIR imaging was not successful in this study, we focus on the differences between micro-FTIR and py-GCMS (Table 5.3). Within the fishmeal samples, micro-FTIR and py-GCMS both identified the presence of polyester and polystyrene, however, micro-FTIR detected the presence of polyethylene within fishmeal samples which was not identified through py-GCMS, and py-GCMS results detected polypropylene within fishmeal samples which was not identified through micro-FTIR, even though spectral libraries and pyrolysis markers allowed for the identification of these two polymers through both techniques. In the soybean results, no polymers were found in common between the two identification techniques; polyester was only identified through FTIR and polyethylene and polystyrene were only identified through py-GCMS, though again spectral/marker information for identifying these polymers was available in both techniques.

Micro-FTIR appears the most popular method for the identification of anthropogenic particles and has many key advantages that make it suitable for these analyses. Primarily, it is a non-destructive method, so the same filters and particles can be scanned multiple times without altering the sample in any way. Particle characteristics such as size, colour, and shape can be recorded which are useful when considering the bioavailability of particles to organisms (Botterell *et al.*, 2020), biological effects e.g. translocation (Wright, Thompson and Galloway, 2013) and the origin of particles (e.g. coloured microfibres are likely from textiles). Use of micro-FTIR equipment has lower training requirements, partly due to the public availability of good quality spectral libraries and lower requirement for data interpretation, however the user must be trained in visual microscopic identification of potential anthropogenic particles, which is open to subjective bias.

For example, brightly coloured, larger particles and fibres that cross the user's field of view will stand out far more than smaller clear, black or white particulates. The requirement for visual identification of particles through microscopy also means that there is a lower limit to the size of particles which can be identified; the smallest particle identified and characterized by micro-FTIR in this study was 24 µm (longest dimension). Particles must also be manually isolated (generally with forceps) for scanning, and this physical manipulation can lead to particle loss during transfer from microscopy to micro-FTIR. Scanning a single particle can take 1-5 minutes depending on how readily a clear signal and spectral match can be achieved. Overall, we estimate microscopic evaluation and spectroscopic analysis took approximately 1 day per sample, but this is heavily dependent on the level of organic and inorganic residue on the filter, the number of anthropogenic particles identified and experience of the analyst.

Py-GCMS has been used to identify microplastics within environmental samples less frequently than FTIR, but is a promising technique which is growing in popularity (Fischer and Scholz-Böttcher, 2019; Yakovenko, Carvalho and ter Halle, 2020; Felline *et al.*, 2022). The use of py-GCMS eliminates user bias, as the whole sample can be homogenized removing the need for visual identification of anthropogenic particles. Py-GCMS is less sensitive to organic soiling hampering identification of particles than micro-FTIR, as the technique identifies plastics based on their breakdown products and does not rely on spectroscopy of the particle surface which can be confounded by organic fouling and weathering (Xu *et al.*, 2019; Ferreiro *et al.*, 2022). It is possible that the disparity in plastic polymer type identified between micro-FTIR and py-GCMS could be due to organic fouling confounding micro-FTIR polymer identification; this should be investigated further in future research. There is no lower size limit for the detection of particles, therefore py-GCMS is a very promising method for the identification of nanoplastics which cannot be visualized through microscopy, and there is the possibility to identify

plastic additives which can have important effects on the toxicity of plastics (Fries *et al.*, 2013). A major limitation of py-GCMS is that the only output, beyond additive content, is particle mass for a limited set of polymers. It has also been suggested that larger particles within a sample can mask the identification of smaller particles due to the greater mass of large particles (Simon, van Alst and Vollertsen, 2018). Size fractionating samples can help alleviate this issue and can be used to determine the mass of plastics in a given size range. Currently, polymer identification by py-GCMS is limited to a small set of polymers for which reliable polymer markers are available, future research should focus on widening this set of polymer markers to allow for the identification of a wide range of plastic polymers; the micro-FTIR results shown here show that a wide range of polymers can be found within environmental and biotic samples. Critically, semi-synthetic and other anthropogenic cellulosic particles such as rayon and cotton cannot be identified by py-GCMS as these particles are primarily cellulose-based and their breakdown products cannot be distinguished from those of naturally-occurring cellulose within environmental samples. It has been mentioned in the literature that a major limitation of py-GCMS is that it is a destructive method, so individually isolated particles cannot be measured twice (Käppler *et al.*, 2018; Primpke, Fischer, *et al.*, 2020; Ribeiro *et al.*, 2020). This is true for individual particles, however in this method we homogenized the samples before analysis, and as each pyrolysis vial only required a 2 mg sub-sample, each sample could be measured multiple times. There is a larger training requirement for the use of this method, as specialist training is required to identify polymers from breakdown markers within results, and calibration curves must be run with every analysis if masses are to be calculated accurately. The processing time for an individual sample is approximately 40 minutes, however calibration standard must be included regularly, increasing analysis time by several hours. To increase throughout, autosamplers can be used to analyse multiple samples with the same calibration standards.

5.4.3 Comparability of masses derived by FTIR and py-GCMS

Coupling microscopy and FTIR produces particle counts and physical characteristics such as morphology, dimensions and colour; however, as these particles are generally too small to weigh via mass balance, mass conversions must be performed to compare data to py-GCMS data. As the images captured of the particles upon identification can only be used to measure anthropogenic particles in two dimensions, and due to the extremely heterogeneous morphologies of anthropogenic particles, an estimation of the third dimension of these particles must be made to calculate particle volume, and therefore particle mass. Here, the identified particles were split into three categories: fragments, fibres and films (though only fragments and fibres were identified); particle masses were calculated by utilizing the corresponding volume equations in the methods section. Particle mass estimation from observable dimensions has been performed in the literature, utilizing similar methods (Simon, van Alst and Vollertsen, 2018; Gomiero *et al.*, 2020; Primpke, Fischer, *et al.*, 2020; Roscher *et al.*, 2022). Simon, van Alst and Vollertsen (2018) used FTIR imaging to quantify microplastic contamination of wastewater, and estimate mass based on two calculations, presuming a cylindrical shape with a void fraction of 40% for fibres, and for all other microplastic particles, the ratio of the major:minor observable dimension was calculated (0.67), and the third dimension was assumed to be smaller than the minor dimension by the same ratio. These three dimensions were then used to calculate volume assuming an ellipsoid shape. This method was also used by Gomiero *et al.*, (2020) who found that FTIR mass calculations reported lower mass compared to py-GCMS data. Primpke, Fischer, *et al.* (2020) also follow this calculation, using a ratio of 0.7, and compare it to a method establishing an average reference particle for each polymer type, from which the number of reference particles identified in the data is found through the corresponding polymer surface area identified via FTIR imaging; polymer mass is subsequently calculated via the calculation of Simon, van Alst and Vollertsen (2018). This decreased the disparity between measured py-

GCMS mass and FTIR mass estimation when tested with three wastewater samples, with FTIR estimated mass 3x higher than py-GCMS calculated mass. Roscher *et al.* (2022) utilised both methods in their estimation of MP mass from FTIR data and found the method of Simon, van Alst and Vollertsen (2018) calculated more comparable masses to py-GCMS data. In our results, the calculations described here resulted in closer masses to those reported by py-GCMS than the method of Simon, van Alst and Vollertsen (2018), with masses larger on average by a factor of 2.1. This may be due to the prevalence of fibres within our samples, which are calculated as a larger mass when volume is calculated as a cylinder than as an ellipsoid.

In comparing FTIR and py-GCMS data there was a similar mass of polystyrene identified, however the mass of polyester was 130x greater in samples analysed using py-GCMS. Given source material was well-mixed, it is unlikely that sample heterogeneity would account for such a large difference. We consider that variations in the sensitivity of each technique for identifying polyester is more likely to play a part in the discrepancy between the two methods. Using microscopy to identify particles for micro-FTIR is subjective, with users selecting likely microplastics based on morphological features. Given polyester is widely used in textile production, it is perhaps unsurprising that the vast majority of polyester particles selected using microscopy were fibrous and brightly coloured (84%). However, it is plausible that polyester microplastics present in colours difficult to distinguish from background particles, would result in an underestimate of its abundance through microscopy. Indeed, the identification of grey polyester fragments within bivalves (Phuong *et al.*, 2018) indicates that polyester fragments could be present and missed when visually screening for anthropogenic particles. The mesh size used in this experiment precluded identification of particles <25 µm, and we consider that particles <50 µm are challenging to identify. While polyester nanoplastics and very small microplastics would be missed used microscopy and micro-FTIR, they would be captured using py-GCMS. Neither FTIR or py-GCMS report mass as a direct output; both

techniques require data conversion which may introduce inaccuracies. In FTIR, estimating particle dimensions based on captured microscopy images, and estimation of the third dimension of particles from a two dimensional image, will lead to inaccuracies in calculated mass, especially when measuring complicated fibrous particle morphologies; this was evident in our study, where we identified a 2.1-fold difference in mass depending on the size to mass conversion calculation applied. Py-GCMS mass is calculated by conversion of peak area to mass using a calibration curve. In our py-GCMS data, some size fractionated polyester results were up to 2.5x greater than the highest calibration standard; higher calibration standards would improve accuracy of the mass calculation. Additionally, the py-GCMS repeatability test showed coefficients of variation up to 16.5% for identical samples run repeated times, suggesting calculated masses can still exhibit variation even with highly homogeneous samples.

To better understand the variance in mass calculated using FTIR and py-GCMS, there is a need to directly compare calculated masses of individual particles using the two methods. This information can then be used to observe whether FTIR or py-GCMS may over- or under-report polymer mass, and identify whether corrective factors applied to FTIR or py-GCMS data can increase the accuracy of mass calculation of microplastic polymers.

Clear differences are observed when comparing the composition of anthropogenic particles identified within micro-FTIR and py-GCMS results (Fig. 5.6). While the majority of the anthropogenic particles identified by micro-FTIR were not plastics, non-plastic anthropogenic particles could not be identified via py-GCMS, as we cannot currently distinguish these predominantly cellulosic particles from other cellulosic particles within organic samples using this technique. The presence of particles that are not distinguishable by users due to physical characteristics, and particles <24 µm within these samples, may account for the far lower plastic mass observed in micro-FTIR than by py-GCMS.

Table 5.3. Considerations when choosing the most appropriate technique (micro-FTIR or py-GCMS) for the analysis of anthropogenic particles within complex samples.

	µFTIR	Py-GCMS
Units	• no. particles per sample/unit mass	• Mass concentration (e.g. µg/kg)
Procedural considerations	• Can identify non-plastics (e.g. rayon, cotton). • Lower limit of size (approx. 20-25 µm) • Nondestructive method • Selection bias from visual identification • Particle loss during manual manipulation • Biofouling can interfere with spectra	• Cannot currently identify non-plastics (e.g. rayon, cotton) • No lower size limit • Destructive method • Limited by availability of plastic breakdown markers • No selection bias • No interaction from biofouling • Calibration curve needs adapting based on polymer concentration within sample
Polymer information	• Number of particles • Size • Colour • Morphology	• Polymer mass • Organic additives
Practical considerations	• Significant time required to visually inspect filters for potential anthropogenic particles • Fast particle scan time (approx. 1 minute) • Simple spectral library comparisons	• Fast preparation time • Requirement for calibration curve takes additional time • Training required for interpretation of results

5.5 Conclusion

The results of this study and that of Primpke, Fischer, *et al.* (2020) show that derived FTIR mass and py-GCMS mass trend together, however there is a disparity in absolute mass values, especially in the polyester results in this study, that must be overcome if the two methods are to be directly comparable in the future. Ultimately, both methods can be used to generate high quality data, and the choice of technique depends on the hypothesis under investigation and the desired outputs. If information on particle number, colour and morphology is required, visual microscopy combined with FTIR should be used. If information on nanoplastics, plastic additives, or high sensitivities are required, py-GCMS may be the most appropriate method.

5.6 Acknowledgements

This work was supported by the Natural Environment Research Council through the EnvEast Doctoral Training Partnership (grant number NE/L002582/1). MC and PL would like to acknowledge funding from the UKRI Global Challenges Research Fund (GCRF) funded project ‘Reducing plastic waste in the Galapagos and Eastern Pacific’. We would like to thank Dr Alexandra ter Halle and Magali Albignac for providing training in py-GCMS methods and their assistance in sample analysis and data processing.

Chapter 6: General discussion

Microplastics are a persistent and prevalent marine pollutant, which pose a risk to marine organisms and ecosystems. In this thesis, I consider whether microplastics pose a risk to marine life and food security, and to investigate this, I: undertook a comprehensive literature review and data synthesis to gauge the state of knowledge on microplastics and food security, and mine existing datasets to determine whether microplastics accumulate in food webs; conducted novel experiments to assess chronic effects of microplastics and cotton fibres on the juvenile life-stage of a commercially important bivalve; explored the extent to which aquaculture feed increases microplastic exposure in farmed fish; and evaluated commonly used microplastic identification techniques to provide recommendations to researchers for the best methods to use when analysing complex environmental samples, investigating whether results utilizing different techniques are directly comparable.

Microplastics and food security

In the early stages of my PhD, I undertook a literature review to assess whether there was sufficient evidence to determine if microplastics pose a risk to marine food security. It was clear that microplastics are a prolific contaminant, present within the intestinal tracts of a vast range of commercially-exploited marine organisms. However, there was no data for several organisms considered critical to global marine food security, such as Alaska pollock, grass carp and whiteleg shrimp (Walkinshaw *et al.*, 2020). These three species alone are responsible for 13.7 million tonnes of food for the global population (FAO, 2018). I surmised that these organisms were likely to ingest microplastics given species with similar feeding strategies have been evidenced to consume plastic (Foekema *et al.*, 2013; Jabeen *et al.*, 2017; Bordbar *et al.*, 2018). Since this review, microplastics have indeed been identified within grass carp (Karbalaei *et al.*, 2019) and in both wild and farmed whiteleg shrimp (Valencia-Castañeda, Ibáñez-Aguirre, *et al.*,

2022; Valencia-Castañeda, Ruiz-Fernández, *et al.*, 2022), but there is still no evidence for microplastic contamination within Alaska pollock. I consider that higher trophic level marine organisms critical to food security, such as Alaska pollock, should be investigated to ascertain specific microplastic-associated risks that may impact upon these organisms, such as contaminant transfer and chronic effects from constant low-dose exposure.

The trophic transfer of microplastics had been demonstrated several times, e.g. from algae to periwinkles, from mussels to crabs, and from mackerel to seals (Farrell and Nelson, 2013; Gutow *et al.*, 2016; Nelms *et al.*, 2018). Due to the evidence that microplastics can be transferred between trophic levels in certain situations, the question of whether microplastics could biomagnify has been posed many times in the literature (Au *et al.*, 2017; Carbery, O'Connor and Palanisami, 2018; Nelms *et al.*, 2018; Setälä *et al.*, 2018). In Chapter 2, I investigated this by data-mining microplastic body burdens for a wide range of commercially-important marine organisms and normalizing microplastic content by organism weight. This highlighted that lower trophic level organisms have much higher body burdens of microplastic than higher trophic level fish, with up to four magnitudes more microplastics per gram wet weight in lower trophic level organisms compared with apex predators (Walkinshaw *et al.*, 2020). Based on this evidence, I surmised that while microplastics could bioaccumulate within the bodies of certain lower trophic organisms which consume microplastics quicker than they can egest them, microplastics are not likely to biomagnify between trophic levels. Since this literature review was published, this theory has been backed up in two studies which investigated plastic accumulation in real world food webs in coastal waters (Covernton *et al.*, 2022) and a freshwater lake (McIlwraith *et al.*, 2021); both studies saw no evidence for biomagnification within food webs. This review (Walkinshaw *et al.*, 2020) has been cited 136 times to date, including one policy citation (United Nations Environment Programme, 2021). Biomagnification is often observed with low molecular weight organic chemicals such as

methylmercury and plastic additives such as bisphenol A (Torres-García *et al.*, 2022), whose low molecular weight and fat-solubility mean they are highly bioavailable, and once ingested they pass into adipose tissue where they cannot be easily excreted. Organic chemicals therefore bioaccumulate within individuals, and biomagnify from predation of contaminated organisms. I hypothesise that microplastics will not biomagnify as their comparatively large size prevents translocation, enabling their excretion. It is possible that microfibres could become tangled within the digestive tracts of organisms and remain for extended periods, but not in high enough concentrations for biomagnification to become an issue. However, microplastics are known to contain toxic chemical additives including organic chemicals such as the persistent organobromine compound PBDE (Turner, 2022). Several chemicals such as PBDEs are known as persistent organic pollutants: a group of toxic chemicals which can bioaccumulate and biomagnify due to their lipophilic nature, they also resist degradation by environmental processes and can travel long distances following release (Fitzgerald and Wikoff, 2014). If these compounds are released from microplastics following ingestion, they may pass into the tissues of marine organisms, such as observed by Scopetani *et al.*, 2018, who detected PBDE (^{13}C-labelled BDE-47) within the amphipod *Talitrus saltator* following feeding with fish food containing PBDE-contaminated microplastics. Microplastics could be facilitating the bioaccumulation and biomagnification of these chemicals, though whether this is significant considering other exposures to persistent organic pollutants is disputed (Bakir *et al.*, 2016; Wang, Guo and Xue, 2021). It is also possible that small microplastics and nanoplastics that have been found in organism tissues (Collard *et al.*, 2017; Al-Sid-Cheikh *et al.*, 2018) could bioaccumulate, therefore the potential for nanoplastics to bioaccumulate and biomagnify should be a focus of future research.

Effects on lower trophic levels

Early research found that microplastics can adversely affect the health of marine organisms, with evidence of significant effects on mortality

(Lee *et al.*, 2013; Gray and Weinstein, 2017), fecundity (Lee *et al.*, 2013; Cole *et al.*, 2015), and growth (Au *et al.*, 2015; Jeong *et al.*, 2016; Foley *et al.*, 2018). However, many such studies have been criticised for using monodisperse, uniform, spherical plastics (e.g. polystyrene microbeads) at high, environmentally-unrealistic concentrations over time-scales <30 days, with experiments largely focussed on adult life-stages (Baroja *et al.*, 2021). Environmental sampling has shown fibres are predominant in coastal waters, and such fibres are made of a range of materials including plastic, cotton and regenerated cellulose. In Chapter 3, I sought to address these criticisms by exposing juvenile mussels to environmentally realistic concentrations of microfibres over a prolonged exposure period. Exposure to polyester microfibres resulted in a significantly lower growth rate, with exposure to 80 polyester fibres per litre resulting in significantly smaller mussels after 32 days exposure. Clearance and respiration rate were not responsible for the decrease in growth observed, therefore I was unable to ascertain the mechanism by which growth rates were decreased from this data alone, though I hypothesise that the decrease is a result of reduced energy available for growth due to one of two mechanisms: homeostatic disruption or reduced energetic intake. Homeostatic disruption, due to immune or stress response, alters energetic flow (Sussarellu *et al.*, 2016), redirecting energy away from growth and reproduction into these maintenance mechanisms, causing an energetic deficit respective to the control group. Reduced energetic intake may be due to a false satiation effect from the ingestion of microfibres, which occupy volumetric space within the gut and cause an energy deficit, as they require energy to be ingested and egested but are nutritionally bereft.

To investigate mussel energy budget throughout the experiment, initial experimental plans involved the calculation of ‘Scope for Growth’. Unfortunately, due to experimental and Covid restrictions I was unable to perform the regular food absorption efficiency measurements required to accurately calculate Scope for Growth throughout the experiment, but was able to determine estimates using measurements

taken at the end of the exposure (Appendix A). I observed a consistent decrease in Scope for Growth in mussels exposed to 80 polyester fibres per litre over the experimental period, which was not observed in the other conditions. The results here suggest that energetics play a part in the reduced growth rates observed, and future research should consider scope for growth, or other dynamic energy budget models (Filgueira, Rosland and Grant, 2011; Sussarellu *et al.*, 2016), as a method of investigating bivalve energetics over chronic timescales as a response to environmentally relevant microplastic concentrations.

This is the first study to consider not only juvenile life stages in a chronic exposure experiment with environmentally realistic microplastic concentrations, but also the first to consider the chronic effect of other anthropogenic particles such as cotton microfibres. Though the observed reduction in growth rate from cotton microfibres was not significant, there is currently a lack of data concerning the concentrations of cotton microfibres in the marine environment, so we cannot conclude that these microfibres do not have an effect at environmentally-realistic concentrations. Future research should aim to accurately quantify these microfibres (elucidated in Chapter 5) in addition to microplastics within the environment and consider the additive effects of all anthropogenic particles. This research illustrates how chronic exposure to environmentally realistic concentrations of microplastic may affect marine organisms and food security. Changes to growth rates in ecologically and commercially important low trophic level organisms such as mussels could have profound consequences on other levels of biological hierarchy. Smaller mussels are less energetically valuable to predators, creating stress within the food chain, and mussels also provide critical ecosystem services such as biofiltration, food provision and carbon sequestration (van der Schatte Olivier *et al.*, 2020) which would be impacted by smaller, less productive organisms. Reduced mussel growth rates would impact upon human producers and consumers, increasing the cost of mussel farming which in turn will make mussels either more costly (due to

longer growth rates) or less nutritionally valuable. Considering that bivalves are a critical food item with over 17 million tonnes farmed annually (FAO, 2020), this could have a significant impact upon food security. I conclude that microplastics research should better consider how effects on commercially important marine organisms may affect food security, in addition to population structure and energy dynamics within the ecosystem as a whole.

Contamination of aquaculture feed

Aquaculture feed represents a potential pathway by which microplastics and other anthropogenic particles might contaminate farmed marine organisms. At the start of this PhD, it was not known whether aquaculture feed was contaminated with microplastics. Given fishmeal is manufactured from lower trophic level organisms that contain microplastics, I hypothesised these microplastics would also be evident in fishmeal (Walkinshaw *et al.*, 2020). I developed and optimised a protocol for isolating anthropogenic particles within aquaculture feeds (Chapter 4, Supplementary information). This method involved a multi-step process including KOH digestion with the addition of a surfactant to solubilize hydrophobic fats and oils, $ZnCl_2$ density separation, and size fractionation, all of which resulted in a mean of 97.5% material removal by mass. In Chapter 4, I applied my method to a range of fishmeals and a soybean meal sample, manufactured from vegetative material that was not expected to contain microplastics. Plastic, semi-synthetic and cotton particles were identified within both fishmeal and soybean meal at similar concentrations. This unexpected result suggests that anthropogenic particle contamination of aquaculture feed is not primarily driven by the level of contamination within the source material. Instead, I surmise that it is likely that post-harvest processes including processing, packaging and transportation are the primary route for contamination of these feeds.

Since the commencement of this study, several other studies have been published exploring microplastic contamination within fishmeal (Karbalaei *et al.*, 2020; Gündoğdu *et al.*, 2021; Thiele *et al.*, 2021; Yao

et al., 2021; Wang *et al.*, 2022). In comparing the results of my experiments with these other studies, I highlighted that semi-synthetic and cotton particles have been overlooked by other research groups. These studies found no contamination within soybean meal (Hanachi et al., 2019) or Antarctic krill (Gündoğdu et al., 2021), whereas I identified 1230 and 1330 particles kg^{-1} in these feeds, respectively. Key methodological differences, including the use of smaller pore size filters, size fractionation of samples, the inclusion of semi-synthetic and cotton polymers within results, and extra contamination controls, may account for these differences, suggesting the methods developed in this study can be used to more effectively isolate anthropogenic particles for identification.

The results of this chapter highlight a novel pathway for the exposure of farmed animals with anthropogenic particles that was not identified before the onset of this PhD. Fishmeal and soybean meal are used in both aquaculture, to feed finfish, and agriculture, to feed cows, pigs and chickens. While in Chapter 2 I concluded that high trophic level organisms will not typically accumulate microplastics from prey, this is not the case for farmed animals. Fish which are farmed in aquaculture include high trophic level organisms such as Atlantic salmon; as these are carnivorous, predatory fish which feed on lower trophic level fish such as smelts and herring, we would expect lower body burdens of microplastics than in the forage fish that they prey on. However, the processing of fishmeal from wild caught fish is intensive, with a processing yield of 22.5% (Tacon and Metian, 2008), meaning that it takes 1000 kg of wild caught fish to manufacture 225 kg fishmeal. This may effectively concentrate the pollutants within individual organisms by a factor of 4.44. There has also been a focus in recent years for the reduction of waste in food supply chains, leading to offal and fish processing waste from wild caught and farmed fish being used in the manufacture of fishmeal. Whilst this is a necessary step to try to utilise all fish tissues and reduce waste as much as possible, we know that microplastics can be retained within the gastrointestinal tract. Therefore these microplastics will contaminate fishmeal unless

cleaning methods can be developed to successfully remove microplastics from these tissues, which would be time-consuming and costly. The manufacture of fishmeal effectively concentrates microplastics within feed pellets which are fed to these animals, therefore this process is akin to biomagnification but via artificial means, and these farmed organisms are likely exposed to a higher number of microplastics through artificial feeds than they would be exposed to through their natural diet. Currently, studies show conflicting results regarding whether farmed or wild marine organisms contain more microplastics (Li *et al.*, 2015, 2016, 2018; Digka *et al.*, 2018; Ding *et al.*, 2018; Phuong *et al.*, 2018; Gomiero *et al.*, 2020), though only Gomiero *et al*. (2020) investigated this within farmed and wild salmon, where they did not observe any difference. It is clear from the study presented here that aquaculture feeds contain anthropogenic particle contaminants, however we do not yet know the additional risk this presents to farmed organisms. Farmed salmon would not only be exposed to anthropogenic particles within their feeds, but also from their surrounding environment, and from contamination during harvest and processing. Through py-GCMS analysis of fishmeal and soybean meal in Chapter 5, I identified a mean total plastic polymer content of 6299 µg kg^{-1} in fishmeal, and 77.8 µg kg^{-1} in soybean meal. Within farmed Atlantic salmon tissues, Gomiero *et al.* (2020) identified 21.3-39.5 µg kg^{-1} polymer in muscle and 18.3-49.9 µg kg^{-1} polymer in liver tissues, with the majority of particles identified in salmon tissues <50 µm. As the mean total plastic polymer identified in aquaculture feed ingredients is markedly higher than the polymer concentration found within farmed salmon tissues, despite being fed on a diet which likely included fishmeal and soybean meal, it is probable that much of the polymer mass identified in our samples is transient within farmed fish, with the possible exception of the size fraction 24-50 µm identified. A proportion of the polymer mass identified in fishmeal and soybean meal could therefore translocate into fish tissues, but whether this is significant compared to other sources is currently unknown. Further research is required to investigate whether the number of anthropogenic particles ingested by

farmed animals through their feed has an effect on apical endpoints which may pose a risk to food security.

It is possible that improved practices and new feeds may prevent anthropogenic particle contamination of aquaculture feeds. Many new feeds are being developed, including microalgal, bacterial, and insect-derived feeds (Hemaiswarya *et al.*, 2011; Rust *et al.*, 2011; Bandara, 2018; Ferrer Llagostera *et al.*, 2019), which can be far better controlled than the harvest of wild fish, and improved manufacture processes could effectively prevent the contamination of these feeds. The move to these new feeds would also ultimately improve food security through reduced dependency upon the capture of huge numbers of small fish for fishmeal manufacture, reducing fishing pressure on these areas and restoring their productivity.

Identifying microplastics in complex samples

As microplastics research progresses, microplastics are being identified in an incredibly diverse range of samples, from Arctic and Alpine snow (Bergmann *et al.*, 2019) to sewage sludge and soil (Corradini *et al.*, 2019). Many of these samples are incredibly complex, containing a wide array of organic and inorganic components. I trialled a series of protocols to identify the most appropriate methods for processing these samples to allow for polymer identification (Chapter 4). However, the identification of anthropogenic particles within these samples was still challenging. As processing methods have developed, a focus has been placed on the techniques used to identify microplastics within processed samples, and while many methods have been developed, there has been little thought into their intercomparability. In Chapter 5, I performed a methods comparison between four commonly used techniques, focussing on their ability to identify microplastics within the complex organic samples often analysed in this research field. I also investigated their ability to identify other anthropogenic particles such as rayon and cotton, as these particles have been identified within aquaculture feeds in this thesis and are of increasing interest to the research community. I quickly discounted the use of Nile red staining as a standalone method for the

identification of anthropogenic particles due to the inability to stain certain plastic and cellulosic polymers and co-staining of organic materials. FTIR imaging is a technique with a huge amount of promise for the research field, allowing non-destructive analysis of particles without human bias, however the complex samples analysed in this chapter were not pure enough to be used with this technology despite having a purification efficacy of 97.5%. Both micro-FTIR and py-GCMS were able to identify and analyse polymers within these complex samples, therefore I focussed on the intercomparability between these two techniques. Some recent studies have found that it may be possible to directly compare the results of FTIR and py-GCMS data through mass conversions (Simon, van Alst and Vollertsen, 2018; Primpke, Fischer, *et al.*, 2020), however the study I performed showed that this is not always the case. There is a lack of the polymer markers used in py-GCMS to identify plastics, so this technique cannot identify as many types of polymer as micro-FTIR which has many publicly-available FTIR spectral libraries, and ultimately the polymers that were identified using both techniques were found at higher concentrations using py-GCMS. While micro-FTIR can be affected by user bias, it can distinguish between anthropogenic and natural cellulosic particles, a major advantage when considering these particles. FTIR imaging can identify semi-synthetic and cotton particles, but current automated analysis software such as siMPle cannot distinguish rayon, cotton or natural cellulose. Likewise, py-GCMS and Nile red staining cannot currently identify cellulosic particles.

Future research should focus on the intercomparability of these methods; here, I found that I could not directly compare results generated by these four methods, however future methods developments may enable this and remove human bias from anthropogenic particle identification. I am currently conducting a direct comparison of the mass (measured by mass balance), calculated mass (based on particle morphology and size) and mass calculated using Py-GCMS for select individual particles which will further elucidate comparability of data from these different techniques. I am

also analysing undigested, ‘raw’ fishmeal and soybean meal samples by py-GCMS to observe whether polymer content can be identified without any preparatory steps; if possible, this would reduce analysis time significantly and may reduce particle loss during processing. The ability to compare results generated from this suite of methods is critical to the research community, as each method is best suited to certain applications. The ability for nanoplastics analysis via py-GCMS is hugely important, and through the combination of nanoplastic identification via py-GCMS and microplastic identification via FTIR, we can gain a more accurate picture of the amount of plastic within different size compartments in the marine environment, revealing information about the breakdown and bioavailability of plastics within marine ecosystems. Furthermore, there is discussion within the community on whether the toxicity of microplastics varies based on their additives and adsorbed contaminants (Hale *et al.*, 2020; Palmer and Herat, 2021). While some publications find a synergistic increase in toxicity when microplastics and chemical pollutants are exposed to organisms together (Barboza, Vieira, *et al.*, 2018; Na *et al.*, 2021), contrasting research illustrates that microplastics either have no synergistic effect on chemical uptake or even reduce contaminant bioavailability and toxicity to marine organisms (Devriese *et al.*, 2017; Scopetani *et al.*, 2018), and the capacity of microplastics to transfer contaminants to organisms may not be significant compared to natural substrates (Browne *et al.*, 2013; Koelmans *et al.*, 2016). Full characterization of microplastics by FTIR and additives by py-GCMS could allow us to study the toxicity of microplastics with known quantities of additives.

Conclusion and Outlook

Microplastics are a globally prevalent and persistent pollutant, and there is growing evidence that cellulosic microfibres are equally prevalent within the marine environment. I identified lower trophic level organisms as most at risk from microplastics, and chronic exposures of juvenile mussels demonstrated environmentally relevant concentrations of microfibres can adversely affect the health of these

organisms. I identified that aquaculture feed represents a novel pathway for the exposure of farmed fish to microplastics and anthropogenic fibres. A conceptual model of the pathways of microplastic ingestion within farmed fish and mussel populations is displayed in Fig. 6.1, highlighting the input of my research into the risks to food security from microplastic ingestion. Lastly, I highlight the challenge of analysing complex organic samples, and advocate for the development of complimentary analytical methods. Collectively, this research expands our knowledge of the risks and exposure routes of microplastics to commercially exploited marine organisms, and highlights a potential risk to marine food security.

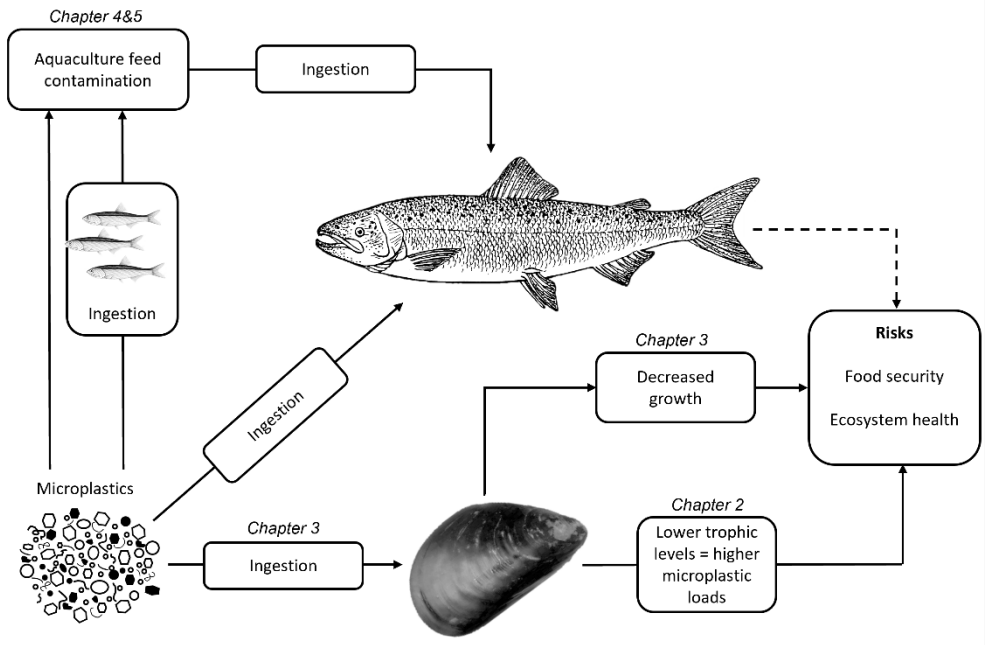


Figure 6.1. A conceptual model of microplastic pathways into lower trophic levels (*Mytilus edulis*) and farmed fish (*Salmo salar*), and subsequent effects of microplastics on organisms, ecosystems and food security identified in this thesis. Dashed lines indicate hypothesised, but as yet untested, pathways.

Throughout this thesis, I have made several recommendations for future microplastics research. Some of these research gaps have since been addressed, but remaining questions include:

From Chapter 2:

1. Do nanoplastics and very small microplastics which are capable of translocating into tissues biomagnify within marine food webs?
2. Do microplastic-associated risks from plastic additives, adsorbed pollutants, and colonizing pathogenic species pose a risk to commercially important marine organisms?
3. Can we calculate the economic costs incurred from microplastic exposure to commercially important marine organisms?

From Chapter 3:

1. How can environmentally-relevant concentrations of microplastics affect the energetics of bivalves and other marine organisms?
2. Which mechanisms or pathways are responsible for the decreased growth rate in bivalves exposed to environmentally relevant levels of microplastic?
3. Is the decrease in growth rate observed in bivalves exposed to anthropogenic particles reflected in other marine organisms at different trophic levels?

From Chapter 4:

1. Is the additional exposure of farmed organisms through contamination of aquaculture feed significant when compared to other sources of contamination?
2. Can anthropogenic particle contamination of aquaculture feed have negative effects on farmed organisms?
3. Can anthropogenic particle contamination of aquaculture feed be decreased through improved post-harvest processes or the introduction of new feed types? What are the cost-benefits of such interventions?

From Chapter 5:

1. Is it possible to further improve anthropogenic particle isolation from complex organic samples to enable processing by FTIR imaging methods?
2. Can method improvements allow for the identification of anthropogenic cellulosic particles by FTIR imaging and py-GCMS?
3. Will method improvements allow for intercomparability between FTIR and py-GCMS data?

My research can be used by the public and policymakers to inform about the effects of microplastics on marine life and food security, and to provide evidence for the requirement to investigate anthropogenic particle contamination of farmed and wild animals. Recent policy changes are reflective of the growing body of evidence that demonstrates the risks plastics pose to marine life. For example, the European Union, Canada, and India have all recently issued bans on specific types of single use plastics, including cigarette packets, earbuds, and cutlery (Council directive 2019/904/EU, 2019; Bhardwaj, 2022; Lindeman, 2022). These policy changes often note risks posed by microplastics in their reasoning for policy change, however, recent studies point to microfibres and tyre particles as being some of the major contributors to marine plastic pollution (Kole *et al.*, 2017; Knight *et al.*, 2020), and therefore bans of single use items are unlikely to reduce microplastic pollution levels. I consider that fundamental changes in the product design stage of plastics manufacturing, to consider end of use and avoid unintended environmental release, are necessary to prioritize the reduction and recycling of plastics, before plastic release can be reduced by any meaningful amount. Without future change in public opinion and policy, the current situation will deteriorate as more anthropogenic particles are released, unregulated and uncontrolled, into the marine environment. Owing to climate change, overfishing and pollution, microplastics pose an additional risk to marine organisms in an environment already exposed to multiple

stressors. Research relating microplastic pollution of the environment to food security and human health, such as that presented within this thesis, is critically important in providing the evidence to push for change and reduce the pollution of our marine environment.

References

Abbasi, S. *et al.* (2018) ‘Microplastics in different tissues of fish and prawn from the Musa Estuary, Persian Gulf’, *Chemosphere*, 205, pp. 80–87. doi: 10.1016/J.CHEMOSPHERE.2018.04.076.

Akhbarizadeh, R., Moore, F. and Keshavarzi, B. (2019) ‘Investigating microplastics bioaccumulation and biomagnification in seafood from the Persian Gulf: a threat to human health?’, *Food Additives & Contaminants: Part A*, pp. 1–13. doi: 10.1080/19440049.2019.1649473.

Al-Sid-Cheikh, M. *et al.* (2018) ‘Uptake, Whole-Body Distribution, and Depuration of Nanoplastics by the Scallop Pecten maximus at Environmentally Realistic Concentrations’, *Environmental Science & Technology*, 52(24), pp. 14480–14486. doi: 10.1021/acs.est.8b05266.

Albentosa, M. *et al.* (2012) ‘First measurements of the scope for growth (SFG) in mussels from a large scale survey in the North-Atlantic Spanish coast’, *Science of the Total Environment*, 435–436. doi: 10.1016/j.scitotenv.2012.07.025.

Amélineau, F. *et al.* (2016) ‘Microplastic pollution in the Greenland Sea: Background levels and selective contamination of planktivorous diving seabirds’, *Environmental Pollution*, 219, pp. 1131–1139. doi: https://doi.org/10.1016/j.envpol.2016.09.017.

Andrady, A. L. (1998) ‘Biodegradation of Plastics: Monitoring what Happens.’, in Pritchard, G. (ed.) *Plastics Additives*. Dordrecht: Springer Netherlands, pp. 32–40. doi: 10.1007/978-94-011-5862-6_5.

Andrady, A. L. (2011) ‘Microplastics in the marine environment’, *Marine Pollution Bulletin*, 62(8), pp. 1596–1605. doi: 10.1016/J.MARPOLBUL.2011.05.030.

Andrady, A. L. (2017) ‘The plastic in microplastics: A review’, *Marine Pollution Bulletin*. doi: 10.1016/j.marpolbul.2017.01.082.

Anestis, A. *et al.* (2007) ‘Behavioral, metabolic, and molecular stress responses of marine bivalve Mytilus galloprovincialis during long-term acclimation at increasing ambient temperature’, *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, 293(2). doi: 10.1152/ajpregu.00124.2007.

Arthur, C., Baker, J. and Bamford, H. (2009) *Proceedings of the international research workshop on the occurrence, effects, and fate of microplastic marine debris, September 9-11, 2008*.

Ašmonaitė, G., Sundh, H., *et al.* (2018) ‘Rainbow trout maintain intestinal transport and barrier functions following exposure to polystyrene microplastics’, *Environmental Science & Technology*. doi: 10.1021/acs.est.8b04848.

Ašmonaitė, G., Larsson, K., *et al.* (2018) ‘Size matters: ingestion of relatively large microplastics contaminated with environmental pollutants posed little risk for fish health and fillet quality’,

Environmental Science & Technology. doi: 10.1021/acs.est.8b04849.

Athey, S. N. and Erdle, L. M. (2022) 'Are We Underestimating Anthropogenic Microfiber Pollution? A Critical Review of Occurrence, Methods, and Reporting', *Environmental Toxicology and Chemistry*, 41(4), pp. 822–837. doi: https://doi.org/10.1002/etc.5173.

Au, S. Y. *et al.* (2015) 'Responses of Hyalella azteca to acute and chronic microplastic exposures', *Environmental Toxicology and Chemistry*, 34(11), pp. 2564–2572. doi: https://doi.org/10.1002/etc.3093.

Au, S. Y. *et al.* (2017) 'Trophic transfer of microplastics in aquatic ecosystems: Identifying critical research needs', *Integrated Environmental Assessment and Management*, 13(3), pp. 505–509. doi: https://doi.org/10.1002/ieam.1907.

Auta, H. S., Emenike, C. U. and Fauziah, S. H. (2017) 'Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions', *Environment International*, 102, pp. 165–176. doi: https://doi.org/10.1016/j.envint.2017.02.013.

Avio, C. G. *et al.* (2015) 'Pollutants bioavailability and toxicological risk from microplastics to marine mussels', *Environmental Pollution*, 198, pp. 211–222. doi: 10.1016/J.ENVPOL.2014.12.021.

Avio, C. G. *et al.* (2020) 'Distribution and characterization of microplastic particles and textile microfibers in Adriatic food webs: General insights for biomonitoring strategies', *Environmental Pollution*, 258, p. 113766. doi: https://doi.org/10.1016/j.envpol.2019.113766.

Avio, C. G., Gorbi, S. and Regoli, F. (2015) 'Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from Adriatic Sea', *Marine Environmental Research*. doi: 10.1016/j.marenvres.2015.06.014.

Azeem, I. *et al.* (2021) 'Uptake and Accumulation of Nano/Microplastics in Plants: A Critical Review', *Nanomaterials* . doi: 10.3390/nano11112935.

Azra, M. N. *et al.* (2021) 'The Contributions of Shellfish Aquaculture to Global Food Security: Assessing Its Characteristics From a Future Food Perspective', *Frontiers in Marine Science*, 8. doi: 10.3389/fmars.2021.654897.

Baillie, J. and Zhang, Y.-P. (2018) 'Space for nature', *Science*, 361(6407), p. 1051. doi: 10.1126/science.aau1397.

Bakir, A. *et al.* (2016) 'Relative importance of microplastics as a pathway for the transfer of hydrophobic organic chemicals to marine life', *Environmental Pollution*, 219, pp. 56–65. doi: 10.1016/J.ENVPOL.2016.09.046.

Bandara, T. (2018) 'Alternative feed ingredients in aquaculture:

Opportunities and challenges', *J. Entomol. Zool. Stud*, 6(2), pp. 3087–3094.

Barboza, L. G. A., Dick Vethaak, A., *et al.* (2018) 'Marine microplastic debris: An emerging issue for food security, food safety and human health', *Marine Pollution Bulletin*. Pergamon, pp. 336–348. doi: 10.1016/j.marpolbul.2018.05.047.

Barboza, L. G. A., Vieira, L. R., *et al.* (2018) 'Microplastics increase mercury bioconcentration in gills and bioaccumulation in the liver, and cause oxidative stress and damage in Dicentrarchus labrax juveniles', *Scientific Reports*. doi: 10.1038/s41598-018-34125-z.

Barker, R. H. (1975) 'Additives in fibers and fabrics.', *Environmental Health Perspectives*, 11, pp. 41–45. doi: 10.1289/ehp.751141.

Barnes, D. K. A. *et al.* (2009) 'Accumulation and fragmentation of plastic debris in global environments', *Philosophical Transactions of the Royal Society B: Biological Sciences*. doi: 10.1098/rstb.2008.0205.

Baroja, E. *et al.* (2021) 'Effects of microplastics on bivalves: Are experimental settings reflecting conditions in the field?', *Marine Pollution Bulletin*, 171, p. 112696. doi: https://doi.org/10.1016/j.marpolbul.2021.112696.

Barrows, A. P. W. *et al.* (2017) 'Grab vs. neuston tow net: a microplastic sampling performance comparison and possible advances in the field', *Analytical Methods*, 9(9), pp. 1446–1453. doi: 10.1039/C6AY02387H.

Barrows, A. P. W., Cathey, S. E. and Petersen, C. W. (2018) 'Marine environment microfiber contamination: Global patterns and the diversity of microparticle origins', *Environmental Pollution*, 237, pp. 275–284. doi: 10.1016/J.ENVPOL.2018.02.062.

Beaumont, N. J. *et al.* (2019) 'Global ecological, social and economic impacts of marine plastic', *Marine Pollution Bulletin*, 142, pp. 189–195. doi: 10.1016/J.MARPOLBUL.2019.03.022.

Bellas, J. *et al.* (2016) 'Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts', *Marine Pollution Bulletin*, 109(1), pp. 55–60. doi: 10.1016/J.MARPOLBUL.2016.06.026.

Béné, C. *et al.* (2015) 'Feeding 9 billion by 2050 – Putting fish back on the menu', *Food Security*. doi: 10.1007/s12571-015-0427-z.

Bergmann, M. *et al.* (2019) 'White and wonderful? Microplastics prevail in snow from the Alps to the Arctic', *Science Advances*, 5(8), p. eaax1157. doi: 10.1126/sciadv.aax1157.

Bessa, F. *et al.* (2018) 'Occurrence of microplastics in commercial fish from a natural estuarine environment', *Marine Pollution Bulletin*, 128, pp. 575–584. doi: https://doi.org/10.1016/j.marpolbul.2018.01.044.

Besseling, E. *et al.* (2015) 'Microplastic in a macro filter feeder: Humpback whale Megaptera novaeangliae', *Marine Pollution Bulletin*,

95(1), pp. 248–252. doi: https://doi.org/10.1016/j.marpolbul.2015.04.007.

Bhardwaj, M. (2022) *India bans single-use plastic to combat pollution*, *Reuters*. Available at: https://www.reuters.com/world/india/india-bans-single-use-plastic-combat-pollution-2022-07-01/ (Accessed: 28 July 2022).

Biginagwa, F. J. *et al.* (2016) 'First evidence of microplastics in the African Great Lakes: Recovery from Lake Victoria Nile perch and Nile tilapia', *Journal of Great Lakes Research*, 42(1), pp. 146–149. doi: 10.1016/J.JGLR.2015.10.012.

Bordbar, L. *et al.* (2018) 'First evidence of ingested plastics by a high commercial shrimp species (Plesionika narval) in the eastern Mediterranean', *Marine Pollution Bulletin*, 136, pp. 472–476. doi: 10.1016/J.MARPOLBUL.2018.09.030.

Borgå, K., Gabrielsen, G. . and Skaare, J. . (2001) 'Biomagnification of organochlorines along a Barents Sea food chain', *Environmental Pollution*, 113(2), pp. 187–198. doi: 10.1016/S0269-7491(00)00171-8.

Botterell, Z. L. R. *et al.* (2019) 'Bioavailability and effects of microplastics on marine zooplankton: A review', *Environmental Pollution*, 245, pp. 98–110. doi: 10.1016/J.ENVPOL.2018.10.065.

Botterell, Z. L. R. *et al.* (2020) 'Bioavailability of Microplastics to Marine Zooplankton: Effect of Shape and Infochemicals', *Environmental Science & Technology*, 54(19), pp. 12024–12033. doi: 10.1021/acs.est.0c02715.

Botterell, Z. L. R. *et al.* (2022) 'Microplastic ingestion in zooplankton from the Fram Strait in the Arctic', *Science of The Total Environment*, 831, p. 154886. doi: https://doi.org/10.1016/j.scitotenv.2022.154886.

Boucher, J. and Friot, D. (2017) *Primary microplastics in the oceans: A global evaluation of sources*, *Primary microplastics in the oceans: A global evaluation of sources*. doi: 10.2305/iucn.ch.2017.01.en.

Boyd, C. E. (2013) 'Aquaculture, Freshwater', *Reference Module in Earth Systems and Environmental Sciences*. doi: 10.1016/B978-0-12-409548-9.03764-7.

Bråte, I. L. N. *et al.* (2016) 'Plastic ingestion by Atlantic cod (Gadus morhua) from the Norwegian coast', *Marine Pollution Bulletin*, 112(1–2), pp. 105–110. doi: 10.1016/J.MARPOLBUL.2016.08.034.

Bråte, I. L. N. *et al.* (2018) 'Mytilus spp. as sentinels for monitoring microplastic pollution in Norwegian coastal waters: A qualitative and quantitative study', *Environmental Pollution*, 243, pp. 383–393. doi: 10.1016/J.ENVPOL.2018.08.077.

Brennecke, D. *et al.* (2015) 'Ingested microplastics (>100 μm) are translocated to organs of the tropical fiddler crab Uca rapax', *Marine Pollution Bulletin*, 96(1–2), pp. 491–495. doi: 10.1016/J.MARPOLBUL.2015.05.001.

Brennecke, D. *et al.* (2016) ‘Microplastics as vector for heavy metal contamination from the marine environment’, *Estuarine, Coastal and Shelf Science*. doi: 10.1016/j.ecss.2015.12.003.

Brown, S. *et al.* (2013) ‘Sea-Level Rise Impacts and Responses: A Global Perspective BT - Coastal Hazards’, in Finkl, C. W. (ed.). Dordrecht: Springer Netherlands, pp. 117–149. doi: 10.1007/978-94-007-5234-4_5.

Browne, M. A. *et al.* (2008) ‘Ingested microscopic plastic translocates to the circulatory system of the mussel, Mytilus edulis (L.)’, *Environmental Science and Technology*. doi: 10.1021/es800249a.

Browne, M. A. *et al.* (2011) ‘Accumulation of microplastic on shorelines woldwide: Sources and sinks’, *Environmental Science and Technology*, 45(21). doi: 10.1021/es201811s.

Browne, M. A. *et al.* (2013) ‘Microplastic Moves Pollutants and Additives to Worms, Reducing Functions Linked to Health and Biodiversity’, *Current Biology*, 23(23), pp. 2388–2392. doi: https://doi.org/10.1016/j.cub.2013.10.012.

Brunner, E. J. *et al.* (2009) ‘Fish, human health and marine ecosystem health: policies in collision’, *International Journal of Epidemiology*, 38(1), pp. 93–100. doi: 10.1093/ije/dyn157.

CA, C. (2013) ‘Plastic for dinner? Observations of frequent debris ingestion by pelagic predatory fishes from the central North Pacific ’, *Marine Ecology Progress Series*, 485, pp. 155–163. Available at: https://www.int-res.com/abstracts/meps/v485/p155-163/.

Cai, M. *et al.* (2018) ‘Lost but can’t be neglected: Huge quantities of small microplastics hide in the South China Sea’, *Science of The Total Environment*, 633, pp. 1206–1216. doi: https://doi.org/10.1016/j.scitotenv.2018.03.197.

Capolupo, M. *et al.* (2018) ‘Uptake and transcriptional effects of polystyrene microplastics in larval stages of the Mediterranean mussel Mytilus galloprovincialis’, *Environmental Pollution*, 241, pp. 1038–1047. doi: https://doi.org/10.1016/j.envpol.2018.06.035.

Carbery, M., O’Connor, W. and Palanisami, T. (2018) ‘Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health’, *Environment International*, 115, pp. 400–409. doi: https://doi.org/10.1016/j.envint.2018.03.007.

Carr, S. A. (2017) ‘Sources and dispersive modes of micro-fibers in the environment’, *Integrated Environmental Assessment and Management*. doi: 10.1002/ieam.1916.

Cashion, T. *et al.* (2017) ‘Most fish destined for fishmeal production are food-grade fish’, *Fish and Fisheries*, 18(5). doi: 10.1111/faf.12209.

Catarino, A. I. *et al.* (2018) ‘Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal’, *Environmental Pollution*, 237, pp. 675–684. doi: 10.1016/J.ENVPOL.2018.02.069.

Van Cauwenberghe, L. *et al.* (2013) ‘Microplastic pollution in deep-sea sediments’, *Environmental Pollution*, 182, pp. 495–499. doi: https://doi.org/10.1016/j.envpol.2013.08.013.

Van Cauwenberghe, L. *et al.* (2015) ‘Microplastics are taken up by mussels (Mytilus edulis) and lugworms (Arenicola marina) living in natural habitats’, *Environmental Pollution*, 199, pp. 10–17. doi: 10.1016/J.ENVPOL.2015.01.008.

Van Cauwenberghe, L. and Janssen, C. R. (2014) ‘Microplastics in bivalves cultured for human consumption’, *Environmental Pollution*, 193, pp. 65–70. doi: 10.1016/J.ENVPOL.2014.06.010.

Chakraborty, S. and Newton, A. C. (2011) ‘Climate change, plant diseases and food security: An overview’, *Plant Pathology*, pp. 2–14. doi: 10.1111/j.1365-3059.2010.02411.x.

Challinor, J. M. (1989) ‘A pyrolysis-derivatisation-gas chromatography technique for the structural elucidation of some synthetic polymers’, *Journal of Analytical and Applied Pyrolysis*, 16(4), pp. 323–333. doi: https://doi.org/10.1016/0165-2370(89)80015-4.

Challinor, J. M. (2001) ‘Review: the development and applications of thermally assisted hydrolysis and methylation reactions’, *Journal of Analytical and Applied Pyrolysis*, 61(1), pp. 3–34. doi: https://doi.org/10.1016/S0165-2370(01)00146-2.

Chamas, A. *et al.* (2020) ‘Degradation Rates of Plastics in the Environment’, *ACS Sustainable Chemistry & Engineering*, 8(9), pp. 3494–3511. doi: 10.1021/acssuschemeng.9b06635.

Chapron, L. *et al.* (2018) ‘Macro- and microplastics affect cold-water corals growth, feeding and behaviour’, *Scientific Reports*, 8(1). doi: 10.1038/s41598-018-33683-6.

Chen, G., Li, Y. and Wang, J. (2021) ‘Occurrence and ecological impact of microplastics in aquaculture ecosystems’, *Chemosphere*, 274, p. 129989. doi: https://doi.org/10.1016/j.chemosphere.2021.129989.

Chen, X. *et al.* (2007) ‘Structure Study of Cellulose Fibers Wet-Spun from Environmentally Friendly NaOH/Urea Aqueous Solutions’, *Biomacromolecules*, 8(6), pp. 1918–1926. doi: 10.1021/bm061186i.

Chen, Y. *et al.* (2020) ‘Identification and quantification of microplastics using Fourier-transform infrared spectroscopy: Current status and future prospects’, *Current Opinion in Environmental Science & Health*, 18, pp. 14–19. doi: https://doi.org/10.1016/j.coesh.2020.05.004.

Cho, Y. *et al.* (2019) ‘Abundance and characteristics of microplastics in market bivalves from South Korea’, *Environmental Pollution*, 245, pp. 1107–1116. doi: 10.1016/J.ENVPOL.2018.11.091.

Choy, C. A. and Drazen, J. C. (2013) ‘Plastic for dinner? Observations of frequent debris ingestion by pelagic predatory fishes from the central North Pacific’, *Marine Ecology Progress Series*, 485, pp. 155–163. Available at: https://www.int-res.com/abstracts/meps/v485/p155-

163.

Christoforou, E. *et al.* (2020) ‘Effects of long-term exposure to microfibers on ecosystem services provided by coastal mussels’, *Environmental Pollution*, 266. doi: 10.1016/j.envpol.2020.115184.

Cohen, A. *et al.* (2016) ‘Technologies for Viable Salmon Aquaculture’, *Aquacultural Engineering*, 74(May).

Cohen, J. E. (2003) ‘Human Population: The Next Half Century’, *Science*, 302(5648), pp. 1172–1175. doi: 10.1126/science.1088665.

Cole, D. W. *et al.* (2009) ‘Aquaculture: Environmental, toxicological, and health issues’, *International Journal of Hygiene and Environmental Health*, 212(4), pp. 369–377. doi: https://doi.org/10.1016/j.ijheh.2008.08.003.

Cole, M. *et al.* (2011) ‘Microplastics as contaminants in the marine environment: A review’, *Marine Pollution Bulletin*, 62(12), pp. 2588–2597. doi: 10.1016/J.MARPOLBUL.2011.09.025.

Cole, M. *et al.* (2014) ‘Isolation of microplastics in biota-rich seawater samples and marine organisms’, *Scientific Reports*. doi: 10.1038/srep04528.

Cole, M. *et al.* (2015) ‘The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod Calanus helgolandicus’, *Environmental Science and Technology*, 49(2), pp. 1130–1137. doi: 10.1021/es504525u.

Cole, M. (2016) ‘A novel method for preparing microplastic fibers’, *Scientific Reports*. doi: 10.1038/srep34519.

Cole, M. *et al.* (2016) ‘Microplastics Alter the Properties and Sinking Rates of Zooplankton Faecal Pellets’, *Environmental Science and Technology*. doi: 10.1021/acs.est.5b05905.

Cole, M. *et al.* (2020) ‘Microplastics, microfibres and nanoplastics cause variable sub-lethal responses in mussels (Mytilus spp.)’, *Marine Pollution Bulletin*, 160. doi: 10.1016/j.marpolbul.2020.111552.

Cole, M. and Galloway, T. S. (2015) ‘Ingestion of Nanoplastics and Microplastics by Pacific Oyster Larvae’, *Environmental Science and Technology*. doi: 10.1021/acs.est.5b04099.

Collard, F. *et al.* (2017) ‘Microplastics in livers of European anchovies (Engraulis encrasicolus, L.)’, *Environmental Pollution*, 229, pp. 1000–1005. doi: 10.1016/J.ENVPOL.2017.07.089.

Conover, R. J. (1966) ‘ASSIMILATION OF ORGANIC MATTER BY ZOOPLANKTON1’, *Limnology and Oceanography*, 11(3), pp. 338–345. doi: https://doi.org/10.4319/lo.1966.11.3.0338.

Coppock, R. L. *et al.* (2017) ‘A small-scale, portable method for extracting microplastics from marine sediments’, *Environmental Pollution*, 230, pp. 829–837. doi: https://doi.org/10.1016/j.envpol.2017.07.017.

Coppock, R. L. *et al.* (2021) ‘Benthic fauna contribute to microplastic sequestration in coastal sediments’, *Journal of Hazardous Materials*, 415, p. 125583. doi: https://doi.org/10.1016/j.jhazmat.2021.125583.

Corami, F. *et al.* (2020) ‘Evidence of small microplastics (<100 μm) ingestion by Pacific oysters (Crassostrea gigas): A novel method of extraction, purification, and analysis using Micro-FTIR’, *Marine Pollution Bulletin*, 160, p. 111606. doi: https://doi.org/10.1016/j.marpolbul.2020.111606.

Cormier, B. *et al.* (2021) ‘Chronic feeding exposure to virgin and spiked microplastics disrupts essential biological functions in teleost fish’, *Journal of Hazardous Materials*, 415, p. 125626. doi: https://doi.org/10.1016/j.jhazmat.2021.125626.

Corradini, F. *et al.* (2019) ‘Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal’, *Science of The Total Environment*, 671, pp. 411–420. doi: https://doi.org/10.1016/j.scitotenv.2019.03.368.

Coughlan, J. (1969) ‘The estimation of filtering rate from the clearance of suspensions’, *Marine Biology*, 2(4), pp. 356–358. doi: 10.1007/BF00355716.

Council directive 2019/904/EU (2019) *Directive (EU) 2019/904 of the European Parliament and of the Council of 5 June 2019 on the reduction of the impact of certain plastic products on the environment*. European Union. Available at: http://data.europa.eu/eli/dir/2019/904/oj (Accessed: 28 July 2022).

Courtene-Jones, W. *et al.* (2017) ‘Optimisation of enzymatic digestion and validation of specimen preservation methods for the analysis of ingested microplastics’, *Analytical Methods*. doi: 10.1039/c6ay02343f.

Covernton, G. A. *et al.* (2019) ‘Size and shape matter: A preliminary analysis of microplastic sampling technique in seawater studies with implications for ecological risk assessment’, *Science of The Total Environment*, 667, pp. 124–132. doi: https://doi.org/10.1016/j.scitotenv.2019.02.346.

Covernton, G. A. *et al.* (2022) ‘Large size (>100-μm) microplastics are not biomagnifying in coastal marine food webs of British Columbia, Canada’, *Ecological Applications*, n/a(n/a), p. e2654. doi: https://doi.org/10.1002/eap.2654.

Critchell, K. and Hoogenboom, M. O. (2018) ‘Effects of microplastic exposure on the body condition and behaviour of planktivorous reef fish (Acanthochromis polyacanthus)’, *PLoS ONE*. doi: 10.1371/journal.pone.0193308.

Davidson, J. *et al.* (2016) ‘Production of market-size North American strain Atlantic salmon Salmo salar in a land-based recirculation aquaculture system using freshwater’, *Aquacultural Engineering*, 74. doi: 10.1016/j.aquaeng.2016.04.007.

Davidson, K. and Dudas, S. E. (2016) ‘Microplastic Ingestion by Wild and Cultured Manila Clams (Venerupis philippinarum) from Baynes

Sound, British Columbia', *Archives of Environmental Contamination and Toxicology*, 71(2), pp. 147–156. doi: 10.1007/s00244-016-0286-4.

Dawson, A. L. *et al.* (2018) 'Turning microplastics into nanoplastics through digestive fragmentation by Antarctic krill', *Nature Communications*. doi: 10.1038/s41467-018-03465-9.

Defossez, J. M. and Hawkins, A. J. S. (1997) 'Selective feeding in shellfish: Size-dependent rejection of large particles within pseudofaeces from Mytilus edulis, Ruditapes philippinarum and Tapes decussatus', *Marine Biology*. doi: 10.1007/s002270050154.

Deng, H. *et al.* (2020) 'Microplastic pollution in water and sediment in a textile industrial area', *Environmental Pollution*, 258, p. 113658. doi: https://doi.org/10.1016/j.envpol.2019.113658.

Desforges, J.-P. W., Galbraith, M. and Ross, P. S. (2015) 'Ingestion of Microplastics by Zooplankton in the Northeast Pacific Ocean', *Archives of Environmental Contamination and Toxicology*, 69(3), pp. 320–330. doi: 10.1007/s00244-015-0172-5.

Desforges, J. P. W. *et al.* (2014) 'Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean', *Marine Pollution Bulletin*, 79(1–2). doi: 10.1016/j.marpolbul.2013.12.035.

Deutsch, L. *et al.* (2007) 'Feeding aquaculture growth through globalization: Exploitation of marine ecosystems for fishmeal', *Global Environmental Change*, 17(2), pp. 238–249. doi: https://doi.org/10.1016/j.gloenvcha.2006.08.004.

Devriese, L. I. *et al.* (2015a) 'Microplastic contamination in brown shrimp (Crangon crangon, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area', *Marine Pollution Bulletin*. doi: 10.1016/j.marpolbul.2015.06.051.

Devriese, L. I. *et al.* (2015b) 'Microplastic contamination in brown shrimp (Crangon crangon, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area', *Marine Pollution Bulletin*, 98(1–2), pp. 179–187. doi: 10.1016/J.MARPOLBUL.2015.06.051.

Devriese, L. I. *et al.* (2017) 'Bioaccumulation of PCBs from microplastics in Norway lobster (Nephrops norvegicus): An experimental study', *Chemosphere*, 186, pp. 10–16. doi: https://doi.org/10.1016/j.chemosphere.2017.07.121.

Digka, N. *et al.* (2018) 'Microplastics in mussels and fish from the Northern Ionian Sea', *Marine Pollution Bulletin*, 135, pp. 30–40. doi: 10.1016/J.MARPOLBUL.2018.06.063.

Ding, J. F. *et al.* (2018) 'Separation and Identification of Microplastics in Digestive System of Bivalves', *Chinese Journal of Analytical Chemistry*, 46(5). doi: 10.1016/S1872-2040(18)61086-2.

Dovidat, L. C. *et al.* (2020) 'Plastic particles adsorb to the roots of freshwater vascular plant Spirodela polyrhiza but do not impair growth', *Limnology And Oceanography Letters*. doi:

10.1002/lol2.10118.

Dris, R. *et al.* (2017) ‘A first overview of textile fibers, including microplastics, in indoor and outdoor environments’, *Environmental Pollution*, 221. doi: 10.1016/j.envpol.2016.12.013.

Ehrlich, P. R., Ehrlich, A. H. and Daily, G. C. (1993) ‘Food Security, Population and Environment’, *Population and Development Review*, 19(1), pp. 1–32. doi: 10.2307/2938383.

Encarnação, P. (2016) ‘5 - Functional feed additives in aquaculture feeds’, in Nates, S. F. B. T.-A. F. (ed.). San Diego: Academic Press, pp. 217–237. doi: https://doi.org/10.1016/B978-0-12-800873-7.00005-1.

Erni-Cassola, G. *et al.* (2017) ‘Lost, but Found with Nile Red: A Novel Method for Detecting and Quantifying Small Microplastics (1 mm to 20 μm) in Environmental Samples’, *Environmental Science & Technology*, 51(23), pp. 13641–13648. doi: 10.1021/acs.est.7b04512.

De Falco, F. *et al.* (2020) ‘Microfiber Release to Water, Via Laundering, and to Air, via Everyday Use: A Comparison between Polyester Clothing with Differing Textile Parameters’, *Environmental Science & Technology*, 54(6), pp. 3288–3296. doi: 10.1021/acs.est.9b06892.

FAO, IFAD, UNICEF, W. and W. (2021) *The State of Food Security and Nutrition in the World 2021. Transforming food systems for food security, improved nutrition and affordable healthy diets for all*, *FAO*.

FAO (2014) *The State of World Fisheries and Aquaculture 2014*. Rome.

FAO *et al.* (2017) *The State of Food Security and Nutrition in the World 2017.Building Resilience for Peace and Food Security.*, *Rome, FAO*. doi: 10.1080/15226514.2012.751351.

FAO (2018) *The State of World Fisheries and Aquaculture Meeting the Sustainable Development Goals*, *Food and Agriculture Organization of the United Nations*. doi: 10.1364/OE.17.003331.

FAO (2020) *The State of World Fisheries and Aquaculture 2020. Sustainability in action.*, *The State of World Fisheries and Aquaculture 2020*. Rome. doi: https://doi.org/10.4060/ca9229en.

FAO, 2015 (2017) *Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety*, *FAO Fisheries and Aquaculture Technical Paper*. doi: 978-92-5-109882-0.

Farrell, P. and Nelson, K. (2013) ‘Trophic level transfer of microplastic: Mytilus edulis (L.) to Carcinus maenas (L.)’, *Environmental Pollution*, 177, pp. 1–3. doi: 10.1016/J.ENVPOL.2013.01.046.

FDA (2017) *Mercury Levels in Commercial Fish and Shellfish (1990-2012)*. Available at: https://www.fda.gov/food/metals/mercury-levels-commercial-fish-and-shellfish-1990-2012 (Accessed: 14 August

2019).

Felline, S. *et al.* (2022) ‘Microplastics’ Occurrence in Edible Fish Species (Mullus barbatus and M. surmuletus) from an Italian Marine Protected Area’, *Microplastics*. doi: 10.3390/microplastics1020021.

Fernlund, J. M. R. (1998) ‘The effect of particle form on sieve analysis: a test by image analysis’, *Engineering Geology*, 50(1), pp. 111–124. doi: https://doi.org/10.1016/S0013-7952(98)00004-0.

Ferreiro, B. *et al.* (2022) ‘Weathering-independent differentiation of microplastic polymers by reflectance IR spectrometry and pattern recognition’, *Marine Pollution Bulletin*, 181, p. 113897. doi: https://doi.org/10.1016/j.marpolbul.2022.113897.

Ferrer Llagostera, P. *et al.* (2019) ‘The use of insect meal as a sustainable feeding alternative in aquaculture: Current situation, Spanish consumers’ perceptions and willingness to pay’, *Journal of Cleaner Production*, 229, pp. 10–21. doi: https://doi.org/10.1016/j.jclepro.2019.05.012.

Filgueira, R., Rosland, R. and Grant, J. (2011) ‘A comparison of scope for growth (SFG) and dynamic energy budget (DEB) models applied to the blue mussel (Mytilus edulis)’, *Journal of Sea Research*, 66(4), pp. 403–410. doi: https://doi.org/10.1016/j.seares.2011.04.006.

Fischer, E. K. *et al.* (2016) ‘Microplastic pollution in lakes and lake shoreline sediments – A case study on Lake Bolsena and Lake Chiusi (central Italy)’, *Environmental Pollution*, 213, pp. 648–657. doi: https://doi.org/10.1016/j.envpol.2016.03.012.

Fischer, M. and Scholz-Böttcher, B. M. (2019) ‘Microplastics analysis in environmental samples – recent pyrolysis-gas chromatography-mass spectrometry method improvements to increase the reliability of mass-related data’, *Analytical Methods*, 11(18), pp. 2489–2497. doi: 10.1039/C9AY00600A.

Fitzgerald, L. and Wikoff, D. S. (2014) ‘Persistent Organic Pollutants’, in Wexler, P. B. T.-E. of T. (Third E. (ed.). Oxford: Academic Press, pp. 820–825. doi: https://doi.org/10.1016/B978-0-12-386454-3.00211-6.

Floerl, O., LM, S. and Bloecher, N. (2016) ‘Potential environmental risks associated with biofouling management in salmon aquaculture ’, *Aquaculture Environment Interactions*, 8, pp. 407–417.

Foekema, E. M. *et al.* (2013) ‘Plastic in north sea fish’, *Environmental Science and Technology*. doi: 10.1021/es400931b.

Foley, C. J. *et al.* (2018) ‘A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates’, *Science of The Total Environment*, 631–632, pp. 550–559. doi: 10.1016/J.SCITOTENV.2018.03.046.

Frias, J. P. G. L. and Nash, R. (2019) ‘Microplastics: Finding a consensus on the definition’, *Marine Pollution Bulletin*, 138, pp. 145–147. doi: 10.1016/J.MARPOLBUL.2018.11.022.

Fries, E. *et al.* (2013) ‘Identification of polymer types and additives in marine microplastic particles using pyrolysis-GC/MS and scanning electron microscopy’, *Environmental Science: Processes & Impacts*, 15(10), pp. 1949–1956. doi: 10.1039/C3EM00214D.

Galloway, T. S. (2015) ‘Micro- and nano-plastics and human health’, in *Marine Anthropogenic Litter*. doi: 10.1007/978-3-319-16510-3_13.

Galloway, T. S., Cole, M. and Lewis, C. (2017a) ‘Interactions of microplastic debris throughout the marine ecosystem’, *Nature Ecology and Evolution*. doi: 10.1038/s41559-017-0116.

Galloway, T. S., Cole, M. and Lewis, C. (2017b) ‘Interactions of microplastic debris throughout the marine ecosystem’, *Nature Ecology and Evolution*. doi: 10.1038/s41559-017-0116.

Gardon, T. *et al.* (2018) ‘Microplastics Affect Energy Balance and Gametogenesis in the Pearl Oyster Pinctada margaritifera’, *Environmental Science & Technology*, 52(9), pp. 5277–5286. doi: 10.1021/acs.est.8b00168.

Gephart, J. A. *et al.* (2021) ‘Scenarios for Global Aquaculture and Its Role in Human Nutrition’, *Reviews in Fisheries Science & Aquaculture*, 29(1), pp. 122–138. doi: 10.1080/23308249.2020.1782342.

GESAMP (2016) *Sources, fate and effects of microplastics in the marine environment: part two of a global assessment*. Edited by C. M. Kershaw, P.J., Rochman. (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/ UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection). Rep. Stud. GESAMP No. 93, 220 p.

Geyer, R., Jambeck, J. R. and Law, K. L. (2017) ‘Production, use, and fate of all plastics ever made’, *Science Advances*. doi: 10.1126/sciadv.1700782.

Goldewijk, K. K. (2005) ‘Three Centuries of Global Population Growth: A Spatial Referenced Population (Density) Database for 1700–2000’, *Population and Environment*, 26(4), pp. 343–367. doi: 10.1007/s11111-005-3346-7.

Goldstein, M. C., Rosenberg, M. and Cheng, L. (2012) ‘Increased oceanic microplastic debris enhances oviposition in an endemic pelagic insect’, *Biology Letters*. doi: 10.1098/rsbl.2012.0298.

Gomiero, A. *et al.* (2020) *Quantification of microplastic in fillet and organs of farmed and wild salmonids - a comparison of methods for detection and quantification - SALMODETECT*.

Gray, A. D. *et al.* (2018) ‘Microplastic in two South Carolina Estuaries: Occurrence, distribution, and composition’, *Marine Pollution Bulletin*, 128. doi: 10.1016/j.marpolbul.2018.01.030.

Gray, A. D. and Weinstein, J. E. (2017) ‘Size- and shape-dependent effects of microplastic particles on adult daggerblade grass shrimp (Palaemonetes pugio)’, *Environmental Toxicology and Chemistry*, 36(11). doi: 10.1002/etc.3881.

Green, D. S. (2016) ‘Effects of microplastics on European flat oysters, Ostrea edulis and their associated benthic communities’, *Environmental Pollution*, 216, pp. 95–103. doi: https://doi.org/10.1016/j.envpol.2016.05.043.

Green, D. S. *et al.* (2019) ‘Exposure to microplastics reduces attachment strength and alters the haemolymph proteome of blue mussels (Mytilus edulis)’, *Environmental Pollution*, 246, pp. 423–434. doi: 10.1016/J.ENVPOL.2018.12.017.

Green, K. (2016) ‘Seafish Insight: The global picture – fishmeal production and trends’, *FAO SOFIA Report*.

Gündoğdu, S. *et al.* (2021) ‘Fish out, plastic in: Global pattern of plastics in commercial fishmeal’, *Aquaculture*, 534. doi: 10.1016/j.aquaculture.2020.736316.

Gutow, L. *et al.* (2016) ‘Experimental Evaluation of Seaweeds as a Vector for Microplastics into Marine Food Webs’, *Environmental Science and Technology*. doi: 10.1021/acs.est.5b02431.

Güven, O. *et al.* (2017) ‘Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish’, *Environmental Pollution*, 223, pp. 286–294. doi: 10.1016/J.ENVPOL.2017.01.025.

Hale, R. C. *et al.* (2020) ‘A Global Perspective on Microplastics’, *Journal of Geophysical Research: Oceans*, 125(1), p. e2018JC014719. doi: https://doi.org/10.1029/2018JC014719.

Halldórsson, H. P., Svavarsson, J. and Granmo, Å. (2005) ‘The effect of pollution on scope for growth of the mussel (Mytilus edulis L.) in Iceland’, *Marine Environmental Research*, 59(1). doi: 10.1016/j.marenvres.2004.02.001.

Ter Halle, A. *et al.* (2017) ‘Nanoplastic in the North Atlantic Subtropical Gyre’, *Environmental Science & Technology*, 51(23), pp. 13689–13697. doi: 10.1021/acs.est.7b03667.

Halstead, J. E. *et al.* (2018) ‘Assessment tools for microplastics and natural fibres ingested by fish in an urbanised estuary’, *Environmental Pollution*, 234. doi: 10.1016/j.envpol.2017.11.085.

Halwart, M., Soto, D. and Arthur, J. R. (Eds. . (2007) *Cage aquaculture Regional reviews and global overview*, *FAO Fisheries Technical Paper*.

Hanachi, P. *et al.* (2019) ‘Abundance and properties of microplastics found in commercial fish meal and cultured common carp (Cyprinus carpio)’, *Environmental Science and Pollution Research*, 26(23). doi: 10.1007/s11356-019-05637-6.

Hantoro, I. *et al.* (2019) ‘Microplastics in coastal areas and seafood: implications for food safety’, *Food Additives & Contaminants: Part A*, 36(5), pp. 674–711. doi: 10.1080/19440049.2019.1585581.

Hanvey, J. S. *et al.* (2017) ‘A review of analytical techniques for

quantifying microplastics in sediments', *Analytical Methods*, 9(9), pp. 1369–1383. doi: 10.1039/C6AY02707E.

Hardesty, B. D. *et al.* (2017) 'Using Numerical Model Simulations to Improve the Understanding of Micro-plastic Distribution and Pathways in the Marine Environment ', *Frontiers in Marine Science* , p. 30. Available at: https://www.frontiersin.org/article/10.3389/fmars.2017.00030.

Hartmann, N. B. *et al.* (2019) 'Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris', *Environmental Science and Technology*. doi: 10.1021/acs.est.8b05297.

Hemaiswarya, S. *et al.* (2011) 'Microalgae: a sustainable feed source for aquaculture', *World Journal of Microbiology and Biotechnology*, 27(8), pp. 1737–1746. doi: 10.1007/s11274-010-0632-z.

Hengstmann, E. and Fischer, E. K. (2019) 'Nile red staining in microplastic analysis—proposal for a reliable and fast identification approach for large microplastics', *Environmental Monitoring and Assessment*, 191(10), p. 612. doi: 10.1007/s10661-019-7786-4.

Henry, B., Laitala, K. and Klepp, I. G. (2019) 'Microfibres from apparel and home textiles: Prospects for including microplastics in environmental sustainability assessment', *Science of the Total Environment*, 652. doi: 10.1016/j.scitotenv.2018.10.166.

Hermsen, E. *et al.* (2017) 'Detection of low numbers of microplastics in North Sea fish using strict quality assurance criteria', *Marine Pollution Bulletin*, 122(1–2), pp. 253–258. doi: 10.1016/j.marpolbul.2017.06.051.

Hernandez, E., Nowack, B. and Mitrano, D. M. (2017) 'Polyester Textiles as a Source of Microplastics from Households: A Mechanistic Study to Understand Microfiber Release During Washing', *Environmental Science & Technology*, 51(12), pp. 7036–7046. doi: 10.1021/acs.est.7b01750.

Hertrampf, J. W. and Piedad-Pascual, F. (2000) 'Fish Meal BT - Handbook on Ingredients for Aquaculture Feeds', in Hertrampf, J. W. and Piedad-Pascual, F. (eds). Dordrecht: Springer Netherlands, pp. 177–191. doi: 10.1007/978-94-011-4018-8_18.

Hidalgo-Ruz, V. *et al.* (2012) 'Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification', *Environmental Science & Technology*, 46(6), pp. 3060–3075. doi: 10.1021/es2031505.

Horton, A. A. *et al.* (2017) 'Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities', *Science of The Total Environment*, 586, pp. 127–141. doi: https://doi.org/10.1016/j.scitotenv.2017.01.190.

Horton, A. A. and Barnes, D. K. A. (2020) 'Microplastic pollution in a rapidly changing world: Implications for remote and vulnerable marine

ecosystems', *Science of The Total Environment*, 738, p. 140349. doi: https://doi.org/10.1016/j.scitotenv.2020.140349.

Horton, A. A. and Dixon, S. J. (2018) 'Microplastics: An introduction to environmental transport processes', *WIREs Water*, 5(2), p. e1268. doi: https://doi.org/10.1002/wat2.1268.

Hurley, R. R. *et al.* (2018) 'Validation of a Method for Extracting Microplastics from Complex, Organic-Rich, Environmental Matrices', *Environmental Science & Technology*, 52(13), pp. 7409–7417. doi: 10.1021/acs.est.8b01517.

Ivar Do Sul, J. A. and Costa, M. F. (2014) 'The present and future of microplastic pollution in the marine environment', *Environmental Pollution*. doi: 10.1016/j.envpol.2013.10.036.

Jabeen, K. *et al.* (2017) 'Microplastics and mesoplastics in fish from coastal and fresh waters of China', *Environmental Pollution*, 221, pp. 141–149. doi: 10.1016/J.ENVPOL.2016.11.055.

Jambeck, J. R. *et al.* (2015) 'Plastic waste inputs from land into the ocean', *Science*. doi: 10.1126/science.1260352.

Jeong, C. B. *et al.* (2016) 'Microplastic Size-Dependent Toxicity, Oxidative Stress Induction, and p-JNK and p-p38 Activation in the Monogonont Rotifer (Brachionus koreanus)', *Environmental Science and Technology*. doi: 10.1021/acs.est.6b01441.

Jiang, P. *et al.* (2018) 'Microplastic-associated bacterial assemblages in the intertidal zone of the Yangtze Estuary', *Science of the Total Environment*. doi: 10.1016/j.scitotenv.2017.12.105.

Johnson, A. C. *et al.* (2020) 'Identification and Quantification of Microplastics in Potable Water and Their Sources within Water Treatment Works in England and Wales', *Environmental Science & Technology*, 54(19), pp. 12326–12334. doi: 10.1021/acs.est.0c03211.

Jones-Williams, K. *et al.* (2020) 'Close encounters - microplastic availability to pelagic amphipods in sub-antarctic and antarctic surface waters', *Environment International*, 140. doi: 10.1016/j.envint.2020.105792.

Kaiser, D., Kowalski, N. and Waniek, J. J. (2017) 'Effects of biofouling on the sinking behavior of microplastics', *Environmental Research Letters*. doi: 10.1088/1748-9326/aa8e8b.

Kanhai, L. D. K. *et al.* (2019) 'Deep sea sediments of the Arctic Central Basin: A potential sink for microplastics', *Deep Sea Research Part I: Oceanographic Research Papers*, 145, pp. 137–142. doi: https://doi.org/10.1016/j.dsr.2019.03.003.

Käppler, A. *et al.* (2016) 'Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or both?', *Analytical and Bioanalytical Chemistry*, 408(29), pp. 8377–8391. doi: 10.1007/s00216-016-9956-3.

Käppler, A. *et al.* (2018) 'Comparison of μ-ATR-FTIR spectroscopy

and py-GCMS as identification tools for microplastic particles and fibers isolated from river sediments', *Analytical and Bioanalytical Chemistry*, 410(21), pp. 5313–5327. doi: 10.1007/s00216-018-1185-5.

Karami, A., Golieskardi, A., Choo, C. K., *et al.* (2017) 'A high-performance protocol for extraction of microplastics in fish', *Science of the Total Environment*, 578. doi: 10.1016/j.scitotenv.2016.10.213.

Karami, A., Golieskardi, A., Ho, Y. Bin, *et al.* (2017) 'Microplastics in eviscerated flesh and excised organs of dried fish', *Scientific Reports*. doi: 10.1002/advs.201700259.

Karami, A. *et al.* (2018) 'Microplastic and mesoplastic contamination in canned sardines and sprats', *Science of The Total Environment*, 612, pp. 1380–1386. doi: 10.1016/J.SCITOTENV.2017.09.005.

Karbalaei, S. *et al.* (2018) 'Occurrence, sources, human health impacts and mitigation of microplastic pollution', *Environmental Science and Pollution Research*, 25(36), pp. 36046–36063. doi: 10.1007/s11356-018-3508-7.

Karbalaei, S. *et al.* (2019) 'Abundance and characteristics of microplastics in commercial marine fish from Malaysia', *Marine Pollution Bulletin*, 148, pp. 5–15. doi: 10.1016/J.MARPOLBUL.2019.07.072.

Karbalaei, S. *et al.* (2020) 'Analysis and inorganic composition of microplastics in commercial Malaysian fish meals', *Marine Pollution Bulletin*, 150. doi: 10.1016/j.marpolbul.2019.110687.

Karlsson, T. M. *et al.* (2017) 'Screening for microplastics in sediment, water, marine invertebrates and fish: Method development and microplastic accumulation', *Marine Pollution Bulletin*, 122(1), pp. 403–408. doi: https://doi.org/10.1016/j.marpolbul.2017.06.081.

Kirstein, I. V. *et al.* (2016) 'Dangerous hitchhikers? Evidence for potentially pathogenic Vibrio spp. on microplastic particles', *Marine Environmental Research*, 120, pp. 1–8. doi: 10.1016/J.MARENVRES.2016.07.004.

Klein, M. and Fischer, E. K. (2019) 'Microplastic abundance in atmospheric deposition within the Metropolitan area of Hamburg, Germany', *Science of The Total Environment*, 685, pp. 96–103. doi: https://doi.org/10.1016/j.scitotenv.2019.05.405.

Knight, L. J. *et al.* (2020) 'Tyre wear particles: an abundant yet widely unreported microplastic?', *Environmental Science and Pollution Research*, 27(15), pp. 18345–18354. doi: 10.1007/s11356-020-08187-4.

Koelmans, A. A. *et al.* (2016) 'Microplastic as a Vector for Chemicals in the Aquatic Environment: Critical Review and Model-Supported Reinterpretation of Empirical Studies', *Environmental Science & Technology*, 50(7), pp. 3315–3326. doi: 10.1021/acs.est.5b06069.

Koelmans, A. A. *et al.* (2017) 'Risks of Plastic Debris: Unravelling Fact,

Opinion, Perception, and Belief', *Environmental Science and Technology*. doi: 10.1021/acs.est.7b02219.

Koelmans, A. A. *et al.* (2020) 'Solving the Nonalignment of Methods and Approaches Used in Microplastic Research to Consistently Characterize Risk', *Environmental Science & Technology*, 54(19), pp. 12307–12315. doi: 10.1021/acs.est.0c02982.

Koelmans, A. A., Besseling, E. and Foekema, E. M. (2014) 'Leaching of plastic additives to marine organisms', *Environmental Pollution*, 187, pp. 49–54. doi: 10.1016/J.ENVPOL.2013.12.013.

Koelmans, A. A., Besseling, E. and Shim, W. J. (2015) 'Nanoplastics in the aquatic environment. Critical review', in *Marine Anthropogenic Litter*. doi: 10.1007/978-3-319-16510-3_12.

Kolandhasamy, P. *et al.* (2018) 'Adherence of microplastics to soft tissue of mussels: A novel way to uptake microplastics beyond ingestion', *Science of The Total Environment*, 610–611, pp. 635–640. doi: 10.1016/J.SCITOTENV.2017.08.053.

Kole, P. J. *et al.* (2017) 'Wear and Tear of Tyres: A Stealthy Source of Microplastics in the Environment', *International Journal of Environmental Research and Public Health* . doi: 10.3390/ijerph14101265.

Konde, S. *et al.* (2020) 'Exploring the potential of photoluminescence spectroscopy in combination with Nile Red staining for microplastic detection', *Marine Pollution Bulletin*, 159, p. 111475. doi: https://doi.org/10.1016/j.marpolbul.2020.111475.

Kooi, M. *et al.* (2017) 'Ups and Downs in the Ocean: Effects of Biofouling on Vertical Transport of Microplastics', *Environmental Science and Technology*. doi: 10.1021/acs.est.6b04702.

Kooi, M. and Koelmans, A. A. (2019) 'Simplifying Microplastic via Continuous Probability Distributions for Size, Shape,and Density', *Environmental Science and Technology Letters*, 6(9). doi: 10.1021/acs.estlett.9b00379.

Ladewig, S. M., Bao, S. and Chow, A. T. (2015) 'Natural Fibers: A Missing Link to Chemical Pollution Dispersion in Aquatic Environments', *Environmental Science and Technology*. doi: 10.1021/acs.est.5b04754.

Larsson, J. *et al.* (2017) 'Regional genetic differentiation in the blue mussel from the Baltic Sea area', *Estuarine, Coastal and Shelf Science*, 195. doi: 10.1016/j.ecss.2016.06.016.

Lavoie, R. A. *et al.* (2013) 'Biomagnification of Mercury in Aquatic Food Webs: A Worldwide Meta-Analysis', *Environmental Science & Technology*, 47(23), pp. 13385–13394. doi: 10.1021/es403103t.

Law, K. L. *et al.* (2010) 'Plastic Accumulation in the North Atlantic Subtropical Gyre', *Science*, 329(5996), pp. 1185–1188. doi: 10.1126/science.1192321.

Law, K. L. and Thompson, R. C. (2014) ‘Microplastics in the seas’, *Science*. doi: 10.1126/science.1254065.

LeBlanc, N. *et al.* (2005) ‘The effect of high air and water temperature on juvenile Mytilus edulis in Prince Edward Island, Canada’, *Aquaculture*, 243(1–4). doi: 10.1016/j.aquaculture.2004.09.035.

Lee, K. W. *et al.* (2013) ‘Size-dependent effects of micro polystyrene particles in the marine copepod tigriopus japonicus’, *Environmental Science and Technology*. doi: 10.1021/es401932b.

Lees, G. (1964a) ‘The measurement of particle elongation and flakiness: a critical discussion of British Standard and other methods’, *Magazine of Concrete Research*, 16(49), pp. 225–230.

Lees, G. (1964b) ‘The measurement of particle shape and its influence in engineering materials’, *Journ. Brit. Granite and Whinstone Federation*, 4(2).

Lees, G. and Sherigold, F. (1965) ‘Discussion: The measurement of particle elongation and flakiness: a critical discussion of British Standard and other methods’, *Magazine of Concrete Research*, 17(52), pp. 161–162.

Lei, L. *et al.* (2018) ‘Polystyrene (nano)microplastics cause size-dependent neurotoxicity, oxidative damage and other adverse effects in Caenorhabditis elegans’, *Environmental Science: Nano*. doi: 10.1039/c8en00412a.

Lenz, R., Enders, K. and Nielsen, T. G. (2016) ‘Microplastic exposure studies should be environmentally realistic’, *Proceedings of the National Academy of Sciences*. doi: 10.1073/pnas.1606615113.

Li, J. *et al.* (2015) ‘Microplastics in commercial bivalves from China’, *Environmental Pollution*. doi: 10.1016/j.envpol.2015.09.018.

Li, J. *et al.* (2016) ‘Microplastics in mussels along the coastal waters of China’, *Environmental Pollution*, 214, pp. 177–184. doi: 10.1016/J.ENVPOL.2016.04.012.

Li, J. *et al.* (2018) ‘Microplastics in mussels sampled from coastal waters and supermarkets in the United Kingdom’, *Environmental Pollution*, 241, pp. 35–44. doi: https://doi.org/10.1016/j.envpol.2018.05.038.

Li, J. *et al.* (2019) ‘Using mussel as a global bioindicator of coastal microplastic pollution’, *Environmental Pollution*, 244, pp. 522–533. doi: 10.1016/J.ENVPOL.2018.10.032.

Li, J., Liu, H. and Paul Chen, J. (2018) ‘Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection’, *Water Research*, 137, pp. 362–374. doi: https://doi.org/10.1016/j.watres.2017.12.056.

Li, L. *et al.* (2020) ‘Effective uptake of submicrometre plastics by crop plants via a crack-entry mode’, *Nature Sustainability*, 3(11), pp. 929–937. doi: 10.1038/s41893-020-0567-9.

Li, Z. *et al.* (2022) 'Is microplastic an oxidative stressor? Evidence from a meta-analysis on bivalves', *Journal of Hazardous Materials*, 423. doi: 10.1016/j.jhazmat.2021.127211.

Liboiron, M. *et al.* (2016) 'Low plastic ingestion rate in Atlantic cod (Gadus morhua) from Newfoundland destined for human consumption collected through citizen science methods', *Marine Pollution Bulletin*, 113(1–2), pp. 428–437. doi: 10.1016/J.MARPOLBUL.2016.10.043.

Lim, P. T. *et al.* (2005) 'First report of Alexandrium taylori and Alexandrium peruvianum (Dinophyceae) in Malaysia waters', *Harmful Algae*, 4(2), pp. 391–400. doi: 10.1016/J.HAL.2004.07.001.

Lim, X. Z. (2021) 'Microplastics are everywhere - but are they harmful?', *Nature*. doi: 10.1038/d41586-021-01143-3.

Lindeman, T. (2022) *Canada lays out rules banning bags, straws and other single-use plastics*, *The Guardian*. Available at: https://www.theguardian.com/environment/2022/jun/20/canada-ban-single-use-plastics (Accessed: 28 July 2022).

Lindeque, P. K. *et al.* (2020) 'Are we underestimating microplastic abundance in the marine environment? A comparison of microplastic capture with nets of different mesh-size', *Environmental Pollution*, 265. doi: 10.1016/j.envpol.2020.114721.

Lock, E.-J., Biancarosa, I. and Gasco, L. (2018) 'Insects as Raw Materials in Compound Feed for Aquaculture BT - Edible Insects in Sustainable Food Systems', in Halloran, A. et al. (eds). Cham: Springer International Publishing, pp. 263–276. doi: 10.1007/978-3-319-74011-9_16.

Löder, M. G. J. *et al.* (2015) 'Focal plane array detector-based micro-Fourier-transform infrared imaging for the analysis of microplastics in environmental samples', *Environmental Chemistry*, 12(5), pp. 563–581. Available at: https://doi.org/10.1071/EN14205.

Löder, M. G. J. and Gerdts, G. (2015) 'Methodology Used for the Detection and Identification of Microplastics—A Critical Appraisal BT - Marine Anthropogenic Litter', in Bergmann, M., Gutow, L., and Klages, M. (eds). Cham: Springer International Publishing, pp. 201–227. doi: 10.1007/978-3-319-16510-3_8.

Lu, Y. *et al.* (2016) 'Uptake and Accumulation of Polystyrene Microplastics in Zebrafish (Danio rerio) and Toxic Effects in Liver', *Environmental Science and Technology*. doi: 10.1021/acs.est.6b00183.

Luo, W. *et al.* (2019) 'Comparison of microplastic pollution in different water bodies from urban creeks to coastal waters', *Environmental Pollution*, 246, pp. 174–182. doi: https://doi.org/10.1016/j.envpol.2018.11.081.

Lusher, A. L. *et al.* (2014) 'Microplastic pollution in the Northeast Atlantic Ocean: Validated and opportunistic sampling', *Marine Pollution Bulletin*. doi: 10.1016/j.marpolbul.2014.08.023.

Lusher, A. L. *et al.* (2017) ‘Sampling, isolating and identifying microplastics ingested by fish and invertebrates’, *Analytical Methods*. doi: 10.1039/c6ay02415g.

Lusher, A. L., McHugh, M. and Thompson, R. C. (2013) ‘Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel’, *Marine Pollution Bulletin*. doi: 10.1016/j.marpolbul.2012.11.028.

MacLeod, M. *et al.* (2021) ‘The global threat from plastic pollution’, *Science*, 373(6550), pp. 61–65. doi: 10.1126/science.abg5433.

Maes, T. *et al.* (2017) ‘A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red’, *Scientific Reports*, 7(1), p. 44501. doi: 10.1038/srep44501.

Mahon, A. M. *et al.* (2017) ‘Microplastics in Sewage Sludge: Effects of Treatment’, *Environmental Science & Technology*, 51(2), pp. 810–818. doi: 10.1021/acs.est.6b04048.

Mardones, J. I. *et al.* (2015) ‘Fish gill damage by the dinoflagellate Alexandrium catenella from Chilean fjords: Synergistic action of ROS and PUFA’, *Harmful Algae*, 49, pp. 40–49. doi: 10.1016/J.HAL.2015.09.001.

Markic, A. *et al.* (2018) ‘Double trouble in the South Pacific subtropical gyre: Increased plastic ingestion by fish in the oceanic accumulation zone’, *Marine Pollution Bulletin*, 136, pp. 547–564. doi: 10.1016/J.MARPOLBUL.2018.09.031.

Masó, M. *et al.* (2003) ‘Drifting plastic debris as a potential vector for dispersing Harmful Algal Bloom (HAB) species’, *Scientia Marina*. doi: 10.3989/scimar.2003.67n1107.

Mateos-Cárdenas, A. *et al.* (2019) ‘Polyethylene microplastics adhere to Lemna minor (L.), yet have no effects on plant growth or feeding by Gammarus duebeni (Lillj.)’, *Science of the Total Environment*, 689. doi: 10.1016/j.scitotenv.2019.06.359.

Maxwell S, H., Melinda K, F. and Matthew, G. (2020) ‘Counterstaining to Separate Nile Red-Stained Microplastic Particles from Terrestrial Invertebrate Biomass’, *Environmental Science & Technology*, 54(9), pp. 5580–5588. doi: 10.1021/acs.est.0c00711.

McIlwraith, H. K. *et al.* (2021) ‘Evidence of Microplastic Translocation in Wild-Caught Fish and Implications for Microplastic Accumulation Dynamics in Food Webs’, *Environmental Science & Technology*, 55(18), pp. 12372–12382. doi: 10.1021/acs.est.1c02922.

McKinney, R. A., Glatt, S. M. and Williams, S. R. (2004) ‘Allometric length-weight relationships for benthic prey of aquatic wildlife in coastal marine habitats’, *Wildlife Biology*. doi: 10.2981/wlb.2004.029.

Meyers, N. *et al.* (2022) ‘Microplastic detection and identification by Nile red staining: Towards a semi-automated, cost- and time-effective technique’, *Science of The Total Environment*, 823, p. 153441. doi: https://doi.org/10.1016/j.scitotenv.2022.153441.

Miles, R. and Chapman, F. (2006) *The Benefits of Fish Meal in Aquaculture Diets*, *FA122*.

Mintenig, S. M. *et al.* (2017) 'Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging', *Water Research*, 108, pp. 365–372. doi: https://doi.org/10.1016/j.watres.2016.11.015.

Mishra, S. *et al.* (2020) 'Synthetic microfibers: Source, transport and their remediation', *Journal of Water Process Engineering*, 38. doi: 10.1016/j.jwpe.2020.101612.

Möller, J. N., Löder, M. G. J. and Laforsch, C. (2020) 'Finding Microplastics in Soils: A Review of Analytical Methods', *Environmental Science & Technology*, 54(4), pp. 2078–2090. doi: 10.1021/acs.est.9b04618.

Moore, M. N. (1985) 'Cellular responses to pollutants', *Marine Pollution Bulletin*, 16(4), pp. 134–139. doi: 10.1016/0025-326X(85)90003-7.

Moore, M. N. *et al.* (2008) 'Chapter Thirty-Three Lysosomes and Autophagy in Aquatic Animals', *Methods in Enzymology*, 451, pp. 581–620. doi: 10.1016/S0076-6879(08)03233-3.

Von Moos, N., Burkhardt-Holm, P. and Köhler, A. (2012) 'Uptake and effects of microplastics on cells and tissue of the blue mussel Mytilus edulis L. after an experimental exposure', *Environmental Science and Technology*. doi: 10.1021/es302332w.

Moriam, K. *et al.* (2021) 'Hydrophobization of the Man-Made Cellulosic Fibers by Incorporating Plant-Derived Hydrophobic Compounds', *ACS Sustainable Chemistry & Engineering*, 9(13), pp. 4915–4925. doi: 10.1021/acssuschemeng.1c00695.

Mouat, J., Lozano, R. L. and Bateson, H. (2010) *Economic Impacts of Marine Litter*, *Kommunenes Internasjonale Miljøorganisasjon (KIMO)*.

Mowi (2021) *Salmon Farming Industry Handbook*. Available at: https://corpsite.azureedge.net/corpsite/wp-content/uploads/2021/05/Salmon-Industry-Handbook-2021.pdf.

Moyen, N. E., Somero, G. N. and Denny, M. W. (2020) 'Mussel acclimatization to high, variable temperatures is lost slowly upon transfer to benign conditions', *The Journal of experimental biology*, 223. doi: 10.1242/jeb.222893.

Murphy, F. *et al.* (2017) 'The uptake of macroplastic & microplastic by demersal & pelagic fish in the Northeast Atlantic around Scotland', *Marine Pollution Bulletin*. doi: 10.1016/j.marpolbul.2017.06.073.

Murray, F. and Cowie, P. R. (2011) 'Plastic contamination in the decapod crustacean Nephrops norvegicus (Linnaeus, 1758)', *Marine Pollution Bulletin*. doi: 10.1016/j.marpolbul.2011.03.032.

Na, J. *et al.* (2021) 'Synergistic effect of microplastic fragments and benzophenone-3 additives on lethal and sublethal Daphnia magna

toxicity', *Journal of Hazardous Materials*, 402, p. 123845. doi: https://doi.org/10.1016/j.jhazmat.2020.123845.

Naidoo, T. and Glassom, D. (2019) 'Decreased growth and survival in small juvenile fish, after chronic exposure to environmentally relevant concentrations of microplastic', *Marine Pollution Bulletin*, 145. doi: 10.1016/j.marpolbul.2019.02.037.

Naidoo, T., Glassom, D. and Smit, A. J. (2015) 'Plastic pollution in five urban estuaries of KwaZulu-Natal, South Africa', *Marine Pollution Bulletin*, 101(1), pp. 473–480. doi: https://doi.org/10.1016/j.marpolbul.2015.09.044.

Nalbone, L. *et al.* (2021) 'Nile Red staining for detecting microplastics in biota: Preliminary evidence', *Marine Pollution Bulletin*, 172, p. 112888. doi: https://doi.org/10.1016/j.marpolbul.2021.112888.

Napper, I. E. *et al.* (2022) 'Potential microplastic release from the maritime industry: Abrasion of rope', *Science of The Total Environment*, 804, p. 150155. doi: 10.1016/J.SCITOTENV.2021.150155.

Napper, I. E. and Thompson, R. C. (2016) 'Release of synthetic microplastic plastic fibres from domestic washing machines: Effects of fabric type and washing conditions', *Marine Pollution Bulletin*, 112(1–2). doi: 10.1016/j.marpolbul.2016.09.025.

Naser, H. A. (2013) 'Assessment and management of heavy metal pollution in the marine environment of the Arabian Gulf: A review', *Marine Pollution Bulletin*. doi: 10.1016/j.marpolbul.2013.04.030.

Naylor, R. L. *et al.* (2009) 'Feeding aquaculture in an era of finite resources', *Proceedings of the National Academy of Sciences of the United States of America*. doi: 10.1073/pnas.0905235106.

Negri, A. *et al.* (2013) 'Transcriptional Response of the Mussel Mytilus galloprovincialis (Lam.) following Exposure to Heat Stress and Copper', *PLoS ONE*, 8(6). doi: 10.1371/journal.pone.0066802.

Nel, H. A. *et al.* (2021) 'Detection limits are central to improve reporting standards when using Nile red for microplastic quantification', *Chemosphere*, 263, p. 127953. doi: https://doi.org/10.1016/j.chemosphere.2020.127953.

Nelms, S. E. *et al.* (2018) 'Investigating microplastic trophic transfer in marine top predators', *Environmental Pollution*, 238, pp. 999–1007. doi: 10.1016/J.ENVPOL.2018.02.016.

Nelms, S. E. *et al.* (2019) 'Microplastics in marine mammals stranded around the British coast: ubiquitous but transitory?', *Scientific Reports*, 9(1). doi: 10.1038/s41598-018-37428-3.

Neumann, B. *et al.* (2015) 'Future Coastal Population Growth and Exposure to Sea-Level Rise and Coastal Flooding - A Global Assessment', *PLOS ONE*, 10(3), p. e0118571. Available at: https://doi.org/10.1371/journal.pone.0118571.

Neves, D. *et al.* (2015) ‘Ingestion of microplastics by commercial fish off the Portuguese coast’, *Marine Pollution Bulletin*, 101(1), pp. 119–126. doi: 10.1016/j.marpolbul.2015.11.008.

Newell, R. and Jordan, S. (1983) ‘Preferential ingestion of organic material by the American oyster Crassostrea virginica’, *Marine Ecology Progress Series*. doi: 10.3354/meps013047.

Newman, S. *et al.* (2015) ‘The economics of marine litter’, in *Marine Anthropogenic Litter*. doi: 10.1007/978-3-319-16510-3_14.

Nunes, L. S. *et al.* (2021) ‘Intake of microplastics by commercial fish: A Bayesian approach’, *Environmental Monitoring and Assessment*, 193(7). doi: 10.1007/s10661-021-09156-1.

Oberbeckmann, S., Löder, M. G. J. and Labrenz, M. (2015) ‘Marine microplastic-associated biofilms - A review’, *Environmental Chemistry*. doi: 10.1071/EN15069.

Ogonowski, M. *et al.* (2017) ‘Ingested microplastic is not correlated to HOC concentrations in Baltic Sea herring’.

Olli, J. J., Krogdahl, Å. and Våbenø, A. (1995) ‘Dehulled solvent-extracted soybean meal as a protein source in diets for Atlantic salmon, Salmo salar L.’, *Aquaculture Research*, 26(3), pp. 167–174.

Olsen, R. L. and Hasan, M. R. (2012) ‘A limited supply of fishmeal: Impact on future increases in global aquaculture production’, *Trends in Food Science and Technology*. doi: 10.1016/j.tifs.2012.06.003.

Ory, N. *et al.* (2018) ‘Low prevalence of microplastic contamination in planktivorous fish species from the southeast Pacific Ocean’, *Marine Pollution Bulletin*. doi: 10.1016/j.marpolbul.2017.12.016.

Ory, N. C. *et al.* (2017) ‘Amberstripe scad Decapterus muroadsi (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre’, *Science of the Total Environment*. doi: 10.1016/j.scitotenv.2017.01.175.

Ory, N. C. *et al.* (2018) ‘Capture, swallowing, and egestion of microplastics by a planktivorous juvenile fish’, *Environmental Pollution*, 240, pp. 566–573. doi: 10.1016/J.ENVPOL.2018.04.093.

Palmer, J. and Herat, S. (2021) ‘Ecotoxicity of Microplastic Pollutants to Marine Organisms: a Systematic Review’, *Water, Air, & Soil Pollution*, 232(5), p. 195. doi: 10.1007/s11270-021-05155-7.

Paul-Pont, I. *et al.* (2016) ‘Exposure of marine mussels Mytilus spp. to polystyrene microplastics: Toxicity and influence on fluoranthene bioaccumulation’, *Environmental Pollution*, 216. doi: 10.1016/j.envpol.2016.06.039.

Peeken, I. *et al.* (2018) ‘Arctic sea ice is an important temporal sink and means of transport for microplastic’, *Nature Communications*, 9(1), p. 1505. doi: 10.1038/s41467-018-03825-5.

Phuong, N. N. *et al.* (2016) ‘Is there any consistency between the

microplastics found in the field and those used in laboratory experiments?', *Environmental Pollution*, 211, pp. 111–123. doi: https://doi.org/10.1016/j.envpol.2015.12.035.

Phuong, N. N. *et al.* (2018) 'Factors influencing the microplastic contamination of bivalves from the French Atlantic coast: Location, season and/or mode of life?', *Marine Pollution Bulletin*, 129(2), pp. 664–674. doi: 10.1016/J.MARPOLBUL.2017.10.054.

PlasticsEurope (2020) 'Plastics − the Facts 2020', *Plastics Europe: Association of Plastics Manufacturers*, pp. 1–64. Available at: https://www.plasticseurope.org/en/resources/publications/4312-plastics-facts-2020.

Plessi, M., Bertelli, D. and Monzani, A. (2001) 'Mercury and Selenium Content in Selected Seafood', *Journal of Food Composition and Analysis*, 14(5), pp. 461–467. doi: 10.1006/JFCA.2001.1003.

Prata, J. C. *et al.* (2019) 'A new approach for routine quantification of microplastics using Nile Red and automated software (MP-VAT)', *Science of The Total Environment*, 690, pp. 1277–1283. doi: https://doi.org/10.1016/j.scitotenv.2019.07.060.

Prata, J. C., Godoy, V., *et al.* (2021) 'Microplastics and fibers from three areas under different anthropogenic pressures in Douro river', *Science of The Total Environment*, 776, p. 145999. doi: https://doi.org/10.1016/j.scitotenv.2021.145999.

Prata, J. C., Sequeira, I. F., *et al.* (2021) 'Preparation of biological samples for microplastic identification by Nile Red', *Science of The Total Environment*, 783, p. 147065. doi: https://doi.org/10.1016/j.scitotenv.2021.147065.

Prata, J. C., da Costa, J. P., *et al.* (2021) 'Selection of microplastics by Nile Red staining increases environmental sample throughput by micro-Raman spectroscopy', *Science of The Total Environment*, 783, p. 146979. doi: https://doi.org/10.1016/j.scitotenv.2021.146979.

Primpke, S. *et al.* (2017) 'An automated approach for microplastics analysis using focal plane array (FPA) FTIR microscopy and image analysis', *Analytical Methods*, 9(9), pp. 1499–1511. doi: 10.1039/C6AY02476A.

Primpke, S. *et al.* (2018) 'Reference database design for the automated analysis of microplastic samples based on Fourier transform infrared (FTIR) spectroscopy', *Analytical and Bioanalytical Chemistry*. doi: 10.1007/s00216-018-1156-x.

Primpke, S., Fischer, M., *et al.* (2020) 'Comparison of pyrolysis gas chromatography/mass spectrometry and hyperspectral FTIR imaging spectroscopy for the analysis of microplastics', *Analytical and Bioanalytical Chemistry*, 412(30), pp. 8283–8298. doi: 10.1007/s00216-020-02979-w.

Primpke, S., Cross, R. K., *et al.* (2020) 'Toward the Systematic Identification of Microplastics in the Environment: Evaluation of a New Independent Software Tool (siMPle) for Spectroscopic Analysis',

Applied Spectroscopy, 74(9), pp. 1127–1138. doi: 10.1177/0003702820917760.

Qu, X. *et al.* (2018) 'Assessing the relationship between the abundance and properties of microplastics in water and in mussels', *Science of The Total Environment*, 621, pp. 679–686. doi: 10.1016/J.SCITOTENV.2017.11.284.

Rebelein, A. *et al.* (2021) 'Microplastic fibers — Underestimated threat to aquatic organisms?', *Science of the Total Environment*. doi: 10.1016/j.scitotenv.2021.146045.

Remy, F. *et al.* (2015) 'When Microplastic Is Not Plastic: The Ingestion of Artificial Cellulose Fibers by Macrofauna Living in Seagrass Macrophytodetritus', *Environmental Science and Technology*, 49(18). doi: 10.1021/acs.est.5b02005.

Revel, M. *et al.* (2019) 'Tissue-Specific Biomarker Responses in the Blue Mussel Mytilus spp. Exposed to a Mixture of Microplastics at Environmentally Relevant Concentrations', *Frontiers in Environmental Science*, 7, p. 33. Available at: https://www.frontiersin.org/article/10.3389/fenvs.2019.00033.

Ribeiro, F. *et al.* (2017) 'Microplastics effects in Scrobicularia plana', *Marine Pollution Bulletin*, 122(1–2), pp. 379–391. doi: 10.1016/J.MARPOLBUL.2017.06.078.

Ribeiro, F. *et al.* (2020) 'Quantitative Analysis of Selected Plastics in High-Commercial-Value Australian Seafood by Pyrolysis Gas Chromatography Mass Spectrometry', *Environmental Science & Technology*, 54(15), pp. 9408–9417. doi: 10.1021/acs.est.0c02337.

Roblin, B. *et al.* (2020) 'Ambient Atmospheric Deposition of Anthropogenic Microfibers and Microplastics on the Western Periphery of Europe (Ireland)', *Environmental Science and Technology*, 54(18). doi: 10.1021/acs.est.0c04000.

Rochman, C. M. *et al.* (2015) 'Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption', *Scientific Reports*. doi: 10.1038/srep14340.

Rochman, C. M. (2018) 'Microplastics research—from sink to source', *Science*, 360(6384), pp. 28 LP – 29. doi: 10.1126/science.aar7734.

Roscher, L. *et al.* (2022) 'Microplastics in two German wastewater treatment plants: Year-long effluent analysis with FTIR and Py-GC/MS', *Science of The Total Environment*, 817, p. 152619. doi: https://doi.org/10.1016/j.scitotenv.2021.152619.

De Ruijter, V. N. *et al.* (2020) 'Quality Criteria for Microplastic Effect Studies in the Context of Risk Assessment: A Critical Review', *Environmental Science and Technology*, 54(19). doi: 10.1021/acs.est.0c03057.

Rummel, C. D. *et al.* (2016) 'Plastic ingestion by pelagic and demersal fish from the North Sea and Baltic Sea', *Marine Pollution Bulletin*, 102(1), pp. 134–141. doi: 10.1016/J.MARPOLBUL.2015.11.043.

Rust, M. *et al.* (2011) ‘The future of aquafeeds NOAA/USDA alternative feeds initiative’. Edited by N. M. F. S. United States. (NOAA tech. memo. NMFS-F/SPO ; 124).

Salin, K. R. *et al.* (2018) ‘Sustainable Aquafeed BT - Sustainable Aquaculture’, in Hai, F. I., Visvanathan, C., and Boopathy, R. (eds). Cham: Springer International Publishing, pp. 123–151. doi: 10.1007/978-3-319-73257-2_4.

Savoca, S. *et al.* (2021) ‘Ingestion of plastic and non-plastic microfibers by farmed gilthead sea bream (Sparus aurata) and common carp (Cyprinus carpio) at different life stages’, *Science of the Total Environment*, 782. doi: 10.1016/j.scitotenv.2021.146851.

van der Schatte Olivier, A. *et al.* (2020) ‘A global review of the ecosystem services provided by bivalve aquaculture’, *Reviews in Aquaculture*, 12(1), pp. 3–25. doi: https://doi.org/10.1111/raq.12301.

Scheld, A. M., Bilkovic, D. M. and Havens, K. J. (2016) ‘The Dilemma of Derelict Gear’, *Scientific Reports*. doi: 10.1038/srep19671.

Schneider, C. A., Rasband, W. S. and Eliceiri, K. W. (2012) ‘NIH Image to ImageJ : 25 years of image analysis HISTORICAL commentary NIH Image to ImageJ : 25 years of image analysis’, *Nature Methods*, 9(7). doi: 10.1038/nmeth.2089.

Science Advice for Policy by European Academies (SAPEA) (2019) *A Scientific Perspective on Microplastics in Nature and Society | SAPEA*, *Evidence Review Report*. doi: 10.26356/microplastics.

Scopetani, C. *et al.* (2018) ‘Ingested microplastic as a two-way transporter for PBDEs in Talitrus saltator’, *Environmental Research*, 167, pp. 411–417. doi: https://doi.org/10.1016/j.envres.2018.07.030.

Scott, N. *et al.* (2019) ‘Particle characteristics of microplastics contaminating the mussel Mytilus edulis and their surrounding environments’, *Marine Pollution Bulletin*, 146, pp. 125–133. doi: https://doi.org/10.1016/j.marpolbul.2019.05.041.

van Sebille, E., England, M. H. and Froyland, G. (2012) ‘Origin, dynamics and evolution of ocean garbage patches from observed surface drifters’, *Environmental Research Letters*, 7(4), p. 44040. doi: 10.1088/1748-9326/7/4/044040.

Senathirajah, K. *et al.* (2021) ‘Estimation of the mass of microplastics ingested – A pivotal first step towards human health risk assessment’, *Journal of Hazardous Materials*, 404, p. 124004. doi: https://doi.org/10.1016/j.jhazmat.2020.124004.

Setälä, O. *et al.* (2018) ‘Chapter 11 - Microplastics in Marine Food Webs’, in Zeng, E. Y. B. T.-M. C. in A. E. (ed.). Elsevier, pp. 339–363. doi: https://doi.org/10.1016/B978-0-12-813747-5.00011-4.

Seuront, L. *et al.* (2019) ‘Decreased thermal tolerance under recurrent heat stress conditions explains summer mass mortality of the blue mussel Mytilus edulis’, *Scientific Reports*, 9(1). doi: 10.1038/s41598-019-53580-w.

Sfriso, A. A. *et al.* (2020) ‘Microplastic accumulation in benthic invertebrates in Terra Nova Bay (Ross Sea, Antarctica)’, *Environment International*, 137, p. 105587. doi: https://doi.org/10.1016/j.envint.2020.105587.

Shah, A. A. *et al.* (2008) ‘Biological degradation of plastics: A comprehensive review’, *Biotechnology Advances*, 26(3), pp. 246–265. doi: https://doi.org/10.1016/j.biotechadv.2007.12.005.

Shannon, L. and Waller, L. (2021) ‘A Cursory Look at the Fishmeal/Oil Industry From an Ecosystem Perspective’, *Frontiers in Ecology and Evolution*, 9. doi: 10.3389/fevo.2021.645023.

Shim, W. J. *et al.* (2016) ‘Identification and quantification of microplastics using Nile Red staining’, *Marine Pollution Bulletin*, 113(1), pp. 469–476. doi: https://doi.org/10.1016/j.marpolbul.2016.10.049.

Shruti, V. C. *et al.* (2022) ‘Analyzing microplastics with Nile Red: Emerging trends, challenges, and prospects’, *Journal of Hazardous Materials*, 423, p. 127171. doi: https://doi.org/10.1016/j.jhazmat.2021.127171.

Simon, M., van Alst, N. and Vollertsen, J. (2018) ‘Quantification of microplastic mass and removal rates at wastewater treatment plants applying Focal Plane Array (FPA)-based Fourier Transform Infrared (FT-IR) imaging’, *Water Research*, 142, pp. 1–9. doi: https://doi.org/10.1016/j.watres.2018.05.019.

Simoneit, B. R. T., Medeiros, P. M. and Didyk, B. M. (2005) ‘Combustion Products of Plastics as Indicators for Refuse Burning in the Atmosphere’, *Environmental Science & Technology*, 39(18), pp. 6961–6970. doi: 10.1021/es050767x.

Song, Y. K. *et al.* (2015) ‘Occurrence and Distribution of Microplastics in the Sea Surface Microlayer in Jinhae Bay, South Korea’, *Archives of Environmental Contamination and Toxicology*, 69(3), pp. 279–287. doi: 10.1007/s00244-015-0209-9.

Spanjer, A. R. *et al.* (2020) ‘Evidence for rapid gut clearance of microplastic polyester fibers fed to Chinook salmon: A tank study’, *Environmental Pollution*, 265, p. 115083. doi: https://doi.org/10.1016/j.envpol.2020.115083.

Stanton, T. *et al.* (2019) ‘Exploring the Efficacy of Nile Red in Microplastic Quantification: A Costaining Approach’, *Environmental Science & Technology Letters*, 6(10), pp. 606–611. doi: 10.1021/acs.estlett.9b00499.

Steer, M. *et al.* (2017) ‘Microplastic ingestion in fish larvae in the western English Channel’, *Environmental Pollution*. doi: 10.1016/j.envpol.2017.03.062.

Stevens, A. and Gobler, C. (2018) ‘Interactive effects of acidification, hypoxia, and thermal stress on growth, respiration, and survival of four North Atlantic bivalves’, *Marine Ecology Progress Series*, 604, pp. 143–161. Available at: https://www.int-

res.com/abstracts/meps/v604/p143-161/.

Stock, F. *et al.* (2019) 'Sampling techniques and preparation methods for microplastic analyses in the aquatic environment – A review', *TrAC Trends in Analytical Chemistry*, 113, pp. 84–92. doi: https://doi.org/10.1016/j.trac.2019.01.014.

Sturm, M. T., Horn, H. and Schuhen, K. (2021) 'The potential of fluorescent dyes—comparative study of Nile red and three derivatives for the detection of microplastics', *Analytical and Bioanalytical Chemistry*, 413(4), pp. 1059–1071. doi: 10.1007/s00216-020-03066-w.

Suaria, G. *et al.* (2020) 'Microfibers in oceanic surface waters: A global characterization', *Science Advances*, 6(23). doi: 10.1126/sciadv.aay8493.

Sundbæk, K. B. *et al.* (2018) 'Sorption of fluorescent polystyrene microplastic particles to edible seaweed Fucus vesiculosus', *Journal of Applied Phycology*. doi: 10.1007/s10811-018-1472-8.

Sussarellu, R. *et al.* (2016) 'Oyster reproduction is affected by exposure to polystyrene microplastics', *Proceedings of the National Academy of Sciences*. doi: 10.1073/pnas.1519019113.

Szewc, K., Graca, B. and Dołęga, A. (2021) 'Atmospheric deposition of microplastics in the coastal zone: Characteristics and relationship with meteorological factors', *Science of The Total Environment*, 761, p. 143272. doi: https://doi.org/10.1016/j.scitotenv.2020.143272.

Tacon, A. G. J. and Metian, M. (2008) 'Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects', *Aquaculture*, 285(1–4). doi: 10.1016/j.aquaculture.2008.08.015.

Tagg, A. S. *et al.* (2017) 'Fenton's reagent for the rapid and efficient isolation of microplastics from wastewater', *Chemical Communications*, 53(2), pp. 372–375. doi: 10.1039/C6CC08798A.

Tagg, A. S. *et al.* (2020) 'Microplastic Monitoring at Different Stages in a Wastewater Treatment Plant Using Reflectance Micro-FTIR Imaging ', *Frontiers in Environmental Science* . Available at: https://www.frontiersin.org/articles/10.3389/fenvs.2020.00145.

Talbot, S. E. *et al.* (2019) 'Adapting the dynamic energy budget (DEB) approach to include non-continuous growth (moulting) and provide better predictions of biological performance in crustaceans', *ICES Journal of Marine Science*, 76(1), pp. 192–205. doi: 10.1093/icesjms/fsy164.

Talvitie, J. *et al.* (2017) 'How well is microlitter purified from wastewater? – A detailed study on the stepwise removal of microlitter in a tertiary level wastewater treatment plant', *Water Research*, 109. doi: 10.1016/j.watres.2016.11.046.

Tamminga, M., Hengstmann, E. and Fischer, E. K. (2018) 'Microplastic analysis in the South Funen Archipelago, Baltic Sea, implementing

manta trawling and bulk sampling', *Marine Pollution Bulletin*, 128, pp. 601–608. doi: https://doi.org/10.1016/j.marpolbul.2018.01.066.

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

Teuten, E. L. *et al.* (2007) 'Potential for plastics to transport hydrophobic contaminants', *Environmental Science and Technology*. doi: 10.1021/es071737s.

Thiele, C. J. *et al.* (2021) 'Microplastics in fish and fishmeal: an emerging environmental challenge?', *Scientific Reports*, 11(1). doi: 10.1038/s41598-021-81499-8.

Thiele, C. J., Hudson, M. D. and Russell, A. E. (2019) 'Evaluation of existing methods to extract microplastics from bivalve tissue: Adapted KOH digestion protocol improves filtration at single-digit pore size', *Marine Pollution Bulletin*, 142. doi: 10.1016/j.marpolbul.2019.03.003.

Thomas, M. *et al.* (2020) 'The world is your oyster: low-dose, long-term microplastic exposure of juvenile oysters', *Heliyon*, 6(1), p. e03103. doi: 10.1016/J.HELIYON.2019.E03103.

Thompson, R. C. *et al.* (2004) 'Lost at Sea: Where Is All the Plastic?', *Science*, 304(5672), pp. 838 LP – 838. doi: 10.1126/science.1094559.

Thompson, R. C. *et al.* (2009) 'Our plastic age', *Philosophical Transactions of the Royal Society B: Biological Sciences*. The Royal Society Publishing, pp. 1973–1976.

Thornton Hampton, L. M. *et al.* (2022) 'Research recommendations to better understand the potential health impacts of microplastics to humans and aquatic ecosystems', *Microplastics and Nanoplastics*, 2(1), p. 18. doi: 10.1186/s43591-022-00038-y.

Torres-García, J. L. *et al.* (2022) 'Bisphenol A in the environment and recent advances in biodegradation by fungi', *Chemosphere*, 303, p. 134940. doi: https://doi.org/10.1016/j.chemosphere.2022.134940.

Treilles, R. *et al.* (2020) 'Impacts of organic matter digestion protocols on synthetic, artificial and natural raw fibers', *Science of The Total Environment*, 748, p. 141230. doi: 10.1016/J.SCITOTENV.2020.141230.

Trestrail, C., Nugegoda, D. and Shimeta, J. (2020) 'Invertebrate responses to microplastic ingestion: Reviewing the role of the antioxidant system', *Science of The Total Environment*, 734, p. 138559. doi: https://doi.org/10.1016/j.scitotenv.2020.138559.

Turner, A. (2022) 'PBDEs in the marine environment: Sources, pathways and the role of microplastics', *Environmental Pollution*, 301, p. 118943. doi: https://doi.org/10.1016/j.envpol.2022.118943.

United Nations Environment Programme (2021) *Global Environment for Cities-GEO for Cities: Towards Green and Just Cities*. Nairobi: UNEP.

Valavanidis, A. *et al.* (2008) 'Persistent free radicals, heavy metals and PAHs generated in particulate soot emissions and residue ash from controlled combustion of common types of plastic', *Journal of Hazardous Materials*, 156(1), pp. 277–284. doi: https://doi.org/10.1016/j.jhazmat.2007.12.019.

Valencia-Castañeda, G., Ibáñez-Aguirre, K., *et al.* (2022) 'Microplastic contamination in wild shrimp Litopenaeus vannamei from the Huizache-Caimanero Coastal lagoon, SE Gulf of California', *Bulletin of Environmental Contamination and Toxicology*. doi: 10.1007/s00128-022-03568-6.

Valencia-Castañeda, G., Ruiz-Fernández, A. C., *et al.* (2022) 'Microplastics in the tissues of commercial semi-intensive shrimp pond-farmed Litopenaeus vannamei from the Gulf of California ecoregion', *Chemosphere*, 297, p. 134194. doi: https://doi.org/10.1016/j.chemosphere.2022.134194.

Veerasingam, S. *et al.* (2021) 'Contributions of Fourier transform infrared spectroscopy in microplastic pollution research: A review', *Critical Reviews in Environmental Science and Technology*, 51(22), pp. 2681–2743. doi: 10.1080/10643389.2020.1807450.

Verla, A. W. *et al.* (2019) 'Microplastic–toxic chemical interaction: a review study on quantified levels, mechanism and implication', *SN Applied Sciences*, 1(11), p. 1400. doi: 10.1007/s42452-019-1352-0.

Viola, S., Arieli, Y. and Zohar, G. (1988) 'Animal-protein-free feeds for hybrid tilapia (Oreochromis niloticus × O. aureus) in intensive culture', *Aquaculture*, 75(1–2). doi: 10.1016/0044-8486(88)90026-9.

Viršek, M. K. *et al.* (2017) 'Microplastics as a vector for the transport of the bacterial fish pathogen species Aeromonas salmonicida', *Marine Pollution Bulletin*, 125(1–2), pp. 301–309. doi: 10.1016/J.MARPOLBUL.2017.08.024.

Waite, H. R., Donnelly, M. J. and Walters, L. J. (2018) 'Quantity and types of microplastics in the organic tissues of the eastern oyster Crassostrea virginica and Atlantic mud crab Panopeus herbstii from a Florida estuary', *Marine Pollution Bulletin*, 129(1), pp. 179–185. doi: 10.1016/J.MARPOLBUL.2018.02.026.

Walkinshaw, C. *et al.* (2020) 'Microplastics and seafood: lower trophic organisms at highest risk of contamination', *Ecotoxicology and Environmental Safety*, 190. doi: 10.1016/j.ecoenv.2019.110066.

Wang, C. *et al.* (2021) 'Comprehensive assessment of factors influencing Nile red staining: Eliciting solutions for efficient microplastics analysis', *Marine Pollution Bulletin*, 171, p. 112698. doi: https://doi.org/10.1016/j.marpolbul.2021.112698.

Wang, J., Guo, X. and Xue, J. (2021) 'Biofilm-Developed Microplastics As Vectors of Pollutants in Aquatic Environments', *Environmental Science & Technology*, 55(19), pp. 12780–12790. doi: 10.1021/acs.est.1c04466.

Wang, Q. *et al.* (2022) 'Microplastics in fish meals: An exposure route

for aquaculture animals', *Science of the Total Environment*, 807. doi: 10.1016/j.scitotenv.2021.151049.

Wang, S. *et al.* (2021) 'Ingestion of nano/micro plastic particles by the mussel Mytilus coruscus is size dependent', *Chemosphere*, 263. doi: 10.1016/j.chemosphere.2020.127957.

Ward, J. E. *et al.* (2019) 'Selective Ingestion and Egestion of Plastic Particles by the Blue Mussel (Mytilus edulis) and Eastern Oyster (Crassostrea virginica): Implications for Using Bivalves as Bioindicators of Microplastic Pollution', *Environmental Science & Technology*, 53(15), pp. 8776–8784. doi: 10.1021/acs.est.9b02073.

Watts, A. J. R. *et al.* (2014) 'Uptake and retention of microplastics by the shore crab carcinus maenas', *Environmental Science and Technology*. doi: 10.1021/es501090e.

Watts, A. J. R. *et al.* (2015) 'Ingestion of Plastic Microfibers by the Crab Carcinus maenas and Its Effect on Food Consumption and Energy Balance', *Environmental Science and Technology*. doi: 10.1021/acs.est.5b04026.

Wegner, A. *et al.* (2012) 'Effects of nanopolystyrene on the feeding behavior of the blue mussel (Mytilus edulis L.)', *Environmental Toxicology and Chemistry*. doi: 10.1002/etc.1984.

Welden, N. A., Abylkhani, B. and Howarth, L. M. (2018) 'The effects of trophic transfer and environmental factors on microplastic uptake by plaice, Pleuronectes plastessa, and spider crab, Maja squinado', *Environmental Pollution*. doi: 10.1016/j.envpol.2018.03.110.

Welden, N. A. C. and Cowie, P. R. (2016a) 'Environment and gut morphology influence microplastic retention in langoustine, Nephrops norvegicus', *Environmental Pollution*, 214, pp. 859–865. doi: 10.1016/J.ENVPOL.2016.03.067.

Welden, N. A. C. and Cowie, P. R. (2016b) 'Environment and gut morphology influence microplastic retention in langoustine, Nephrops norvegicus', *Environmental Pollution*, 214, pp. 859–865. doi: 10.1016/J.ENVPOL.2016.03.067.

Welden, N. A. C. and Cowie, P. R. (2016c) 'Long-term microplastic retention causes reduced body condition in the langoustine, Nephrops norvegicus', *Environmental Pollution*, 218. doi: 10.1016/j.envpol.2016.08.020.

Widdows, J. (1973) 'Effect of temperature and food on the heart beat, ventilation rate and oxygen uptake of Mytilus edulis', *Marine Biology*, 20(4), pp. 269–276. doi: 10.1007/BF00354270.

Widdows, J. (1976) 'Physiological adaptation ofMytilus edulis to cyclic temperatures', *Journal of comparative physiology*, 105(2), pp. 115–128. doi: 10.1007/BF00691115.

Widdows, J. *et al.* (2002) 'Measurement of stress effects (scope for growth) and contaminant levels in mussels (Mytilus edulis) collected from the Irish Sea', *Marine Environmental Research*. doi:

10.1016/S0141-1136(01)00120-9.

Widdows, J. and Staff, F. (2006) ‘Biological Effects of contaminants: measurement of scope for growth in mussels’, in *ICES Techniques in Marine Environmental Sciences*.

Wiggin, K. J. and Holland, E. B. (2019) ‘Validation and application of cost and time effective methods for the detection of 3–500 μm sized microplastics in the urban marine and estuarine environments surrounding Long Beach, California’, *Marine Pollution Bulletin*, 143, pp. 152–162. doi: https://doi.org/10.1016/j.marpolbul.2019.03.060.

Willett, W. *et al.* (2019) ‘Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems’, *The Lancet*, 393(10170), pp. 447–492. doi: https://doi.org/10.1016/S0140-6736(18)31788-4.

Willis, S. (2003) ‘The use of soybean meal and full fat soybean meal by the animal feed industry’, in *12th Australian soybean conference. Soy Australia, Bundaberg*.

Windsor, M. L. (2001) *Fishmeal. Torry advisory note no. 49.*, *FAO in partnership with SIFAR*.

De Witte, B. *et al.* (2014) ‘Quality assessment of the blue mussel (Mytilus edulis): Comparison between commercial and wild types’, *Marine Pollution Bulletin*, 85(1), pp. 146–155. doi: 10.1016/J.MARPOLBUL.2014.06.006.

Wójcik-Fudalewska, D., Normant-Saremba, M. and Anastácio, P. (2016) ‘Occurrence of plastic debris in the stomach of the invasive crab Eriocheir sinensis’, *Marine Pollution Bulletin*, 113(1–2), pp. 306–311. doi: 10.1016/J.MARPOLBUL.2016.09.059.

Wollenberg, E. *et al.* (2016) ‘Reducing risks to food security from climate change’, *Global Food Security*. doi: 10.1016/j.gfs.2016.06.002.

Woodall, L. C. *et al.* (2014) ‘The deep sea is a major sink for microplastic debris’, *Royal Society open science*, 1(4), p. 140317.

Woods, M. N. *et al.* (2018) ‘Microplastic fiber uptake, ingestion, and egestion rates in the blue mussel (Mytilus edulis)’, *Marine Pollution Bulletin*, 137, pp. 638–645. doi: 10.1016/J.MARPOLBUL.2018.10.061.

Wootton, N., Reis-Santos, P. and Gillanders, B. M. (2021) ‘Microplastic in fish – A global synthesis’, *Reviews in Fish Biology and Fisheries*, 31(4), pp. 753–771. doi: 10.1007/s11160-021-09684-6.

Wright, S. L. *et al.* (2013) ‘Microplastic ingestion decreases energy reserves in marine worms’, *Current Biology*, 23(23), pp. R1031–R1033. doi: https://doi.org/10.1016/j.cub.2013.10.068.

Wright, S. L., Thompson, R. C. and Galloway, T. S. (2013) ‘The physical impacts of microplastics on marine organisms: A review’, *Environmental Pollution*, 178, pp. 483–492. doi: 10.1016/J.ENVPOL.2013.02.031.

Xu, C. *et al.* (2020) ‘Are we underestimating the sources of microplastic pollution in terrestrial environment?’, *Journal of Hazardous Materials*. doi: 10.1016/j.jhazmat.2020.123228.

Xu, J.-L. *et al.* (2019) ‘FTIR and Raman imaging for microplastics analysis: State of the art, challenges and prospects’, *TrAC Trends in Analytical Chemistry*, 119, p. 115629. doi: https://doi.org/10.1016/j.trac.2019.115629.

Xu, X.-Y. *et al.* (2017) ‘Microplastic ingestion reduces energy intake in the clam Atactodea striata’, *Marine Pollution Bulletin*, 124(2), pp. 798–802. doi: 10.1016/J.MARPOLBUL.2016.12.027.

Xue, B. *et al.* (2020) ‘Underestimated Microplastic Pollution Derived from Fishery Activities and “Hidden” in Deep Sediment’, *Environmental Science and Technology*, 54(4). doi: 10.1021/acs.est.9b04850.

Yakovenko, N., Carvalho, A. and ter Halle, A. (2020) ‘Emerging use thermo-analytical method coupled with mass spectrometry for the quantification of micro(nano)plastics in environmental samples’, *TrAC Trends in Analytical Chemistry*, 131, p. 115979. doi: https://doi.org/10.1016/j.trac.2020.115979.

Yao, C. *et al.* (2021) ‘Occurrence of Microplastics in Fish and Shrimp Feeds’, *Bulletin of Environmental Contamination and Toxicology*, 107(4). doi: 10.1007/s00128-021-03328-y.

Yu, X. *et al.* (2018) ‘Occurrence and distribution of microplastics at selected coastal sites along the southeastern United States’, *Science of The Total Environment*, 613–614, pp. 298–305. doi: https://doi.org/10.1016/j.scitotenv.2017.09.100.

Yuan, W. *et al.* (2019) ‘Microplastic abundance, distribution and composition in water, sediments, and wild fish from Poyang Lake, China’, *Ecotoxicology and Environmental Safety*, 170, pp. 180–187. doi: 10.1016/J.ECOENV.2018.11.126.

Zambrano, M. C. *et al.* (2019) ‘Microfibers generated from the laundering of cotton, rayon and polyester based fabrics and their aquatic biodegradation’, *Marine Pollution Bulletin*, 142, pp. 394–407. doi: https://doi.org/10.1016/j.marpolbul.2019.02.062.

Zambrano, M. C. *et al.* (2020) ‘Aerobic biodegradation in freshwater and marine environments of textile microfibers generated in clothes laundering: Effects of cellulose and polyester-based microfibers on the microbiome’, *Marine Pollution Bulletin*, 151. doi: 10.1016/j.marpolbul.2019.110826.

Zarfl, C. (2019) ‘Promising techniques and open challenges for microplastic identification and quantification in environmental matrices’, *Analytical and Bioanalytical Chemistry*, 411(17), pp. 3743–3756. doi: 10.1007/s00216-019-01763-9.

Zettler, E. R., Mincer, T. J. and Amaral-Zettler, L. A. (2013) ‘Life in the “plastisphere”: Microbial communities on plastic marine debris’, *Environmental Science and Technology*. doi: 10.1021/es401288x.

Zhang, F. *et al.* (2020) ‘Direct and indirect effects of microplastics on bivalves, with a focus on edible species: A mini-review’, *Critical Reviews in Environmental Science and Technology*, 50(20), pp. 2109–2143. doi: 10.1080/10643389.2019.1700752.

Zhang, Y. *et al.* (2022) ‘Current status and future perspectives of microplastic pollution in typical cryospheric regions’, *Earth-Science Reviews*, 226, p. 103924. doi: https://doi.org/10.1016/j.earscirev.2022.103924.

Zhu, X. (2021) ‘The Plastic Cycle – An Unknown Branch of the Carbon Cycle ’, *Frontiers in Marine Science* . Available at: https://www.frontiersin.org/articles/10.3389/fmars.2020.609243.

Ziajahromi, S. *et al.* (2017) ‘Impact of Microplastic Beads and Fibers on Waterflea (Ceriodaphnia dubia) Survival, Growth, and Reproduction: Implications of Single and Mixture Exposures’, *Environmental Science and Technology*, 51(22). doi: 10.1021/acs.est.7b03574.

Appendix C: Research dissemination

Throughout my PhD, I have disseminated my research through peer-reviewed publication, presenting at domestic and international conferences and workshops, and educational outreach:

Peer-reviewed publications:

- Walkinshaw *et al.* (2020). Microplastics and seafood: lower trophic organisms at highest risk of contamination. *Ecotoxicology and Environmental Safety*, Volume 190, 110066. https://doi.org/10.1016/j.ecoenv.2019.110066.
- Walkinshaw *et al.* (Submitted). Impact of polyester and cotton microfibres on growth and sublethal biomarkers in juvenile mussels.
- Walkinshaw *et al.* (Submitted). Detection and characterization of microplastics and microfibres in fishmeal and soybean meal

Awards:

- PlyMSEF travel bursary of £400 to conduct a laboratory research project at Université Paul Sabatier, Toulouse. (2019)
- Walkinshaw *et al.* (2020) publication listed as a highly cited paper, placed in the top 1% of environment/ecology papers in Web of science based on citations in year of publication

Conferences:

- MICRO2018. Fate and impacts of microplastics: knowledge, actions and solutions
    - Exploring the impact of microplastic on the Humboldt large marine ecosystem (2018, Poster)
- PlyMSEF 2019.
    - Exploring the impact of microplastic on the Humboldt large marine ecosystem (2019, Poster)
- PharmaQ 2019.

        - Microplastics and aquaculture: current understanding and future research concerns (2019, Oral)
    - MICRO2020
        - Assessment of juvenile mussel growth and health following chronic exposure to anthropogenic fibres (2020, Oral)
    - PRIMO21
        - Impact of polyester and cotton microfibres on growth and sublethal biomarkers in juvenile mussels (2022, Oral)

Workshops:

- (2018) International Training Workshop on Microplastic Debris. Lima, Peru.
- (2018) ARCH-UK workshop – Microplastics, Aquaculture and Fisheries – a Risk Assessment. London, UK.
- (2021) Online workshop on Microplastic Research.
- (2021) Workshop on the microbial hazards of microplastics in food. Cefas (Online)

Fieldwork:

- (2019) Secondment under NERC Changing Arctic Ocean programme. I undertook a five-week research cruise on board RRS James Clark Ross in the Barents Sea, where I was part of the research team seeking to comprehend how benthic communities, biogeochemical processes and ecosystems are responding to changing sea ice cover due to climate change. Changing Arctic Ocean: Seafloor (ChAOS) project, lead investigator Dr Christian März

Educational outreach and media:

- (2018-2022) Educational outreach at schools in Plymouth, educating pupils in plastic pollution and effects on the marine environment
- (2019-2022) Mentored students undertaking research placements at Plymouth Marine Laboratory

- Following publication of my literature review, I was interviewed by *Environmental health news* about my research into marine microplastics and food security
- (2021) Invited to present a seminar on microplastic risks to food security to MSc students at Plymouth University
- (2022) Invited to present a seminar entitled “Microplastic in the food chain: current understanding and future research concerns” at Université Paul Sabatier, Toulouse