OCCURRENCE OF MICROPLASTIC IN FISH: PRESENCE, KNOWLEDGE AND SOLUTIONS

Nina K. Wootton

School of Biological Sciences
The University of Adelaide

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“Knowing is the key to caring, and with caring there is hope that people will be motivated to take positive actions. They might not care even if they know, but they can’t care if they are unaware.”

- Sylvia A. Earle

DECLARATION

I, Nina K. Wootton, certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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ETHICS

Human ethics for this project were obtained from the University of Adelaide Human Research Ethics Committee Approval (H-2020-142). Available on request.

All fish samples were obtained from markets and had been previously caught by fishers as part of their routine fishing operations, therefore no animal ethics were required.

SUMMARY

Globally, plastic pollution is becoming a major anthropogenic environmental issue. Since plastic was first invented in the early 20th century, plastic production has exponentially increased, with its durability and longevity causing problems for the environment. Plastic pieces enter the ocean via runoff, wastewater, or incorrect disposal, and physical forces such as wave action and weather cause it to break-down into microplastic (plastic pieces <5mm). Microplastic can be ingested by marine life, including fish that are commonly caught and sold for human consumption. I investigate the extent of plastic in fish globally, and uncover how this may be impacting the seafood industry. I focus specifically on Oceania, a heavily under researched region in regard to plastic pollution, to explore the abundance and type of microplastic that fish species are ingesting. I use a combination of approaches to characterise and explore the extent and effect of microplastic on the seafood industry.

Fish have been documented to ingest microplastic as early as the 1970's, and there has been an influx of research investigating fish ingestion of microplastics globally. I systematically reviewed all published literature on fish and microplastic, outlining that plastic is present in 49% of all fish world-wide, with an average amount of over 3.5 pieces per fish. I identified areas which require further research, finding limited information on fish caught and sold for human consumption in the Oceania region, which was an important pre-cursor for my subsequent research.

In the next two chapters, I explore the microplastic abundance and type in fish purchased from fish markets across southern Australia and in Fiji. Although I found that microplastic was ubiquitous across fish species, the abundance and type varied depending on the location, species and ecological traits. In southern Australia fish had less plastic than the global average, with only 36% of fish found to be ingesting microplastic. Fish from northern areas such as Queensland, had more microplastic (61%). When comparing countries, the plastic type and abundance differed significantly between similar species sampled in Australia and Fiji, with more plastic in fish from Australia, and a higher presence of microplastic fibres; sources of these are likely from synthetic clothing and fishing equipment. This research

ascertains that microplastic is present in fish species across the Oceania region, with potential implications for the seafood industry.

Subsequently, I surveyed key stakeholders in the fishing and seafood industries to gain information on their knowledge and perspectives of marine plastic pollution. Commercial and recreational fishers, as well as fishmongers, were interviewed to identify starting points to inform management suggestions to limit plastic pollution within the industry. By including stakeholder engagement, I ensured that the needs of fishers and fish mongers are addressed, and management options have stakeholder support, increasing the likelihood of compliance. My surveys suggested the implementation of education tools, provision of better disposal facilities and exploration of plastic-free fishing equipment options would all be appropriate mitigation strategies to lower the use and disposal of plastic. These suggestions highlight the scope for change within the seafood industry and identify potential steps forward in lowering the risks caused by this global problem.

In this thesis, I confirm the presence of microplastic from fish species across the Oceania region, and although there is less plastic than fish globally, it can still pose problems for seafood industry stakeholders. Global awareness of plastic pollution is increasing, and this thesis provides a substantial contribution to the scientific body of plastic pollution work. As well as providing concrete evidence of microplastic presence, all chapters of this thesis provide evidence to inform the seafood industry, as well as the general public about the potential presence, impacts and mitigation strategies of microplastic pollution. Ultimately this research provides tangible data to continue building global awareness, and empower management to effectively address plastic pollution, with solutions and education strategies that will be embraced by the seafood industry and the general public alike.

PUBLICATIONS AND CONTRIBUTIONS

CHAPTER TWO: Microplastic in fish – A global synthesis

Authors: Wootton N, Reis-Santos P, Gillanders BM.

Status: Published in *Reviews in Fish Biology and Fisheries,* Vol. 31, Issue 4, pp. 753-771. (Wootton *et al.* 2021c)

CHAPTER THREE: A comparison of microplastic in fish from Australia and Fiji

Authors: Wootton N, Ferreira M, Reis-Santos P, Gillanders BM.

Status: Published in *Frontiers in Marine Science*, Vol. 8, Article 690911. (Wootton *et al.* 2021a)

CHAPTER FOUR: Low abundance of microplastics in commercially caught fish across southern Australia

Authors: Wootton N, Reis-Santos P, Dowsett N, Turnbull A, Gillanders BM.

Status: Published in *Environmental Pollution*, Vol. 290, Article 118030. (Wootton *et al.* 2021b)

CHAPTER FIVE: Perceptions of plastic pollution in a prominent fishery: Building strategies to inform management

Authors: Wootton N, Nursey-Bray M, Reis-Santos P, Gillanders BM.

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CO-AUTHOR AFFILIATIONS

Bronwyn M Gillanders
School of Biological Sciences
University of Adelaide
Adelaide, South Australia, Australia

Patrick Reis-Santos
School of Biological Sciences
University of Adelaide
Adelaide, South Australia, Australia

Marta Ferreira
School of Marine Studies
University of the South Pacific
Suva, Fiji

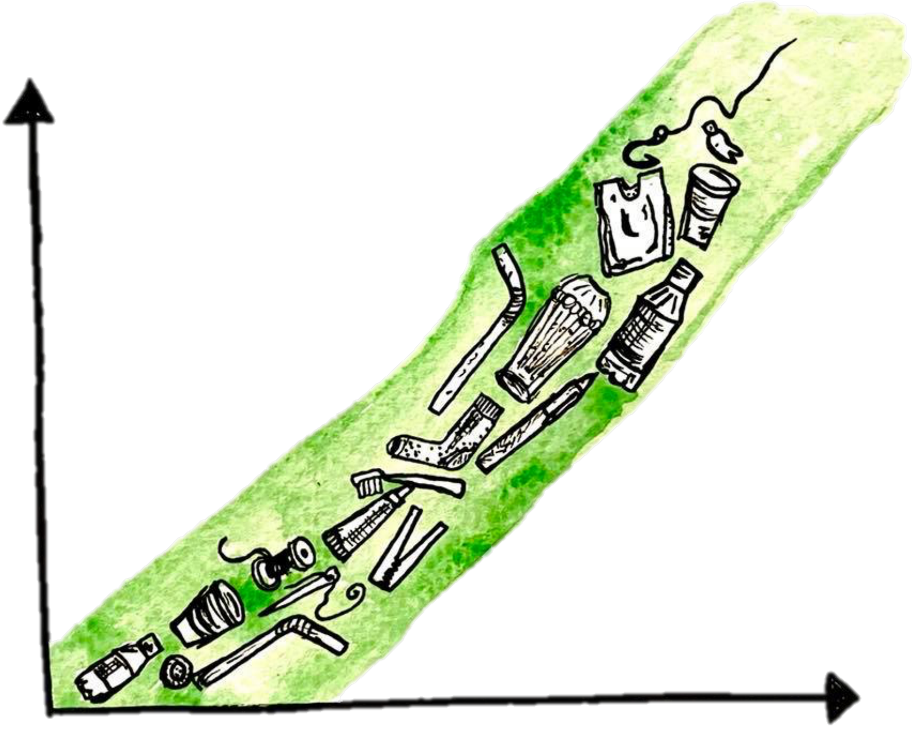
Natalie Dowsett
South Australia Research and
Development Institute (Food Sciences)
Adelaide, South Australia, Australia

Alison Turnbull
South Australia Research and
Development Institute (Food Sciences)
Adelaide, South Australia, Australia

Melissa Nursey-Bray
School of Social Sciences
University of Adelaide
Adelaide, South Australia, Australia

CHAPTER 1

GENERAL INTRODUCTION



1. General Introduction

1.1 Plastic presence and production

Humans have adapted naturally occurring materials including clay, stone, metal, mineral and plants for their own benefit since Palaeolithic times. Come the twentieth century, an entirely different adaption entered the market; the synthetic polymer (Laufer 1947). Originating in 1907, as Leo Baekeland mixed phenol and formaldehyde under intense heat and pressure, Bakelite was formed and the term ‘plastic’ was coined (Baekeland 1909). Here began the ‘Age of Plastics’ – a universal trillion-dollar industry, positioned to modify the way humans lived all aspects of life (Worm *et al.* 2017).

Plastic was first produced on a global scale following World War 2, and production has steadily increased since (Barnes *et al.* 2009; Andrady 2011). On an annual basis, we produce more than 380 million tonnes of plastic globally (Geyer *et al.* 2017), with this number set to escalate in conjunction with world-wide population growth. Plastics have become so popular that geologists are now considering pieces of plastic in the soil sediment to be the main indicator of the current geological time period, the Anthropocene (Waters *et al.* 2016; Zalasiewicz *et al.* 2016). For many daily uses, plastic has replaced historically orthodox materials, such as glass, pottery and wood, by providing a cheaper, lighter and longer-lasting substitute.

The term ‘plastic’ encompasses a large and diverse group of materials with distinct properties, uses and applications (Laufer 1947). Structurally, plastic is formed by many repeated chains of monomers, which combined with chemical additives form the base unit of all plastic – a polymer (Naka 2015). A variety of different polymers are now commercially available, allowing their function and application to be equally as variable. Plastic is used in abundance throughout the building, automotive, construction, medical, textile, packaging, agriculture and food industries (Geyer *et al.* 2017). Different sectors demand more plastic than others, with nearly 36% of plastic production effort focused on single-use packaging, 16% on building and infrastructure, and 14% on textiles (Geyer *et al.* 2017). The benefits that plastic materials provide to health and safety, energy, infrastructure and material preservation make them almost irreplaceable in the current global market (Andrady and Neal 2009). Many of the aforementioned industries would not have seen their recent advances without the widespread development of plastic.

1.2 Plastic as an emerging environmental threat

When plastics first rose to prominence in the 1950's they were thought to be harmless, but decades of overproduction, mismanaged waste disposal and consumerism have resulted in a large array of environmental concerns, with plastic pollution now recognised as one of the planet's largest environmental problems (Rochman *et al.* 2013; Worm *et al.* 2017). Globally there are multiple issues with the disposal of plastics, with plastic waste filling landfill and consequently polluting the land sediment (Hoornweg *et al.* 2013). Incinerating plastics does not address the problem either, as it produces toxic chemicals which leach into the air, land and water (Brereton 1996; Ágnes and Rajmund 2016; Verma *et al.* 2016). The durability and strength of plastic as a material is the main contributor to their survival and persistence in the natural world (Andrady and Neal 2009; Wesolowski *et al.* 2020). Such hard-wearing, resilient and hydrophobic compounds do not mix well with natural environments, causing major physical and chemical impacts on the environment.

Plastics differ in chemical composition, causing variability in their potential environmental impact (Hahladakis *et al.* 2018). The most common plastic polymers are polypropylene and polyethylene, both of which are utilised in film and sheets for packaging, structural building materials and plastic bags. Due to their insolubility in water and larger molecular weight, polypropylene and polyethylene have lower environmental risk (Al-Sammerrai and Al-Nidawy 1989; Maddah 2016). Other polymers such as polyvinyl chloride, polyurethane, polystyrene and polycarbonate are more problematic as they contain increased chemicals and additives which may cause health problems in both humans and other organisms (Lithner *et al.* 2011; Worm *et al.* 2017). These include plasticisers such as phthalates and other synthetic compounds like Bisphenol A (BPA) and Bisphenol S (BPS), all of which can act as hormone disruptors. The chemical nature of these plastics limits their potential to be appropriately reused and recycled, challenging future goals of limited plastic use and the 'circular economy’. Further to this, plastics in the marine environment can act as a ‘chemical cocktail,’ where the chemicals added during manufacturing combine with those already present in seawater to accumulate on the plastic (Rochman 2015). This causes concern surrounding the complex combination of chemicals that are binding to marine plastics, and the potential these have to negatively affect marine life which consume them (Rochman 2015).

1.3 Microplastic

Size categories are also frequently used to classify types of plastic, including macroplastic (<200mm), mesoplastic (5-200mm), microplastic (1μm-5mm) and nanoplastic (<1μm) (Worm *et al.* 2017). However, these categories are disputed and there is yet to be a global standard protocol for defining plastic size (Provencher *et al.* 2017; Gigault *et al.* 2018). Nevertheless, for the remainder of this thesis we will use the aforementioned size categories and ranges, with particular focus on microplastic.

Microplastic, the virtually invisible pieces of plastic, have managed to spread themselves extensively throughout the environment (Thompson 2015). By nature, microplastic is further classified as either primary or secondary microplastic (Cole *et al.* 2011). Primary microplastic are specifically manufactured to be of this size; they typically come in the form of microbeads in cosmetics, nurdles (the raw building blocks of plastic products), or synthetic fibres from clothing or fishing gear (Cole *et al.* 2011). Larger plastic pieces can be broken down into smaller parts by chemical, biological and physical mechanisms such as sunlight, wave action and weather (Figure 1.1). This type of microplastic is coined a secondary microplastic and occurs eventually to all plastic as it degrades in the environment.

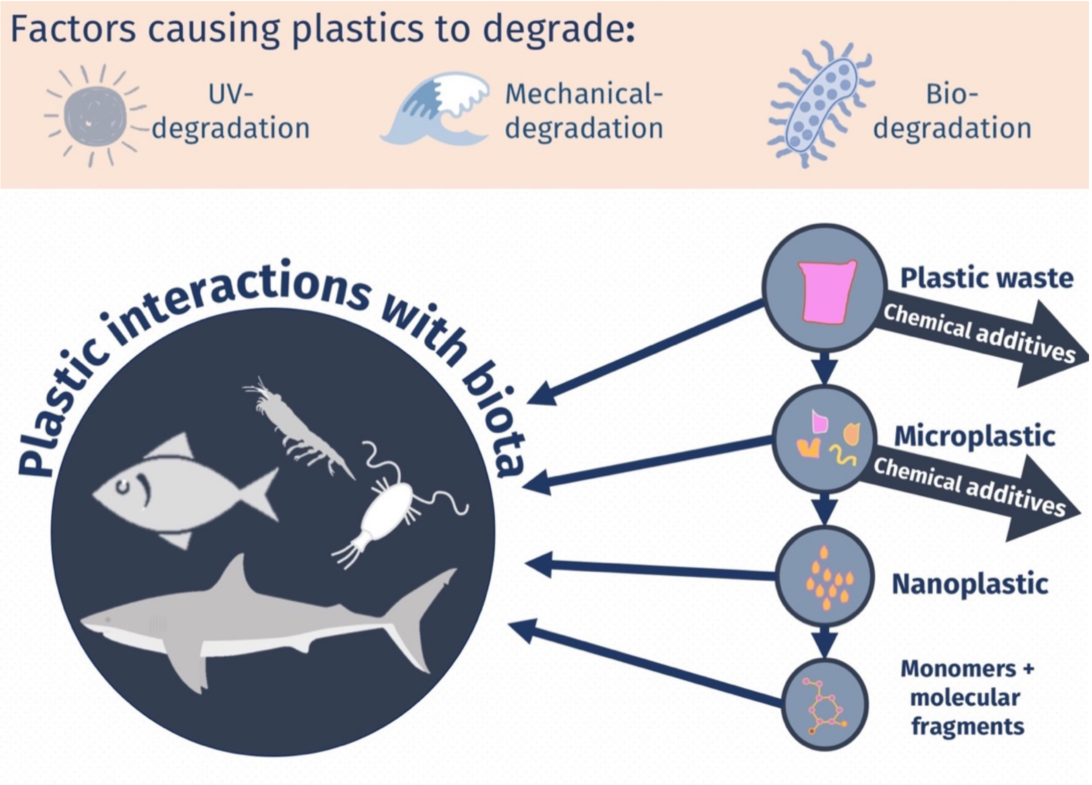


Figure 1.1: Plastic degradation in marine environments

1.4 Microplastic in aquatic environments

Microplastic are ubiquitous in the ocean; they are found around the coastlines, embedded in the Arctic ice, deep in the sediment and floating on the sea surface (Law *et al.* 2010; Obbard *et al.* 2014; Thompson 2015; Barrett *et al.* 2020). There are extensive problems associated with microplastic in ocean ecosystems – removal is difficult (Jambeck *et al.* 2015b), and as degradation occurs the presence of plastic in the ocean is perpetual (Derraik 2002; Thompson *et al.* 2004). Anthropogenic sources dispense between 4.8 and 12.7 million tonnes of plastic waste into the marine environment annually (Jambeck *et al.* 2015a). Humans viewing water as the universal solvent and an ‘out of sight, out of mind’ attitude is likely the largest contributor. This outflow of plastic waste is expected to grow alongside the current exponential growth in plastic production, with worst case estimates suggesting the cumulative amount of plastic may reach 250 million tonnes by 2025 (Jambeck *et al.* 2015a).

1.5 Microplastic affecting marine life

In the past half-century microplastic has entered the marine animal food chain. It's presence is both challenging and destructive, and its ingestion is now contributing to organism fatalities in the ocean (Gall and Thompson 2015). Over 700 marine species are reported to be affected by microplastic ingestion (Kuhn and van Franeker 2020), including turtles (e.g. Duncan *et al.* 2019), fish (e.g. Steer *et al.* 2017), seabirds (e.g. Provencher *et al.* 2018), barnacles (e.g. Goldstein and Goodwin 2013) and amphipods (e.g. Jamieson *et al.* 2019). As the plastic degrades into micro-pieces, the likelihood of it entering food webs increases (Browne *et al.* 2008). It travels up the food chain, from tiny organisms to larger commonly consumed fish species, via trophic transfer (Carbery *et al.* 2018)(Figure 1.2). From an ecotoxicological perspective, there are also other indirect effects on the organisms such as a decrease in reproductive performance, growth and behaviour changes (Wang *et al.* 2019; Wang *et al.* 2020). Current studies have mostly investigated these effects in a laboratory setting - Wang *et al.* (2019) found that fish fed polystyrene displayed reproductive endocrine disruption, oxidative stress and problems with prenatal development. Other studies have shown microplastic causing blockages throughout the digestive system (e.g. Lusher *et al.* 2013), nutritional and growth problems (e.g. Pedà *et al.* 2016; Jabeen *et al.* 2018) and inflammatory responses (e.g. Lu *et al.* 2016). In the wild, it is predicted that those affected by plastic may then be more susceptible to predators and more likely to catch diseases in their weakened state (Laist 1987; Derraik 2002; Worm *et al.* 2017).

Recent studies also show that consumed microplastic can migrate to tissues and other parts of the organism, including the liver and muscle (Browne *et al.* 2008; Courtene-Jones *et al.* 2017; Ding *et al.* 2018). Furthermore, particular toxic substances contained in plastic can be released when ingested by the organism, accumulating in their fatty tissue, including plasticisers, colouring agents and monomer residues (Lithner *et al.* 2011; Lavers *et al.* 2014; Lavers and Bond 2016; Worm *et al.* 2017). Microplastic is capable of adsorbing to several classes of organic pollutants due to the hydrophobic properties, which could be transferred to organisms and enter the food-web (Setälä *et al.* 2014; Woodall *et al.* 2014). Despite this, the effects of toxic substances from plastics on organisms is less well known than entanglement and ingestion, most likely due to difficulties in methodology (Worm *et al.*

2017) and less media coverage (Henderson and Green 2020). It is fundamental that the presence of microplastic in marine life is documented, as knowledge on the types and abundance of microplastic will provide consequential information on other potential toxic effects on affected marine species.

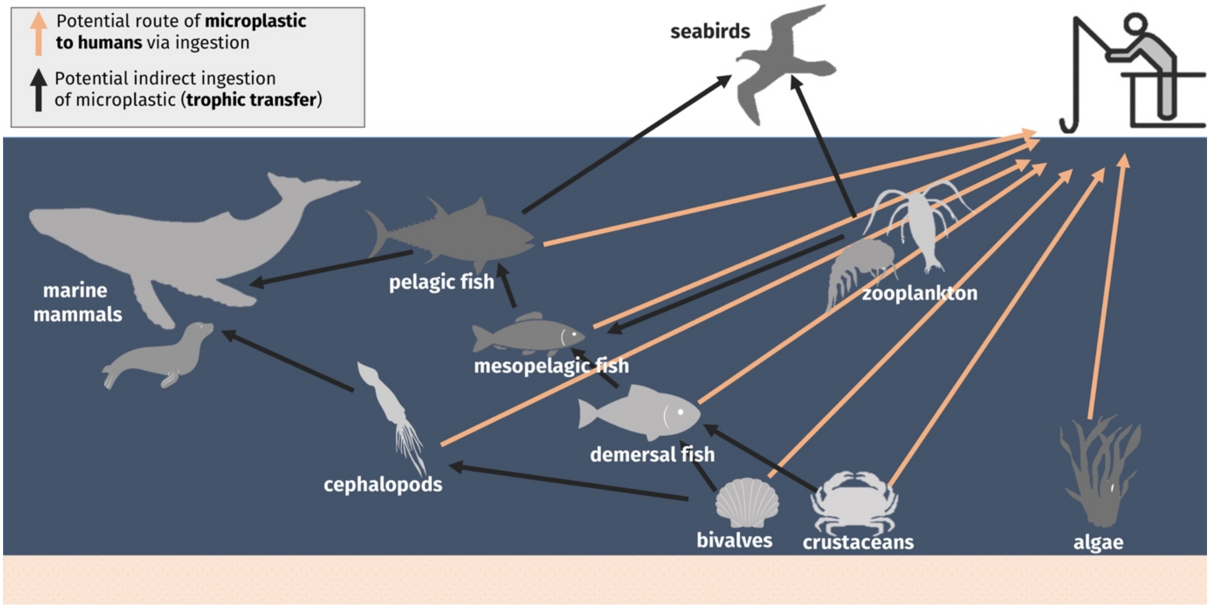


Figure 1.2: Interactions of marine organisms with microplastic, potential routes for trophic transfer and microplastic ingestion by humans.

1.6 Seafood safety

Microplastic has been found in much of what is regularly in our fridge and pantry – salt (Kim *et al.* 2018), beer (Liebezeit and Liebezeit 2014), honey (e.g. Diaz-Basantes *et al.* 2020), sugar (Liebezeit and Liebezeit 2013), drinking water (Pivokonsky *et al.* 2018) and seafood (Rochman *et al.* 2015). In regards to seafood, ocean plastic pollution provides a likely uptake mechanism for microplastic consumption in marine species, and hence a potential route for human contamination (Smith *et al.* 2018; Mercogliano *et al.* 2020). Investigating the extent and spread of microplastic across seafood species is necessary, as baseline information in this realm is essential to create steadfast solutions in the future. It is important that we go above the assessments of biota and environment contamination and evaluate wild marine seafood that is being harvested for human consumption. It is essential that we provide tangible evidence that microplastic is present in seafood species, particularly in locations where we currently have limited information, such as Oceania (Sequeira *et al.* 2020).

Fisheries and aquaculture products have well-known health benefits, providing an essential protein source for a large portion of the global population as well as fatty acids that presently cannot be sourced alternatively (Tacon and Metian 2013). In 2017, fish products accounted for 17 percent of the global population's animal protein intake, providing some developing countries such as Bangladesh, Cambodia and some small island developing states with more than 50 percent of their animal protein (FAO 2020). Globally, seafood consumption is on the rise, increasing by 1.5 percent per year (FAO 2020). In 1961, the world population consumed on average 9.0kg of seafood per person; by 2018, this had increased to 20.5kg (FAO 2020). These consumption patterns vary regionally, with Oceania having the highest seafood intake per capita of 24.8kg annually (FAO 2017). With the seafood industry providing food sources for such a large portion of the population, it is essential that any threats that could negatively impact on fisheries sustainability or safety are identified. With the parallel increase in plastic production, poor waste management and seafood consumption globally, knowledge surrounding the risks that plastic and microplastic may pose to seafood safety is pertinent.

Perhaps the most unnerving aspect of microplastic ingestion by human consumers is the unknowns around long term exposure, whether by seafood pathways or other sources (Mathur *et al.* 2014; Vethaak and Legler 2021). Considering the period between the commercialisation of plastic and present-day concerns is yet to reach the length of the average western human lifetime, there is likely still further information to be uncovered. Whether this information holds large or small implications to long term health, it is prudent for efforts to be made to minimise plastic consumption (Vethaak and Legler 2021).

1.7 Plastic in the fishing industry

Statistics show that the fishing industry itself is one of the biggest contributors to marine plastic waste, with more than 10% of ocean plastics traced back to fishing equipment (Thomas *et al.* 2019). In certain oceanic regions these contributions can be even higher, such as the Great Pacific Garbage Patch, where almost half the plastic originates from fishing sources (Lebreton *et al.* 2018). Fishing equipment is commonly made of plastic materials, as a result of plastics' longevity and durability. Trawl nets, long lines, hand lines, gillnets and pots are popular fishing gear, the majority of which are produced from plastic materials. Fishing equipment can be accidentally lost due to weather conditions causing the gear to become brittle and fragment. Further, loss of plastic fishing equipment can be a result of fishers purposely abandoning gear (e.g. due to illegal fishing activities), operational pressure (e.g. illegal overuse of limited gear in certain time periods), weather and habitat conditions (e.g. a line getting snagged on a rock or stuck in a seabed), or lack of onshore disposal sites for gear due to price or access problems (FAO 2017). The wide application of plastic in aquaculture, from traditional rope cultures to intensive cage farming, provides another source of marine plastic pollution (FAO 2017); with inadequate waste management and staff awareness providing ample opportunities for waste and loss (Skirtun *et al.* 2022). Likely seafood species housed in aquaculture and mariculture facilities are exposed to microplastic from their living environment, causing additional concern for these industries (Mathalon and Hill 2014).

Irrespective of the entry pathway, plastic waste is clearly penetrating the marine system via fisheries and therefore ascertaining knowledge gaps and education potential from the people who work within the fishing industry is an essential first step in providing ongoing solutions to limit the presence of this problematic and persistent contaminant. From here, policy and management strategies can be moulded to help limit plastic pollution in the seafood industry, with the support of the industry itself.

Thesis aims and scope

This thesis investigates the global presence of microplastic in fish, focusing on its occurrence in species purchased through fish markets in the Oceania region. It places these data in an international context and identifies potential solutions within the fishing industry to help lessen the impact of marine plastics. The thesis is organised as four data chapters (Chapters 2 – 5), which are written as scientific papers that have been either published or submitted for publication in peer-reviewed journals. Hence, each chapter has the structure of a stand-alone study with its own targeted introduction and discussion. Data chapters are presented in such a way that previous information and data are built upon, drawing on findings in earlier chapters and using that knowledge to guide future research (Figure 1.3). I begin by synthesising current literature on the presence of microplastic in fish globally (Chapter 2), discovering that there are limited data surrounding fish from Oceania, particularly those purchased through seafood markets. I then present two case studies from this region; one comparing the quantity and type of microplastic in fish between two different Oceania countries (Chapter 3); the other investigating the presence of microplastic in fish across all of southern Australia and investigating the potential ecological drivers of increased microplastic ingestion (Chapter 4). Finally, I identify potential solutions to limit future use of plastic in a prominent Australian fishery, providing suggestions for management and policy to limit plastic use within the seafood industry (Chapter 5). Chapter 6 synthesises my main findings, highlighting the importance of quality microplastic research, as well as directions for future research.

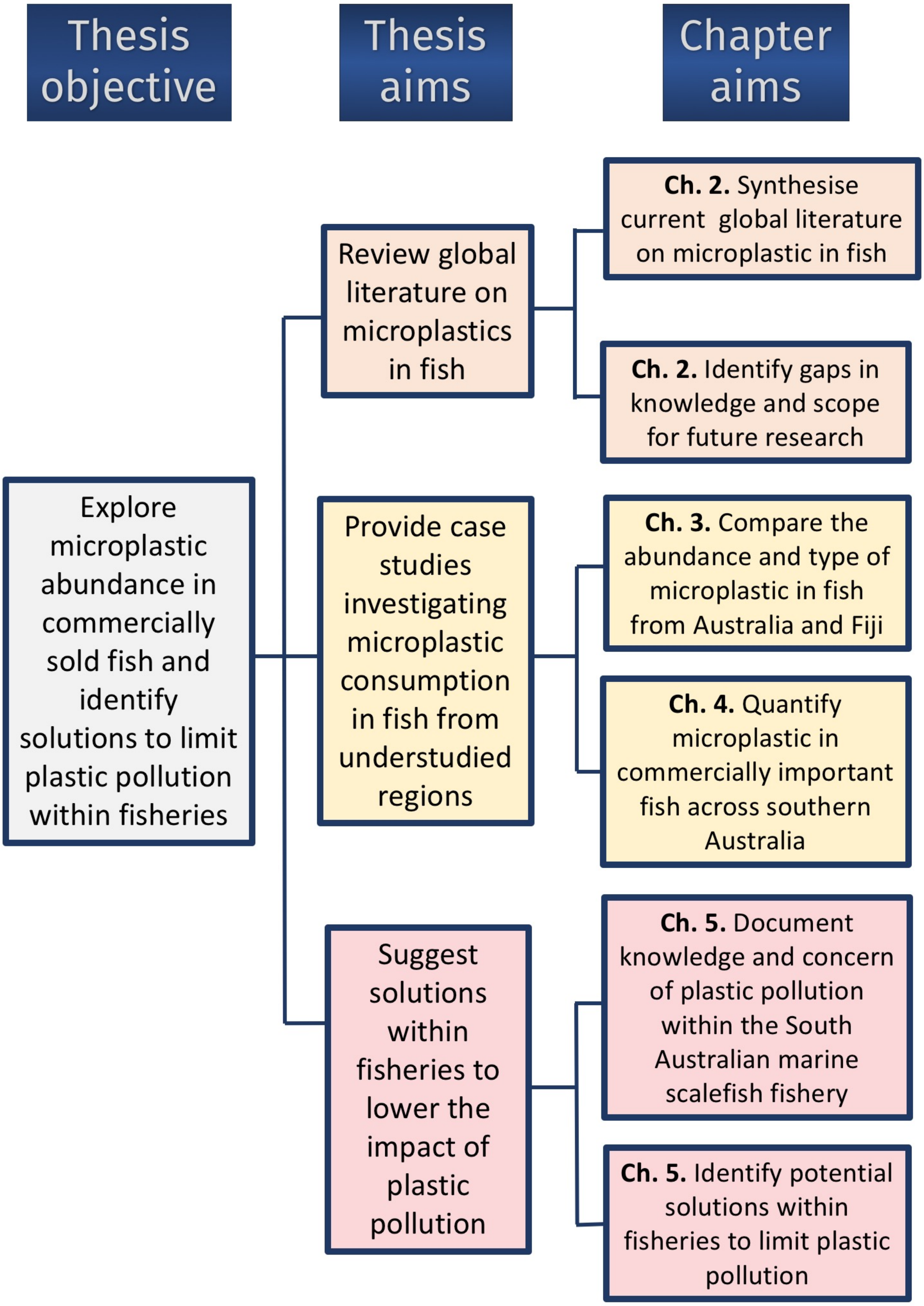


Figure 1.3: Schematic representation of overarching thesis objective and aims and individual chapter aims for this thesis.

An overview of each chapter and its objectives is detailed below.

Chapter One: Introduction

This chapter is a broad overview of research on plastic in the environment, with relevant information from previous studies.

Chapter Two: Microplastic in fish – A global synthesis

Objective: Systematically synthesise the available literature investigating microplastic presence in fish to discover global drivers of plastic consumption and highlight gaps in knowledge.

Literature on ingestion of microplastic by fish is rapidly growing, yet there is no systematic review of this in fish from all aquatic environments. This chapter uses a systematic review approach to compile the literature investigating consumption of microplastic by fish. Subsequently, using only studies where comparable and high standard methodological techniques are utilised, this chapter investigates which ecological drivers (e.g. diet, habitat), regional location, or source of capture (e.g. wild-caught or aquaculture) may be triggering higher levels of microplastic contamination. This chapter was an important precursor in highlighting gaps in information which were then filled in chapters three and four.

This chapter has been published in *Reviews in Fish Biology and Fisheries* (Wootton *et al.* 2021c)

Chapter Three: A comparison of microplastic in fish from Australia and Fiji

Objective: Quantify the presence of microplastic in the gastro-intestinal tract of fish purchased from seafood markets in Australia and Fiji, comparing how differing lifestyles, waste management strategies and population sizes could be influencing plastic consumption.

Data on microplastic presence in fish commonly caught and sold for human consumption in the South Pacific is limited. This chapter fills this gap by sampling four popular eating fish from both Fiji and Australia, counting their plastic abundance and testing their polymer type using Fourier Transform Infrared Spectroscopy. I use these data to compare the variations in microplastic amount and type, and draw on any potential influences on these differences.

This chapter has been published in *Frontiers in Marine Science* (Wootton *et al.* 2021a)

Chapter Four: Low abundance of microplastics in commercially caught fish across southern Australia

Objective: Quantify and compare the amount of plastics present in fish sold for human consumption across southern Australia (Western Australia, South Australia, Victoria, Tasmania and New South Wales).

In this chapter, I sampled nine commercially important fish species from five southern Australian states. By collecting this information, I was able to compare differences in plastic abundance between regions within Australia, as well as compare them to international studies. Further, I investigated ecological drivers causing microplastic ingestion, comparing diet and habitat of species sampled to see how this could be influential in an increase in plastic consumption in particular fish species.

This chapter has been published in *Environmental Pollution* (Wootton *et al*. 2021b)

Chapter Five: Perceptions of plastic pollution in a prominent fishery: Building strategies to inform management

Objective: Document the knowledge of plastic pollution within the South Australian marine scalefish industry, identifying future solutions to limit the impact of plastic pollution.

My final data chapter combines the knowledge I gained from the previous three data chapters, acknowledging the potential impact microplastic could have on fisheries. I interviewed key stakeholders in the South Australian Marine Scalefish Fishery, including recreational and commercial fishers and fish mongers, to understand how plastic pollution is perceived within the industry. Using this platform, I was able to isolate their differing levels of concern and suggest some solutions to help lower plastic in our oceans in the future, contributing to fishery and waste management, and informing policy.

This chapter has been published in *Marine Policy* (Wootton *et al.* 2022)

Chapter Six: General Discussion

This chapter provides a broad discussion and synthesis of the key results from the data chapters of this thesis, suggesting options for future research. I discuss the importance of quality microplastic research, and how this will help guide industry and key stakeholders to take action.

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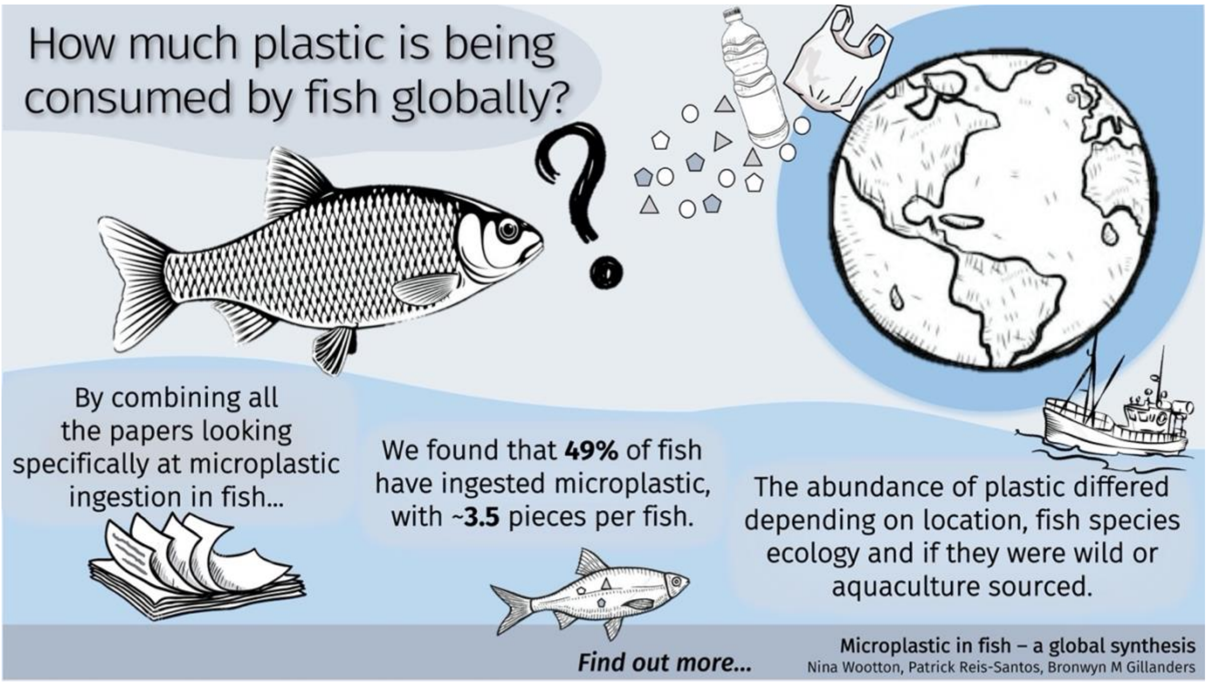
CHAPTER 2

MICROPLASTIC IN FISH – A GLOBAL SYNTHESIS



How much plastic is being consumed by fish globally?

By combining all the papers looking specifically at microplastic ingestion in fish...

We found that **49%** of fish have ingested microplastic, with ~**3.5** pieces per fish.

The abundance of plastic differed depending on location, fish species ecology and if they were wild or aquaculture sourced.

Find out more...

Microplastic in fish – a global synthesis
Nina Wootton, Patrick Reis-Santos, Bronwyn M Gillanders

Statement of Authorship

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Principal Author

Name of Principal Author (Candidate)	Nina Wootton		
Contribution to the Paper	Contributed to design of the study, reviewed literature, collected and analysed the data, applied statistical analyses, wrote the manuscript and acted as corresponding author.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	5/10/21

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Patrick Reis-Santos		
Contribution to the Paper	Contributed to design and development of the study, advice and suggestions throughout, provided comments and feedback on manuscript.		
Signature		Date	06/10/2021

Name of Co-Author	Bronwyn Gillanders		
Contribution to the Paper	Contributed to design and development of the study, advice and suggestions throughout, provided comments and feedback on manuscript.		
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REVIEWS

Microplastic in fish – A global synthesis

Nina Wootton · Patrick Reis-Santos · Bronwyn M. Gillanders

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Abstract Plastic contamination is ubiquitous, with plastic found in hundreds of species of aquatic wildlife, including fish. Lacking a broad and comprehensive view of this global issue across aquatic environments, we collated and synthesised the literature that focuses on microplastic ingestion in fish from marine, freshwater and estuarine environments. First, we assessed how the approaches used to investigate microplastic in fish have changed through time, comparing studies globally. A greater understanding of this changing landscape is essential for rigorous and coherent comparisons with only 42% of published studies following recommended approaches of chemical digestions and verifying plastic via polymer identification. Then, using this subset of studies, we found that 49% of all fish sampled globally for microplastic ingestion had plastic (average of 3.5 pieces per fish), with fish from North America ingesting more plastic than fish from other regions. We then evaluated the role of environment, habitat, feeding strategy and source (i.e. aquaculture or wild-caught) in the ingestion of microplastic. Research from marine environments dominated (82% of species) but freshwater fish ingested more plastic, as did detritivores, fish in deeper waters and those from aquaculture sources. By collating global microplastic research we identified regional disparities and key knowledge gaps that support research towards freshwater environments and aquaculture sources. Overall, we highlight the need for consistent guidelines in methods used to evaluate microplastic in fish, to ensure data are unambiguous, comparable and can be widely used to support mitigation and management strategies, inform potential policy actions, and evaluations of environmental, food safety, and human health goals.

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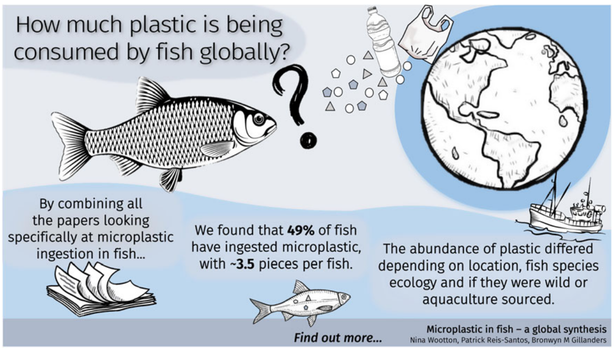
N. Wootton (✉) · P. Reis-Santos · B. M. Gillanders
Southern Seas Ecology Laboratories, School of Biological Sciences, University of Adelaide, Adelaide,
SA 5005, Australia
e-mail: nina.wootton@adelaide.edu.au

Graphic abstract



Keywords Microplastic · Fish · Ingestion · Contamination · Fisheries · Aquaculture

Introduction

Plastic was first synthesised in the early 1900s, with plastic production and usage becoming increasingly popular from the 1950s, and now ubiquitous in all aspects of modern life (Worm et al. 2017). It was not until the 1970s that we see the first official reports on the presence of plastic debris in the marine environment (Rochman 2018), although it is likely that it was occurring well before then. Since then, the scientific literature recording plastic in aquatic environments and wildlife has grown exponentially (Dris et al. 2015; Worm et al. 2017), with increasing focus on the presence and effects of microplastic (plastic $\leq$ 5 mm) (Borja and Elliott 2019). Overall, microplastic ingestion has been documented in over 700 marine species and there are many studies showing plastic in sea turtles, sea birds and other charismatic megafauna, such as whales and dolphins (Besseling et al. 2015; Gall & Thompson 2015; Germanov et al. 2018; Kuhn & van Franeker 2020; Worm et al. 2017). In fish, microplastic has been found in a suite of species from shallow to deep waters, and from freshwater, marine and estuarine aquatic systems (Kasamesiri & Thaimuangphol 2020; Talley et al. 2020; Valente et al. 2019; Yuan et al. 2019). Moreover, fish collected from markets, wild-caught and those bred for aquaculture purposes have all been found with varying amounts of microplastic present in their guts, gills and tissues (Rochman et al. 2015; Wootton et al. 2021b; Wu et al. 2020).

Ingestion of microplastic in fish can occur either via primary ingestion, meaning fish are ingesting the plastic directly, usually by mistaking it for food or accidentally ingesting it (Worm et al. 2017); or by secondary digestion when prey that already contains plastic is ingested (Watts et al. 2014). The latter is referred to as trophic transfer and can lead to bioaccumulation and biomagnification (Farrell & Nelson 2013; Provencher et al. 2019a; Zhang et al. 2019), and potentially to increased accumulation of microplastic in higher trophic level predators, although this is still underevaluated (Carbery et al. 2018; Miller et al. 2020). Alternative exposure routes, such as gills, can also allow microplastic uptake in fish. In addition to trophic level, feeding strategy, biogeography, habitat and ecological niche likely impact the amount and type of plastic ingested by fishes, as plastic pollution varies globally, and there is, for instance, more plastic contamination in the sediment than the water column (Harris 2020; Woodall et al. 2014). Despite growing evidence of microplastic ingestion in fish, we still lack a quantitative comparison of microplastic ingestion across aquatic environments, from fresh to marine water. Identifying potential ecological and geographical drivers of microplastic ingestion in fish is essential in determining how plastic may be affecting fish globally, and spreading across aquatic food webs. Ultimately, a greater understanding of global plastic contamination and how ecology or biogeography may exacerbate or buffer the risk or propensity for fish to be exposed to microplastics will go a long way in informing coherent research strategies to safeguard not only environmental, but also food safety and human health requirements, considering the commercial value of many fish and fisheries.

Commercial fish species face a myriad of anthropogenic threats, including climate change, overfishing, habitat degradation and pollution (Baechler et al. 2020). From a global food perspective, fisheries and aquaculture are essential for sources of dietary protein, providing a critical proportion of the world-wide food supply (Béné et al. 2015), and up to 10% of the population with important income and livelihood (Béné 2006; FAO 2020). As global seafood consumption continues to rise, understanding the potential threats of microplastic pollution is crucial.

With growing global interest in plastic pollution in fish, a range of laboratory methods and sampling techniques have been used (Hermsen et al. 2018; Vandermeersch et al. 2015; Wesch et al. 2016). The range of applied methods make it difficult to accurately compare studies (Provencher et al. 2019b). For example, separate studies in Newfoundland, Canada report vastly different results. One found that none of the fish sampled ingested microplastic (Liboiron et al. 2018), whilst another study found 73% of fish contained plastic (Wieczorek et al. 2018). It is difficult to ascertain whether these differences are driven by the species or the methods used because Liboiron et al. (2018) used a 1 mm sieve and the naked-eye to identify plastic, whereas Wieczorek et al. (2018) undertook a more rigorous approach, including a chemical digestion and microscope identification with a smaller sieve size (0.7 μm), as well as Fourier Transfer Infrared Spectroscopy (FTIR) to validate plastic identification. Thus, differences among these and other studies likely echo the accuracy of the applied methodologies. Ultimately, it has been demonstrated that to produce accurate and robust results, microplastic studies need a large sample size of specimens (minimum $\geq$ 10), must use chemical digestion and as small as possible sieves to remove organic matter, adopt a range of quality control measures, and chemically verify the polymers (Hermsen et al. 2018; Lusher et al. 2017; Markic et al. 2019; Wesch et al. 2016).

Despite the growing interest in microplastic pollution, we lack a global and comprehensive synthesis of microplastic ingestion in fish that encompasses all aquatic environments. In particular, an evaluation that contributes to understanding the underlying drivers of microplastic ingestion in fish and allows for accurate comparisons on the abundance of microplastic by focusing on studies that followed standardised and recommended practices in reporting microplastic contamination (Markic et al. 2019). Reviews to date generally only investigate the presence of plastic in marine life broadly, with a brief component covering fish (Gall & Thompson 2015; Kühn et al. 2015; Ryan 2019; Worm et al. 2017), or focused only on limited literature and period of time (Sequeira et al. 2020; Wang et al. 2020). Other studies only addressed marine fish (Markic et al. 2019; Santos de Moura & Vianna 2020; Savoca et al. 2021), exclusively freshwater and estuarine environments (Collard et al. 2019; Parker et al. 2021), or address the potential effects that microplastic in seafood could have on human health (De-la-Torre 2020; Kwon et al. 2020). While some studies have focused on both marine and freshwater environments (e.g. Coverton et al. 2021) they have been less exhaustive in their literature search. Thus, there is a significant gap in the literature summarising microplastic ingestion in fish across all aquatic environments globally. Therefore, we analysed the current global literature specifically investigating microplastic ingestion in fish to produce a critical synthesis comparing global plastic loads among environments, including fish from aquaculture. Simultaneously we evaluate geographic, taxonomic and ecological patterns of microplastic presence in fish species. Importantly, considering the abundance of diversity in methodological approaches in the literature we also synthesise the prevalence of studies that apply the recommended quality practices (Markic et al. 2019). By using methodologies which are clearly reproducible, with reduced operator dependency on separation and identification of microplastic, the estimation of microplastic contamination is strengthened (Hidalgo-Ruz et al. 2012; Lusher et al. 2017; Provencher et al. 2017). In doing so, we are able to increase our understanding of the variation in microplastic contamination in fish globally, across all environments, and reinforce our ability to evaluate the future health risks to humans due to dietary exposure. Overall, by comparing literature using standardised methods we reduce conflicting standards and create a robust and reproducible approach that provides an accurate representation of the current state of the art and an essential baseline for future comparisons.

This study aims to provide a global synthesis of microplastic ingestion in fish, across all aquatic environments. Specifically, we (1) evaluate if the methods used to determine microplastic in fish have improved; then (2) using only studies that apply recognised quality practices, we compare the differences in microplastic abundance in fish from different geographical regions, environments and habitat zones, with different feeding strategies, and collected from different sources (wild versus aquaculture); and finally (3) we identify key knowledge gaps, and outline future perspectives and research priorities that are needed to foster the robust cross-disciplinary evidence required to understand the impacts of marine plastic contamination, inform potential management and policy actions.

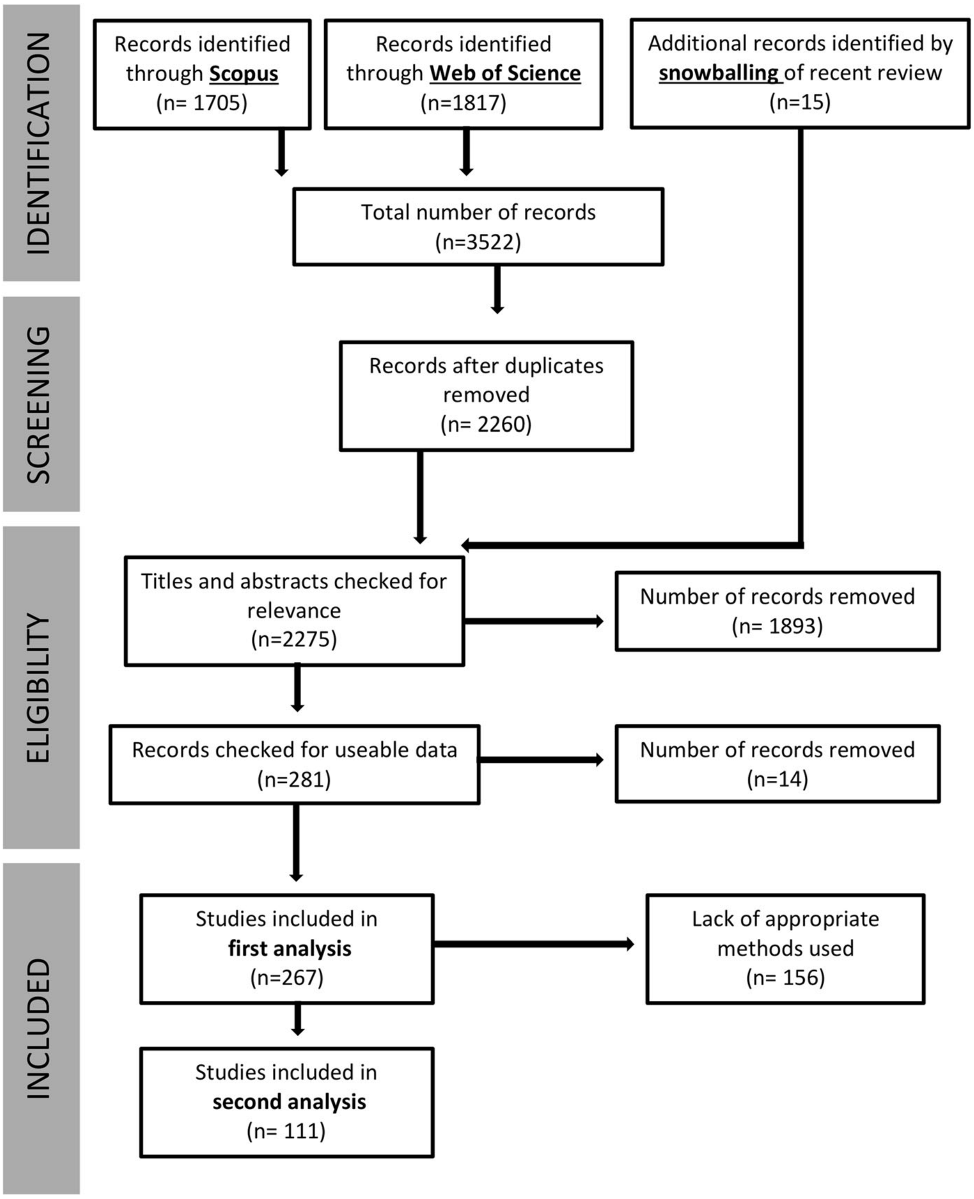


Fig. 1 Flow chart outlining the criteria for the inclusion of studies in the systematic literature review following the PRISMA (Preferred Reporting Items for Systematic Reviews) framework

Methods

Study selection and assessment

We conducted a systematic literature search investigating microplastic presence in fish. Given its preference and wide acceptability in the scientific community, we followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Moher et al. 2010). The literature search was undertaken using both the Web of Science and Scopus scientific citation databases (June 16th, 2021). The title, abstract and keywords were searched using the following search criteria: (*plastic*) AND (fish*) AND (consum* OR ingest* OR eat*) where the asterisk acts as a wildcard allowing all derivatives of the words to be identified (e.g. *plastic* also searches for microplastic, microplastics, plastics). These search terms resulted in 1705 studies in the Scopus database, and 1817 studies in the Web of Science database, which, once duplicates were removed, represented a total of 2260 studies that were investigated further (Fig. 1). Additionally, we identified ten recent broad review studies investigating themes related to fish and microplastic (Azevedo-Santos et al. 2019; Collard et al. 2019; Danopoulos et al. 2020; Markic et al. 2019; Provencher et al. 2019a; Santos de Moura & Vianna 2020; Savoca et al. 2021; Sequeira et al. 2020; Walkinshaw et al. 2020; Wang et al. 2020), and implemented a snowballing method, where a further 15 studies were identified in the reference lists (total number of studies 2275). Snowballing involves using the reference list of review papers to identify additional papers, and allowed the identification of studies that used species specific names in their title and abstracts.

The title, abstract and keywords of the 2275 studies were initially scanned and 281 studies selected as they sampled fish stomachs and/or gastro-intestinal tracts for microplastic content. Further eligibility checks removed 14 studies as they were not relevant, because they did not investigate microplastic in fish, focused on laboratory controlled exposures or did not have the required information (Table S1, Fig. 1). A total of 267 studies were included in our first analysis to analyse if methods used to determine microplastic in fish and how methods have changed over time (Table S1).

Due to the large variation in the methodologies used in microplastic research, we took particular care to select studies that had consistent, comparable methodological approaches, and followed the recommended quality practices for microplastic research. Only studies that met four critical criteria, and followed recommended quality practices for microplastic research, were included in our second analyses to assess differences in microplastic abundance in fish. These included:

1. A minimum sample size of ten or more individuals per species used to quantify microplastic contamination
2. A chemical digestion step (e.g. KOH, NaOH or other), and used a microscope and sieve (less than 200 μm) to initially identify the microplastic.
3. A chemical analysis procedure to validate microplastic identification and polymer type (e.g. Raman-Spectroscopy or Fourier Transfer Infrared Spectroscopy).
4. Use of quality assurance and quality controls

We chose these criteria based on recent literature examining the methods used to investigate the prevalence of microplastic in biota, and outlined recommendations and quality practices for microplastic research (Hermsen et al. 2018; Lusher et al. 2017; Markic et al. 2019). Overall, 111 studies met our criteria and were included in our global evaluation of the ingestion of microplastic by fish (Table S1). Our framework ensures that all selected studies followed standardised procedures and methodologies, and thus extracted data met minimum quality criteria.

Data and information on a suite of variables, including study environment (marine, estuarine or freshwater), species, and microplastic contamination were taken directly from the text, graphs or data tables of individual studies (See Table 1). Studies were organised by regional groups based on where the fish were caught or purchased, and information was collected from the methods section of the study. Countries from Central America were grouped with North America, and Oceania included the South Pacific and Australia. The method used to source the fish (e.g. Wild-caught, Aquaculture, Fish-market) was obtained from the methods section of the study. Although we assumed that fish-market sourced fish were wild-caught species, it was not stated in most studies so we chose to keep them as a separate group. Microplastic ingestion in fish is most commonly measured as the frequency of occurrence, and expressed as a percentage (%) of individual fish within a species containing at least one piece of microplastic. Plastic load, the mean amount of microplastic per fish from all fish sampled in a species, is another key measure used in microplastic research. Due to a large number of individual fish that consumed no microplastic pieces (and therefore had a microplastic count of zero), it is possible for plastic loads to be less than one for a species (e.g. of 20 fish sampled 10 individuals consumed one piece of microplastic each, the plastic load for this species is 0.5). This information was collected from the species in each study, so mean and medians of the plastic load can be non-integer values as the plastic load had previously been calculated in the study. Also, in cases where studies reported plastic load using only fish which had consumed microplastic, the load was recalculated to include all fish sampled, even those with no microplastic present. All studies contained either the frequency of occurrence (or an average across the study) as well as plastic load data, with the exception of three studies (Akoueson et al. 2020; Bagheri et al. 2020; Ferreira et al. 2020; Garcia et al. 2021), which were removed from the plastic load data set. Where necessary, data

Table 1 Summary and description of variables extracted from studies. The trophic level, habitat and feeding strategy information for each species were sourced from FishBase (Froese and Pauly 2019). Fish were further categorised based on the guild approach (Elliott et al. 2007)

Variables	Description and range
Publication year	1982–2021
Year the fish were caught	1982–2020
Search engine	Web of Science, Scopus, Review snowballing
Environment	Marine, Estuarine, Freshwater
Method used to source fish	Wild-caught, Fish-market, Aquaculture
Location, country and the longitude and latitude	e.g. Melbourne, Australia, (− 37.8136, 144.9631)
Region and their regional longitude and latitude	North America, South America, Europe, Middle-East, Africa, Asia, Oceania
Life history stage	Larvae, Juvenile, Sub-adult, Adult
Method	Digestion, Naked-eye, Microscope
Sieve size	e.g. 36 µm, 1 mm, no sieve
Verification test	FT-IR, Raman-spectroscopy, Hot needle test
Number of species examined in study	1–51
Sample size (number of fish per species)	< 10, ≥ 10
Family, species and common name	e.g. Mugilidae, *Mugil cephalus,* sea mullet
Exact sample size per species	1–884
Trophic level	2–4.5
Habitat	Reef-associated, Pelagic, Benthic-demersal, Deep sea
Feeding strategy	Detritivore, Herbivore, Omnivore, Carnivore
Frequency of occurrence (%)	Percentage of fish of each species containing at least one piece of plastic
Plastic load	Amount of plastic per individual fish averaged for all individuals of that species examined

were extracted from published figures using the desktop version WebPlotDigitizer (Rohatgi 2020). For every species present in the examined studies, trophic level, feeding strategy (e.g. detritivore, herbivore, omnivore and carnivore) and habitat (e.g. reef-associated, pelagic, benthic-demersal, deep-sea) classifications were sourced from FishBase (Froese and Pauly 2019).

Statistical analysis

In the first part of our analysis, to evaluate the use of quality practices in microplastic literature, we used all 267 studies with information on microplastic in fish gastrointestinal tracts, regardless of applied methodologies. The number of studies using different identification methods (sample size of more or less than 10; digestion, microscope or naked eye; and presence or absence of a verification test) were counted per publication year. This allowed us to understand the global distribution of the studies, if particular methods were being used in some regions more than others, and ascertain where information to date is still ambiguous or lacking validation. Then, to carry out a global comparison of the ingestion of microplastic by fish, we focused on the comparable, quality practices dataset. Data were collected from every species examined in the 111 studies, totalling 506 observations from 338 different species (Table S2). The differences in species and observations is due to some species being reported over multiple studies (e.g. *Engraulis encrasicolus* were reported in seven different studies, sampled in different locations and different years (Bakir et al. 2020; Collard et al. 2017; Filgueiras et al. 2020; Kazour et al. 2019; Lopes et al. 2020; Renzi et al. 2019; Savoca et al. 2020)).

Due to the data not meeting the normality assumptions of ANOVA tests, Kruskal–Wallis H tests were applied to the frequency of occurrence and plastic load data to determine if there were any statistically significant differences between variables. If a difference was detected a pairwise Wilcox test was used, to determine which specific variables differed. All statistical analyses were performed in R Studio (Version 1.2.5019) using the maps (Becker et al. 2018), ggplot2 (Wickham 2016), doBy (Højsgaard & Halekoh 2020), scatterpie (Guangchuang 2019) and rgeos (Bivand and Colin 2020) packages.

Results

Prevalence of quality practices in microplastic literature

In total there have been 267 peer-reviewed studies published globally on the ingestion of microplastic by fish, with the first study published in 1972 (Carpenter et al. 1972). In the last 10 years, there has been a steady increase in the number of studies on microplastic in fish, with 87 studies published in 2020, compared to one in 2010 (Fig. 2). Over this time period, there is an evident trend regarding the adoption of the recommended quality practices regarding microplastic research, with higher ratios of studies using larger sample sizes, i.e., of 10 or more individuals (89% of studies since 2018 compared to 80% prior to 2018); an increase in the use of chemical digestions and microscope methods (70% since 2018 compared to 22% prior to 2018); and a chemical verification and validation of the identified microplastic particles (70% since 2018 compared to 40% prior to 2018) (Fig. 2). Overall, 91% of the studies which passed the three criteria to use in our global comparison were published from 2018 onwards.

Regionally, there is more research from Asia (27% of all studies) and Europe (35%), compared to other locations (Fig. 3, Table S3). The Middle-East, Oceania and Africa combined contribute to less than 14% of the global research (Fig. 3, Table S3). When investigating the quality of methodology, Asia and the Middle East, have largely adopted the quality methods and approaches (54 and 80% of studies passed our method criteria, respectively), followed by Europe, Oceania and Africa (46, 40 and 38% of studies), whilst North and South America show a disproportionate number of studies lacking the recommended approaches for microplastic research (22 and 13% of studies) (Fig. 3). These trends also align with the year the studies were published (e.g. 94% of studies from Asia and the Middle East are published from 2018 onwards).

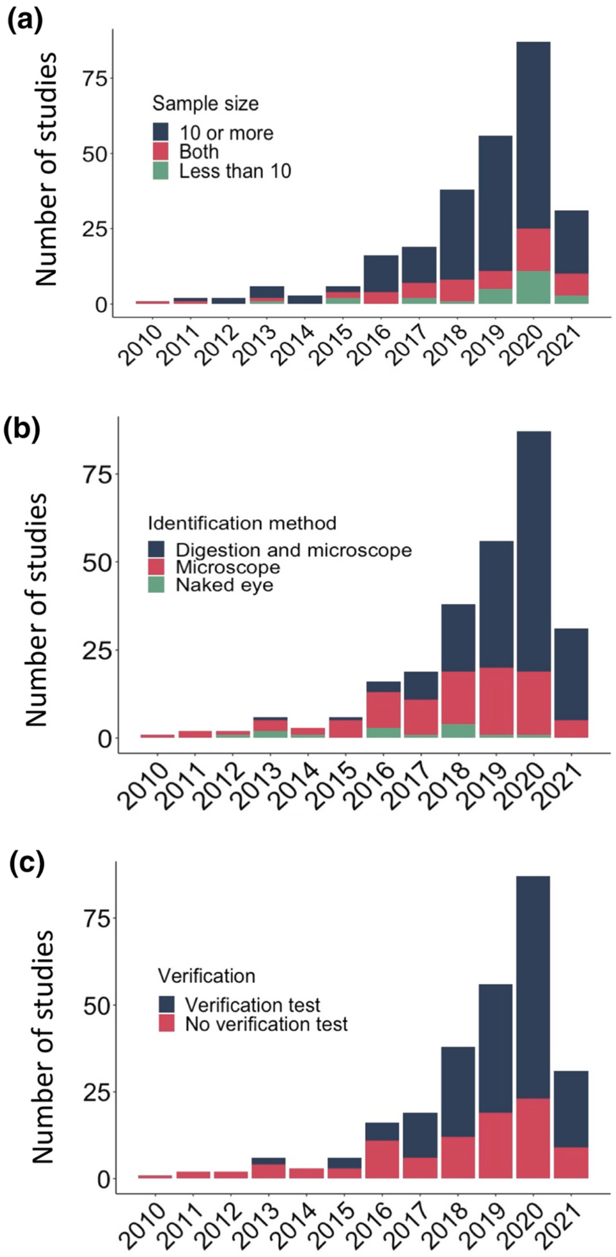


Fig. 2 Number of published studies since 2010 on the occurrence of microplastic in fish, according to **a**) sample size, **b**) plastic identification method, and **c**) tests validating plastic identification. Navy blue in all graphs represents the recommended quality practices for microplastic research in marine biota. In **a**) 'both' refers to studies in which numerous species are sampled with some sample sizes of more than 10 and some less than 10

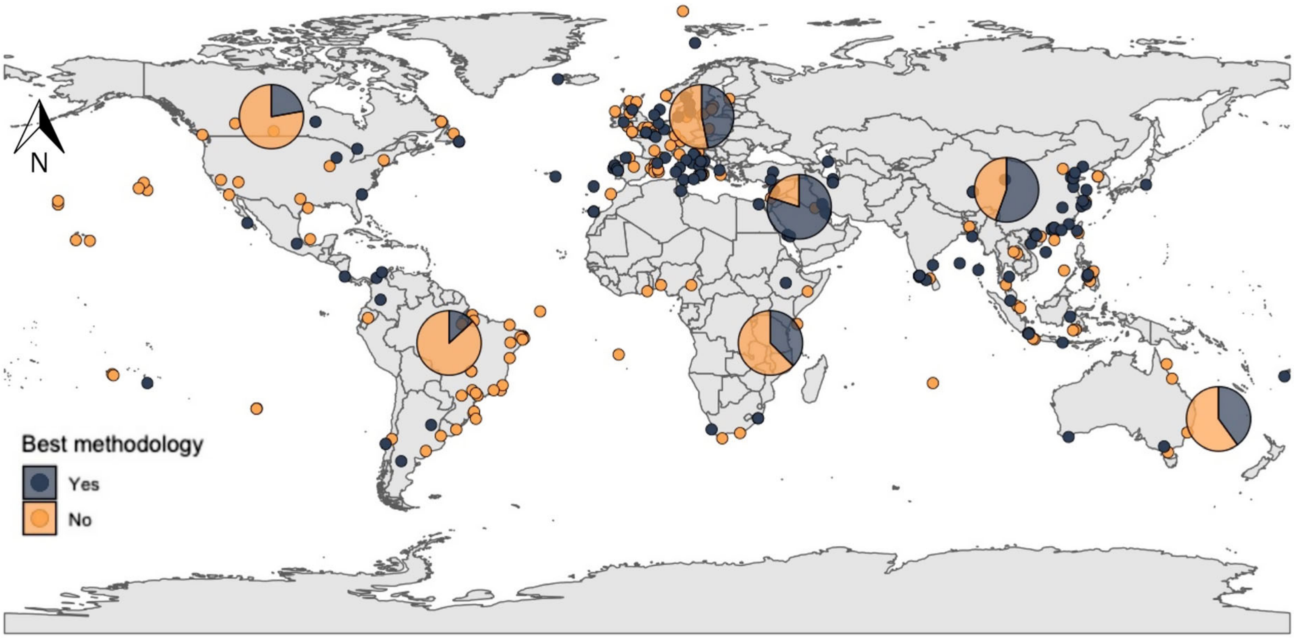


Fig. 3 Global distribution of studies evaluating microplastic in fish that used the recommended quality approaches. Each point represents one study, and pie charts show the number of studies per region of the globe. Number of studies per region—North America (n = 27), South America (n = 38), Europe (n = 94), Middle East (n = 10), Africa (n = 16), Asia (n =72), Oceania (n = 10)

Global comparison of microplastic ingestion by fish

Analysing the information compiled from the 111 studies that followed the recommended quality practices for microplastic research, globally, on average, 49% of fish from 506 observations (338 different species) had microplastic present in their gastrointestinal tract, but with variations in the frequency of occurrence among regions (x^2 53.19·, df 6, $p < 0.01$; Fig. 4a). Regional data showed that the percentage of individual fish that ingested at least one piece of microplastic was significantly higher in North America (mean = 82.9%, median = 99%) than all other regions, excluding Africa ($p < 0.01$). Africa was the only region where all the species sampled had at least one fish with microplastic, hence its higher frequency of occurrence (mean = 61.6%, median = 58%; Fig. 4a). In contrast, South America (mean = 23.2%, median = 10.5%) had significantly fewer individual fish with microplastic ($p < 0.01$) compared to all regions excluding Oceania. The latter was the only region where there were no species with 100% frequency of occurrence (Fig. 4a).

The average plastic load across the globe was 3.5 (± 0.8) pieces of microplastic per fish from observed species, though we also found significant variations among regions (x^2 47.78·, df 6, $p < 0.01$; Fig. 4b). North America (mean = 15.5, median = 2.4, Table S4) had the highest mean values compared to all other regions ($p < 0.05$) excluding Africa, which had the second highest mean plastic load with 5.9 pieces per fish from observed species (Fig. 4b). South America had the lowest plastic load of individual fish (mean = 0.73, median = 0.15, Table S4, $p < 0.01$), with 75% of all observations of species sampled having a mean plastic load of 0.3 or less. Nonetheless, studies from Asia had the highest plastic loads per individual (different species from separate studies recorded average plastic loads up to 366, 88, 45.2, and 27.4), and these drive the variation between the median and mean plastic load in the region (0.93 and 3.98 respectively). Likewise, high individual plastic load values per fish species in North America (species recorded plastic loads of 82.6, 60.8, 47.7 and 36.7) and Middle East (plastic load = 21.8) skewed distribution and drove the variation between mean and medians (North America, see above; Middle East (mean = 3.22, median = 1.6, Table S4).

Marine studies were generally more abundant and encompassed 82% of the total observations of species. A significant difference in the frequency of occurrence

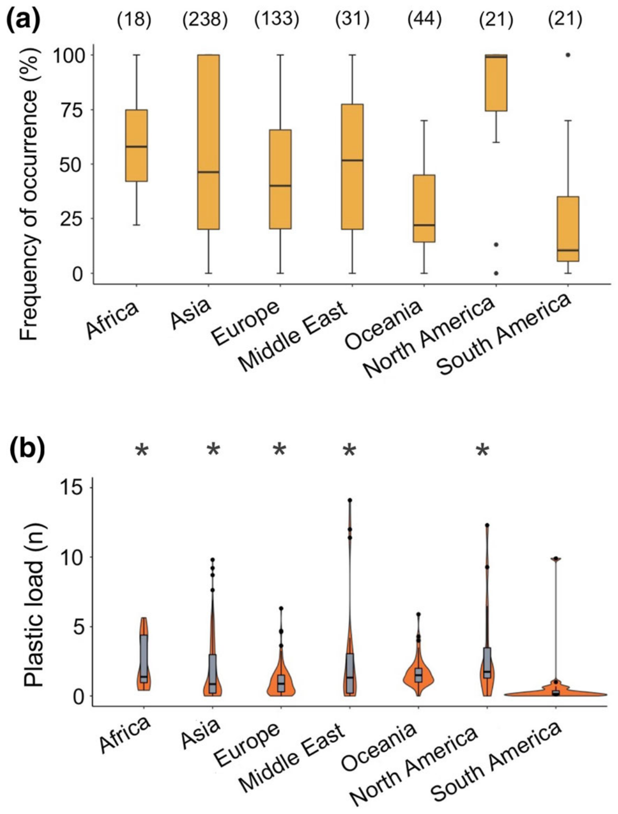


Fig. 4 **a**) frequency of occurrence and **b**) microplastic load in fish from different regions. Numbers in brackets at top are the total number of species sampled. In the box plots (**a**) the horizontal black line is the median, the bar shows the inter-quartile range, and the whiskers show 1.5 × interquartile range. The violin plot (**b**) shows the kernel density estimation and is a representation of the distribution of the data. The number in brackets represent the quantity of observed species. • represents outlier; * represents extreme outliers. There are two outliers from South America (**a**); and four extreme outliers in Asia, one in the Middle East and two in North America (**b**)

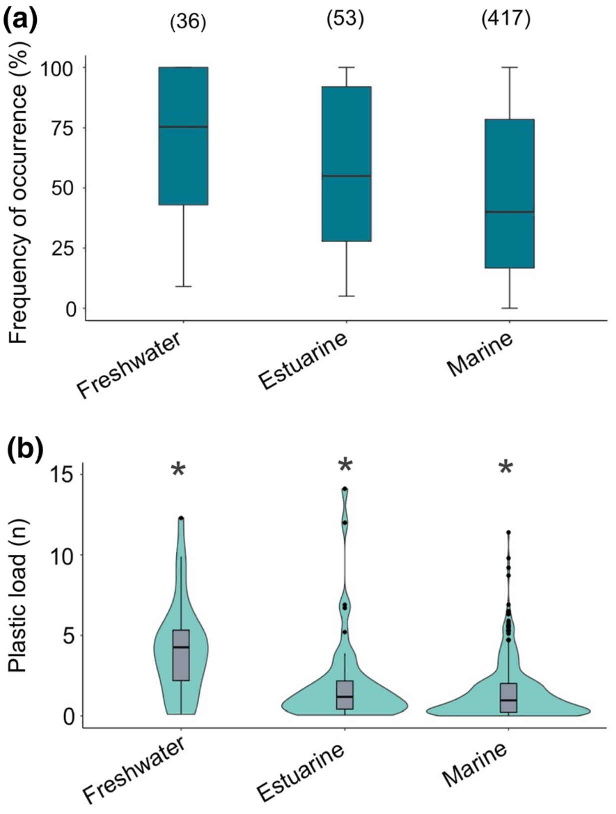


Fig. 5 **a**) frequency of occurrence and **b**) plastic load of microplastic in fish from different environments. In the box plots (**a**) the horizontal black line is the median, the bar shows the inter-quartile range, and the whiskers show 1.5 × interquartile range. The violin plot (**b**) shows the kernel density estimation and is a representation of the distribution of the data. The number in brackets represent the quantity of observed species. * represents extreme outliers. There were three extreme outliers in both freshwater and marine, and one in estuarine (**b**)

of microplastic in individual fish among aquatic environments was found (x^2 18.604·, df 2, $p < 0.01$; Fig. 5a), with the occurrence of plastic decreasing from freshwater (mean = 68.5%, median = 75.4%) to marine environments (mean = 46.2%, median = 40.0%, $p < 0.01$). Similarly, there were differences in the plastic load between environments (x^2 43.445·, df 2, $p < 0.01$; Fig. 5b). This was driven by freshwater (mean = 8.0, median = 4.4) fish ingesting higher quantities of plastic than those from both marine (mean = 2.7, median = 1.0) and estuarine (mean = 6.5, median = 1.2) environments (Table S5, $p < 0.01$).

The presence of plastic differed among fish occupying different habitats (x^2 24.064, df 3, $p < 0.01$; Fig. 6a); which was caused by individual fish from reef-associated areas (mean = 32.2%, median = 22.2%) having less microplastic than those which reside in pelagic (mean = 53.7%, median = 50%) and benthic-demersal zones (mean = 51.1%, median = 45.5%) ($p < 0.01$). Overall, individual fish from the deep sea had the highest median frequency of occurrence of plastic, but there were no species in which plastic was found in all fish (i.e. 100% frequency of occurrence). There were significant differences in the plastic load between habitats (x^2 10.555, df 3, $p < 0.05$; Fig. 6b), with the only differences occurring between fish from pelagic and reef-associated habitats ($p < 0.01$).

We found that feeding strategy influenced the frequency of occurrence of microplastic in individual fish (x^2 20.289, df 3, $p < 0.01$; Fig. 7a), with more detritivorous fish (mean = 71.1%, median = 75.1%)

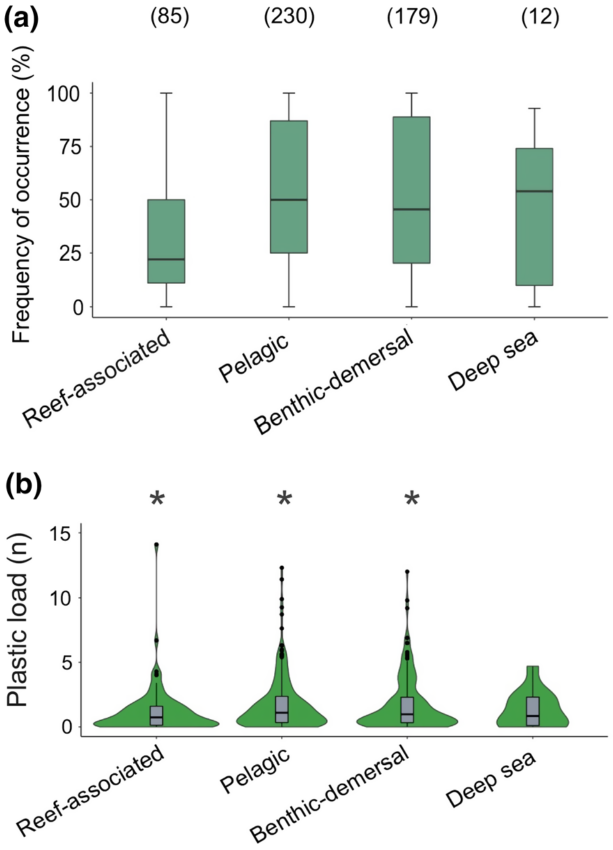


Fig. 6 **a**) frequency of occurrence and **b**) plastic load of microplastic in fish from different habitat zones. In the box plots (**a**) the horizontal black line is the median, the bar shows the inter-quartile range, and the whiskers show 1.5 × interquartile range. The violin plot (**b**) shows the kernel density estimation and is a representation of the distribution of the data. The number in brackets represent the quantity of observed species. • represents outlier; * represents extreme outliers. There was one outlier in reef-associated (**a**) and five extreme outliers in pelagic and one in both reef-associated and benthic-demersal (**b**)

having ingested plastic in comparison to carnivorous fish (mean = 46.2%, median = 38.5%, $p < 0.01$). The plastic load was also dependent on feeding strategy (x^2 37.456, df 3, $p < 0.01$; Fig. 7b). Carnivorous (plastic load mean = 3.3, median = 0.9) and omnivorous fish (mean = 1.8, median = 1.0) ingested less plastic pieces than detritivorous fish (mean = 5.7, median = 3.7, $p < 0.01$). Ninty-four percent of the observations of detritivorous fish species had a plastic load of one or more pieces (Fig. 7b). Though carnivorous fish had the lowest frequency of occurrence, they had the highest maximum values of plastic loads, with *Stolephorus* spp. (Engraulidae) from separate studies in Asia having averages up to 88 and 366 pieces of plastic per individual (Fig. 7b, Outliers) (Ningrum et al. 2019; Ningrum & Patria 2019). Furthermore, when comparing trophic level and frequency of occurrence of microplastic in fish, no relationship was found (Fig. S1).

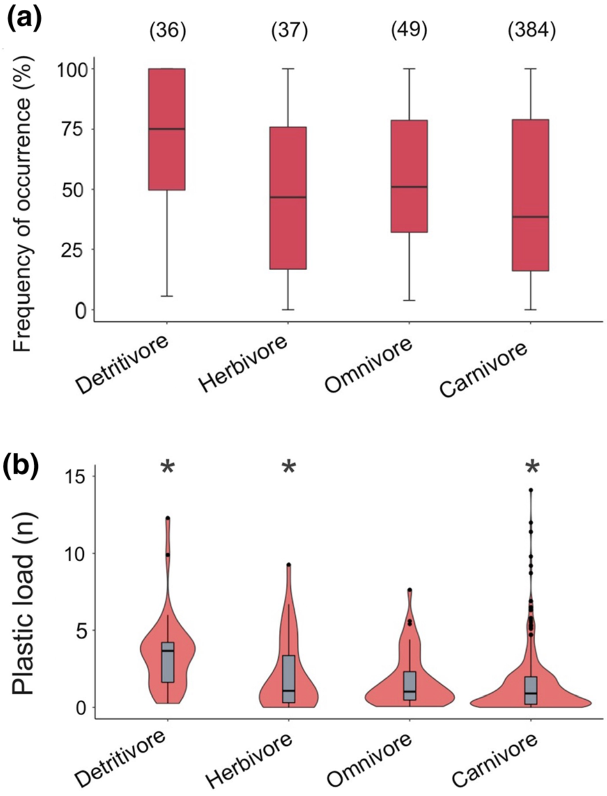


Fig. 7 **a**) frequency of occurrence and **b**) plastic load of microplastic in fish with different feeding strategies. In the box plots (**a**) the horizontal black line is the median, the bar shows the inter-quartile range, and the whiskers show 1.5 × interquartile range. The violin plot (**b**) shows the kernel density estimation and is a representation of the distribution of the data. The number in brackets represent the quantity of observed species. * represents extreme outliers. There were three extreme outliers for herbivores and four for carnivores (**b**)

Finally, the frequency of occurrence varied strongly with fish source, i.e. whether fish were wild-caught, aquaculture bred or collected at fish markets (x^2 20.481, df 2, $p < 0.01$; Fig. 8a). The frequency of occurrence of plastic in individual fish from aquaculture (mean = 81.6%, median = 100%) was almost double that of fish caught in the wild (mean = 45.2%, median = 40%) or purchased through fish markets (mean = 55.4%, median = 42.4%, $p < 0.01$). Overall, more than 50% of the observations of species sampled from aquaculture contained frequency of occurrence of 100%. For market and wild sourced fish only 16% of the observations of species had microplastic in every individual fish from that species (Fig. 8a).

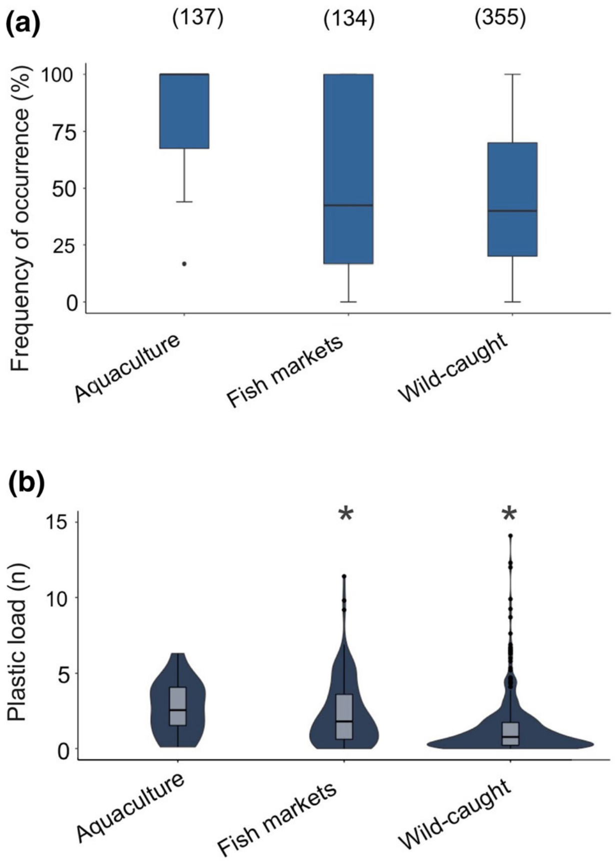


Fig. 8 a) frequency of occurrence and **b**) microplastic load of fish from different sources. In the box plots (**a**) the horizontal black line is the median, the bar shows the inter-quartile range, and the whiskers show 1.5 × interquartile range. The violin plot (**b**) shows the kernel density estimation and is a representation of the distribution of the data. The number in brackets represent the quantity of observed species. • represents outlier; * represents extreme outliers. There was a low outlier in aquaculture (**a**) and five extreme outliers in wild-caught and two in fish market fish (**b**)

Similar patterns were seen in the plastic load, with aquaculture individual fish having more plastic on average than other fish (x^2 27.078, df 2, $p < 0.01$; Fig. 8b). However, whilst aquaculture sourced individual fish had the highest median (2.6 pieces of plastic), fish sourced from markets had the highest mean value (5.9 pieces of plastic) due to the very high maximum plastic loads found in anchovy *Stolephorus* spp. collected in markets in south east Asia (up to 388 piece of plastic). In contrast, 59% of observations of wild caught species had one or less pieces of plastic (Fig. 8b). There were 17 different observations of species from ten separate studies that were from aquaculture sources, and covered a range of habitats and feeding types, and were predominantly from marine sources in Asia (Table S2).

Discussion

Globally, 49% of individual fish ingested microplastic; with an average of 3.5 pieces per individual. In recent years, studies have increasingly adopted higher quality and readily comparable approaches to the ingestion of microplastic by fish, with over 90% of studies meeting our criteria for quality research being published from 2018 onwards. The use of similar approaches among 111 studies, allowed us to effectively compare the presence of microplastic in fish from different regions across the globe. We found that fish from freshwater environments had higher microplastic frequencies and loads than marine environments, despite contributing to less than 15% of current research. We discovered there was more microplastic in fish with detritivorous feeding strategies, and in those that reside in deep-sea habitats. We also compared fish sourced from markets, aquaculture or collected in the wild, finding aquaculture fish had higher levels of microplastic. Ultimately, our analyses and data compilation underpin the importance of standardisation and of defining clear guidelines for microplastic research, as from all research on fish ingestion of microplastic we were only able to use 41% of studies in our global comparison as a means to ensure robust and coherent data comparison. Controlling for methodological approaches is paramount, and all microplastic in biota research should strive to follow strict guidelines on quality standard of methods, to cut down on potential shortcomings arising from how different methodologies can impact results.

Regionally, fish from North America had the highest amount of microplastic. The United States generated 37.83 million tonnes of plastic waste in 2010, the second highest of any country (Jambeck et al. 2015). Furthermore, high levels of microplastic have been found in the sediment of North America's marine and estuarine environments (e.g. 1410 pieces per dry kilogram of sediment in Dodson et al. (2020)). Other regions also have high quantities of microplastic, so it is difficult to know what exactly is causing these high numbers, but results also reflect the fact nearly half of the species investigated from North America are from freshwater and estuarine systems, which in general have higher amounts of plastic (Collard et al. 2019; Schmidt et al. 2017). The only other region which had such a high proportion of species from freshwater systems was Africa, which had the second highest amount of plastic in fish by region. In agreement with previous studies, North America is a key area where more microplastic research is needed, particularly to determine the abundance and population level risks in commercial species (Baechler et al. 2020). Other regions, such as South America, Africa and Oceania also lack quality quantification of microplastic in fish and would benefit from more approaches following stringent protocols to assess microplastic ingestion in fish from all aquatic environments.

The frequency of occurrence that we found in individual marine fish (46%) was similar to the data compiled by Markic et al. (2019), where 38% of fish had ingested plastic. Likewise, the plastic load value from marine fish in our study (2.8 ± 1.3) was similar than the average plastic load in Markic et al. (2019) (2.6 ± 0.2). We expected to see microplastic consumption in fish increasing overtime, due to both higher levels of microplastic in the environment, as well as better methodologies to detect them (Savoca et al. 2021). However, the similarity in results between our study and Markic et al. (2019) further highlights the importance of repeatable, long-term sampling to identify statistically supported, clear and robust trends in microplastic research. In fact, future initiatives based on consistent methods and routine sampling from markets (e.g. Wootton et al. 2021a) could underpin the establishment of an easy to implement, broad scale monitoring to detect trends in microplastic contamination with appropriate statistical power. Both our study, and Markic et al. (2019) use similar approaches by including studies that met standard methods criteria, although Markic et al. (2019) did not require a chemical verification of polymer type. Despite this, our study compiled information from 506 observations of fish while Markic et al. (2019) had 199, with only 70 shared between the two studies. From this perspective, our study has provided a more in depth, up-to-date and detailed dataset than previously published review based literature.

Individual fish from freshwater and estuarine environments had more than two times the plastic load than those from marine environments. These results are not unexpected as river systems have 40–50 times more plastic than the maximum concentration recorded in the open ocean (Schmidt et al. 2017), with rivers being the major pathway for pollution via estuaries into the sea (Collard et al. 2019). Ultimately, 88% of microplastic pollution in the ocean comes from land, with between 1.15 and 2.41 million tonnes of plastic waste estimated to currently flow from river systems into the ocean every year (Jambeck et al. 2015; Lebreton et al. 2017). Additionally, microplastic research is growing in freshwater and estuarine environments, therefore the higher microplastic levels could also be driven by methodological advances in microplastic detection (Savoca et al. 2021). Despite the evidence that freshwater systems are a major source of marine pollution, and despite increased research over the last few years, there is still a shortage of literature researching microplastic in freshwater environments compared with marine systems. This is a key gap that needs to be filled globally.

We hypothesised that feeding type would influence the amount of microplastic ingested by fish. Microplastic was found more frequently in detritivores, and in higher abundance than carnivores. These findings differ from predictions where higher trophic level biota, such as carnivorous fish, accumulate more plastic via both biomagnification and bioaccumulation across the food web (Batel et al. 2016; Carbery et al. 2018; Watts et al. 2014). However, most studies quantifying trophic transfer of plastics have taken place in laboratory conditions (Farrell & Nelson 2013; Gouin 2020), or on high trophic level taxa (Nelms et al. 2018) and we still need to understand how these results relate to field observations of fish (Miller et al. 2020). With limited research and knowledge addressing bioaccumulation and biomagnification in trophic webs, our study does support that the lower trophic organisms are also at risk. Other studies demonstrate that large numbers of microplastic are accumulating in the sediment, with an estimated 14 million tonnes of microplastic currently present on the seafloor, more than double what is found on the ocean's surface (Barrett et al. 2020; Woodall et al. 2014). So, it is logical that fish which are feeding solely in the sediment have higher levels of microplastic contamination. Although our data suggests that habitats (e.g. reef-associated, pelagic, demersal, deep-sea) are not having an effect on microplastic ingestion (Table S2). Therefore, this lack of significance perhaps shows that feeding strategy is more influential on microplastic ingestion than the habitat fish occupy (Miller et al. 2020). Nonetheless, there are still many species and groups of species understudied and a need to further clarify the potential for bioaccumulation/magnification to occur in wild caught, namely predatory, fish is needed. Additionally, inconsistencies between the data regarding habitat of a species obtained from FishBase (Froese and Pauly 2019), and the actual collection point of the particular fish, could explain the reduced trends between microplastic ingestion and habitats.

Significantly more individual fish from aquaculture systems have microplastic than both wild-caught fish and those purchased from fish markets. Similar results have been found in bivalves, where farmed mussels had double the microplastic content compared to wild-caught (Mathalon and Hill 2014). Fish produced in aquaculture systems are likely ingesting microplastic from the pens, tanks or nets where they are raised, as these are commonly made of plastic materials. Further, aquaculture fish may be inefficient visual feeders, and are likely to forage on microplastic if food sources become unavailable (Roch et al. 2020). This is an area that would benefit from additional research, both in terms of numbers of studies assessing microplastic prevalence in aquaculture but also to address the causes and trends that drive the increased levels of plastic that we found. Moreover, as aquaculture is a critical source of protein for the world's growing population knowledge on potential plastic-related risks is paramount. When fish were purchased from fish markets, it was commonly not recorded if the fish were wild-caught or aquaculture sourced (e.g. Calderon et al. 2019; Ding et al. 2019). Considering that most of these studies are looking into the environmental effects of plastic, it is assumed they were wild-caught species, and although this is not stated in most studies, the similarity in microplastic results suggest this was mostly the case. It should be noted, that despite the high plastic load and frequency of occurrence values from aquaculture fish, this is unlikely skewing any of our other comparisons as the aquaculture fish were from a range of habitats and feeding strategies. Although the majority of all aquaculture species investigated were from marine environments in Asia, neither of these groups had particularly high amounts of plastic so having their source as aquaculture is not weighting the results. One key issue to resolve, considering the extensive literature documenting plastic found in fish, is to unravel if, and how much, microplastic is due to the pollution currently present in the aquatic environment, or may be accumulated during the fishing process. Overall, further insight on microplastic contamination derived from aquaculture production or fisheries activities is needed, so mitigation strategies towards reduction of plastic in production systems can be implemented.

Future directions

The majority of the studies that met our method criteria were published from 2018 onwards; this is indicative that recent guideline papers with microplastic research recommendations are having an impact. To generate more comparable literature, it is essential that all research on microplastic in biota follow a set of standardised methods. Namely, sample size of at least ten per species, chemical digestions, chemical verification of polymers and high standard quality controls (Markic et al. 2019). Within this synthesis, the selection criteria was tightly set, encompassing only studies which used the four prementioned methods. However, even then, within the chosen criteria there were still some variability with approaches, such as the use of different chemical digestions, which could potentially affect the recovery rate of some polymers (Karami et al. 2017), and differences in the reporting of the lower size limit of microplastic pieces, or no mention of this at all. This further highlights the importance of having a narrow set of high standard methods for microplastic investigation in the future. Standardisation is vital for studies trying to depict trends, or species impacts, and without high quality standards set it is difficult for this research to carry robustness. Futhermore, without standardised methods the likelihood of region bias is high, as most studies follow methods that are produced among research groups, or within a similar geographic location, thus amplifying local or regional bias and further impairing comparisons in the long term. Overall, it is essential that we strive towards defining clear and strict guidelines that boost comparable and reproducible microplastic research (Cowger et al. 2020; Provencher et al. 2020a, 2020b).

Research on the effects of microplastic on marine organisms is increasing, however there is still limited knowledge on the role that microplastic is playing on food security and safety (Walkinshaw et al. 2020). As global consumption of seafood rises, it is essential that we understand potential threats and challenges that microplastic pollution may pose to the fishery and aquaculture industries, across fish, ecosystem and human health perspectives. Individual fish are likely accumulating both physical and chemical sublethal effects from microplastic ingestion, including ecotoxicological and behavioural impacts (Foley et al. 2018; McCormick et al. 2020; Rochman et al. 2013), however as our knowledge on microplastic risks increase, there needs to be a focus on the impacts of microplastic on fish populations, and more broadly on aquatic ecosystems, considering fish health is necessary in ensuring a sustainable fishing stock. In particular, how impacts of plastic are interacting with other anthropogenic stressors, such as climate change, ocean acidification, overfishing and habitat degradation is required.

Moreover, an additional hazard from microplastic is their associated chemicals, that are either added during production or can be adsorbed to the plastic surface from the environment (e.g. plasticizers, organic compounds, heavy metals) (Gassel et al. 2013; Teuten et al. 2007). The link between the long-term exposure of plastic and their associated chemicals to impact fish or humans via consumption are still understudied but a clear research priority, (Barboza et al. 2018; Smith et al. 2018; Vethaak & Legler 2021) as microplastic derived chemicals have been found in fish muscle tissue (Barboza et al. 2020). Most research investigates the gastrointestinal tract of the fish; and whilst most fish are not eaten whole, there are several instances, dependent on species, size and human consumption behaviours in different regions of the world where fish are consumed in entirety. Additionally, micro and nano plastics have the potential to translocate from gut and gills to internal tissues, and thus be transferred via consumption to other trophic levels and humans. It is possible that a large quantity of consumed microplastic remain undetected due to egestion, with suggestions that it takes on average seven days for the microplastic to pass through a fish (Ory et al. 2018), with this period likely species-specific. Thus, toxicological data investigating both micro and nano plastics, and their potential to translocate in both fish and humans will be imperative for food safety risk assessment (FAO 2017).

Overall, we identify regions, environments and community-level information where focused ongoing research is needed, to ensure we create an all-encompassing profile of microplastic risk in fish populations for the future. Regionally, North America, South America, Oceania and Africa all lack rigorous data on plastic ingestion in fish species, so we suggest these regions should be prioritised. In addition, further focus on freshwater and estuarine ecosystems is required. Community-level information, particularly surrounding trophic transfer, bioaccumulation and biomagnification may elucidate fishing, production or consumption trade-offs between environmental, human health and food security risks. Finally, we suggest researchers should focus on aquaculture raised seafood, to disentangle the potential sources of microplastic presence in these environments.

Conclusion

This study has demonstrated the ubiquity of microplastic presence in fish globally, with plastic being found in fish from all regions, environments, habitat zones and feeding strategies. Our synthesis identified ecological and geographical drivers of microplastic ingestion, with fish from North America, freshwater environments, deep-sea habitats and with detritivorous diets presenting higher plastic contamination. Fish from aquaculture sources also had increased plastic than those wild-caught. As global fisheries and aquaculture are a critical component of a large portion of the global population's diet and livelihoods, the problem of plastic contamination in the environment and commercially important fish is becoming a major issue for wild fisheries, aquaculture and those that depend on them (Béné et al. 2015; FAO 2020). Despite the concern of microplastic contamination impacting human consumers of seafood, there is still very limited information supporting these claims, and we suggest an increase of quality information of microplastic ingestion in fish species from understudied regions and species (Provencher et al. 2020b). Many efforts have been put into awareness of plastic use to limit an individual's use of plastic, but more policies and regulations are needed to ensure we move into a circular economy in regards to plastic use, which will benefit the fishing industry, the fish and their entire ecosystems (Borrelle et al. 2017; Rochman et al. 2016). Ultimately, a better understanding of plastic contamination in fish globally, and the ecological drivers such as habitat, diet and environment which are influencing such plastic, will enhance future research strategies to protect environmental and food safety requirements, particularly considering the commercial value of global fisheries.

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Data availability Data are available in the supporting information (Appendix A).

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Supplementary Information S2

Microplastic in fish – a global synthesis

Nina Wootton, Patrick Reis-Santos, Bronwyn M. Gillanders
Reviews in Fish Biology and Fisheries (2021)

Table S2.1: Spreadsheet listing all studies investigating microplastic in fish identified by the literature search, including detailed reasons for exclusion, as well as other extracted information, such as location and methodological approach.

Available online: https://static-content.springer.com/esm/art%3A10.1007%2Fs11160-021-09684-6/MediaObjects/11160_2021_9684_MOESM2_ESM.pdf

Table S2.2: Spreadsheet listing individual species with species specific information from studies which passed method criteria and were included in the second analysis.

Available online: https://static-content.springer.com/esm/art%3A10.1007%2Fs11160-021-09684-6/MediaObjects/11160_2021_9684_MOESM2_ESM.pdf

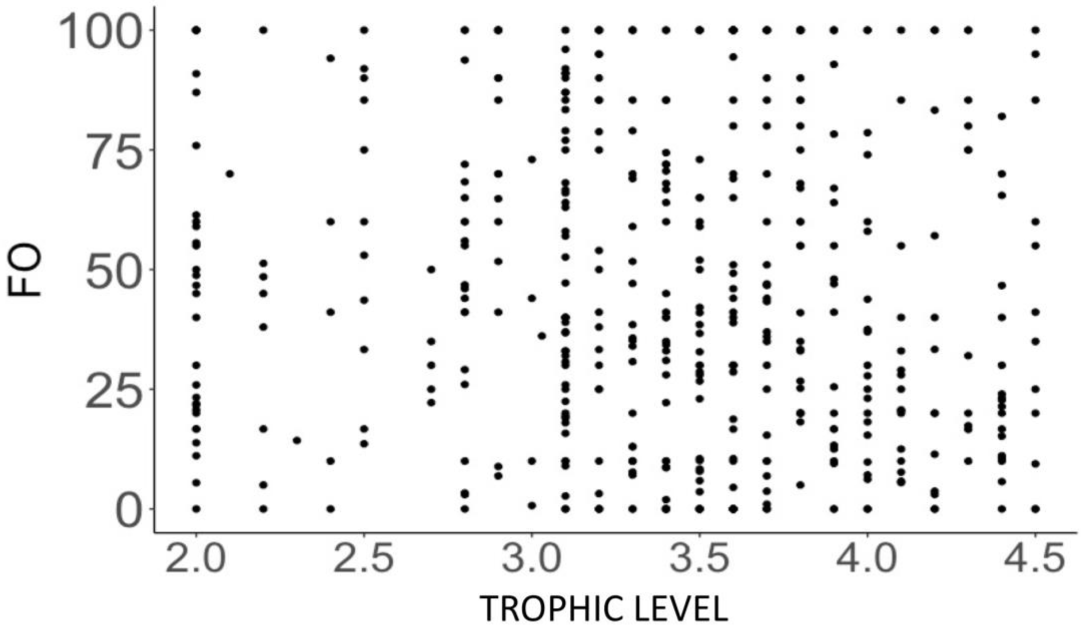


Fig S2.1: Frequency of occurrence of microplastic in fish species from different trophic levels. Each dot represents one species.

Table S2.3: Geographic distribution of number of studies in different environments. The numbers in brackets represent the percentage of studies per environment (columns), apart from the last row, where the numbers in brackets are the percentage across environments.

Region	Total number of studies (%)	Marine	Estuarine	Freshwater
Africa	16 (6.0)	11 (5.9)	1 (3.2)	4 (8.2)
Asia	72 (27.0)	49 (26.2)	7 (22.6)	16 (3.3)
Oceania	10 (3.75)	8 (4.3)	1 (3.2)	1 (2.0)
Europe	94 (35.2)	76 (40.6)	5 (16.1)	13 (26.5)
Middle East	10 (3.75)	9 (4.8)	1 (3.2)	0 (0)
North America	27 (10.1)	18 (9.6)	2 (6.5)	7 (14.3)
South America	38 (14.2)	16 (8.6)	14 (45.2)	8 (16.3)
Global	267	187 (70)	31 (12)	49 (18)

Table S2.4: Geographic distribution of number of studies and species used in quantitative analysis and the mean and median frequency of occurrence (FO) and plastic load (PL) of microplastics in fish species for each region. Species mean and median FO are % values.

Region	# of studies	# of species	Species mean FO (±SE) %	Species mean PL (±SE)	Median FO (%)	Median PL
Africa	6	18	61.6 (±5.54)	5.87 (±2.50)	58	1.58
Asia	39	238	53.7 (±2.40)	3.98 (±1.59)	46.3	0.93
Oceania	4	44	28.6 (±3.08)	1.72 (±0.17)	21.9	1.50
Europe	43	133	43.7 (±2.56)	1.45 (±0.40)	40	0.90
Middle East	8	31	51.2 (±6.03)	3.22 (±0.92)	51.7	1.6
North America	6	21	82.9 (±6.23)	15.50 (±5.06)	99	2.36
South America	5	21	23.2 (±5.53)	0.73 (±0.47)	10.5	0.15
Total	111	506	49.0 (±1.53)	3.50 (±0.80)		

Table S2.5: Distribution of number of studies and species used in quantitative analysis and the mean and median frequency of occurrence (FO) and plastic load (PL) of microplastics in fish species for each environment.

Environment	# of studies	# of species	Species mean FO (±SE)	Species mean PL (±SE)	Median FO	Median PL
Estuarine	12	53	57.5% (±4.51)	6.45 (±2.14)	55.0	1.23
Freshwater	16	36	68.5 (±5.26)	7.98 (±1.80)	75.4	4.4
Marine	85	417	46.2 (±1.68)	2.75 (±0.92)	40.0	0.99

Table S2.6: Distribution of number of species used in quantitative analysis and the mean and median frequency of occurrence (FO) and plastic load (PL) of microplastics in fish species for each habitat.

Habitat	# of species	Species mean FO (±SE)	Species mean PL (±SE)	Median FO	Median PL
Reef-associated	85	32.2% (±3.01)	1.43 (±0.32)	22.2%	0.76
Pelagic	230	53.7 (±2.27)	4.96 (±1.70)	50	1.20
Benthic-demersal	179	51.1 (±2.63)	2.76 (±0.59)	45.5	0.99
Deep sea	12	45.0 (±9.78)	1.31 (±0.42)	54.0	0.84

Table S2.7: Distribution of number of species used in quantitative analysis and the mean and median frequency of occurrence (FO) and plastic load (PL) of microplastics in fish species for each feeding strategy.

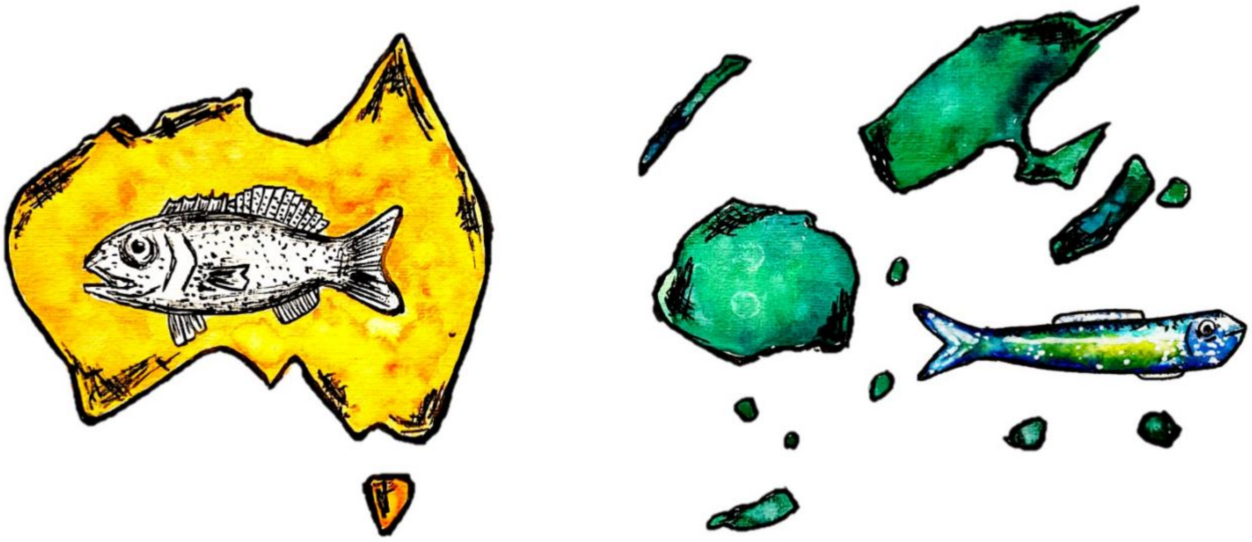
Feeding strategy	# of species	Species mean FO (±SE)	Species mean PL (±SE)	Median FO	Median PL
Detritivore	36	71.1% (±4.78)	5.67 (±1.73)	75.1%	3.70
Herbivore	37	48.4 (±5.71)	6.08 (±1.94)	46.7%	1.45
Omnivore	49	55.0 (±4.20)	1.75 (±0.25)	51.0%	1.00
Carnivore	384	46.2 (±1.78)	3.28 (±1.03)	38.5%	0.93

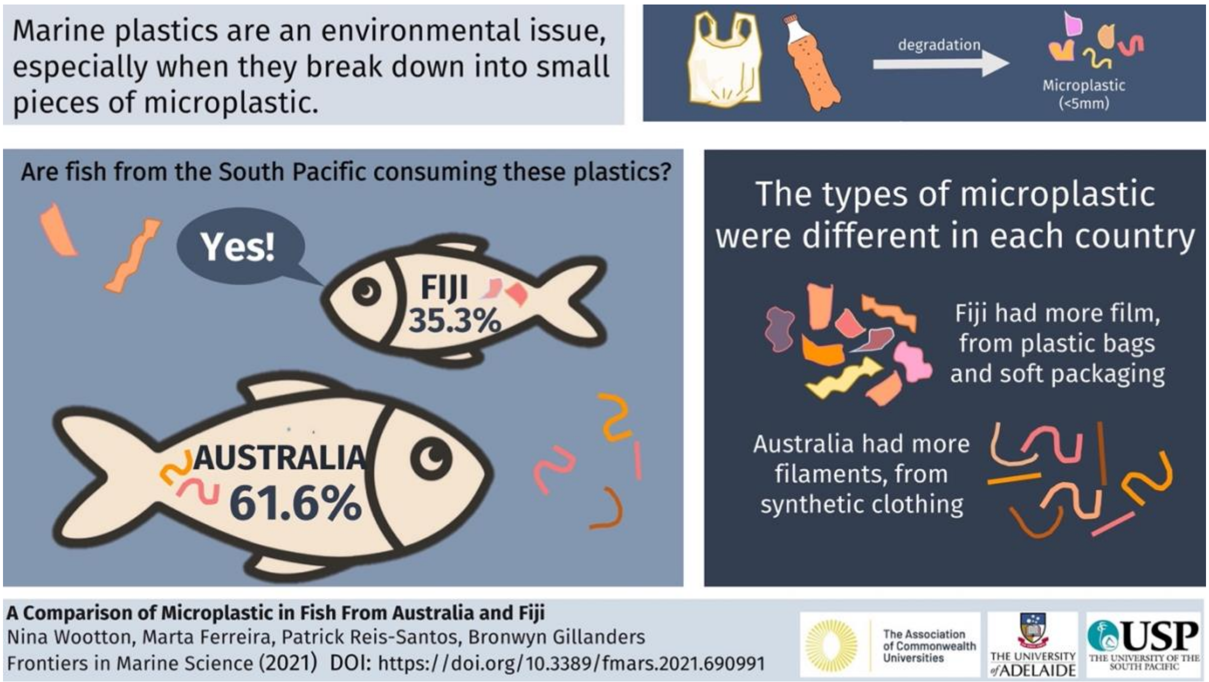
Table S2.8: Distribution of number of studies and species used in quantitative analysis and the mean and median frequency of occurrence (FO) and plastic load (PL) of microplastics in fish species for each source.

Source	# of species	Species mean FO (±SE)	Species mean PL (±SE)	Median FO	Median PL
Aquaculture	17	81.6% (±6.31)	2.70 (±0.44)	100%	2.55
Fish markets	134	55.4 (±3.46)	5.93 (±2.83)	42.4	1.80
Wild-caught	355	45.2 (±1.67)	2.64 (±0.43)	40.0	0.82

CHAPTER 3

A COMPARISON OF MICROPLASTIC IN FISH FROM AUSTRALIA AND FIJI





Marine plastics are an environmental issue, especially when they break down into small pieces of microplastic.

Are fish from the South Pacific consuming these plastics?

The types of microplastic were different in each country

Fiji had more film, from plastic bags and soft packaging

Australia had more filaments, from synthetic clothing

A Comparison of Microplastic in Fish From Australia and Fiji
Nina Wootton, Marta Ferreira, Patrick Reis-Santos, Bronwyn Gillanders
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Principal Author

Name of Principal Author (Candidate)	Nina Wootton		
Contribution to the Paper	Contributed to design of the study, reviewed literature, conducted the fieldwork, collected samples, completed laboratory processing, operated the FT-IR, analysed the data and applied statistical analyses, wrote the manuscript and acted as corresponding author.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	5/10/21

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Marta Ferreira		
Contribution to the Paper	Contributed to design and development of the study, advice and suggestions throughout initial data collection stages in Fiji.		
Signature	Deceased	Date	15/10/21

Name of Co-Author	Patrick Reis-Santos		
Contribution to the Paper	Contributed to design and development of the study, advice and suggestions throughout, provided comments and feedback on manuscript.		
Signature		Date	06/10/2021

Name of Co-Author	Bronwyn Gillanders		
Contribution to the Paper	Contributed to design and development of the study, advice and suggestions throughout, acquisition of funding, provided comments and feedback on manuscript.		
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A Comparison of Microplastic in Fish From Australia and Fiji

Nina Wootton[1]*†, Marta Ferreira[2]†‡, Patrick Reis-Santos[1]† and Bronwyn M. Gillanders[1]†

[1] Southern Seas Ecology Laboratories, School of Biological Sciences, University of Adelaide, Adelaide, SA, Australia,
[2] Faculty of Science Technology and Environment, School of Marine Studies, University of the South Pacific, Suva, Fiji

Awareness surrounding plastic pollution has increased significantly in the past decade, leading to concerns on potential adverse effects on biota, including the consumption of microplastic by fish. Globally, plastic has been found in many species of fish, but little research has been undertaken in the southern hemisphere. We assessed the abundance and type of plastic in fish captured and sold for human consumption in Australia and Fiji. Fish (goatfish, sea mullet, paddletail, and common coral trout) had their gastrointestinal tracts dissected and microplastic quantified under a microscope. Plastic polymer types were confirmed using μ-FTIR. In Australia, plastic was found in 61.6% of fish gastrointestinal tracts, while in Fiji, 35.3% of fish had plastic. Fish from Australia had almost double the amount of plastic on average than fish caught in Fiji, with 1.58 (± 0.23) pieces per fish in Australia compared to 0.86 (± 0.14) in fish caught in Fiji. The types of plastic differed between countries, with fibers comprising 83.6% of microplastic pieces in fish from Australia whereas 50% of microplastic found in fish from Fiji was film. Polyolefin was the most abundant polymer type in both fibers from Australia and film from Fiji. We hypothesize variations in abundance and plastic type are a reflection of the population density and coastal geomorphology, but may also be a result of legislation and waste management strategies in the two countries. This work adds evidence to the pervasive presence of plastic in fish gastrointestinal tracts, reinforcing the urgent need for efficient plastic waste management, but also a better understanding of the impacts of microplastic on marine biota.

Keywords: microplastic, ingestion, fish research, South Pacific, plastic pollution, contamination

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Gabriel Machovsky-Capuska,
The University of Sydney, Australia

Reviewed by:
Muhamed Ashraf P,
Central Institute of Fisheries
Technology (ICAR), India
Olga Pantos,
Institute of Environmental Science
and Research (ESR), New Zealand

***Correspondence:**
Nina Wootton
nina.wootton@adelaide.edu.au

†ORCID:
Nina Wootton
orcid.org/0000-0001-5624-8441
Marta Ferreira
orcid.org/0000-0003-2098-066X
Patrick Reis-Santos
orcid.org/0000-0001-9843-9465
Bronwyn M. Gillanders
orcid.org/0000-0002-7680-2240

‡Deceased

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INTRODUCTION

Plastic debris is accumulating in marine environments at a rapid rate, with recent research finding plastic to be ubiquitous in oceans globally (Barnes et al., 2009; Wilcox et al., 2015; Worm et al., 2017). Plastics are highlighted as a major environmental hazard and can have a variety of health impacts on marine organisms, including suffocation, entanglement and contamination throughout all trophic levels (Page et al., 2004; Pierce et al., 2004; Stamper et al., 2009; Rochman et al., 2013). A highly prevalent type of plastic in the environment are microplastic, which are defined as pieces of plastic less than 5 mm in size (Eriksen et al., 2014; da Costa et al., 2016). Microplastic is either manufactured to be this size (primary microplastic) or the result of environmental weathering and forces breaking down pieces of larger plastic (secondary microplastic) (Worm et al., 2017). Recently, there has been a rise in research surrounding microplastic as an environmental contaminant, initiated by an increase in concern and awareness from the scientific community, policymakers and the general public on the impacts these small particles are having on marine environments and biota (Rochman et al., 2013). Studies have now found microplastic in all areas of the water column, including deep-sea floors, coastal sediments and the ocean surface (Reisser et al., 2013; Eriksen et al., 2014; Peng et al., 2018). Furthermore, they have been identified to be ingested by a range of marine organisms, including whales, fish and larvae (Besseling et al., 2015; Steer et al., 2017; Burkhardt-Holm and N'Guyen, 2019).

Microplastic ingestion in marine fish is well documented (Markic et al., 2019; Sequeira et al., 2020; Savoca et al., 2021), with field studies reporting microplastic ingestion in wild-caught fish of both commercial and non-commercial interest from a broad range of trophic levels, habitats and benthic zones (Foekema et al., 2013; Nadal et al., 2016; Murphy et al., 2017; Baalkhuyur et al., 2018; Burkhardt-Holm and N'Guyen, 2019; Garnier et al., 2019). Despite this, the literature surrounding microplastic in fish sold through seafood markets and supermarkets is limited. With seafood consumption increasing worldwide, understanding the potential risks of human consumption of microplastic needs further attention. Apart from the reported physical impacts of microplastic when ingested by marine organisms (Wright et al., 2013), there is also concern that the small particles could act as a vector for toxic chemicals either added during manufacturing stages, or pollutants [e.g., persistent organic pollutants (POPs), flame retardants and heavy metals] sorbed onto the surface of the microplastic (Teuten et al., 2009; Bakir et al., 2014). Laboratory observations show these chemicals are capable of causing adverse impacts on fish (e.g., Rochman et al., 2014; Pedà et al., 2016), however, the extent to which microplastic ingestion is exposing individuals to chemical pollutants or its potential implications to seafood safety is far from well understood (Hermabessiere et al., 2017; Hantoro et al., 2019; Walkinshaw et al., 2020).

Land-based activities, such as unprotected landfill, mismanagement of household and commercial waste, sewage, littering, and industrial pollution are major sources of marine plastic (Jambeck et al., 2015; Li et al., 2016; Turrell, 2020). Therefore, to some extent the plastic found in the ocean is a reflection of the waste produced and how it is managed in different countries. Australia and Fiji have different waste management strategies, as well as differing population sizes, cultures, and lifestyles. Marine litter generation is predicted to be high in countries that have under-performing waste management systems. Small Island Developing States (SIDS), such as Fiji, have unique problems in waste collection and disposal due to their lack of space for landfill, inadequate expertise and technology and particularly their remote locations creating exhaustive costs to transport waste and recycling (Mohee et al., 2015). Furthermore, Fiji's increase in urbanization alongside growth in tourism have amplified the imbalance between the abundance of waste produced and the country's ability to manage it correctly (Kelman and West, 2009; Lachmann et al., 2017). Although Jambeck et al. (2015) estimated that Australia produces over eleven times the amount of plastic waste that Fiji does (1,902,591 kg per day compared to 168,430 kg per day in 2010), the amount of mismanaged waste in Fiji is almost four times that of Australia (13,889 tonnes in Australia compared to 49,257 tonnes in Fiji) (Jambeck et al., 2015).

In this study, we investigate the abundance and type of plastic found in the gastrointestinal tracts of a suite of species of fish captured and sold for human consumption in two regions of the South Pacific (Australia and Fiji) with distinct economic development levels and waste management strategies. In doing so, we provide essential information on microplastic contamination in fish from a vastly understudied region (Markic et al., 2019; Savoca et al., 2021), which can be used as key baseline data for future studies to monitor the presence and patterns in microplastic type and abundance in the South Pacific. Overall, the extent of microplastic contamination in commercially sold fish from the South Pacific is unknown, as are the types of plastics and polymers present, and how they compare among different sized countries, regarding landmass, population, economy and waste management strategies.

MATERIALS AND METHODS

Sample Collection

Fish samples (193 total) from five species of commercially important species were collected from fish markets in Suva, Fiji, and Brisbane and Sydney, Australia in 2019 (**Figure 1**). Fish were selected based on local availability, as well as covering a range of trophic levels and habitats (e.g., reef-associated, benthic, pelagic) (**Table 1**). Three species were collected in both countries (common coral trout, paddletail, sea mullet), the other species were closely related (Family Mullidae) and chosen based on similarity in feeding habits, habitats and trophic levels. All 73 fish (∼20 individuals from 4 species) purchased in Australia were from the same commercial fishing areas in Queensland and were purchased in June 2019. In Fiji, 120 fish (∼30 individuals from 4 species, see **Table 1**) were purchased from the Suva Municipal Markets in February and March 2019. All fish were locally fished. Most fish were purchased whole, although some samples (eight coral trout from Australia) were collected as frames, with the gastrointestinal tracts still intact, following filleting for human consumption. All fish were transported on ice to the laboratory and stored in the freezer at −20°C until further processing.

Laboratory Methods

All processing of fish occurred inside a laminar flow cabinet. Fork length of the whole fish or fish frame was measured (**Table 1**). The fish were rinsed with ultrapure (Milli-Q) water, dissected, and the gastrointestinal tracts removed. The fish that were collected as frames had the outside rinsed thoroughly with ultrapure water to ensure any external contamination was removed. The entire gastrointestinal tracts were weighed, rinsed in ultrapure water, and placed in individual previously cleaned polypropylene sample jars (**Table 1**). Due to interstate travel restrictions, dissections of some species (common coral trout, sea mullet, paddletail) from Queensland occurred in the field, under open airflow, and all surfaces cleaned thoroughly. In these cases, dissected gastrointestinal tracts were rinsed with ultrapure water, stored and sealed in previously cleaned vials, and transported to the laboratory until further processing, as described above.

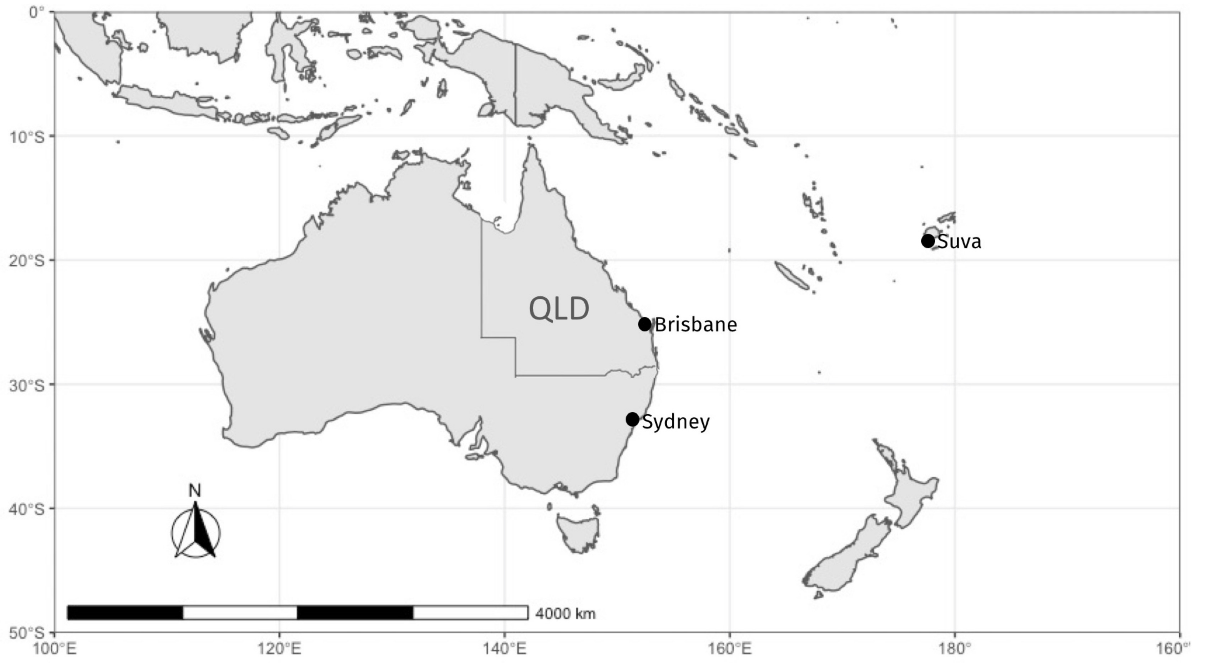

FIGURE 1 | Map of sampling locations in Australia and Fiji. Samples in Australia were fished in the coastal region of Queensland (QLD), and purchased from fish markets in Sydney and Brisbane. Samples from Fiji were fished and purchased locally at the Suva fish markets.

TABLE 1 | Fish purchased in Australia and Fiji.

Common name	Scientific name	Feeding strategy	Habitat	Australia fork length	Fiji fork length	Australia gastrointestinal tract weight	Fiji gastrointestinal tract weight
Common coral trout	*Plectropomus leopardus*	Carnivore	Reef-associated	468.9 (± 18.1)	308.8 (± 8.8)	45.1 (± 5.1)	20.4 (± 2.8)
Bluestriped goatfish	*Upeneichthys lineatus*	Carnivore	Demersal	159.8 (± 5.2)		2.7 (± 0.24)	
Paddletail	*Lutjanus gibbus*	Carnivore	Benthopelagic	430.8 (± 8.5)	214.5 (± 3.5)	39.3 (± 4.8)	9.9 (± 0.65)
Yellowspot goatfish	*Parupeneus indicus*	Carnivore	Demersal		254.5 (± 3.3)		12.6 (± 0.6)
Sea mullet	*Mugil cephalus*	Detrivore	Benthopelagic	343.8 (± 5.6)	245.0 (± 2.2)	24.8 (± 7.7)	13.9 (± 1.8)

The table shows the common name, scientific name, feeding strategy, habitat, mean fork length (mm ± SE), and mean gastrointestinal tract weight (grams ± SE) of each fish species from each country.

Organic material in the gastrointestinal tracts was digested with 10% potassium hydroxide (KOH) solution in ultrapure water (Foekema et al., 2013; Rochman et al., 2015). The samples were then left to digest in closed vials overnight at 60°C in an oven. The digestion method using 10% KOH has been documented as the best method to extract microplastics with the highest isolation efficiency (Dehaut et al., 2016; Lusher et al., 2017; Thiele et al., 2019).

The resultant liquified samples were sieved through two sieves (1 mm and 38 μm), catching any hard objects, including microplastic. All of the sieving process was completed in the laminar flow cabinet. The sieves were examined under a stereo microscope (Leica M80) and any objects thought to be plastic were recorded and collected for further chemical analysis. Microplastic color, size group (>38 μm and <1 mm or >1 mm) and type (i.e., fibers, fragments or film; **Figure 2**) were all recorded.

Contamination Controls

All surfaces, vials and utensils were cleaned beforehand with ultrapure water and dried in a laminar flow. Throughout all processing and analysis, strict protocols were undertaken to ensure that contamination risk was minimized (Lusher et al., 2017; Provencher et al., 2017; Hermsen et al., 2018). The laboratory work area was cleaned methodically before dissections occurred, and between each fish. All fish were rinsed and dissections performed in a laminar flow cabinet to avoid external contamination. Bright pink lab coats and clothing made from natural fibers were worn at all times. Only two pink fibers were found in the samples, and they were excluded. Both procedural and environmental blank samples were prepared during every stage of the methodology (open vials during dissection, polypropylene jars with 10% KOH during digestion and open Petri dishes during sieving and microscope analysis) (Hermsen et al., 2017; Kroon F. et al., 2018). Blank samples

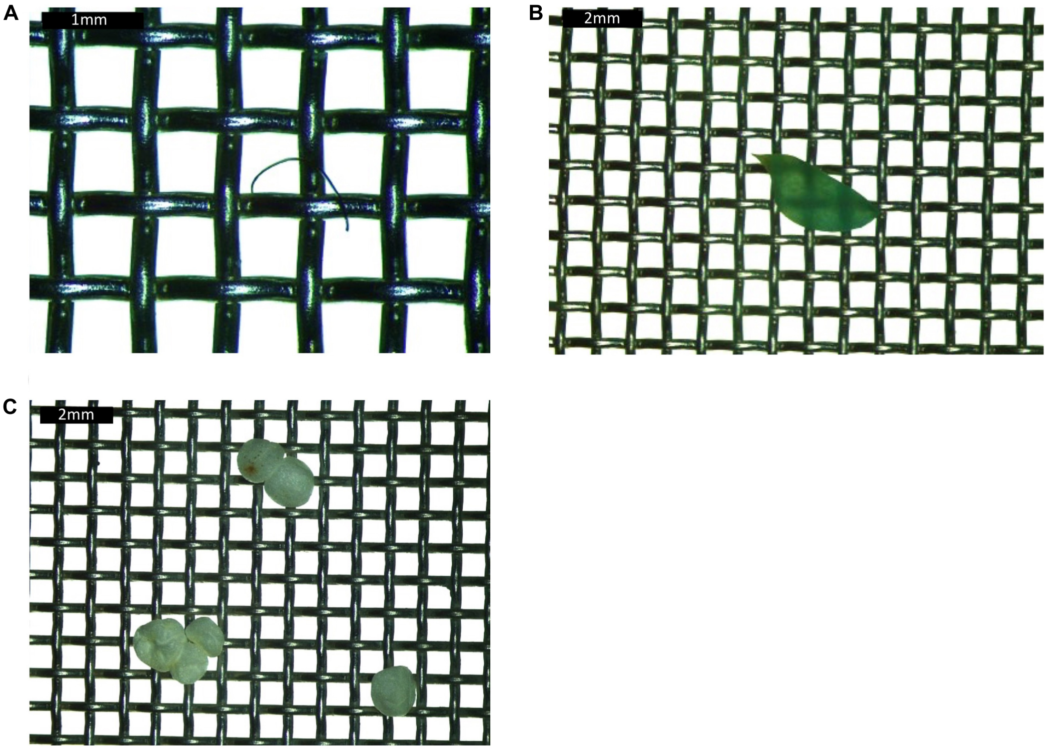

FIGURE 2 | Images of **(A)** fiber, **(B)** film, and **(C)** fragment collected from the gastrointestinal tract of fish. Each grid is 1 × 1 mm square.

were placed directly alongside the work area and processed, filtered and analyzed using the same methods we used for fish gastrointestinal tracts. No evidence of contamination was found in the blank samples.

Characterization and Identification of Microplastics

We tested microplastic pieces using micro-Fourier Transformed Infrared Spectroscopy (μ-FTIR) (Bruker Hyperion) to identify polymer type (Jung et al., 2018). Attenuated Total Reflection (ATR) was applied to all cleaned samples at a resolution of 4 cm^{-1}. Aperture size was readjusted to as small as necessary depending on sample size. Three randomly selected measurement positions were chosen within each sample, and 64 co-scans for each measurement were taken. The spectrum range was set between 3,900 and 650 cm^{-1}, with the atmospheric water/CO_2 region between 2,500 and 1,900 cm^{-1} excluded when compared to spectral libraries, as recommended by Primpke et al. (2018) and Jensen et al. (2019). All spectra outputs were compared to the libraries of reference (Bruker ATR Library for Polymers, Bruker ATR Library for Chemicals, Bruker ATR Library for Pharma, Bruker ATR Library for Forensics) to verify the polymer type (**Supplementary Figure 1**). A hit quality value (i.e., percent match) between the sample and the library reference spectrum was obtained for each item. The polymer was only confirmed if the match was >50% and additionally if the stringent visual analysis of the peaks were identified as the same.

We aimed to test all pieces of microplastic, however, some pieces were too small to analyze on the μ-FTIR slide (24% of all samples) so we did not include these in our final plastic counts. When there were multiple pieces of microplastic from the same fish that visually appeared to be the same plastic piece and polymer type, we tested a portion of the pieces first (e.g., if there were three pieces of plastic that visually appeared the same we would test two of them). If polymer type was found to be the same, we assumed that the remaining pieces were of the same polymer type.

Data Interpretation and Statistical Analysis

The color, type and size of microplastics were quantified for each individual fish. The average amount of microplastic per fish of each species and country was calculated; this is referred to as the plastic load (PL). This value includes all fish sampled, even those which were found to have no plastic present, hence the average PL can be a value less than one. The percentage of fish with at least one piece of plastic was quantified for each species and country. This value represents the frequency of occurrence of plastic ingestion (FO).

We tested for a relationship between plastic load and fork length or gastrointestinal tract weight but none were found. The data were analyzed using a negative binomial generalised linear model (GLM) to investigate the influence of location of capture (country) and species on the estimated frequency of occurrence of plastic and plastic load per fish. We used the Akaike's information criterion value corrected for small sample sizes (AICc) to select the best model fit. For the best fit model (Location + Species), estimates of the species means were made, followed by ANOVA and pairwise tests to determine any differences between species and countries. Residual plots were analyzed to ensure data met assumptions. Graphical outputs were produced from the model predictions. Statistical analyses were conducted using R studio software (Version 3.6.1), including the maps (Becker et al., 2018), ggplot2 (Wickham, 2016), emmeans (Length et al., 2020), doBy (Højsgaard and Halekoh, 2020), rgeos (Bivand and Rundel, 2020), rnaturalearth (South, 2017a), rnaturalearthdata (South, 2017b), sf (Pebesma, 2018), treemap (Tennekes, 2017), tidyverse (Wickham et al., 2019), and sunburstR (Bostock et al., 2020) packages.

Subsequently, Primer-e was used to run Canonical Analysis of Principal Coordinates (CAP) on the multivariate polymer dataset. CAP was used to assess the ability to classify fish to their country of origin based on the types of microplastic they contain. CAP is a constrained ordination that allows an unbiased measure of how different groups are in multivariate space (Anderson and Willis, 2003). Variables were log(x + 1) transformed and unrestricted random permutations of the transformed data were applied. Cross-validated classification accuracies were analyzed for all species combined.

RESULTS

Differences in Abundance of Plastic Between Countries

A total of 296 pieces of microplastic were collected using visual microscope techniques (148 from Fiji and 148 from Australia), of which 212 were confirmed as microplastic using the μ-FTIR (102 from Fiji and 110 from Australia). The majority of microplastic pieces were larger than 1 mm in size (88.9% of pieces from Australia and 86.4% of pieces from Fiji), with the remainder of pieces being between 38 μm and 1 mm.

There were no trends between fish size, or the weight of the gastrointestinal tract, and the amount of microplastic counted. Overall, the frequency of occurrence of plastic ingestion in fish from Australia was higher than Fiji (61.6% in Australia compared to 35.3% in Fiji) and there was a significant difference between countries ($p < 0.001$, **Figure 3**). The overall plastic load found in fish was higher in Australia compared to Fiji (1.58 ± 0.23 and 0.86 ± 0.14 pieces per fish, respectively) ($p < 0.05$). Both countries had a large percentage of fish (38.4% in Australia and 64.7% in Fiji) that had zero pieces of microplastic (**Figures 3A,B**). The highest number of microplastic found in any one fish was eight pieces, found in a common coral trout and paddletail in Australia, and a common coral trout from Fiji (**Figures 3A,B**, respectively).

All species from both countries had microplastic present, however, the predicted frequency of occurrence of plastic ingestion varied depending on the species and which location they were captured (**Figure 4**). In Fiji, the common coral trout was the species with the most fish with at least one piece of plastic, whilst yellowspot goatfish had the least (**Figure 4**). In Australia, the paddletail had the most fish with microplastic and the sea mullet had the least (**Figure 4**).

The plastic load also changed between species and locations. In Fiji, the highest expected microplastic load was in paddletail and the lowest in sea mullet (**Figure 5**), whereas, in Australia, common coral trout had the most pieces of microplastic on average in their gastrointestinal tract and similarly to Fiji, sea mullet had the least (**Figure 5**).

Differences in Types of Plastic Between Countries

Of the 296 pieces initially identified under the microscope as microplastic, we analyzed 124 pieces using the μ-FTIR (**Figure 5**). This number of analyses in the μ-FTIR is due to, first, some pieces of microplastic being too small (e.g., not visible to the naked eye) and thus we were unable to transfer and verify their polymers onto the μ-FTIR slide, with these pieces removed from the reported total counts of plastic ($n = 71$). Second, when multiple pieces of plastic in the same fish visually appeared identical we tested a portion of microplastic pieces, with the μ-FTIR confirming these pieces were all the same polymer. Overall, of the 124 pieces we tested, 111 were confirmed as microplastic, an 89.5% accuracy. After the pieces of plastic that were visually identified, and confirmed by the μ-FTIR as being the same were combined back into our total ($n = 101$), we had a total of 212 confirmed pieces of microplastic.

There were differences in the types of microplastic found in fish between the two countries. Half of the microplastics found from fish in Fiji were film (50.0%), with the remaining split between fragments (25.5%) and fibers (24.5%) (**Figure 6A**). In contrast, in Australia, fibers dominated (82.4%), with fewer fragments (10.2%) and film (7.4%) present (**Figure 6B**).

The polymer type of plastic pieces varied between countries and within the type of microplastic (fiber, fragment and film, **Figure 6**). In fish from Australia, pieces of microplastic were dominated by polyolefin (48.2% of all fibers, film and fragments from Australia; **Figure 6A**). Polyolefins are a broad polymer group that includes polyethylene (75% of all Australian polyolefins), ethylene-vinyl acetate (11%), synthetic rubber (5.5%), polypropylene (5.5%), and polystyrene (3%) (see **Supplementary Table 1**). In fish from Fiji, polyolefin was also the most abundant polymer group identified, with 38% of all plastic falling in this category. This included polyethylene (36% of Fiji polyolefins), ethylene-vinyl acetate (26%), polypropylene (15%), polystyrene (10%), and other polymers such as polyurethane and synthetic rubber (**Supplementary Table 1**).

Within the fiber morphotype, polyolefin fibers (49.5% of all Australian fibers) were the most prominent, followed by polyester with 20% (**Figure 6A** and **Supplementary Table 1**). Synthetic fibers (10.1% of Australian fibers), were also substantial and were composed of a combination of elastane, lycra, rayon and spandex mixed with natural materials such as cotton and wool. In Fiji, the fibers were dominated by paint (36.0% of fibers from Fiji). Despite this, a total of seven polymer groups were identified in fibers from both countries, with five present in both (pure nylon, paint, polyester, polyolefin and synthetic fiber). Fiji additionally

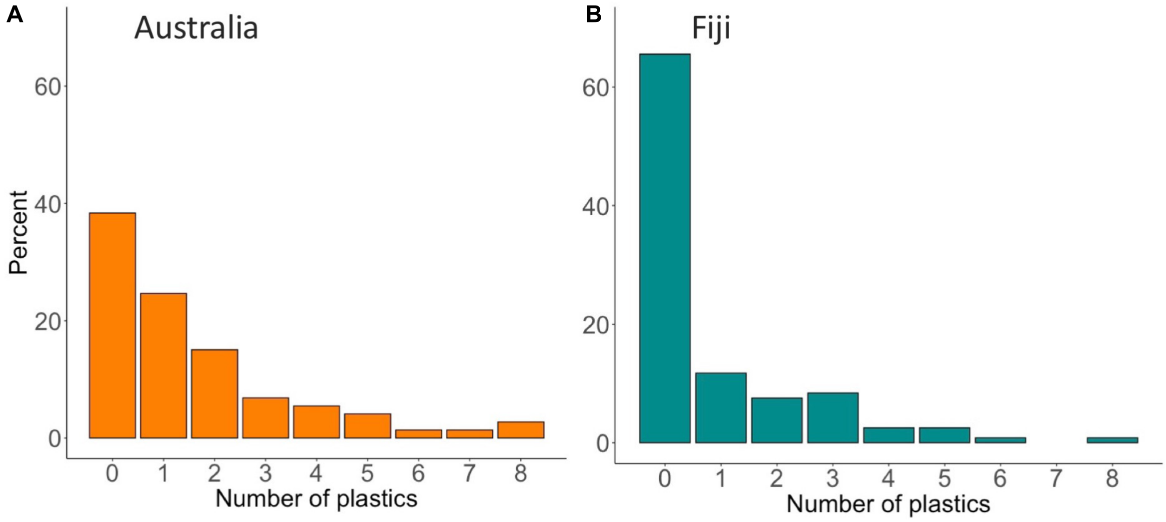

FIGURE 3 | Percentage of the total count of plastics found in the gastrointestinal tract of individual fish from **(A)** Australia (*n* = 73 fish) and **(B)** Fiji (*n* = 120 fish).

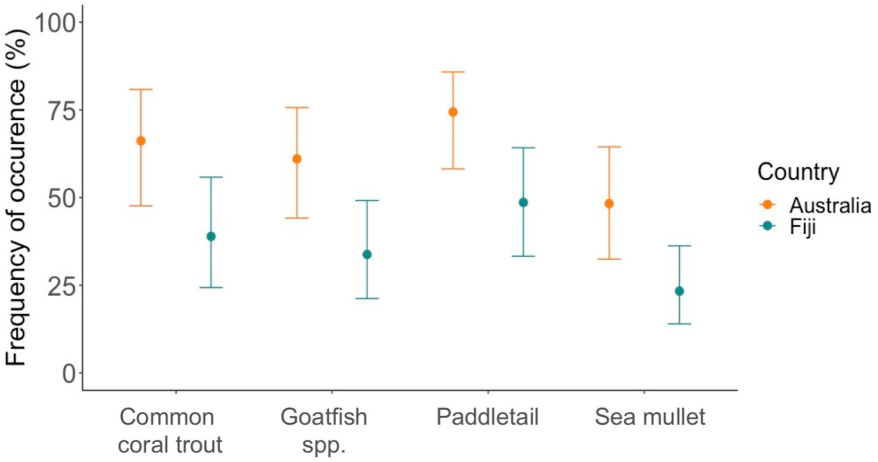

FIGURE 4 | Frequency of occurrence of plastic ingestion (FO). The graph shows the expected percentage of fish from each species that had at least one piece of microplastic present in their gastrointestinal tract based on a negative binomial GLM. The error bars show the lower and upper 95% confidence intervals. The blue points and lines represent fish purchased in Fiji and the orange points and lines represent those purchased in Australia. Refer to **Table 1** for scientific species names, where goatfish is yellowspot goatfish for Fiji and bluestriped goatfish for Australia.

had small numbers of acrylate and polyurethane filaments (*n* = 2, *n* = 1, respectively) and Australia had PVC (Poly Vinyl Chloride) and polymer resin filaments identified (*n* = 4, *n* = 3, respectively).

In general, film pieces from Australia had less diversity in their polymer types compared to Fiji, with only three polymers identified (**Supplementary Table 1**) while Fiji had six different polymer groups, including polyolefin (37.3%), acrylate (19.6%), paint, nylon, and silicone (**Figure 6B** and **Supplementary Table 1**). Within these six polymer groups there were 14 polymers identified, with polyethylene dominating (24% of all film pieces from Fiji). Fragments were less common in both countries but when present were mostly polyolefin and paint. Additionally, Australia had one piece of both polymer resin and PVC; while Fiji had one piece of nylon.

Results of the CAP analysis revealed an overall high level of accuracy of classification of fish to their country of origin, based on the amount and type of plastic found in their gastrointestinal tracts. Cross-validated classification results varied between countries, with an overall correct classification of 73.96% to the respective country where the fish were caught.

DISCUSSION

All of our five species of fish (*L. gibbus, M. cephalus, P. indicus, P. leopardus, U. lineatus)* consumed microplastic. Overall, 61.6% of fish from Australia and 35.3% of fish from Fiji had microplastic in their gastrointestinal tract contents, with higher average plastic

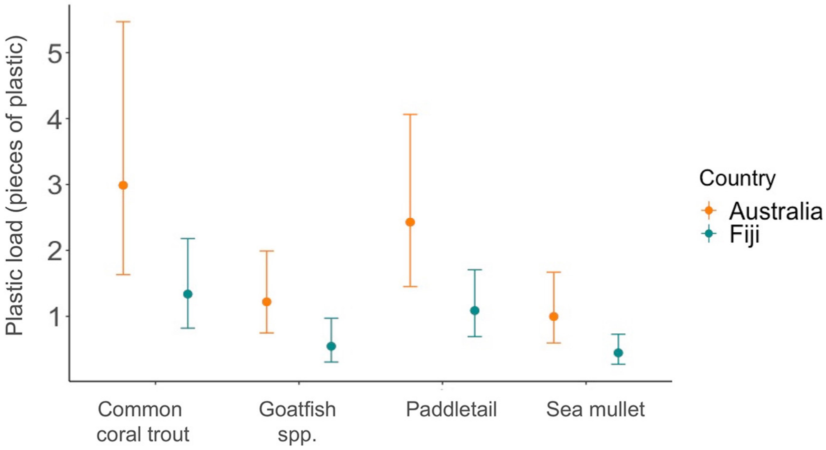

FIGURE 5 | Average plastic load per fish. The points show the expected mean number of microplastics found in the gastrointestinal tract of each species from a negative binomial GLM fitted to the data. The vertical lines represent the lower and upper 95% confidence intervals. The blue points and lines represent fish purchased in Fiji, while the orange points and lines are those purchased in Australia. **Table 1** provides scientific species names, where goatfish is yellowspot goatfish for Fiji and bluestriped goatfish for Australia.

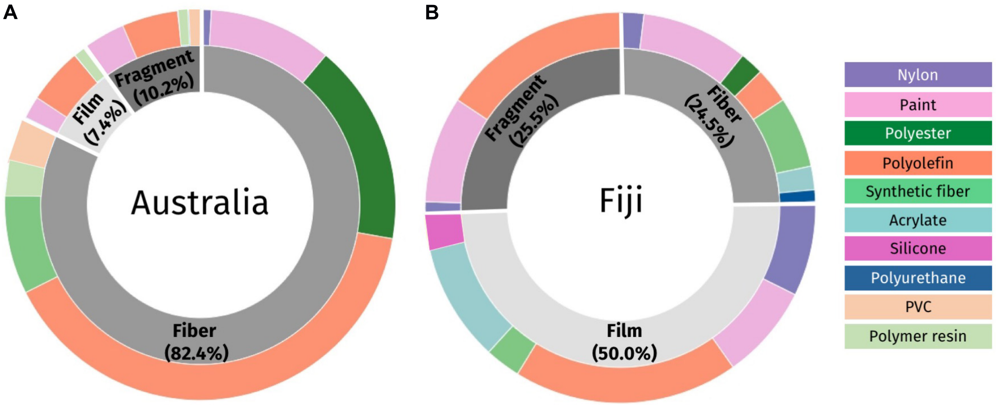

FIGURE 6 | Different types of microplastic found in the gastrointestinal tract of fish in Australia **(A)** and Fiji **(B)**. The inner layer shows the plastic types identified under the microscope (fiber, fragment, and film) in shades of gray; the outer layer shows the plastic polymer groups confirmed using the μ-FTIR (**Supplementary Table 1**).

loads in Australian (1.58 pieces) fish compared with those from Fiji (0.86 pieces). Microplastic was found in fish species across different trophic levels (carnivores, detrivores) and habitats (reef, seagrass, sediment/sand, open seas, and rocky reef). All five species sampled are important fishery species in both countries, supporting the economy, employment, and food sources of their respective populations. Therefore, quantifying the base levels of microplastic in these species provides leverage for future risk assessment and an understanding of the levels of potential contamination present in seafood to evaluate potential risks to human consumption.

Research on microplastic ingestion in fish from the South Pacific is limited. One of the more recently published studies (Markic et al., 2018), collected fish from markets across four South Pacific countries (New Zealand, Samoa, Tahiti and Rapa Nui), finding that across all regions on average 24.3% of fish ingested plastic, much lower than the 61.6% in our Australian fish and 35.3% in Fijian fish that we found in our study. The range between countries is broad, but encompasses values similar to those in which we found, with 49.2% of fish from Rapa Nui containing microplastic. Another study investigating fish from a variety of small islands across the South Pacific (Lord Howe Island, French Polynesia and Henderson Island) also found lower numbers of fish with plastic than what our study identified, with Forrest and Hindell (2018) only detecting ten fish from the 126 individuals (7.9%) collected to have microplastic present. Both studies (Forrest and Hindell, 2018; Markic et al., 2018) include species from similar habitats (benthic and rocky reef) to the fishes in our study, however, plastic contamination still varied to our results, with both higher and lower amounts of plastic found in similar species. The only study to our knowledge investigating fish from Fiji found 68% of fish to ingest microplastic, with an average of 5.5 pieces per fish (Ferreira et al., 2020), and these findings are much higher than what we found in the fish we sampled from Fiji (FO–35.3%, PL–0.86). Research is more prominent in Australia where at least six separate studies have identified microplastic in fish from the region (Cannon et al., 2016; Halstead et al., 2018; Kroon J. F. et al., 2018; Jensen et al., 2019; Su et al., 2019; Crutchett et al., 2020). Kroon J. F. et al. (2018) and Jensen et al. (2019) found much higher levels of contamination than our fish, with both studies finding 95% of fish sampled from the Great Barrier Reef had micro debris present. Clearly, there are discrepancies across currently published data investigating microplastic in fish from the South Pacific; with differences potentially caused by the locations sampled and the waste management from these regions, population sizes of countries, currents and environments retaining or depositing microplastic from offshore, or due to the methodology implemented.

Methods used for detection of microplastic have varied over time, with improvements in technical abilities since microplastic research has increased in popularity (Savoca et al., 2021). Some researchers used only naked-eye (e.g., Forrest and Hindell, 2018) or microscope detection (e.g., Cannon et al., 2016) to visualize the gastrointestinal tract content. Although these methods were initially accepted in microplastic research, the trend in analytical methods are moving toward more robust and standardized laboratory procedures, including a digestion of the gastrointestinal tract content and chemical verification of the polymers (Markic et al., 2019; Savoca et al., 2021). In our study, we ensured we followed the highest standard of methodology, using a chemical digestion, large sample sizes, contamination controls and confirmation of polymers using a chemical verification, such as FTIR (Wesch et al., 2016; Hermsen et al., 2017; Lusher et al., 2017). Although we understand the difficulty with implementing a more complex procedure, particularly in countries were resources are limited, it is likely that previous studies may have underestimated the abundance of microplastic in fish from the South Pacific (e.g., Cannon et al., 2016 where only 0.3% of fish sampled contained microplastic). It is thought that increasing counts of plastic in recent studies is a combination of an improvement in analytical methodology, as well as a likely increase in the fish ingesting plastic more frequently as it becomes more abundant in the environment (Savoca et al., 2021).

Initially, it was predicted that Australia may have less microplastic in fish than Fiji because in recent years Australia has made positive moves forward in its waste management and legislation surrounding plastic use. Specifically, in 2018 the Queensland state government released a comprehensive "Waste Management and Resource Recovery Strategy," including the implementation of "Queensland's Plastic Pollution Reduction Plan"(Queensland Government, 2018a,b). These legislations included the execution of a single-use plastic bag ban, a container deposit scheme, as well as stricter enforcement on illegal dumping and littering (Queensland Government, 2018a). In contrast, in Fiji, waste management strategies are rare, and at the time of sampling the fish in our study, there were no bans on plastic bags, and very limited waste management facilities. In a positive move, there have been recent advances in the management of plastics in Fiji, with a plastic bag ban being enforced at the beginning of 2020, and prohibitions on other plastic items (e.g., Styrofoam and plastic straws) commencing at the start of 2021. With our sampling occurring at the beginning of 2019, these policies had not yet been implemented or had an effect. Despite our initial predictions that Fiji would have higher levels of microplastic in their fish, this was not found to be evident, as overall Australia had both higher frequency of occurrence of plastic ingestion and the plastic load in their fish compared to Fiji. It is likely that neither Australia nor Fiji's recent plans to reduce their plastic output would have had substantive effect on the microplastic presence in such a short time period. Due to the long breakdown time of plastic, the microplastic identified in this study may have been introduced in the environment before the new regulations, nevertheless we expect to see positive effects of these plastic reduction plans over the years and in future assessments. This perpetual microplastic may have been amassing in oceanic accumulation zones, where ocean currents and gyres cause microplastic to gather in particular marine regions. Fish with exposure to these regions are likely to consume more microplastic as a consequence of higher likelihood of microplastic interactions, as well as a limitation to food availability due to the oligotrophic nature of the water (Sigman and Hain, 2012). Although neither Fiji or Australia are located specifically within a microplastic gyre, the currents surrounding both regions suggest high levels of microplastic present in the water, which are likely linked to the microplastic found in fish (Lebreton et al., 2012; Eriksen et al., 2014).

Overall, differences in in population size between the two countries could explain the results, with highly populated coastal regions generally associated with higher densities of plastic debris (Lebreton et al., 2012). Fiji is relatively sparsely populated, with a population size of roughly 900,000, of which around 896,000 live on the coastline (Jambeck et al., 2015). In contrast, in Australia the population is 25 million people, of which 80% reside on the coast (Yang and Kelly, 2015). The east coast, where the fish from this study were caught is particularly dense. The differences in population sizes also mean there is a stark difference in the amount of waste produced each day, with Australia predicted to produce eleven times more plastic waste than Fiji (Jambeck et al., 2015). Land-based effluent discharges are a contributor of microplastic in our waterways, with quality sewage treatment systems acting as a filter to limit their outflow (Siegfried et al., 2017). Depending on the treatment type, one treatment plant in Sydney, on Australia's eastern coast, is thought to discharge between 3.6 and 460 million microplastic pieces into marine systems daily (Ziajahromi et al., 2017). Australia's increase in plastic waste production, as well as land-based effluent discharges, could be influencing the difference in plastic abundance between the countries, however waste management strategies and differing lifestyles could also be having an impact, particularly in the type of plastics identified.

The microplastic found in Fiji fish were dominated by sheets of plastic film, which are commonly secondary microplastic, broken down from original larger pieces of plastic. These could be from a range of sources such as polypropylene or polyethylene (29% of film from Fiji), potentially from plastic bags and soft food packaging, or acrylate and paint chips (also 29% of film from Fiji) possibly from boats. The issues surrounding waste management strategies in small island developing states such as Fiji could be contributing to this, as incorrect use of landfill and disposal could mean larger plastic items are entering the waterways and subsequently eroded into microplastic (Mohee et al., 2015; Hardesty et al., 2017). In contrast, the landfill management in Australia, as well as legislation, policies and education programs, could be contributing to the lower numbers of fragments and film, as the likelihood of hard or larger pieces of plastic are prevented from entering the ocean in the first place (Willis et al., 2018). In Australia, over 80% of the microplastic identified were fibers, a pervasive microplastic in the marine environment, commonly formed from synthetic clothing and fishing gear (Browne et al., 2011; De Falco et al., 2019). Australia's larger population, as well as the fact that one load of washing may contribute up to 1.5 million pieces of microplastic, may both be contributing to these large numbers of fibers (De Falco et al., 2019). High levels of fibers in fish from developed countries with large populations is evident in other studies. Markic et al. (2018) found New Zealand fish had higher quantities of fibers compared to fish from Samoa, which had more fragments and film. Further, a study from Rochman et al. (2015) comparing fish and bivalves purchased from markets in Indonesia and the United States found that the United States had much larger proportions of microplastic fibers in their fish compared to Indonesia. These comparison studies showing high quantities of fibers in developed countries are noteworthy, and perhaps indicative of a further link between differing lifestyles of populations and environmental plastic contamination.

With global consumption of seafood on the rise, understanding the potential risks and challenges that could transpire from microplastic contamination in seafood is more important than ever (FAO, 2020). The physical and toxicological harm that microplastic could potentially cause to fish and their ecosystems could be a threat to local food security, particularly in communities that rely on seafood as a key source of protein (Béné, 2006; Rochman et al., 2016). The long term exposure of microplastic, and the chemicals associated with them, have the ability to negatively affect fish health, potentially impacting the long term sustainability of fisheries (Smith et al., 2018). The spread of microplastic throughout global marine ecosystems have generated concern about whether microplastic ingestion in seafood could penetrate the food web and eventually be consumed by humans. The fish species sampled in this study are mostly eaten after their gastrointestinal tracts are removed, thus the chance of human consumption of the microplastic in this case is low (Dawson et al., 2021). It is important consumers ensure fish are appropriately gutted prior to consumption. In doing so, they are lowering the risk of microplastic contamination from their diets. However, there is still a potential risk to humans as a result of the uptake and translocation of microplastics and their associated chemicals into the flesh of the fish (Teuten et al., 2009). The mechanisms behind this uptake, and the link between long term exposure of microplastic, and the potential for chemicals to translocate is still understudied, and requires more research effort in the future (Ory et al., 2018; Smith et al., 2018). Furthermore, the risk of consumption of microplastic from a human health perspective is still far from being well understood (Smith et al., 2018; Vethaak and Legler, 2021).

Linking variations in the abundance and type of microplastic present in coastal environments and local biota is an area that requires further investigation. Likewise, understanding the type of plastic present, their location of origin and unraveling the pathways these plastics take to reach the marine environment is crucial to developing solutions for microplastic contamination (Kane and Clare, 2019; Petersen and Hubbart, 2021). While plastic presence across coastal environments is widespread, there are a suite of reasonable intervention points that could help lower its impact, from initial production, to disposal, which would assist in reducing plastic entering the marine environment in the first place (Tibbetts et al., 2018; Prata et al., 2019; Petersen and Hubbart, 2021). By further investigating these avenues, we can begin to create answers to limit the presence of microplastic in marine wildlife and fish species. As yet, we do not yet know how these microplastic may be negatively affecting fish health (Ory et al., 2018), how plastic may be impacting human health or if bioaccumulation and biomagnification of chemicals are occurring (Walkinshaw et al., 2020). However, for now, we can say that fish in the South Pacific are consuming microplastics, and the numbers differ between the two countries. Our results provide important baseline data which can be combined with future data to give a broad picture of microplastic contamination in seafood in the South Pacific.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because samples were purchased through seafood markets.

AUTHOR CONTRIBUTIONS

NW collected the data, undertook the data analysis, wrote the manuscript, and prepared the figures and tables. BG and PR-S reviewed the manuscript, figures, and tables. BG, PR-S, and MF provided advice and guidance throughout the study. All authors conceived the project idea and designed the data analysis.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.690991/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary information S3

A comparison of microplastic in fish from Australia and Fiji

Nina Wootton, Marta Ferreira, Patrick Reis-Santos, Bronwyn M Gillanders

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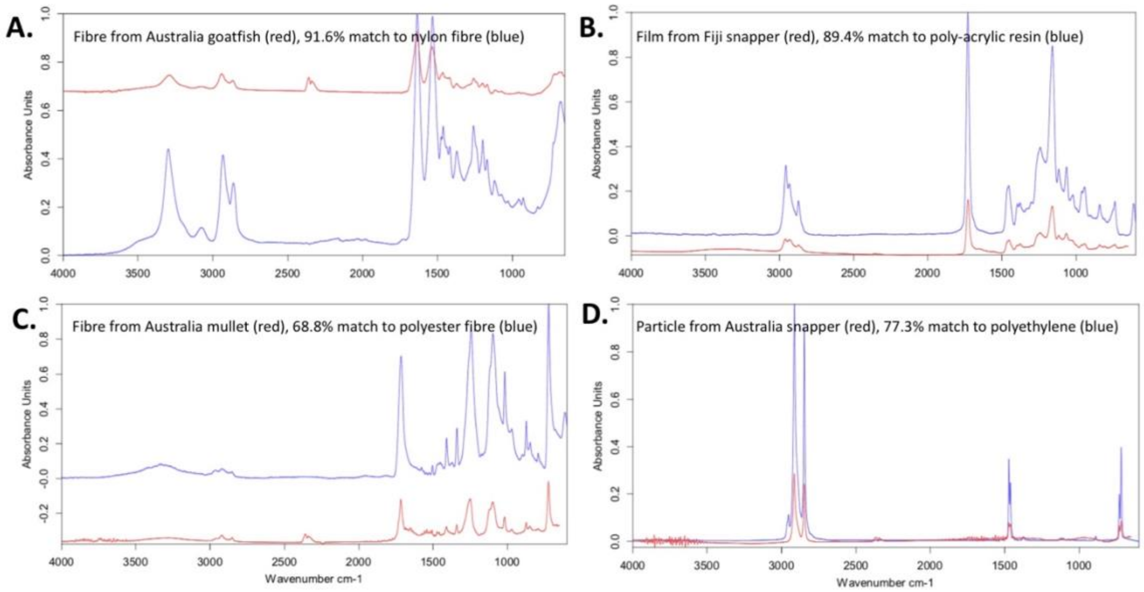


Figure S3.1: A sample of ATR-FTIR spectral matches from microplastic sourced from fish from Fiji and Australia. This includes (A) nylon fiber from Australian bluestriped goatfish, (B) poly-acrylic resin from Fijian paddletail, (C) polyester fiber from Australian sea mullet and (D) polyethylene fragment from Australian paddletail. Red spectra represent the FTIR output from the plastic samples, blue spectra are the polymer library spectral matches, (A) 91.6% hit quality, (B) 89.4%, (C) 68.8%, (D) 77.3%. The range of spectrum was set between 3,900 and 650 cm^{-1}, with the region between 2,500 and 1,900 cm^{-1} excluded due to atmospheric water and CO_2 interferences.

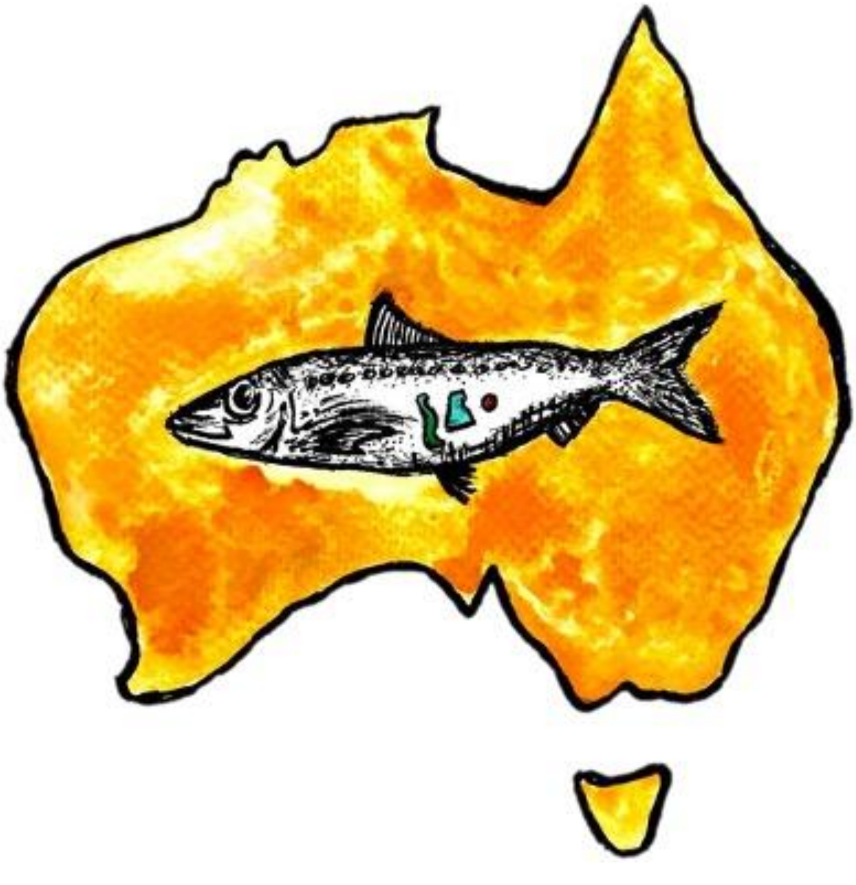
Table S3.1: Counts of different types of plastic, polymers and general groups of polymers found in fish from Australia and Fiji. PVC is poly-vinyl chloride

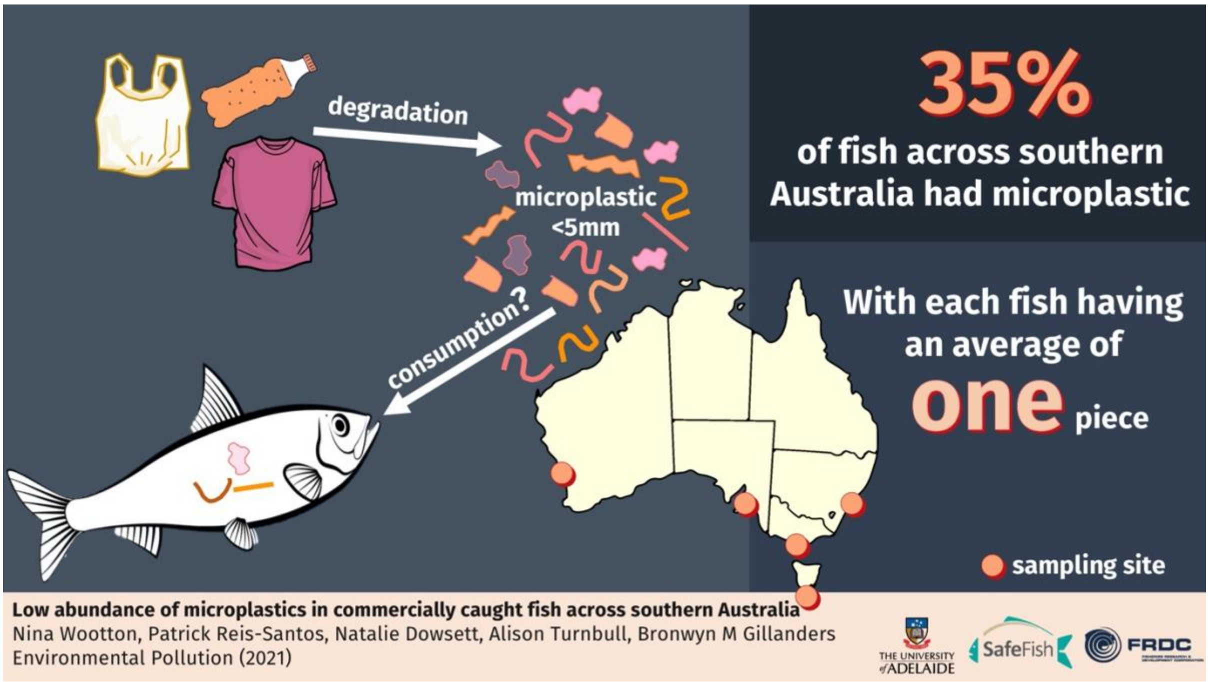
Country	Type of plastic	Polymer	General polymer group	Number of polymer pieces
Australia	**Fiber**	Copolymer (beach shoe) – ethylene vinyl acetate	Polyolefin	4
		Ethylene vinyl acetate	Polyolefin	2
		Hostalen GM 6255	Polyolefin	1
		Polyethylene	Polyolefin	34
		White rubber	Polyolefin	2
		Polypropylene	Polyolefin	2
			Polyolefin total	**45**
		Inorganic plaster	Paint	6
		Paint	Paint	1
		Primer paint	Paint	4
			Paint total	**11**
		Rayon polyester fiber	Polyester	11
		Poly (ethylene terephthalate)	Polyester	1
		Polyester fiber	Polyester	6
			Polyester total	**18**
		Polyvinyl	PVC	2
		PVC based fiber	PVC	2
			PVC total	**4**
		Cotton and elastane	Synthetic fiber	5
		Modal and spandex semi synthetic fiber	Synthetic fiber	4
			Synthetic fiber total	**9**
		Nylon fiber	Nylon	1
			Nylon total	**1**
		Polymer resin	Polymer resin	3
			Polymer resin total	**3**
			TOTAL	**91**
Australia	**Film**	Hydroxypropyl cellulose	Polymer resin	1
			Polymer resin total	**1**
		Polyethylene	Polyolefin	5
			Polyolefin total	**5**
		Primer paint	Paint	2
			Paint total	**2**
			TOTAL	**8**
Australia	**Fragment**	Polyethylene	Polyolefin	2
		Polystyrene	Polyolefin	2
		White rubber	Polyolefin	1
			Polyolefin total	**5**
		Acrylic paint	Paint	2
		Primer paint	Paint	2
			Paint total	**4**
		PVC Plasticizer	PVC	1
			PVC total	**1**
		Polymer resin	Polymer resin	1
			Polymer resin total	**1**
			TOTAL	**11**

Fiji	**Fiber**	Clear varnish seidenmatt with polyurethane	Polyolefin	1
		Ethylene vinyl acetate	Polyolefin	1
		Polypropylene	Polyolefin	2
			Polyolefin total	**4**
		Acrylic paint	Paint	4
		Primer paint	Paint	5
			Paint total	**9**
		Polyacrylate	Acrylate	1
		Polyacrylonitrile (copolymer)	Acrylate	1
			Acrylate total	**2**
		Poly (ethylene terephthalate)	Polyester	1
		Rayon polyester fiber	Polyester	1
			Polyester total	**2**
		Spandex	Synthetic fiber	1
		Cotton lycra fiber	Synthetic fiber	5
			Synthetic fiber total	**6**
		Nylon fiber	Nylon	2
			Nylon total	**2**
			TOTAL	**25**
Fiji	**Film**	Black rubber	Polyolefin	2
		Polystyrene	Polyolefin	2
		Polyethylene	Polyolefin	12
		Polypropylene	Polyolefin	3
			Polyolefin total	**19**
		Polyacrylate	Acrylate	3
		Acrylic fiber	Acrylate	1
		Acrylic resin	Acrylate	6
			Acrylate total	**10**
		Acrylic paint	Paint	2
		Primer paint	Paint	5
		Paint	Paint	1
			Paint total	**8**
		Mixed nylon fiber	Nylon	5
		Nylon	Nylon	3
			Nylon total	**8**
		Cotton lycra fiber	Synthetic fiber	3
			Synthetic fiber total	**3**
		Silicone	Silicone	3
			Silicone total	**3**
			TOTAL	**51**
Fiji	**Fragment**	Ethylene vinyl acetate	Polyolefin	9
		Polyethylene	Polyolefin	2
		Polypropylene	Polyolefin	1
		Polystyrene	Polyolefin	4
			Polyolefin total	**16**
		Primer paint	Paint	8
		Paint	Paint	1
			Paint total	**9**
		Nylon	Nylon	1
			Nylon total	**1**
			TOTAL	**26**

CHAPTER 4

LOW ABUNDANCE OF MICROPLASTICS IN COMMERCIALLY CAUGHT FISH ACROSS SOUTHERN AUSTRALIA





35% of fish across southern Australia had microplastic

With each fish having an average of **one** piece

Low abundance of microplastics in commercially caught fish across southern Australia
Nina Wootton, Patrick Reis-Santos, Natalie Dowsett, Alison Turnbull, Bronwyn M Gillanders
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Principal Author

Name of Principal Author (Candidate)	Nina Wootton		
Contribution to the Paper	Contributed to design of the study, reviewed literature, conducted the fieldwork, collected samples, completed laboratory processing, operated the FT-IR, analysed the data and applied statistical analyses, wrote the manuscript and acted as corresponding author.		
Overall percentage (%)	75%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	5/10/21

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Patrick Reis-Santos		
Contribution to the Paper	Contributed to design and development of the study, advice and suggestions throughout, provided comments and feedback on manuscript.		
Signature		Date	06/10/2021

Name of Co-Author	Natalie Dowsett		
Contribution to the Paper	Funding acquisition, provided comments and feedback on manuscript.		
Signature		Date	12/10/2021

Name of Co-Author	Alison Turnbull		
Contribution to the Paper	Funding acquisition, provided comments and feedback on manuscript.		
Signature		Date	7/10/2021

Name of Co-Author	Bronwyn Gillanders		
Contribution to the Paper	Contributed to design and development of the study, advice and suggestions throughout, acquisition of funding, provided comments and feedback on manuscript.		
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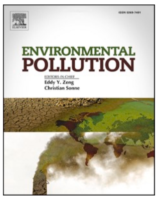

Low abundance of microplastics in commercially caught fish across southern Australia☆

Nina Wootton[a,*], Patrick Reis-Santos[a], Natalie Dowsett[a,b], Alison Turnbull[b,c], Bronwyn M. Gillanders[a]

[a] School of Biological Sciences, University of Adelaide, SA, 5005, Australia
[b] South Australian Research and Development Institute, Food Sciences Division, GPO Box 397, Adelaide, SA, 5001, Australia
[c] Institute of Marine and Antarctic Studies, University of Tasmania, Taroona, Tasmania, 7053, Australia

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ABSTRACT

Plastic pollution has increased significantly in the past decades and is now a major global environmental issue. Plastic objects enter the ocean and are broken down into smaller pieces, while wastewater and runoff also carry microplastics (plastics <5 mm) into the ocean. Plastic has been found in over 700 different species of marine wildlife but little research has examined fish sold for human consumption. We determined the microplastic abundance in nine commercially important, wild-caught fish species purchased from seafood markets across 4000 km of Australia (Western Australia, South Australia, Victoria, Tasmania, New South Wales). For microplastic quantification, fish gastro-intestinal tracts were chemically digested and the amount and type of microplastic identified under a microscope and Fourier transform infrared spectrometer. Across all states, an average of 35.5% of fish samples had at least one piece of microplastic in their gastro-intestinal tract. South Australia had the highest percentage of fish with plastic (49%) and Tasmania the lowest (20%). The average microplastic load was 0.94 piece per fish but ranged from 0 to 17 pieces, with polyolefin identified as the dominant polymer group. Overall, the ingestion of microplastic was widespread across species, locations, diets and habitat niches of fish species investigated, but the average plastic ingestion was less than other similar global studies. This study provides novel insights on the use of fish species from seafood markets to assess environmental contamination by microplastic, as well as an important perspective of the potential for microplastic contamination to enter the human food chain.

1. Introduction

Since its creation in the early 20th century, plastic has become a widespread and popular material due to its durability, convenience and low-cost (Dehaut et al., 2019). Of the 8300 million metric tonnes of virgin plastics produced to date, only 9% have been recycled (Geyer et al., 2017), and it is estimated that 80% of all plastic ever produced is in landfill or in our natural environments (Geyer et al., 2017). Between 4.8 and 12.7 million tonnes of plastic enter the ocean each year (Jambeck et al., 2015), with vast amounts of microplastics accumulating globally (i.e. plastic pieces less than 5 mm in size) (Eriksen et al., 2014; Rochman et al., 2013). Microplastic can either be manufactured to be that size (primary microplastics), or formed from larger pieces of plastic that have broken down due to weathering and physical forces such as wave and wind action (secondary microplastic). Microplastics are ubiquitous throughout the ocean and are commonly identified in marine life, including marine mammals (e.g. Burkhardt-Holm and N'Guyen, 2019), turtles (e.g. Duncan et al., 2019), teleost fish (e.g. Collicutt et al., 2019; Steer et al., 2017), sharks (e.g. Valente et al., 2019) and a variety of bivalves and crustaceans (e.g. Bour et al., 2018; Cho et al., 2019; Courtene-Jones et al., 2017). Globally microplastic presence has been documented in over 386 marine fish species (Savoca et al., 2021). However, the majority of these studies have not examined fish obtained from seafood markets and destined for human consumption. Additionally, whilst research on plastic contamination has been undertaken globally, it is understudied in the South Pacific and Southern Ocean regions.

Microplastic ingestion in fish can occur via primary ingestion, where

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☆ This paper has been recommended for acceptance by Eddy Y. Zeng.

* Corresponding author.

E-mail address: nina.wootton@adelaide.edu.au (N. Wootton).

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fish consume the plastic directly, mistaking it for food or inhaling it accidently; or through secondary ingestion, when prey that has consumed plastic is taken by a predator (Nelms et al., 2018). This trophic transfer can lead to both bioaccumulation and biomagnification in organisms of a higher trophic level (Chagnon et al., 2018; Farrell and Nelson, 2013; Provencher et al., 2019). However, the accumulation of microplastics in fish and other trophic levels, and how this could potentially impact human health via the consumption of seafood is not well understood (Carbery et al., 2018; Vethaak and Legler, 2021). The transfer of microplastic and their potential contaminants into the marine food web could have serious consequences for the seafood industry as an essential protein source (Carbery et al., 2018). Therefore, understanding the presence of microplastic in fish gastro-intestinal tracts is also key to evaluate the implications of microplastics and their associated chemicals transferring to other edible tissues (Ribeiro et al., 2020; Zitouni et al., 2020). Seafood produce could also be contaminated with microplastic through adherence to the exterior surface of the seafood species, or even as a result of cross-contamination during the processing of seafood (Cole et al., 2013; EFSA, 2016). Despite these risks, and the possibility that seafood could be a source of microplastic contamination for human consumers (especially if eaten whole), microplastic in fish sold through seafood markets is lacking quantification (Ziccardi et al., 2016).

The Australian seafood industry, including fishing, aquaculture and associated processing industries, was worth more than five billion dollars to the national economy in 2018 (FRDC, 2019). The average Australian consumes approximately 14 kg of seafood each year (ABARES, 2018), and with increased studies identifying the health benefits of seafood, this is likely to increase (Hosomi et al., 2012), replicating the pattern observed globally (FAO, 2020). Australia's thriving seafood industry emphasises the importance of investigating the impact of microplastic in commercially important fish species. Fish species are recognised to consume microplastic globally, with fish commonly dying from suffocation, or losing condition and growth prior to being caught (Foley et al., 2018; McCormick et al., 2020). Considering fish health is essential in maintaining a sustainable fishing stock, it is vital that we advance knowledge surrounding microplastic presence and its potential impacts on fish, and hence fisheries productivity. Baseline knowledge of microplastic in seafood, including fish species, would allow appropriate and timely mitigation actions, without a negative impact on the economics and success of the seafood industry (Farady, 2019).

Whilst research on microplastic has spread globally, there is a noticeable lack of studies in the Oceania region (Savoca et al., 2021). In particular, there are no studies exploring microplastics in seafood destined for human consumption in Australia, and this gap in research must be filled. In this study, we purchased locally caught fish species to quantify the presence of microplastic in fish sold for human consumption across Australia. We aim to compare the differences in the frequency and level of microplastic contamination among locations. Furthermore, we examine the differences in microplastic contamination among species, and evaluate if dietary/foraging or habitat preferences are associated with increased microplastic ingestion, and assess ecological or biological factors that may lead to specific fish species consuming more plastic. Overall, we deliver a set of important data on microplastic in fish from seafood markets, covering a highly underreported region. This enhances our understanding of the microplastic in fish sold for human consumption, and helps to inform future assessments of associated risk to food security due to potential impacts on fish health, and thus fisheries success.

2. Methods

2.1. Sampling methods

Fish were collected from fish markets and seafood stores in Australian capital cities from March to August 2019, specifically in Perth, Western Australia; Adelaide, South Australia; Melbourne, Victoria; Hobart, Tasmania; and Sydney, New South Wales (Fig. 1, Table 1). Reliable information of the source of the fish were provided from the fish monger when fish were purchased, and all fish were confirmed as wild-caught and local (i.e. within each state). Fish species were selected based on local availability, and to cover a range of habitats (e.g. reef, benthic, pelagic; Table 2). The same species were collected from each state where possible; otherwise, we collected similar species from the same family. Fish were bought in small sub-samples, from a variety of different fishmongers and fish stores, over a variety of days to ensure that samples were not caught from the same school of fish. The majority of fish were purchased whole, although a small number were sampled as frames, with intact gastro-intestinal (GI) tracts. All fish samples and GI samples were stored in the freezer at −20 °C prior to analysis for microplastics.

2.2. Laboratory methods

2.2.1. Sample preparation, digestion and microplastic extraction

All processing of fish occurred inside the laminar flow cabinet. Fish were identified to species level, and fork length of the whole fish or fish frame was measured (Table 2). The whole fish and fish frames were rinsed thoroughly with ultrapure (Milli-Q) water, to guarantee any exterior microplastic contamination was removed. The fish were dissected, the GI tracts weighed, and then rinsed in ultrapure water. Due to limitations associated with interstate travel restrictions, dissections of some large species occurred in the field, using open air flow outdoor areas, cleaning all areas before and between fish dissections thoroughly. When this occurred, dissected GI tracts were rinsed thoroughly with ultrapure water, tightly sealed in previously cleaned sample jars, and transported on ice back to the laboratory to undergo the next stage of processing. Procedural blanks consisting of open vials with water were also used to evaluate any contamination from field dissections (detailed below in section 2.2.2 Quality assurance and quality control).

The entire GI tract was covered with 10% potassium hydroxide (KOH) solution in ultrapure water to digest any organic material (Foekema et al., 2013; Rochman et al., 2015). Samples were then placed in a 60 °C oven overnight (12–14 h), allowing the majority of the organic material to be digested.

The resulting samples were drained in the laminar flow cabinet through two sieves (1 mm and 38 μm) allowing microplastic pieces to be caught in the sieve. The two sieves were observed under a stereomicroscope (Leica M80) with an attached digital camera. All potential microplastics were recorded by sieve size, photographed and collected for further chemical analysis. The colour and type of microplastic (fibre, film, fragment) were also recorded. Pieces of sand, bone fragment and rock were all found in samples, however we ruled them out as potential microplastic due to physical features under the microscope or by touch.

2.2.2. Quality assurance and quality control

Contamination of microplastic from the laboratory environment can influence the accuracy of data in microplastic research, therefore, strict procedures were established to ensure that contamination risk was minimised (Azad et al., 2018; Lusher et al., 2017b; Provencher et al., 2017). The area where dissections occurred was cleaned methodically and all sample jars and dissection equipment were rinsed three times with ultrapure water and dried in the laminar flow cabinet prior to use. Dissections and preparation of GI tracts in the laboratory were completed inside the laminar flow cabinet, to avoid the risk of exterior contamination.

All fish processing and stages of laboratory work were completed wearing bright pink lab coats and underclothing of natural fibres was worn at all times. If any pink fibres were found in samples they were considered as contamination from the lab coats, although no pink fibres were found. Procedural, field and environmental blank controls were used at each stage of sample processing, from dissection (open vial filled with ultrapure water, inside laminar flow or at the outdoor dissecting

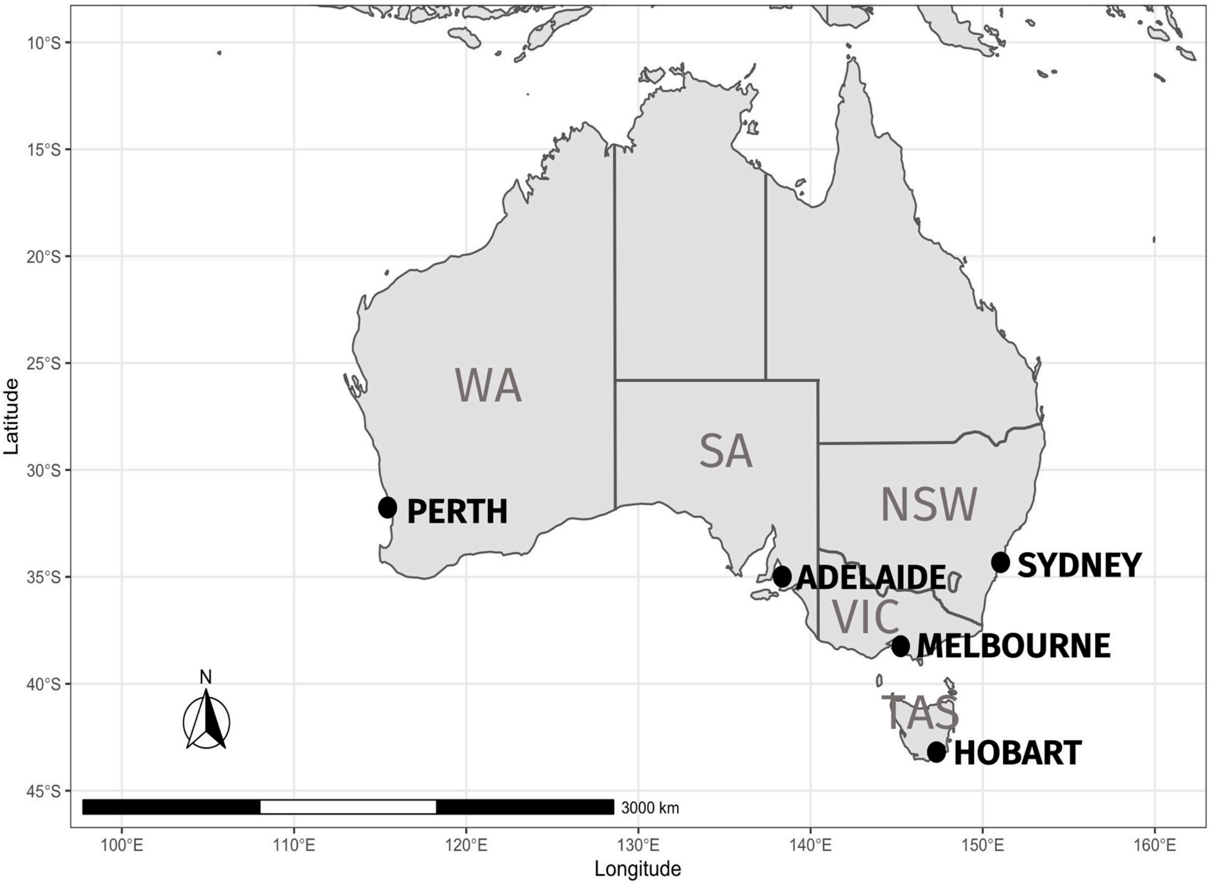

Fig. 1. Map of sampling locations in Australia. Samples were purchased from fish markets and seafood stores in Perth (Western Australia – WA), Adelaide (South Australia – SA), Melbourne (Victoria – VIC), Hobart (Tasmania – TAS), and Sydney (New South Wales – NSW).

Table 1
Summary of samples per species and collection location (Western Australia – WA, South Australia – SA, Victoria – VIC, Tasmania – TAS, New South Wales – NSW).

Common name	Scientific name (Family)	WA	SA	VIC	TAS	NSW	Total
Australian herring	*Arripis georgianus* (Arripidae)	20	20				40
Australian salmon (Eastern and Western)	*Arripis trutta/ Arripis truttaceus* (Arripidae)		45	20	21		86
Australian sardine	*Sardinops sagax* (Clupeidae)	20	17	28	20	20	105
Snapper	*Chrysophrys auratus* (Sparidae)	19	15	20		21	75
Dusky flathead	*Platycephalus fuscus* (Platycephalidae)			10		18	28
Garfish	*Hyporhamphus melanochir* (Hemiramphidae)		23	25	21	21	90
King George whiting	*Sillaginodes punctatus* (Sillaginidae)	24	108	19		10	161
Sea mullet	*Mugil cephalus* (Mugilidae)	20	17			17	54
Tiger flathead	*Platycephalus richardsoni* (Platycephalidae)		27	14	22		63

table), to digestion (polypropylene jar with 10% KOH, with lid on placed in oven at 60 °C overnight), and microscope identification (open vial with ultrapure water, next to microscope). The procedural blanks were placed directly next to the workplace and examined in the same manner as the fish samples, sieving through both sieves and analysing under the microscope. One blank for each group of samples being examined was used for each stage of the laboratory procedure, and was examined at the end of each session (total blanks = 105). No evidence of contamination was found in the control samples.

2.2.3. *Verification and identification of microplastic*

The current literature recommends a minimum sub-sample of 10% of microplastic pieces to be tested for verification of microplastic (Galgani et al., 2013; Garcia-Garin et al., 2019). We selected a proportion (20%, or 135 of 674 pieces) of all microplastic pieces to test using micro-Fourier Transformed Infrared Spectroscopy (μ-FTIR) (Bruker Hyperion) to confirm that they were plastic and to identify polymer type (Jung et al., 2018). A relative proportion of the type of plastic (e.g. fragment, fibre, film) we found was tested (e.g. ~13% of total microplastic were fragments, so 13% of FTIR tested samples were fragments) and samples of all sizes and colours were randomly chosen to ensure an accurate representation. We applied Attenuated Total Reflection (ATR) to all cleaned pieces of microplastic, with a resolution of 4 cm^{-1}. The size of the aperture was modified to be as small as needed for the sample being tested (e.g. for a small fibre the aperture was readjusted to be the long, narrow shape of the sample being tested). Measurement positions were randomly selected at three points within the sample and 64 co-scans were run for each measurement. The spectrum range was fixed between 3900 and 650 cm^{-1}, however, when comparing to the spectral libraries the atmospheric water/CO_2 region between 2500 and 1900

Table 2
Summary of fish species sampled, their biological data and collection location (Western Australia – WA, South Australia – SA, Victoria – VIC, Tasmania – TAS, New South Wales – NSW). Feeding strategy and habitat information was sourced through FishBase (Froese and Pauly, 2019).

Common name	States	Feeding strategy	Habitat	Fork length (mm±SE)	Gastro-intestinal weight (grams±SE)
Australian herring	WA, SA	Carnivore	Pelagic	199 (±3)	8.43 (±0.69)
Australian salmon	SA, VIC, TAS	Carnivore	Pelagic	309 (±8)	29.29 (±2.43)
Australian sardine	WA, SA, VIC, TAS, NSW	Omnivore	Pelagic	155 (±2)	2.77 (±0.11)
Snapper	WA, SA, VIC, NSW	Carnivore	Reef	370 (±14)	72.71 (±10.54)
Dusky flathead	VIC, NSW,	Carnivore	Demersal	431 (±11)	17.54 (±2.23)
Southern garfish	SA, VIC, TAS, NSW	Herbivore	Pelagic	312 (±3)	4.85 (±0.25)
King George whiting	WA, SA, VIC, NSW	Carnivore	Demersal	320 (±3)	8.34 (±0.43)
Sea mullet	WA, SA, NSW	Detrivore	Pelagic	339 (±9)	50.68 (±5.35)
Tiger flathead	SA, VIC, TAS	Carnivore	Demersal	455 (±6)	26.10 (±1.82)

cm^{-1} was excluded as recommended by Primpke et al. (2018) and Jensen et al. (2019). All readings were compared to the libraries of reference (Bruker ATR Library for Polymers, Bruker ATR Library for Chemicals, Bruker ATR Library for Pharma, Bruker ATR Library for Forensics) to verify the polymer type, and confirm the presence of plastic (Figure S1). Percentage match, known as "hit quality" was required to be above 50% between the tested sample and the reference library. This value is slightly lower than other studies which suggest a percentage match value of more than 60% (Kroon et al., 2018), due to some of the pieces measured being particularly small (100 μm). To further confirm the polymer type all major peaks in the spectra were stringently identified, and visually confirmed on all pieces tested.

2.3. Data analysis

The percentage of fish found with at least one piece of plastic present is defined as the frequency of occurrence of plastic ingestion. The frequency of occurrence was evaluated for differences between state, and differences between species. The average amount of microplastic found was quantified, and will be referred to as the plastic load.

A negative binomial generalised linear model (GLM) was used to investigate if location of capture (state), species, weight of GI tract and individual length (fork length) were influencing the plastic load and frequency of occurrence of plastic. All combinations of terms were tested and ranked based on Akaike's information criterion value corrected for small sample sizes (AICc) to select the best model fit (Table S1). For the frequency of occurrence of plastic only species and state were determined to be influencing results, while the plastic load was influenced by both species and state as well as the weight of the GI tract and the fork length of the fish. Using the respective best fit models, estimates of species means were conducted, followed by ANOVA (one-way analysis of variance) and pairwise tests to determine any differences between location (i.e. state) and species. Residual plots were created to ensure that all data met the assumptions of the model, and then graphical outputs using the model predictions were produced. Due to the aims and scope of our study, we chose to model (negative binomial) how the plastic varied between states, with all species combined; and between species with the data collected from all states combined. Additional data are available in the supporting information (Figure S2, S3). To investigate if habitat and feeding type of the fish were having an impact on the likelihood of fish consuming microplastic, negative binomial GLM were also fit to the data, with only habitat and feeding type included as the tested variable.

Data and statistical analyses and graphical outputs were completed using R studio software (Version February 1, 5019) and the ggplot2 (Wickham, 2016), doBy (Højsgaard and Halekoh, 2020), rgeos (Bivand and Colin, 2020), MASS (Venables and Ripley, 2002), emmeans (Length et al., 2020), tidyverse (Wickham et al., 2019), rnaturalearth (South, 2017a), rnaturalearthdata (South, 2017b), sf (Pebesma, 2018), and maps (Becker et al., 2018) packages. We used Microsoft excel to produce the donut graph.

3. Results

3.1. Descriptive statistics of microplastic presence in fish

In total, 702 fish were collected and 674 pieces of microplastic were extracted from the GI tracts, ranging from zero to 17 pieces per individual fish (Fig. 2). Overall, 35.5% of fish contained microplastic (249 of the 702 fish sampled, Table S2), with an average of 0.96 (±0.08) piece of microplastic per fish (Table S3). The majority of microplastic pieces were larger than 1 mm in size (66%), with the remainder of pieces being between 38 μm and 1 mm.

3.2. Differences between locations

There were significant differences in the estimated frequency of occurrence (χ^2 16.18, df 4, $p < 0.01$) and estimated plastic load (χ^2 16.67, df 4, $p < 0.01$) between states (Fig. 3). South Australia had the highest number of fish with at least one piece of microplastic while Tasmania had the lowest (Fig. 3). Similarly, South Australia had the highest average plastic load, more than four times that of the lowest average load found in Tasmania (Fig. 3). New South Wales, Victoria and Western Australia all had similar estimated percentages of fish with at least one piece of microplastic (Fig. 3) and microplastic loads (Fig. 3).

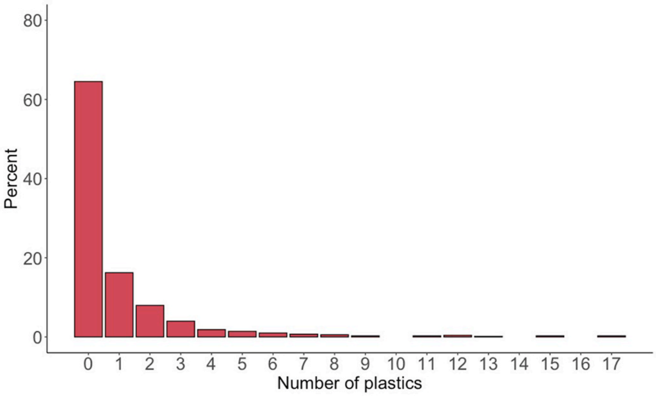

Fig. 2. Frequency distribution of percentage of fish with different numbers of microplastic in their GI tract.

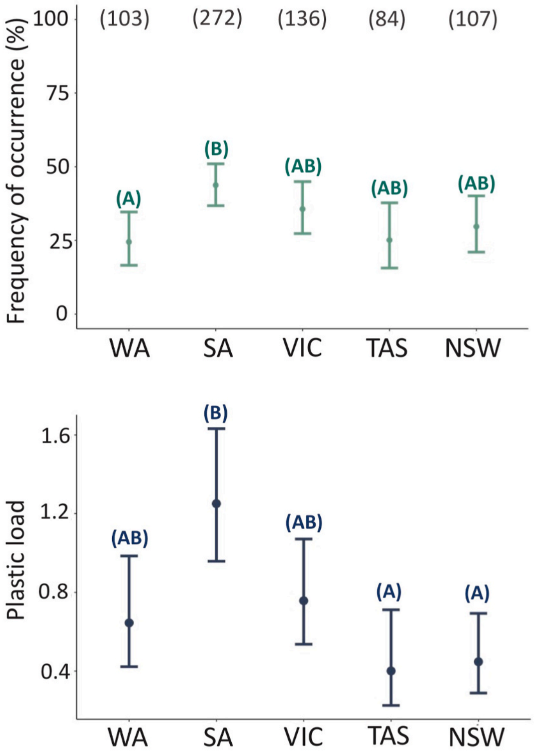

Fig. 3. Estimated (top) frequency of occurrence (%) and (bottom) plastic load, by state for Australia. The graphs show the expected percentage of fish from each state that had at least one piece of microplastic in their GI tract, and the predicted mean number of microplastics found in all species between states. All data are fitted to a negative binomial GLM, with location and species as a fixed effect for frequency of occurrence (top); and location, species, fork length and weight of GI tract as a fixed effect for plastic load (bottom). Error bars represent the lower and upper confidence intervals of the model and letters indicate significant differences. Refer to Table 2 for state names. Sample sizes are shown at the top of the figure.

3.3. Differences between species

There were also significant differences in the estimated frequency of occurrence (χ^2 38.16, df 8, $p < 0.001$) and estimated plastic load (χ^2 48.65, df 8, $p < 0.001$) between species (Fig. 4). This was driven by a low number of Australian sardines and southern garfish estimated to have consumed microplastic (14% and 21% respectively), and a higher number of sea mullet (50%), dusky flathead (42%) and King George whiting (42%; Fig. 4). Similarly, Australian sardines and southern garfish were also estimated to have the lowest plastic load, with less than 0.3 piece found on average in each species (Fig. 4). Australian snapper, dusky flathead, Australian salmon and sea mullet were all predicted to have more than one piece of plastic on average in their GI tract (Fig. 4).

3.4. Differences between fish feeding strategy and habitat

There were significant differences in the estimated frequency of occurrence (χ^2 40.83, df 3, $p < 0.001$) and plastic load (χ^2 52.16, df 3, $p < 0.001$) between fish with different feeding strategies (Fig. 5). Both detrivores and carnivores had a higher estimated frequency of occurrence and plastic load than herbivores and omnivores. Detrivores had the highest estimated frequency of occurrence and omnivores the lowest (Fig. 5). Carnivorous fish had the highest estimated plastic load and herbivores the lowest (Fig. 5).

The habitats where fish reside also significantly affected the frequency of occurrence (χ^2 27.16, df 2, $p < 0.001$) and plastic load (χ^2 12.81, df 2, $p < 0.005$) of microplastic (Fig. 6). Demersal fish had a significantly higher estimated frequency of occurrence than pelagic and reef associated fish (Fig. 6). Similarly, demersal fish had a higher estimated plastic load than pelagic fish (Fig. 6).

3.5. Differences in the type of microplastic

When inspected under the microscope, plastic type was dominated by fibres, with 81.8% (551 of 674 pieces) being identified within this category. The remaining pieces were recorded as fragments (12.7%), and film (5.5%). There were no significant differences between state, species, feeding strategy or habitat observed. We used FTIR spectrometry to identify the polymer types of a proportion (20% of the total) of microplastic pieces (135 of 674 pieces). Samples were selected randomly within the relative portion of fibres, film and fragments of all the pieces identified. Of the 135 pieces tested, 99.3% were originally from anthropogenic sources, with 121 identified as plastic polymers, an 89.6% accuracy. Of the 14 remaining pieces not categorised as plastic polymers, 13 were still anthropogenic debris, as they were made of natural materials (e.g. cotton, wool or hemp) but modified for human use (e.g. a cotton clothing item). These pieces were identified as from anthropogenic sources through the forensic libraries used in tandem with the FTIR spectrometer. The piece classified as non-anthropogenic was a piece of oyster shell.

When investigating the microplastic pieces tested under the FTIR spectrometer, polyolefin made up almost half of the total polymers (Fig. 7); this is a broad group of polymers including polyethylene (40.8% of all the polyolefin samples) and polypropylene (46.9% of all polyolefin samples). Synthetic fibres, also called ‘poly-blends’, are a mixture of polyester, nylon or elastane combined with natural fibres such as rayon, cotton or wool, and were the second most prominent polymer group, with more than a quarter of the microplastic pieces falling into this category. The remaining polymers included acrylate, pure nylon, paint, pure polyester and poly-vinyl. Due to the small sample size of examined polymers we only investigated any differences in polymers between states, of which there were none.

4. Discussion

Overall, this study shows that approximately one third of all commercially important fish being sold in Australian fish markets had microplastic in their GI tract contents. All fish species purchased and from all states sampled had microplastic, however, there were differences in the frequencies of occurrence and plastic load between states and species. Further, fish from different trophic levels (carnivores, detrivores, herbivores, omnivores) and those which reside in different habitat zones (reef, pelagic and demersal) had differing levels of plastic. Overall, our study provides a baseline of microplastic contamination in nine important fishery and popular eating species of fish from across 4000 km of southern Australia. These data provide a key step to understanding the implications surrounding the transfer of microplastic and their associated chemicals into other organs and tissues of fish, hence how much plastic is potentially being ingested by seafood consumers.

Our results are directly comparable to international literature investigating presence of microplastic in commercially caught and sold fish. We found that in total 35.5% of fish in Australian states sampled had at least one piece of microplastic present in their GI tract, which is lower but close to the global average of 37.6% (Markic et al., 2019). However, the average plastic load across all states was 0.96 piece of microplastic, which is nearly three times less than the global average of 2.6 pieces (Markic et al., 2019). Individual studies using similar methods and sampling similar species to ours, had varied abundances of microplastic; with some finding similar amounts of plastics to our study [e.g. 26% of pelagic fish from Moroccan-Atlantic waters (Maaghloud et al., 2020), and 49% of fish had microplastic in the South China Sea (Koongolla et al., 2020), respectively]. While others had more

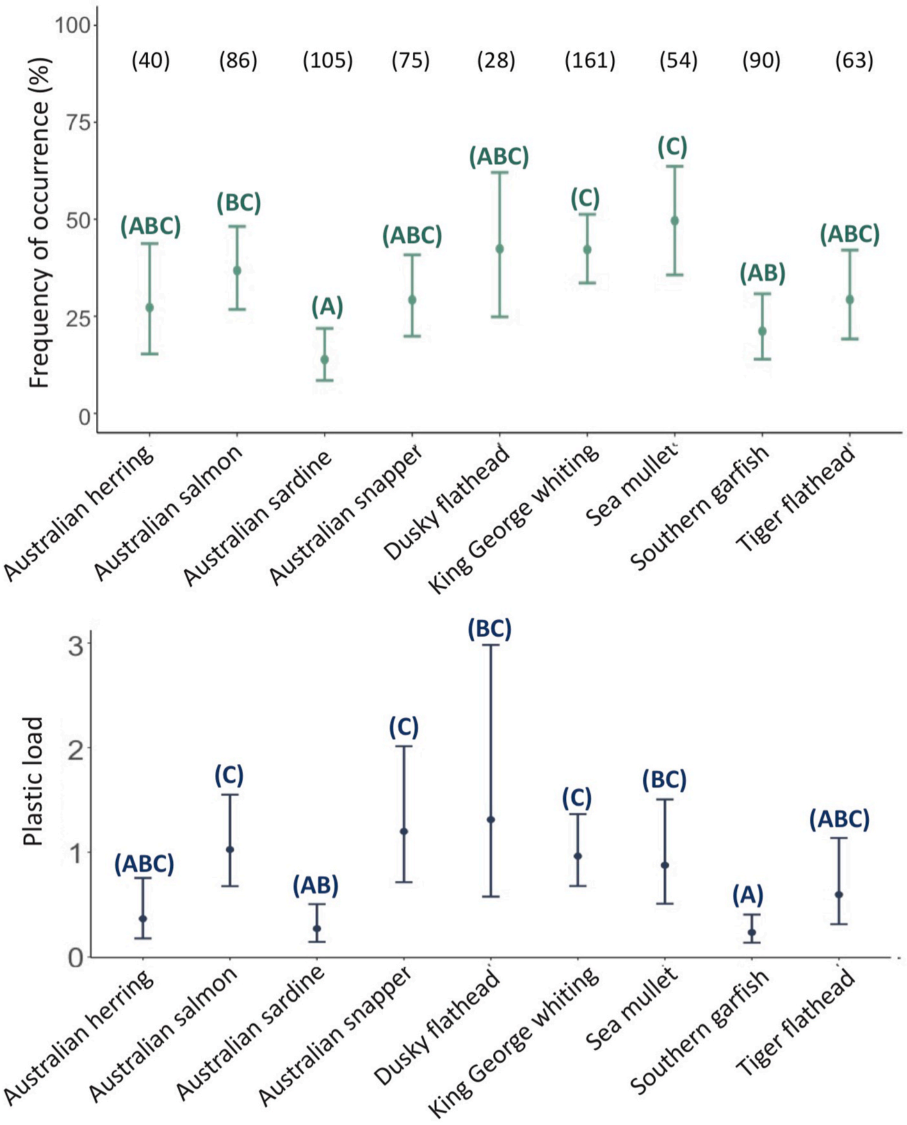

Fig. 4. Estimated (top) frequency of occurrence (%) and (bottom) plastic ingestion load, by species for Australia. The graphs show the expected percentage of fish from each species that had at least one piece of microplastic in their GI tract, and the predicted mean number of microplastics found in all species. All data are fitted to a negative binomial GLM, with location and species as a fixed effect for frequency of occurrence (top); and location, species, fork length and weight of GI tract as a fixed effect for plastic load (bottom). Error bars represent the lower and upper confidence intervals of the model and letters indicate significant differences. Refer to Table 1 for scientific names of species. Sample sizes are shown at the top of the figure.

microplastic, including fish sampled in China, where 92.5% contained microplastic and off the coast of Morocco where 76% of fish had microplastic (Maaghloud et al., 2021). Lower microplastic contamination was found in Brazil, where using the same methodologies, only 13.9% of the fish sampled had microplastic (Neto et al., 2020). It is difficult to ascertain the reason for these varying results, with many factors such as location, species, methodology, or size of fish all potentially responsible for impacting the levels.

Approaches used for detection of microplastic in biota vary greatly, which is why it is important to take the methodology into consideration both when doing microplastic research, and when making comparisons between studies (Provencher et al., 2017). There is a large move in microplastic research towards standardised methodology protocols, although at times this may be impractical, depending on the focus of the research questions being explored (Provencher et al., 2020). Despite this, it is vital that we harmonise the varying outputs of research, to allow repeatability, comparability and accuracy (Provencher et al., 2020). Recently, the quality and rigorousness of methodology has improved in microplastic research, increasing the detection of microplastic in fish, as we are now able to detect smaller fragments of microplastic than previously possible. Our study follows what is currently defined as high standard methodology, with the use of a chemical digestion, large sample sizes, contamination controls, and confirmation of polymer type using chemical techniques (Hermsen et al., 2018; Lusher et al., 2017b; Wesch et al., 2016). The chemical digestion used in our study (10% KOH at 60°) is recognised as one of the most efficient and universally applied methods of extraction of microplastic (Dehaut et al., 2016; Lusher et al., 2017b; Thiele et al., 2019). There are concerns that KOH left at this temperature for extended periods of time (24 h plus) has the potential to alter the tenacity and integrity of some fibres (Karami et al., 2017; Prata et al., 2019), however literature has shown that its digestion efficiency and recovery rate of polymers is still appropriate, particularly considering the benefits of shorter digestion times and the universal application of the method (Dehaut et al., 2016).

Within Australia there are only six other studies to date investigating microplastic ingestion in fish (Table 3). Two of these studies sampled estuarine or freshwater fish, while the other four sampled marine species (Cannon et al., 2016; Crutchett et al., 2020; Jensen et al., 2019; Kroon et al., 2018). None of the fish sampled in these studies were purchased through commercial fish markets. Of the marine studies, there were a range of results (FO, 0.3%–95%), but as mentioned above, these dissimilarities may relate to differences in methodology (Table 3). Cannon et al. (2016) found less plastic than any of the other studies in Australia, but also sieved stomach contents through a set of large sieves, the smallest being 0.33 mm (330 μm). Two of the other marine studies both

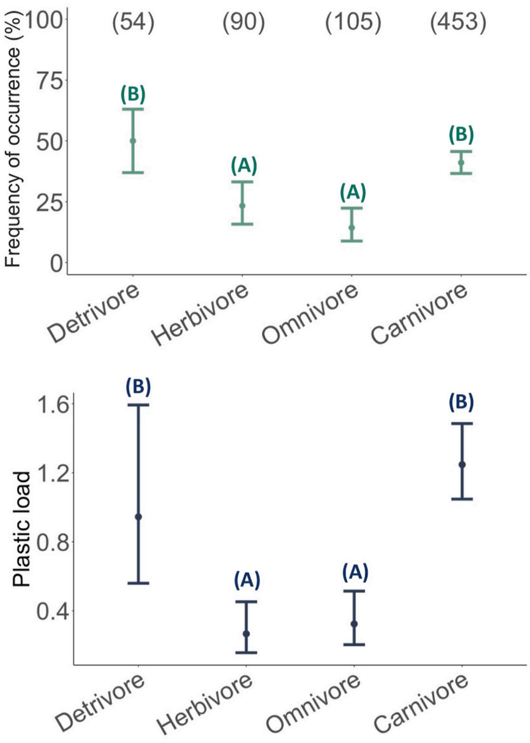

Fig. 5. Estimated (top) frequency of occurrence (%) and (bottom) plastic load, by feeding strategy for Australia. The graphs show the expected percentage of fish from each feeding strategy that had at least one piece of microplastic in their GI tract, and the predicted mean number of microplastics found in each feeding strategy, both fitted to a negative binomial GLM, with feeding strategy as a fixed effect. Error bars represent the lower and upper confidence intervals of the model and letters indicate significant differences. Refer to Table 2 for species classifications of feeding strategies. Sample sizes are shown at the top of the figure.

used a 37 µm sieve (similar to the 38 µm sieve our study used), and all found much higher levels of plastic in their fish (Jensen et al., 2019; Kroon et al., 2018). The disparities in microplastic are potentially caused by smaller pieces of microplastic passing through the larger sieve grids in Cannon et al. (2016), which is supported by the results from our study, where 34% of the microplastic pieces were caught in the smaller sieve size. Nevertheless, location could also be impacting results, with high abundance of microplastic in the Great Barrier Reef matching other studies from this region, such as Wootton et al. (2021) where 61.6% of fish from Queensland contained plastic. It is possible that fish from the northern parts of Australia are consuming higher abundances of microplastic due to transport of plastic waste from neighbouring countries north of Australia renowned for higher plastic contamination, e.g. China and Indonesia (Jambeck et al., 2015). Further, the Great Barrier Reef could be acting as a trapping region for the microplastic, as reefs globally have been documented to capture more plastic than other environments (Huang et al., 2021). Likely, it is a combination of geographic location, but also differences in methods, that are causing the observed differences in abundance of microplastic in other Australian fish studies.

Globally environmental levels of microplastic contamination are linked to the presence of microplastic in fish and biota (Ferreira et al., 2020; Tien et al., 2020; Zhang et al., 2020). In our study, fish sampled from markets in South Australia had both the highest frequency of occurrence and load compared to all of the other states. This aligns with the high levels of microplastic found in the sediment of the Great Australian Bight, a large open bay off the southern coastline of Australia, and hypothesised to be due to ocean currents and water flow in the region (Barrett et al., 2020). Plastic waste could potentially be reaching

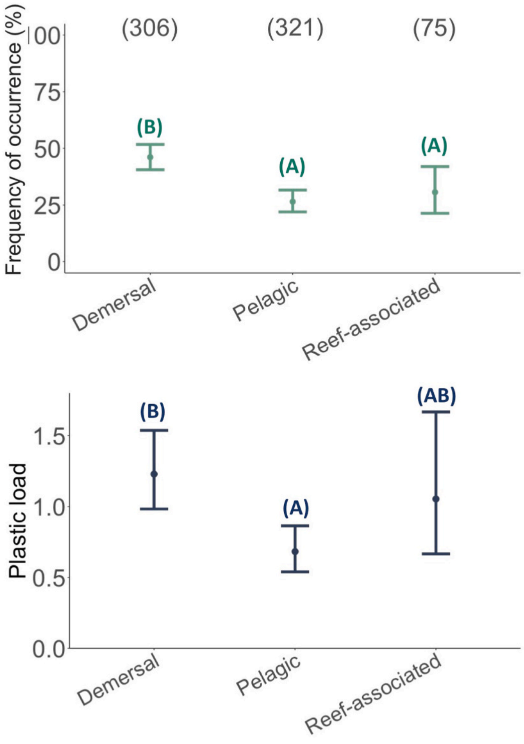

Fig. 6. Estimated (top) frequency of occurrence (%) and (bottom) plastic load, by habitat zone for Australia. The graphs show the expected percentage of fish from each habitat zone that had at least one piece of microplastic in their GI tract, and the predicted mean number of microplastics found in each habitat zone, both fitted to a negative binomial GLM, with habitat zone as a fixed effect. Error bars represent the lower and upper confidence intervals of the model and letters indicate significant differences. Refer to Table 2 for species classifications of habitat zones. Sample sizes are shown at the top of the figure.

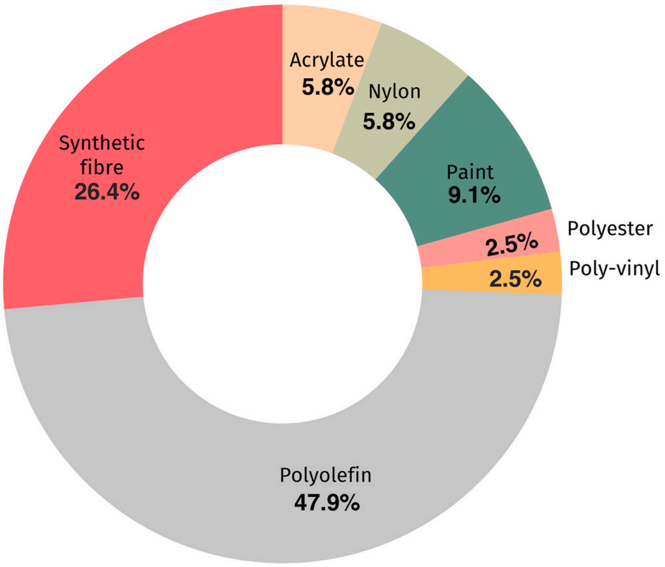

Fig. 7. Polymer groups of all microplastics pieces (fibres, films and fragments combined) found in fish from Australia. Polymer groups were confirmed using the µ-FTIR and then categorised depending on their broad polymer family. Synthetic fibres are a blend of natural and synthetic materials (e.g. poly-blend such as cotton-polyester).

Table 3
Summary of microplastic ingestion in fish in Australia. The summary of methods includes if a digestion was used; microscope identification, sieve size, and the use of chemical verification.

Study	Location	Environment	Summary of methods	Total fish sample size	Frequency of occurrence (%)	Plastic load
Halstead et al. (2018)	Sydney Harbour	Estuarine	Microscope identification, FT-IR verification	93	43	2.677
Kroon et al. (2018)	Great Barrier Reef	Marine	Microscope identification, 37 μm sieve, FT-IR verification	20	95	5.8
	Greater Melbourne Area	Freshwater	Chemical digestion, microscope identification, 20 μm sieve, FT-IR verification	180	19.4	0.6
Cannon et al. (2016)	South-east Australia	Marine/ Freshwater	Microscope identification, 0.33 mm sieve, FT-IR verification	342	0.3	0.0058
Jensen et al. (2019)	Great Barrier Reef	Marine	Microscope identification, 37 μm sieve, FT-IR verification	60	95	7.583
Crutchett et al. (2020)	Western Australia	Marine	Chemical digestion, microscope identification, 22 μm sieve, FT-IR verification	27	26	0.26
Wootton et al. (2021)	Queensland	Marine	Chemical digestion, microscope identification, 38 μm sieve, FT-IR verification	73	61.6	1.58
This study	Australia (NSW, SA, Tas, Vic, WA)	Marine	Chemical digestion, microscope identification, 38 μm sieve, FT-IR verification	702	35.5	0.96

the south of Australia from as far as Africa and Asia via the Leeuwin current, which wraps around the southwest side of Australia (Lebreton et al., 2012; Van Sebille et al., 2015). Furthermore, the Spencer Gulf, where more than 80% of our South Australian fish were caught, is known for having high water retention (Nunes Vaz et al., 1990), which could be impacting the accumulation of plastic, and contribute to higher quantities of plastic in South Australian fish. In a study investigating microplastic presence in the coastal seafloor across southern Australia, microplastic was found in the highest abundance in South Australia's sediment, with Tasmania having the lowest abundance, which matches our findings in fish (Ling et al., 2017). In contrast, Tasmania's isolation from mainland Australia, and their lack of urbanisation may be influencing the low numbers of plastic we found in fish sourced from these waters.

Microplastics identified in this study were dominated by fibres, likely from synthetic clothing or fishing equipment, with more than 80% of plastic pieces identified as this morphotype. Wastewater systems are likely to be contributing to this, considering more than 1.5 million pieces of microplastic are predicted to enter our waterways through a single load of washing (De Falco et al., 2019). Additionally, wastewater discharges are a significant contributor of microplastic in our water systems, and although filters in treatment plants attempt to lessen their impact, there is still microplastics being released via these pathways (Ngo et al., 2019). One wastewater treatment plant in Sydney discharges between 3.6 million and 460 million pieces of microplastic into the ocean daily, the majority of which are fibres (Ziajahromi et al., 2017). Another potential source of fibres in the marine environment is via fishing equipment, with some studies suggesting more than 10% of plastic in the ocean is from fishing gear (Thomas et al., 2019). Fishing nets and ropes are commonly made of polyolefin (e.g. polypropylene and polyethylene), which were a dominant polymer identified in the microplastic pieces tested in this study. Furthermore, the type of plastic found in fish from our study match the results from Reisser et al. (2013), where sea surface samples across Australia where dominated by polyolefin.

The feeding strategy of fish influenced the likelihood of microplastic being consumed with carnivorous diets having higher plastic load than omnivores or herbivores. This is consistent with the literature which suggests higher trophic level organisms (such as carnivores) could be accumulating more microplastic via bioaccumulation or biomagnification across the food web (Batel et al., 2016; Carbery et al., 2018). Furthermore, we found detrivorous fish (e.g. sea mullet), which feed predominantly by extracting food from the sediment, to have higher frequency of occurrence and plastic load than other feeding types. This is consistent with literature which document that microplastics accumulate on the ocean floor (e.g. Barrett et al., 2020; Woodall et al., 2014), so it is likely a consequence that fishes which feed in this habitat are likely to encounter more microplastic, and accidently consume it. Our habitat data further substantiates the likelihood of fish being exposed to microplastic while in the benthos, as demersal fish, which depend on the benthos had higher amounts of plastic than pelagic fish. Other studies have further supported this, with fish from demersal habitats having higher abundances of plastic than those residing in pelagic zones (Jabeen et al., 2017; Murphy et al., 2017). Therefore, it is suggested that fish which reside in the demersal or benthic habitats are more likely to encounter plastic by accidently consuming it than those which dwell in the water column.

It is important to acknowledge that by analysing the GI tract, this only provides a glimpse of what the individual fish has recently ingested (Cortés, 1997). Fish over their lifetime will be consuming microplastic, with individuals either dying due to suffocation or plastic-induced satiation prior to being caught, but also egesting smaller plastics (Foley et al., 2018; Ory et al., 2018). Once ingested, microplastic can remain in the digestive tract of fish for anywhere from days to weeks (Batel et al., 2016; Cedervall et al., 2012). From here, microplastic can transfer to other aquatic organisms and up the food web (Carbery et al., 2018; Farrell and Nelson, 2013), and may in part explain the higher amounts of microplastic in carnivorous fish. Despite the presence of microplastic in fish species globally, knowledge regarding the physical or chemical implications microplastic may have on fish is still understudied, with high variability across taxa (Foley et al., 2018). Notwithstanding this, there is evidence to suggest that microplastic may pose a deleterious effect to marine organisms globally, impacting organisms' behaviour, growth, reproduction and even survival (Ding et al., 2018; Jacob et al., 2020). Therefore, the increasing levels of microplastic contamination, and their persistence in organisms may pose a series of threats and challenges to fishery and aquaculture industries.

Lifestyle choices, such as abundance and popularity of fish consumed, and how the fish is prepared and cooked, means that the chance Australian consumers are ingesting microplastic via consumption of fish is low (Dawson et al., 2021). Although we have been able to confirm that microplastic is present in the GI tract of fish in Australia, the relevance of this to human wellbeing may be reduced compared with other cultures, as in Australia we often discard the GI tract of the fish species prior to consumption (Dawson et al., 2021). However, it should be noted that some species, such as white bait and sardines are consumed in their entirety by many cultures in Australia, so the risks of microplastic in the gastrointestinal tracts cannot be ruled out completely. Furthermore, while there have already been some studies investigating the presence of microplastic and the chemical contaminants that derive from microplastic (both from absorption from the environment, and those present in the plastic from manufacture) (e.g.

Ribeiro et al., 2020; Su et al., 2019), this is a key knowledge gap (Lusher et al., 2017a). Determining the implications between the presence of microplastic in the GI tract, and how microplastics or their associated chemical contaminants could be transferring into the edible tissues is a crucial area of research, and one that needs to be informed first by the presence of microplastic in the GI tract (Savoca et al., 2021).

Measuring the negative effect of microplastic on fish health and hence fisheries success is necessary. Additional research investigating microplastic presence in other seafood species, such as crustaceans and molluscs, that are commonly consumed in entirety, would be beneficial considering the higher likelihood of humans consuming microplastic. With the increasing abundance of plastic in our environment, there is the chance that humans are eating microplastic from a suite of contaminated food sources, such as seafood, salt, honey and water (Grigorakis et al., 2017; Kim et al., 2018; Pivokonsky et al., 2018; Van Cauwenberghe and Janssen, 2014). A recent study found that there are higher amounts of microplastic in the air we breathe than any of the previously mentioned food sources (Mohamed Nor et al., 2021). Despite this, the understanding surrounding potential health risks that microplastics may pose to human health needs further consideration (Barboza et al., 2018; Vethaak and Legler, 2021). Finally, toxicological data, surrounding micro and nano plastic contamination, and their abilities to translocate across fish and humans, is imperative for future food safety risk assessments (Lusher et al., 2017a).

5. Conclusion

This research quantified and compared the presence of ingested microplastic in nine fish species from seafood markets across the south of Australia. All the analysed species from all the states had microplastic present in their GI tract, with variations depending on location and feeding strategy. This information provides important knowledge on microplastic contamination in Australian fish species sold for human consumption, and can be built upon, both temporally and spatially, to see how contamination levels change into the future. Although we are yet to expand our knowledge on how microplastics might be negatively impacting fish health (Ory et al., 2018), or health impacts to humans (Walkinshaw et al., 2020), these findings deliver important information to the seafood industry, from fishers to consumers.

Author statement

Nina Wootton: Conceptualization, Visualization, Methodology, Validation, Formal analysis, Investigation, Writing – Original Draft, Funding acquisition **Patrick Reis Santos:** Writing - Review & Editing, Methodology, Visualization, Supervision **Natalie Dowsett:** Writing - Review & Editing, Funding acquisition **Alison Turnbull:** Writing - Review & Editing, Funding acquisition **Bronwyn M. Gillanders:** Supervision, Conceptualization, Writing - Review & Editing, Visualization, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.118030.

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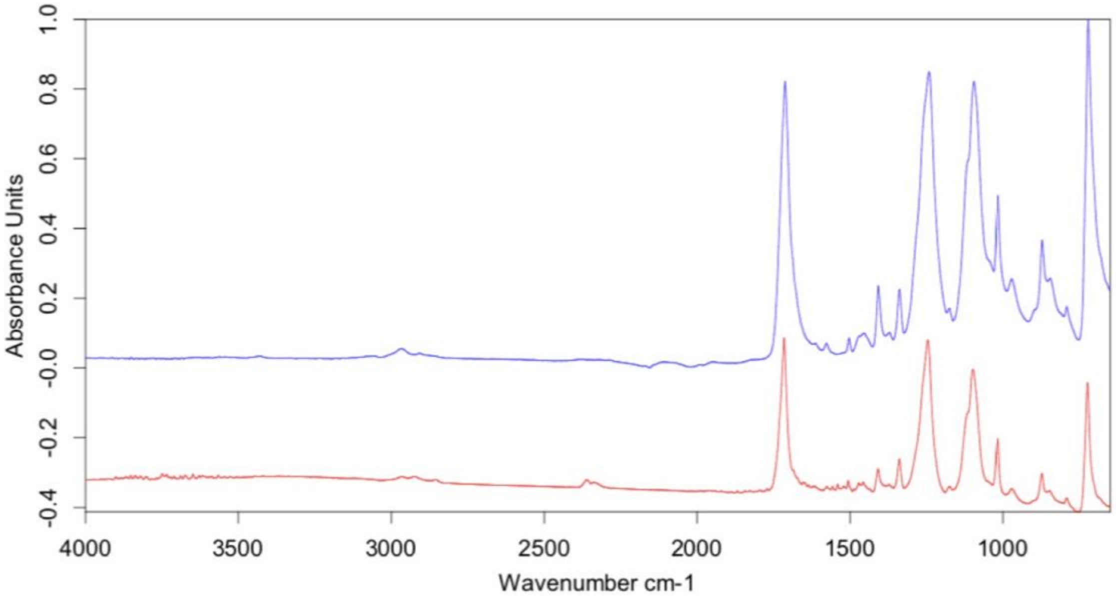
Supplementary Information S4

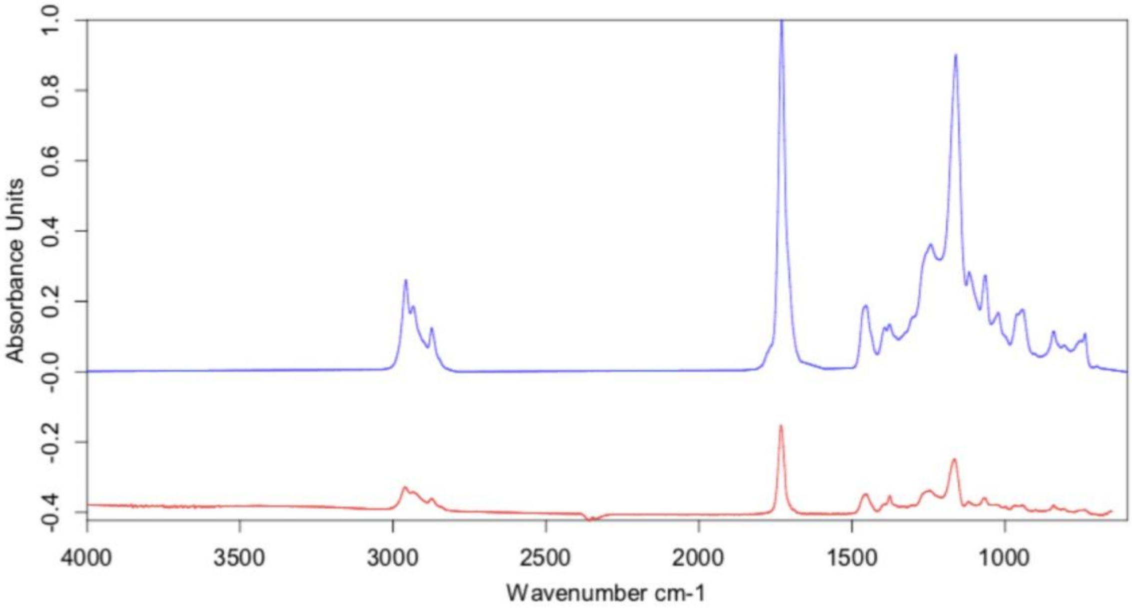
Low abundance of microplastics in commercially caught fish across southern Australia

Nina Wootton, Patrick Reis Santos, Natalie Dowsett, Alison Turnbull, Bronwyn M. Gillanders

Environmental Pollution (2021) Vol. 290, Article 118030



A. Filament from eastern Australian salmon (red), 96.7% match to polyester (blue)



B. Film from King George whiting (red), 96.3% match to acrylate resin (blue)

Figure S4.1: A sample of ATR-FTIR spectral matches from microplastic sourced from fish in southern Australia. This includes (A) polyester fibre from eastern Australian salmon and (B) acrylate resin film from King George whiting. Red spectra represent the FTIR output from the plastic samples, blue spectra are the polymer library spectral matches, (A) 96.7%, (B) 96.3%.

Table S4.1: Summary results of negative binomial generalised linear model. The top ranked model and adjusted weight after selection for ΔAIC ≤ 2. The best model fit it highlighted in bold.

Response variable	**Fixed effects**	**Residual deviance**	**d.f.**	**AIC**	**ΔAIC**
Frequency of occurrence	**Species + Location**	**43.015**	**17**	**164.55**	**0.00**
	Species + Location + Weight of Gut	41.833	16	165.37	0.82
	Species + Location + Fork Length	42.88	16	166.41	1.86
	Species + Location + Weight of Gut + Fork Length	41.595	15	167.13	2.58
	Species + Fork Length	56.42	20	171.96	7.41
	Species + Weight of Gut	58.038	20	172.58	8.03
	Weight of Gut + Fork Length + Species	56.42	19	173.96	9.41
	Location + Fork Length	71.81	24	179.34	14.79
	Weight of Gut + Fork Length + Location	69.96	23	179.50	14.95
	Location + Weight of Gut	81.078	24	188.62	24.07
	Weight of Gut + Fork Length	104.27	27	205.81	41.26
Plastic load	**Species + Location + Weight of Gut + Fork Length**	**536.53**	**674**	**1698.9**	**0.0**
	Species + Location + Fork Length	538.54	676	1704.6	5.7
	Weight of Gut + Fork Length + Species	531.35	678	1712.1	13.7
	Species + Location + Weight of Gut	540.58	687	1712.8	13.2
	Species + Fork Length	532.66	680	1719.3	20.4
	Weight of Gut + Fork Length + Location	530.06	682	1720.7	21.8
	Species + Weight of Gut	535.79	691	1720.9	22.0
	Species + Location	541.69	689	1722.9	24.0
	Location + Fork Length	532.69	684	1728.9	30.0
	Location + Weight of Gut	534.48	695	1742.4	43.5
	Weight of Gut + Fork Length	525.09	686	1763.8	64.9

Table S4.2: Frequency of occurrence (%) of plastic ingestion for each state and species of fish. Blank cells represent no data collection of fish in those states.

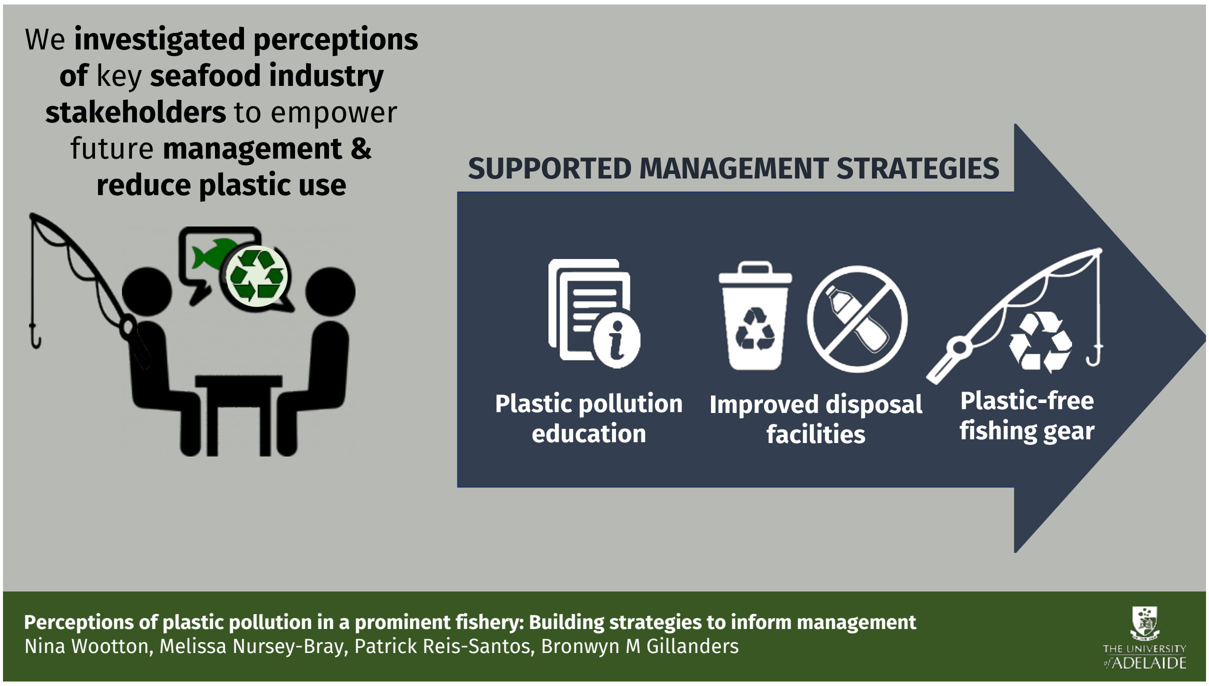
Common name	**WA**	**SA**	**VIC**	**TAS**	**NSW**	**Combined states**
Australian herring	25.0	35.0				30.0
Australian salmon		60.0	25.0	23.8		43.0
Australian sardine	10.0	5.9	17.9	10.0	25.0	14.3
Snapper	36.8	20	35.0		28.6	30.7
Dusky flathead			80.0		22.2	42.9
Garfish		43.5	20.0	9.5	19.0	23.3
King George whiting	12.5	61.1	36.9		50.0	50.3
Sea mullet	50.0	52.9			47.1	50.0
Tiger flathead		22.2	50.0	36.4		33.3
Combined species	26.2	47.4	32.4	20.2	29.9	

Table S4.3: Average plastic load (± SE) separated by state and species of fish. Blank cells represent no data collection from fish in those states.

Common name	**WA**	**SA**	**VIC**	**TAS**	**NSW**	**Combined states**
Australian herring	0.50 (±0.24)	0.70 (±0.29)				0.60 (±0.18)
Australian salmon		2.31 (±0.50)	1.20 (±0.76)	0.43 (±0.24)		1.60 (±0.76)
Australian sardine	0.80 (±0.75)	0.06 (±0.06)	0.25 (±0.11)	0.20 (±0.16)	0.30 (±0.13)	0.32 (±0.15)
Snapper	1.32 (±0.64)	0.80 (±0.52)	1.45 (±0.60)		0.62 (±0.27)	1.05 (±0.26)
Dusky flathead			2.70 (±1.09)		0.28 (±0.14)	1.14 (±0.44)
Garfish		0.57 (±0.15)	0.20 (±0.08)	0.10 (±0.07)	0.19 (±0.09)	0.27 (±0.05)
King George whiting	0.13 (±0.08)	2.21 (±0.30)	0.53 (±0.18)		0.60 (±0.22)	1.60 (±0.21)
Sea mullet	0.70 (±0.18)	1.06 (±0.37)			1.12 (±0.40)	0.94 (±0.18)
Tiger flathead		0.30 (±0.13)	1.14 (±0.46)	0.50 (±0.17)		0.56 (±0.13)
Combined species	0.66 (±0.20)	1.50 (±0.16)	0.87 (±0.18)	0.31 (±0.09)	0.50 (±0.10)	

CHAPTER 5

PERCEPTIONS OF PLASTIC POLLUTION IN A PROMINENT FISHERY: BUILDING STRATEGIES TO INFORM MANAGEMENT



We **investigated perceptions of** key **seafood industry stakeholders** to empower future **management & reduce plastic use**

SUPPORTED MANAGEMENT STRATEGIES

- **Plastic pollution education**
- **Improved disposal facilities**
- **Plastic-free fishing gear**

Perceptions of plastic pollution in a prominent fishery: Building strategies to inform management
Nina Wootton, Melissa Nursey-Bray, Patrick Reis-Santos, Bronwyn M Gillanders

THE UNIVERSITY of ADELAIDE

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Principal Author

Name of Principal Author (Candidate)	Nina Wootton		
Contribution to the Paper	Contributed to design of the study, reviewed literature, conducted the interviews, analysed the data and applied thematic analyses, wrote the manuscript and acted as corresponding author.		
Overall percentage (%)	75%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	5/10/21

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Melissa Nursey-Bray		
Contribution to the Paper	Contributed to design and development of the study, advice and suggestions throughout, thematic analysis, provided comments and feedback on manuscript.		
Signature		Date	7.10.2021

Name of Co-Author	Patrick Reis-Santos		
Contribution to the Paper	Contributed to design and development of the study, advice and suggestions throughout, thematic analysis, provided comments and feedback on manuscript.		
Signature		Date	06/10/2021

Name of Co-Author	Bronwyn Gillanders		
Contribution to the Paper	Contributed to design and development of the study, advice and suggestions throughout, provided comments and feedback on manuscript.		
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Perceptions of plastic pollution in a prominent fishery: Building strategies to inform management

Nina Wootton [a],[*], Melissa Nursey-Bray [b], Patrick Reis-Santos [a], Bronwyn M. Gillanders [a]

[a] Southern Seas Ecology Laboratories, School of Biological Sciences, University of Adelaide, SA 5005, Australia
[b] School of Social Sciences, University of Adelaide, SA 5005, Australia

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ABSTRACT

Plastic ingestion in seafood species, and human consumers, are thought to have potential harmful impacts. However, there is limited information from seafood industry stakeholder groups, including processors, traders and fishers, about how they perceive plastic pollution, its impacts and potential management. Using the South Australian Marine Scalefish Fishery as a case study, we uncover levels of awareness, knowledge and concern, as well as impacts and potential solutions of marine plastic pollution from commercial and recreational fishers and fishmongers. Stakeholders highlight key areas of concern and motivations to champion the mitigation of plastic pollution, and promote management pathways with increased stakeholder support, including the implementation of education resources, improvement of waste disposal facilities and exploration of plastic-free fishing equipment. Understanding the perceptions of stakeholder groups will inform policy makers and empower managers to build awareness strategies, shift opinions through communication and reduce plastic pollution.

1. Introduction

1.1. Plastic pollution, fisheries and knowledge

Marine plastic pollution is quickly becoming one of the most widely acknowledged environmental problems of the century [1,2]. The pervasive nature of plastic, as well as its ability to break down into small microplastic (plastic less than 5 mm in length) and travel unknown distances is causing major issues for marine ecosystems globally [2–4]. Impacts of plastic on marine life include suffocation or starvation due to ingestion or entanglement, both of which have been documented to occur commonly in fish worldwide [5–7]. These issues are likely to increase as we continue to pollute the ocean with plastics [2]. The discovery of microplastic in fish and throughout the marine food chain has also led to concerns about the consumption of seafood by humans [8–10], although the direct effects of exposure on human health are still relatively unknown [11–13].

The marine environment currently faces a long list of threats; with pollution, overexploitation, climate change and habitat destruction all causing major concern for the fishing industry. Alongside the loss of marine biodiversity comes trepidation of the oceans ability to continue to provide rising global populations with essential ecosystem services, including food and protein sources [14]. Further, these pressures mitigate the ocean's ability to recover from increasingly common extreme weather events and changes. Worldwide, marine fisheries are not immune to these threats; fish stocks are in decline and face various pressures surrounding management and sustainability [15]. The recent increase of plastic pollution also jeopardises the fishing industry, and those who rely on it (e.g. fishmongers, consumers) [16]. Marine plastics limit the productivity of commercial fisheries by compromising fish health, impacting marine wildlife, and contributing to depleting fish stocks [17,18]. Additionally, marine litter causes damage to fishing equipment and vessels, by getting stuck in their propellers or tangled in nets [19], triggering financial impacts, as equipment needs to be replaced, and time is lost cleaning and repairing, limiting the fishers catch [18,19]. It is estimated that marine litter costs the European fishery sector 61.7 million USD annually, or just under 5000 USD per vessel [18–20]. Therefore, the productivity, profitability and longevity of the fishing industry is vulnerable and highly susceptible to risks associated with marine plastic, particularly if paired with other stressors [17]. In this context, understanding how stakeholders including commercial, recreational and seafood traders view plastic and microplastic

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[*] Corresponding author.

E-mail addresses: nina.wootton@adelaide.edu.au (N. Wootton), melissa.nursey-bray@adelaide.edu.au (M. Nursey-Bray), patrick.santos@adelaide.edu.au (P. Reis-Santos), bronwyn.gillanders@adelaide.edu.au (B.M. Gillanders).

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pollution as a threat will help inform management to curb pollution, and champion education to build awareness and trust.

It is estimated that ocean-based sources account for more than twenty percent of all plastics in the ocean [21–23]. The fishing and aquaculture industries are major contributors, with an estimated 640, 000 tonnes of plastics from fishing gear entering the ocean each year (∼ 10% of all marine debris) [19,24,25]. The past few decades have seen the fishing industry increasingly rely on plastic in the form of nets, lines, ropes, traps and other fishing equipment, with plastic's lightness, durability, buoyancy and affordability making it an ideal resource [26, 27]. Fishing equipment is often lost by accident, as rough weather conditions and worn gear cause the equipment to break while in use [28], or in illegal and unregulated fisheries, fishing vessels deliberately release their equipment into the environment [29]. Further, recreational fishers are also contributors to plastic waste, either from fishing materials breaking or discarded, but also from food and drink packaging that is disposed of incorrectly [30]. Once plastic has been broken down to microplastic, it is increasingly difficult to identify the source of the plastic waste. Regardless of the cause, plastic and microplastic waste from the fishing industry is pervasive in the marine system, as larger macroplastics can cause entanglement with wildlife [31,32], and smaller microplastic can be ingested by species, causing decreased health and toxicity in animals that consume them [33–35]. With the fishing industry both contributing to, and negatively affected by marine plastics, raising awareness and education in commercial and recreational fishers is an essential first step towards lowering the risks created from fishing equipment entering the environment.

In light of this increase in public awareness of ocean health, industry and individuals who rely on the ocean for their livelihoods must be equipped with the appropriate information and education to help safeguard the ocean, and share knowledge with those around them [36]. Progressive action will likely shine favourably upon the fishing industry, with potential benefits to individuals involved and the seafood industry's overall economic strength. The application of this knowledge is no easy feat, as messaging and intervention needs to be finely constructed to target different communities and audiences [37,38]. Cultural and educational biases shape opinions and perceptions [39]. Priorities towards different environmental and social concerns are likely to influence people's knowledge, as well as the willingness to take proactive action towards issues that are perceived as a lower priority. Further, the nature of global news and social media allows individuals to shift the blame and guilt associated with plastic use to other groups, either to international countries, regions or metropolitan highly populated areas. It is important to understand the rationale, causes and consequences behind these thoughts, as well as assess perceptions, so that appropriate mitigation strategies can be designed [40]. It is pointless to design management policies that will not be embraced by the community they target, hence studies investigating the opinions of this sector are pertinent before such protocols are planned and eventually implemented [41].

Marine scalefish fisheries worldwide are encountering challenges surrounding sustainability and management [15], and the South Australian Marine Scalefish fishery (MSF) is an example of one of these fisheries. The South Australian MSF is a multi-species, multi-gear, multi-sector fishery, and is considered the most diverse and complex fishery in South Australia [42]. Overall, three of the key stakeholders within the fishery, i.e. recreational fishers, commercial fishers and fishmongers, have different perspectives and concerns on the fishery's management [43]. Additionally, all pre-mentioned stakeholders combined provide important dynamics and context behind the fisher. The South Australian MSF captures a diverse group of people and locations, and provides an interesting case-study for information to be collated and used to compare across similar fisheries globally. The broad diversity of target species and fishing gear allows comparisons to most global fisheries, as at least some similarities can be drawn, particularly compared to single species and gear fisheries [42].

Recent research investigates the public perception of plastic pollution [e.g. [44–47]], however there has been limited research specifically examining knowledge within the fishing industries. Therefore, this study had three main aims: (i) to document perceptions, awareness and concern of plastic pollution within a prominent fishery, including of microplastics; (ii) to analyse potential causes and reasons to why views and knowledge surrounding plastic pollution within the fishery may differ, and (iii) to identify potential solutions that stakeholders are willing to incorporate within the fishing industry to lower the impact of plastic pollution on the marine environment. With awareness about plastic pollution increasing globally, particularly in relation to the seafood industry, obtaining knowledge of the potential risks and solutions within fisheries is pertinent. Understanding the perceptions the seafood industry has on plastic pollution will underpin strategies to build awareness globally, trust and empower management to effectively address plastic pollution, with solutions that are more easily embraced by the multiple stakeholders.

2. Methods

2.1. Case study profile: South Australian marine scalefish fishery

The South Australian MSF operates in the coastal waters of the state of South Australia (SA), from the border of Western Australia to the border of Victoria (Fig. 1). The MSF is a multi-species fishery, with over 60 species on the commercial fishing list. Fish species are dominated by King George whiting, southern garfish, southern calamari and Australian snapper (Table S1), which together account for 70% of the total fishery value [42,48]. Other species targeted include snook, octopus, Australian herring, leather jackets and West Australian salmon (Table S1). Further, the MSF is a multi-gear fishery, covering 21 gear types, most commonly hook and line, longline, haul nets, mesh nets and jigs [48]. Following commercial fishing efforts, seafood are often sold either directly from fishers to fishmongers and commercial fish stores, or through the South Australian Fisherman's Co-operative Limited (SAFCOL), a centralised fish market where fishers can sell their produce

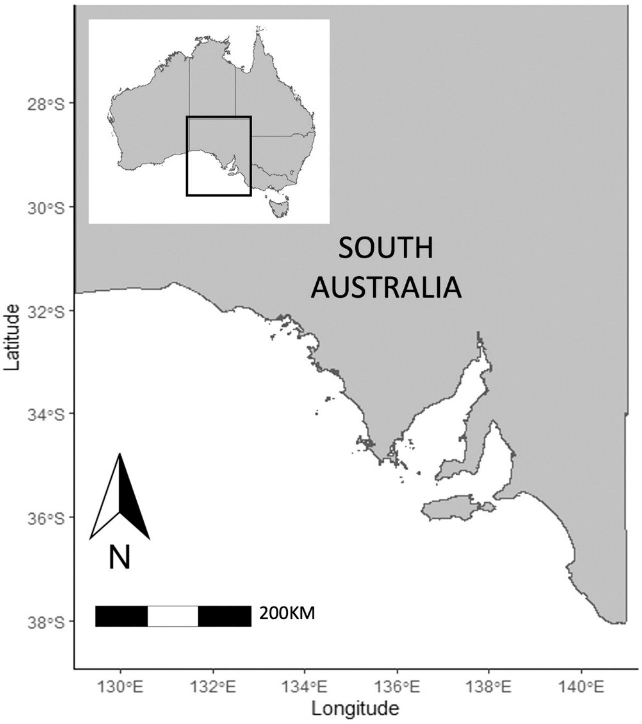

Fig. 1. Map of South Australia, where the South Australian Marine Scalefish Fishery is fished.

directly to wholesalers. Marine scalefish are also commonly targeted by recreational fishers, with 277,000 recreational fishers estimated to fish in SA [49].

The MSF has undergone multiple management shifts over time, and this combined with the divergent types of fishers within the industry has likely led to the historic conflicts between different stakeholders. The MSF is managed by the Department of Primary Industries and Regions South Australia (PIRSA). In total, it includes 309 commercial MSF licences, and additionally has other fishery license holders (e.g. 149 Southern Rock Lobster, 61 Northern Rock Lobster and 36 Lakes and Coorong) who have some commercial access to marine scalefish species [50]. Due to large numbers of licenses, as well as lowering stocks and reduced profitability, the SA state government announced a reform of the MSF in 2021 [51]. It hopes a reduction of commercial fishers will allow the once vibrant and profitable industry to take a positive turn, increasing sustainable fish stocks and improve opportunities for recreational fishers [51]. The reform includes the 'voluntary license surrender program', where the SA state government has committed to removing up to 150 licenses from the fishery; as well as splitting the fishery into four broad regional fisheries management zones [51].

The fishery operates with a mixture of both metropolitan and regionally based individuals, from a diverse range of socio-economic backgrounds. Within the commercial component of the fishery, there is a combination of family-owned and corporate based fishing licenses.

2.2. *Semi-structured interviews*

The study was undertaken over a five-month period (November 2020–March 2021). We conducted qualitative semi-structured interviews (see Table S2 for model interview), of three major groups within the MSF in SA. This included interviews with commercial fishers who work within the MSF, with recreational fishers who commonly target scalefish, and fishmongers, who sell and market the scalefish produce. Semi-structured interviews provide more extensive detail than traditional surveys, allowing smaller sample sizes as facts, attitudes and opinions are gathered [52]. Commercial fisher and fishmonger respondents were recruited using representative sampling [53,54], while recreational fishers were randomly approached along fishing jetties in Metropolitan Adelaide and via snowball sampling (i.e. when existing study subjects are used to recruit future subjects) [54]. Data collection of the interviews continued until the research reached 'information saturation' – meaning the point at which no new data will reveal itself, allowing the researcher to conclude that the research has achieved its goals [55,56]. This occurred after nine recreational fishers, six commercial fishers, and five fishmongers were interviewed. Due to time and travel restraints, as well as Covid-19 restrictions, the majority of commercial fisher and fishmonger interviews were conducted by phone while the recreational fishers were interviewed face-to-face. Throughout our analysis, we ensured that we were consistent with the renowned criteria for establishing trustworthiness in qualitative research [57]. These criteria include credibility, transferability, dependability and confirmability in the data and analysis of the information obtained [57].

The areas of focus for the interviews included: (i) fishers and fishmongers' knowledge on plastic pollution and microplastic, (ii) fishers and fishmongers' perceptions of plastic pollution and microplastic and its potential impact on their fishery and livelihoods, and (iii) fishers and fishmongers' ideas for reduction of plastic use and changes within the fishery or region.

2.2.1. *Commercial fishers*

Of the commercial fisher respondents, five were male and one was female. They had between five and forty years of experience in the commercial MSF in SA, with an average of 25 years. The fishers resided and worked across a large spread of the SA coastline. Most fishers targeted marine scalefish specifically, although some had changed industries recently to also target either rock lobster, or prawns and abalone. Most fishers were either currently or had previously been part of business partnerships with family members as license holders within the MSF.

2.2.2. *Recreational fishers*

All nine recreational fisher respondents were male and on average had been fishing in a recreational setting for more than 25 years. This included one fisher who had been actively recreationally targeting marine scalefish in SA for more than 60 years, as well as an individual who had only begun fishing the previous year. The fishers' main target species were within the MSF. None of the respondents were members of RecFish SA (nationally recognised peak body of recreational fishing in SA).

2.2.3. *Fishmongers*

Three of the fishmonger respondents were female and two were male, and they had on average owned or worked at fish and seafood shops for more than 25 years. As is the nature of seafood shops, the fishmongers questioned sold a variety of species but all included popular marine scalefish (commonly King George whiting, southern garfish, southern calamari and shark species).

2.3. *Thematic analysis*

Thematic analysis was used to code and then categorise the key findings from the semi-structured interviews. This style of analysis allows patterns to be identified across and within the dataset, providing solutions to the questions being considered [55]. Thematic analysis provides an adaptable method, which can be broadly used across different questions and methods to assist in understanding the interviewees' positions, insights, values and experiences regarding the topic under analysis. We used an inductive approach with our analysis, meaning that the coding and theme development was dictated by the data collected rather than prior conceived perceptions of the data. The analysis took place in a step by step nature, with five main steps. This included: (i) familiarisation with the data, by interviewing, transcribing and reading the interviews, (ii) searching for themes, (iii) coding the themes, (iv) reviewing and amending of identified themes, and (v) writing up and analysing themes.

2.4. *Triangulation*

Triangulation of data obtained through the semi-structured interviews ensured the validity of the thematic analysis findings. Triangulation is a popular technique utilised within social sciences to guarantee validation of data by verifying it across multiple sources, reducing bias, and creating greater confidence in the final results [58,59]. In our study, we achieved triangulation by including three investigator perspectives when completing the thematic analysis of the semi-structured interviews.

2.5. *Data analysis*

Following triangulation, results were synthesised into overarching themes (e.g. awareness and concern of plastic, plastic education of the general public, 'plastic is not a problem in this region', conflict between recreational and commercial fishers, willingness to change behaviour, sceptism towards council/government and priorities towards other problems) and direct and representative quotes were taken from the interviews to support the themes. Statistics were gathered on the interviewees demographic information (time they have been involved in the fishing industry for and sex). Their levels of knowledge were grouped into qualitative categories low, medium and high (based on if they knew what microplastics were and if they thought there was plastic in fish from SA). Statistical analysis were run on R studio (Version 1.4.1717), using the 'ggplot2' and 'sf' packages [60,61].

3. Results

The original focus of this study was to outline the opinions and knowledge of microplastic and plastic pollution held by individuals within the SA marine scalefish fishery. However, results also highlight additional concerns fishers hold and may prioritise ahead of mitigation strategies against plastic pollution. Although there was some alignment in perspectives between the three stakeholder groups (recreational fishers, commercial fishers and fishmongers), there were also conflicting ideas in some areas. Following synthesis of the interviewee answers, results are presented under three overarching themes. First, the awareness and concern towards marine plastic and microplastic pollution between individuals and groups, and how other problems often take priority. Second, the perceived responsibility that regional locations, stakeholder groups or even individuals are contributing towards the presence and impact of litter, and how this is redirected to others is summarised. Finally, we outline the willingness of respondents to apply changes within the fishing industry to reduce plastic use.

3.1. Awareness and concern varies between individuals and groups

This study emphasises the variation in knowledge that currently exists in information surrounding plastic pollution, and specifically microplastic, as only 55% of all interviewees were able to unquestionably define what microplastic was. Only 20% of individuals had a high level of knowledge surrounding the issue, with 40% of interviewees having a low level of knowledge. The levels of knowledge were spread across the stakeholder groups, with all three sub-groups having varied awareness of microplastic and plastic pollution (Fig. 2).

Variation surrounding knowledge and awareness is influencing the level of concern which individuals have towards marine plastic and microplastic pollution, as well as its impact on their industry in the future. Across all groups, there was a link between interviewees who had high levels of knowledge about plastic and microplastic pollution, and the concern they had for the problem, with all four interviewees classed as having high levels of knowledge also expressing concern towards plastic pollution. Some fishers and fishmongers acknowledged that the abundance of plastic in the marine systems was "*shocking*" (Fishmonger 4), "*the worst*" (Recreational Fisher 6), "*terrible*" (Commercial Fisher 1) and "*horrifying*" (Fishmonger 1). Others had lower levels of concern, suggesting that marine plastic presence is "*nothing to make me concerned*" (Fishmonger 3) and that the ocean's health is "*in a pretty good state*" (Recreational Fisher 9) and "*real clean*" (Commercial Fisher 2).

In relation to the presence of microplastic in fish from the MSF, across all groups, 50% of interviewees thought that there could be microplastic present in fish caught and sold in SA (Fig. 3). The remaining 50% held views that aligned with the idea that "*it's not really relevant here because we have such clean oceans*" (Commercial Fisher 2). However, when looking only at the fishmonger group, all five interviewees suggested that there was no microplastic in fish in SA (Fig. 3). When one fishmonger was asked what the effect would be if microplastic were found inside fish which were sold to consumers they responded: "*Strife, it would wipe us out*" (Fishmonger 4).

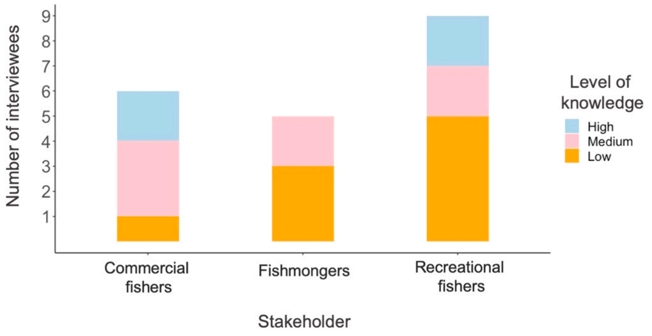

Fig. 2. Number of interviewees (from each stakeholder group) with different levels of knowledge. Knowledge was assigned based on if interviewees could define what a microplastic was, and if they thought there were plastic in fish in South Australia.

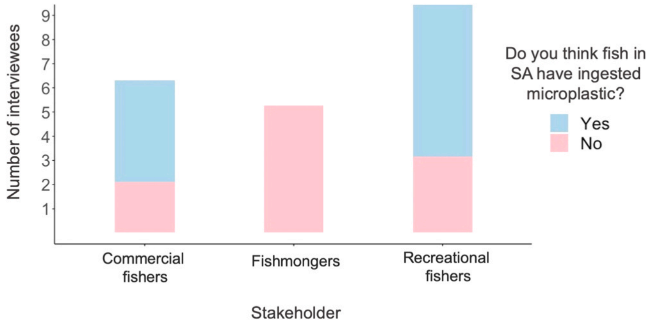

Fig. 3. Number of interviewees (from each stakeholder group), who thought fish from South Australian marine waters could have ingested plastic.

3.1.1. Priorities to other problems

Interviews, particularly in the commercial and recreational fisher stakeholder groups, showed that while plastic pollution was a tangible threat to the MSF, people prioritised other problems. For example, individuals from all stakeholder groups identified declining fish stocks and overfishing as the "*number one*" (Commercial Fisher 3) problem, with one fishmonger even claiming "*I reckon we have another 30 years left on fishing*" (Fishmonger 4). Trawling was also identified as another major problem within the fishing sector, described as "*very destructive and wasteful*" (Commercial Fisher 3) and "*it is not the rubbish that is making the ocean bad, it's the trawler that's the problem*" (Fishmonger 4). Other individuals stressed that pollution from sources other than plastic had more of a negative effect on the ocean health – "*most people have got two cars and using fossil fuels, so it is not just the plastic issue, it is more a broad pollution perspective*" (Commercial Fisher 2). Undersized fish, dying seagrass and Covid-19 were also identified as threats to the longevity of the fishery.

The MSF reform process was raised as a major recurring problem by the commercial fishers. The commercial fishers discussed how the reform was "*a mess really*" (Commercial Fisher 2) and "*very complicated*" (Commercial Fisher 2). The general conclusion was that they "*have all had an absolute gutful of it*" (Commercial Fisher 3). However, there was some hope that the government agencies in charge of the reform were "*working through it, hopefully reducing some red tape and strict regulations that are in place*" (Commercial Fisher 2).

3.2. Perceived responsibility

A widely held view across all stakeholder groups that were interviewed was that although marine plastic pollution was a problem, it was not something of concern locally in SA. Further, the individuals who had accepted that it was an issue in Australia often implied that it was not a fault of their own particular stakeholder group, but rather the fault of somebody else, such as the general public, opposing recreational or commercial fishing groups, or the local council and government.

3.2.1. Plastic pollution is not a local issue

A common opinion expressed by all three groups was that marine plastic and microplastic pollution was "*not in this country*" (Commercial Fisher 5) as "*our ocean* [was] *too pristine*" (Commercial Fisher 3). The general consensus was that plastic is more present and problematic in other regions globally, and even if plastic and microplastic was found in SA waters, it is likely entering the system from international waters. All groups attributed the responsibility of plastic pollution elsewhere, shifting the blame to other countries or regions. The following quote is illustrative: "*A lot of it comes from elsewhere, people dump it in oceans overseas and it finds its way here quickly… Here in SA we have good recycling practices and have had them for years. SA is more aware than the others.*" (Recreational Fisher 4).

Examples were given frequently, and from all stakeholder groups, that countries other than Australia were more to blame in the plastic pollution issue:

> *"The thing that springs to my mind is a lot of poor Asian countries – they don't have recycling but even that they don't have rubbish collection systems in place"* (Commercial Fisher 3)

> *"In our fish there would be no plastic. But in Thailand, all the beaches in Thailand are now covered in rubbish*" (Recreational Fisher 1)

> *"I understand that China etc. would have more [plastic in fish], all relative to the population of the different countries"* (Commercial Fisher 2)

> *"Do you know what, not in this country. I would more think of Indonesia and Asia and what not"* (Commercial Fisher 5)

> *"I imagine in places like India"* (Commercial Fisher 6)

> *"When I first started travelling to Fiji and Bali pollution was not as noticeable, but in the last five years it is a lot more noticeable"* (Fishmonger 2)

> *"It is mainly the Japanese, the Spanish, in the Mediterranean, up that way"* (Fishmonger 4)

> *"Plastic inside of them? I would not imagine so, something from the northern hemisphere, or Asia or the Pacific but not here no"* (Fishmonger 5)

Additionally, there were views from regionally located commercial and recreational fishers that the metropolitan regions are higher contributors to plastic pollution within SA. The following quote is illustrative from across the regionally based individuals:

> *"We are very fortunate here, cause the effort and concentration of effort is very limited compared to what you get in the CBD, I would say the closer you get to Adelaide [SA's capital city] the more concentrated the plastic and debris you can get in the ocean*" (Commercial Fisher 2)

It was suggested this was due to the fact metropolitan beaches "*have a lot of jetties*" and the population there has "*a slightly different mentality*" (Recreational Fisher 6).

3.2.2. General public education

Within the commercial fisher group, there were strong trends throughout interviews that they perceived that the general public is contributing more to plastic pollution in the environment than fishers, with four of the six interviewees (67%) making comments indicating this. There were particular concern from the commercial fishers that education surrounding plastic pollution in the general public was lacking, with suggestions of better signage at popular fishing locations and documentaries highlighting the local presence of marine plastics a proactive starting point. Furthermore, the management required to implement education programs and enforce public compliance was discussed. Examples of the comments from commercial fishers confirming these points were plentiful:

> *"It is all about management, and managing people. It is the general public that need more education"* (Commercial Fisher 2)

> *"I would say it's an educational thing. I think a good start would be to have some educational signs at the start of the jetty for the rec fishers. Have some signs saying how to dispose of their rubbish"* (Commercial Fisher 2)

> *"Rule and regulate anything, doesn't necessary solve anything. Education is the main thing. Just an appreciation of what you are doing" (*Commercial Fisher 3)

> *"I think it would be good if there were more local documentaries about it and people could see what you see under the microscope, it would make it a bit easier to believe. Like you could show them what you see under the microscope and say 'ooh that's plastic no that's not that's the gut' and you could really see that would be good for everyone including the education for the children coming up through it"* (Commercial Fisher 6)

> *"My biggest opinion that there needs to be more information given to the general public"* (Commercial Fisher 1)

> *"Everything is so focused on like fish size, fish catch, bag limits. There should be a little bit more pollution education coming from those industries, from fisheries or PIRSA coming to the general public"* (Commercial Fisher 1)

3.2.3. Recreational and commercial fishers conflict

Analysis shows that there was a clear division between recreational and commercial fishers, with recreational fishers commenting on the "*criminal side*" (Recreational Fisher 6) of commercial fishing; and commercial fishers noting the "*highly irresponsible*" (Commercial Fisher 3) fishing methods that recreational fishers implement. Fifty percent of the commercial fishers discussed the "*damage being done by our recreational fishers*" (Commercial Fisher 4), claiming that "*the commercial sector is much more careful than any of them, it's our industry*" (Commercial Fisher 4). One commercial fisher had particular issues with recreational tackle shops, claiming they were major contributors to the marine plastic pollution issue as "*recreational fishers lose so much gear as it is designed to break*" and that the recreational tackle shops were "*selling plastic down the throat of the fish*" (Commercial Fisher 3). One said that because commercial fishers have professional training in boat operations (such as MARPOL, the International Convention for the Prevention of Pollution from Ships), they "*know the rules and penalties*" and are "*trying to protect our income and profession*" (Commercial Fisher 1).

While there was less mention from the recreational fishers about the commercial fisheries impact on the environment, one recreational fisher did suggest that "*what father and son does on the end of the wharf or in their boat makes no difference whatsoever*" (Recreational Fisher 6). Further recreational fishers had concern that commercial fishing was causing other environmental problems, and lowering local fish populations.

3.2.4. Sceptism towards government

A distrust in government organisations to manage the fishery was a recurring theme through both the commercial and recreational fishing groups, and how "*half the rules and regulations [enforced by management] make no sense*" (Commercial Fisher 3). This is further seen in relation to sustainability within the fishery, including marine pollution, as sustainability is not considered a "*primary concern for the government, PIRSA, SARDI [South Australia Research and Development Industry] and fish operators*" (Commercial Fisher 3). Additionally, both commercial and recreational fishers spoke of issues with local councils removing bins from conveniently located jetties, meaning now "*people just dump it (rubbish)*" (Recreational Fisher 1). There were multiple reports of rubbish bin removal at both metropolitan and regional beach jetties, as councils "*don't want fishermen or anyone putting their stuff in the bins there*" (Commercial Fisher 6). This accumulation of events has caused distrust from both recreational and commercial fishers in the council, with fishers claiming they "*don't trust the council to do the right thing anyway*" (Commercial Fisher 6).

3.3. Willingness to adapt behaviour

Across all three stakeholder groups there was a general positive willingness to change in regards to broader plastic use, both personally and within their respective industries (Fig. 4). The one caveat that recurred consistently across groups was that change would only occur if it was convenient, easy, and cheap. The following quote is illustrative: "*we would consider using non-plastic equipment if it came down to the same cost, that is our main reason for not using something. Plastic is strong, light*" (Commercial Fisher 4). Within the recreational fisher group, eight of the nine fishers interviewed (89%) indicated they would change to "*natural equipment if it existed*" (Recreational Fisher 2). The only fisher who was critical of using non-plastic fishing claimed "*the feel of the plastic is better*" (Recreational Fisher 4).

All five of the fishmongers interviewed indicated they would be willing to change behaviour within their industry, highlighting some of the proactive choices they have already made to limit their plastic use within their respective workplaces. Examples of these include:

> "*We actively encourage people to bring their own containers and charge people for the foam boxes*" (Fishmonger 5)
>
> "*Getting rid of plastic bags… we just leave the product on the counter, and we don't ask them if they want a plastic bag. We just only give them if they ask for it*" (Fishmonger 4)
>
> "*People bring in their plastic containers. We are trying to educate a little bit to do it that way, don't have to use paper and plastic at the same time*" (Fishmonger 4)
>
> "*We try to use as small bags as possible. Different sized bags to make sure we fit the bag. So that we don't use a big bag*" (Fishmonger 3)
>
> "*If they don't need it (plastic bags) than don't give it. Or put multiple bits of seafood in the one produce bag*" (Fishmonger 1)

Although the general consensus among the fishmongers was that change was possible and in many ways was already being implemented, there were still some concerns surrounding the nature of seafood products "*cause they are wet*" (Fishmonger 3). Fishmongers voiced their questions about "*how else are we going to wrap the fish up?*" (Fishmonger 4) with seafood "*not being a dry product… like if you need to buy mushrooms you can use paper, or hessian bags for fruit and veggies*" (Fishmonger 3). Solutions were suggested that "*somebody needs to investigate a true biodegradable*" (Fishmonger 2), and educate customers to "*wash their container and bring it back again*" (Fishmonger 4).

4. Discussion

Marine plastic pollution is a global environmental problem, affecting fish health, biodiversity and ecosystem services [1,14]. The plastic pollution issue is multisectoral and diverse, and will continue to be pervasive in the marine environment in the absence of appropriate mitigation measures [62]. Interdisciplinary solutions are key in resolving complex marine plastic issues, including high quality data collection, utilising education to promote changes in awareness, and strengthening management and policy processes [63,64]. Understanding the knowledge, perceptions and motivations of key stakeholder groups that rely on marine environments is essential in developing applicable management strategies and shifting opinions through communication and education [65]. By analysing stakeholder perceptions, one can identify current opinions and barriers within sectors that may inhibit future change, and these can be used as a point of reference prior to implementing policy. Further, by working alongside stakeholders, we ensure that strategies are developed that will encourage wide compliance, and will be accepted by the community. This study is novel in examining opinions related to marine plastic pollution across stakeholder groups within a fishery, and can be used to see if similar patterns and thoughts are reflected across other fisheries globally. We identify a number of key considerations which have potential to impact upon the success of future mitigation strategies for plastic reduction. First, there is a clear lack of knowledge, and hence concern, within the MSF in SA surrounding marine plastic pollution, which may in the future impede the effectiveness of management actions. Second, our results identified that there were opinions within the fishery that attributed the blame of marine plastic pollution to international countries or metropolitan regions, hence there was less urgency to create change. However, broadly there were positive steps into how a fishing industry with less plastic would function, and be accepted, and ideas for lowering plastic use within the fishery were discussed.

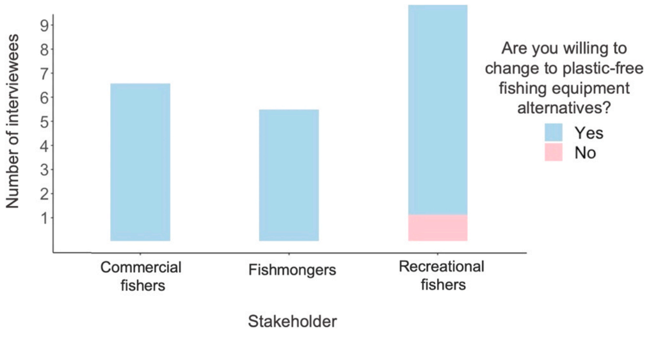

Fig. 4. Number of interviewees (from each stakeholder group) who were willing to implement plastic-free fishing equipment in their respective work places.

The results from our study align with previous social research focusing on solutions to environmental issues globally. Public behaviour and concern regarding plastic use is influenced by awareness, communication and education [44–46], analogous with knowledge levels in fishers and fishmongers and their concern of plastic pollution found in our study. Further, extensive enquiries into perceptions of other environmental problems, such as climate change, suggest that knowledge on the topic, or lack of it, is an essential indicator of how concerned or aware of risks individuals are [66–68]. The link between individuals shifting blame of an environmental problem, whether that be to other regions [69,70] or to the government [71–73] is a common social occurrence, which has been confirmed throughout our interviews. In general Australians considered themselves to have relatively sound recycling and plastic disposal compared to other less developed countries, and interviewees were quick to shift blame towards these other regions as larger contributors of the plastic problem. Furthermore, there is a link between the physiological distance from a problem and the tendency to shift the responsibility, as individuals tend to detach themselves from issues, and hence solutions, if they cannot physically visualise the negative effects of such problems [74,75].

4.1. Recommended management strategies

This study has underlined the necessity and opportunity for targeted education and awareness agendas to be implemented throughout all sectors of the fishing industry. Enriched awareness and deeper knowledge surrounding the risks associated with marine plastic are a crucial first step in changing the attitudes of the stakeholder groups towards accepting management changes and adapting individual choices [76–79]. There are obvious uncertainties, knowledge gaps and misreported data related to marine plastics, particularly surrounding their entry point, fate within the marine environment and ecotoxicological effects [80,81]. From this perspective, the potential for misconceptions to occur, through media outlets, documentaries and the general public is likely. Therefore, clear, scientifically sound data must be used in education packages to inform and engage key stakeholders groups, as well as the general public [76]. In this regard, stakeholders suggest in the first instance for management to focus on creating education tools and strategies that will actively inform fishers and fishmongers of the risks associated with plastic, with particular focus on local examples.

Throughout the interviews, there was a clear requisite for better quality and local information within the sector. Fisher education and outreach are key in limiting the deleterious effects that fishing equipment may have on marine environments, particularly concerning discarded fishing gear [82]. Considering change is not possible nor probable without acceptance of the risk of the problem, education must be used as the first tool in creating positive change within the industry.

Our interviews also suggest there were a lack of appropriate disposal locations, such as rubbish bins, throughout SA jetties and popular fishing spots. There was a call throughout the interviews for councils to supply better bins, and signage outlying regulations with littering at these locations. Individuals are less likely to illegally litter if there are disposal facilities available, especially in cases where the bins are easily accessible and in convenient locations [83]. Likewise, the addition of appropriate notices at popular fishing locations reminding fishers of the potential harmful effects of disposing both fishing and non-fishing waste into the ocean would encourage fishers and the general public to refrain from littering [82]. We recommend that fishery management work alongside local councils to ensure that appropriate disposal facilities are available, signage is up to date and clear, and policing of these rules are active.

The final recommendation drawn from the stakeholder opinions involved striving to find and implement alternate fishing equipment made of non-plastic materials for both recreational and commercial fishers, as well as plastic-free substitutes (e.g. biodegradable bags) for fishmongers. There is positive scope for the infiltration of some simple and cheap plastic-free fishing equipment to become available for recreational and commercial fishers: as our interviews highlighted, there is willingness and capacity within the industry for these changes to be accepted [84]. This aligns with findings from other studies, where generally fishers recognised they held some responsibility for marine plastics and were willing to accept and promote change within the industry [85,86]. The feasibility of reducing the use of plastic within the fishing industry broadly needs more research, however components of our recommendations are applicable globally and across fisheries [86]. In regards to limiting plastic fishing equipment, focus should initially be given to creating appropriate and effective gear to replace gear which has a high risk of becoming derelict and causing damage, such as seine nets and drift gill nets [87,88]. The vast majority of current fishing and aquaculture equipment is recyclable, so when gear is weathered to a point where it is unusable recycling should be mandated [89]. From a seafood transport and fish monger perspective, plastic is currently crucial for food safety and longevity, therefore appropriate substitutes need to be carefully developed. Biodegradable bags made from corn and starch are increasing in popularity, and new initiatives such as 'MarinaTex', a compostable film made from fish scales, are entering the commercial market [90]. Incentives to both initially create, and then eventually use plastic-free alternatives, via economic stimuli and marketing tactics would create opportunity for growth within this sector [91]. Further, we recommend that management begins this process on a small scale, allowing the appropriate equipment to be trialled and accepted rather than flooding the market which could create sceptism and delay progress [92]. Finally, we suggest the introduction of non-plastic fishing equipment would ideally occur alongside educational campaigns, enabling awareness and acceptance.

4.2. Future directions

Fishers, scientists and fishery management collaborations can enable the generation of potential pathways within institutions that will allow fisher knowledge to contribute towards ongoing conversations in the marine pollution scope. Global studies have shown that the integration of fisher knowledge into policy decisions encourages the acceptance of new management ideas within the industry [93,94]. Additionally, there is widespread acceptance that to manage and prevent marine litter, a co-responsibility between management and different fishing sectors is necessary, alongside improved interactions between stakeholders [76]. These points are crucial in ongoing future work and management implementations, if successful mitigation is going to transpire.

Research that highlights the concerns and awareness within a group are only representative of views from a particular time, thus could change in the future, therefore we suggest that ongoing research, as mitigation strategies are enforced, would be beneficial. Larger scale studies across Australia are required, to compare if fishers from other states and regions have similar concerns to what was found in SA. It would be valuable to investigate whether the perceptions surrounding SA having clean coastal waters are linked to the recycling system in SA, or if there are other factors playing a role. Moreover, broadscale and international comparisons on the knowledge and attitudes towards marine plastic pollution of key stakeholders are also recommended. Finally, accurate audits of plastic use and disposal throughout fisheries globally would guide future research and mitigation focus, and inspire proactive ideas and change within the industry.

5. Conclusion

The South Australian MSF is likely to encounter social and economic changes as a result of marine plastic pollution in the future [62]. With plastic production set to continually increase, and waste management strategies unable to cope, the ongoing existence of marine plastic pollution presents a persistent problem, one which is only going to get worse moving forward. In this study, a sample of key stakeholders in the fishery – commercial fishers, recreational fishers and fishmongers, revealed their levels of knowledge regarding marine plastic pollution, highlighting education gaps, awareness and concern. All three stakeholder groups had different priorities which they ranked higher than marine pollution, including overfishing, the MSF reform and trawling. Plastic pollution was also misperceived as less of an issue locally in Australia, and that it was having higher impacts on international countries and fisheries. In synthesising information from the interviews, we suggested some mitigation strategies to help grow awareness and education within the fishery, and hopefully limit plastic use and pollution from the fishery in the future.

CRediT authorship contribution statement

Nina Wootton: Conceptualisation, Methodology, Data curation, Investigation, Visualization, Writing – original draft. **Melissa Nursey-Bray:** Conceptualisation, Visualization, Validation, Methodology, Writing – review & editing. **Patrick Reis-Santos:** Methodology, Visualization, Validation, Writing – review & editing. **Bronwyn Gillanders:** Conceptualisation, Visualization, Supervision, Writing – review & editing.

Declarations of interest

None.

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Ethics declaration

Collection of the data in this study was approved by the Human Research Ethics Committee "Microplastic perceptions in the South

Australian marine scalefish industry" (H-2020-142) granted for the period July 31, 2020–2023.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.marpol.2021.104846.

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Supplementary Information S5

Perceptions of plastic pollution in a prominent fishery: Building strategies to inform management

Nina Wootton, Melissa Nursey-Bray, Patrick Reis-Santos, Bronwyn M. Gillanders

Table S1: Table of common and scientific names of fish species regularly caught in the South Australian Marine Scalefish Fishery.

Common name	**Scientific name**
King George whiting	*Sillaginodes punctatus*
Southern garfish	*Hyporhamphus melanochir*
Southern calamari	*Sepioteuthis australis*
Australian snapper	*Chrysophrys auratus*
Snook	*Sphyraena novaehollandiae*
Octopus	*Octopus* spp
Australian herring	*Arripis georgianus*
Leather jackets	Family Monacanthidae
West Australian salmon	*Arripis truttaceus*

Table S2: Example of the interview questions asked to fishers (commercial and recreational) and fishmongers. Please note that this guide is a representation of the main themes to be discussed in this semi-structured interview. It doesn't include the various prompts and non-leading general questions that may be used such as "Can you tell me a bit more about that?" and "Do you have any examples of that?" There were slight variations depending on the group interviewed, indicated by the alternate options in parenthesis.

Part 1: Personal details

Name:
Email/phone number:
Affiliation:

Part 2: Background

Tell me a bit about yourself? (tailor to specific person)
How long have you been working as a fisher (fishmonger, or involved in recreational fishing)?

Part 3: Awareness

What state do you think the ocean is currently in? What changes have you observed in the ocean during your time fishing (or working as a fishmonger)?

When you hear the term 'plastic pollution' what do you think of?

When you hear the word 'microplastic' what comes to mind?

Part 4: Concern/Impacts

What is your view on plastic pollution generally?

What is your view on the issue of plastic pollution in the seafood industry?

Do you think there are microplastics in fish in South Australia? Why or why not?

If there were microplastics found in fish in SA what impacts do you think would occur? Do you think the presence of microplastics in SA is a risk to you or your industry?
If yes, how, if no, why not?

Have you heard or seen in the media anything about the general public discussing plastic pollution/microplastics? If yes, can you describe this? What did you feel in response to this media?

Have you heard anyone in your industry (fellow fishers, workmates, friends who you fish with etc) discussing plastic pollution? What did they say?

Part 5: Solutions

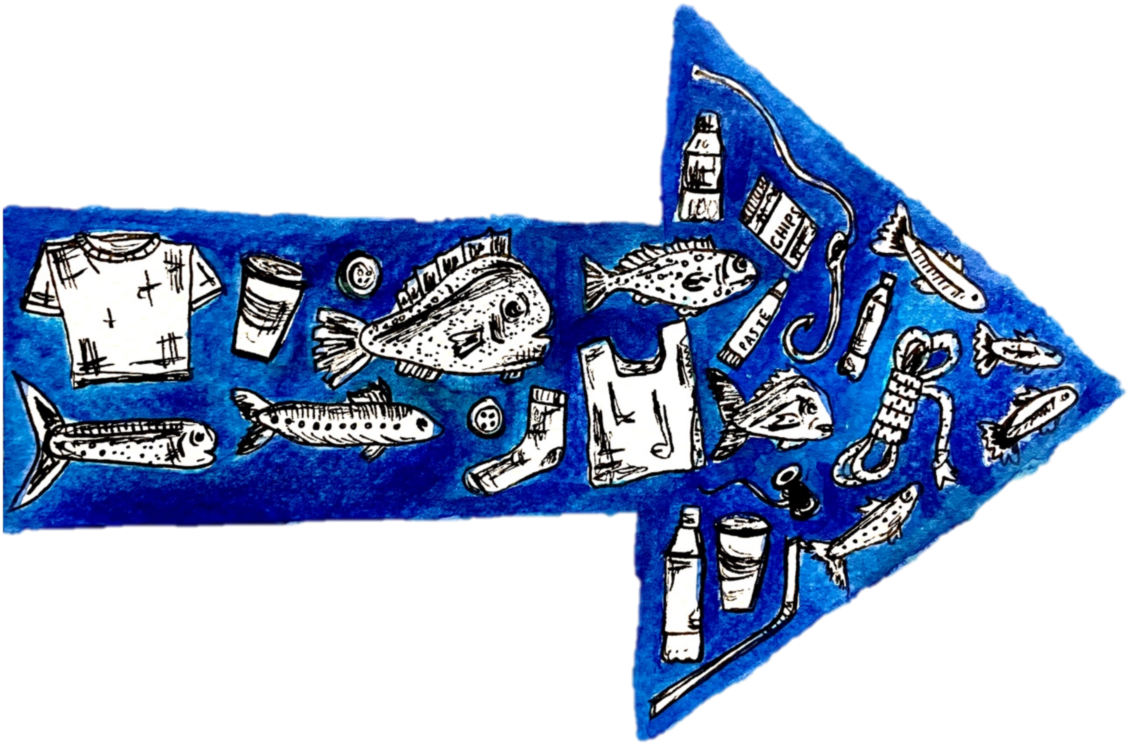
What types of measures would you suggest as possible solutions to address the impact of microplastics in fish?

At a personal level, would you do anything to change the use of plastic within the fishing industry?

If yes, what would you do? If not, why not?

CHAPTER 6

GENERAL DISCUSSION



6. General discussion

Knowledge on the presence of microplastic pollution in marine species provides further evidence of the widespread effect plastic is having on the planet. This understanding can be applied in guiding fisheries and waste management and creating education tools to ultimately lower our overall use of plastic. Throughout the research for this thesis, I collected detailed information on the presence of microplastic in key components of the Australian seafood industry, summarising how this may affect fisheries and uncovering potential steps to limit the negative impact of marine plastic pollution. Specifically:

(i) I found that microplastic presence is ubiquitous across fish sampled worldwide, with more than 49% of individual fish sampled ingesting microplastic, at an average of 3.5 pieces per fish. This was discovered by combining the global literature in a systematic review, which further highlighted regions and groups of fish that are understudied, and in particular a large gap in research regarding microplastic ingestion in fish from the Oceania region (Chapter 2) (Figure 6.1);

(ii) I filled this gap by sampling over 900 commercial fish across Oceania, and documented the abundance and type of microplastic ingested in commonly consumed species. Microplastic were found in 35% to 61% of fish sampled, with an average amount of 0.9 - 1.6 pieces per fish. Although this is less than the global average, it still represents a potential threat to seafood safety and the seafood industry in the region (Chapters 3 and 4) (Figure 6.1);

(iii) Interviews with key stakeholders in fisheries provided information on the varying levels of knowledge and concern surrounding plastic pollution within the seafood industry. I have created management and policy advice for future actions that are supported by key stakeholders, such as increased education and improved disposal facilities, which in turn will help raise awareness of plastic use and disposal within the fishing sector (Chapter 5);

In this closing chapter, I examine the main outcomes of my research, discussing future directions and how this work can contribute to the ongoing goal of clean oceans.

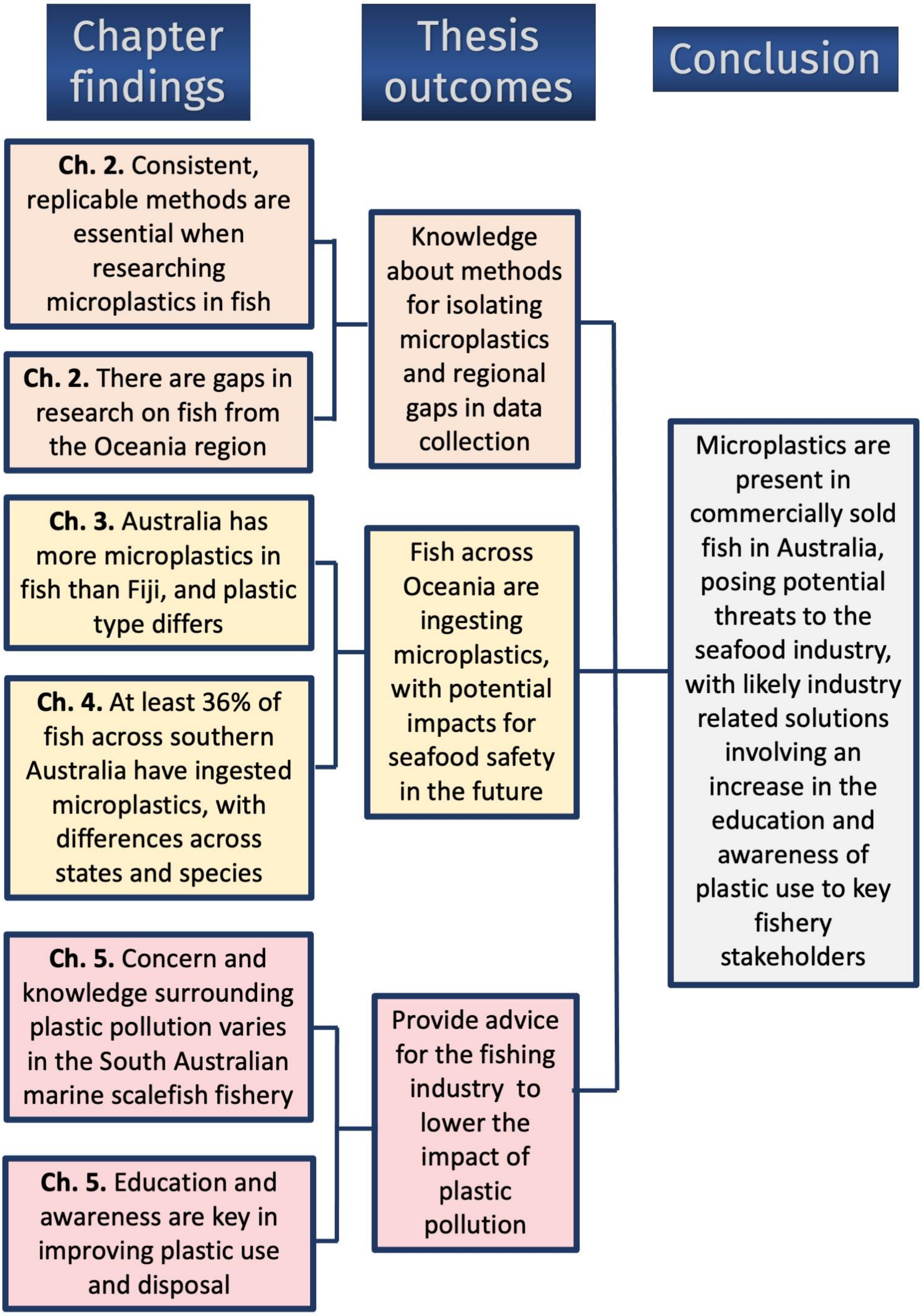


Figure 6.1: Schematic representation of chapter findings, thesis outcomes and overarching conclusion.

6.1 Global plastic in seafood – knowledge presence and absence

Microplastic has been found in seafood species throughout the 21st century (e.g. Van Cauwenberghe and Janssen 2014; Rochman *et al.* 2015; Smith *et al.* 2018), and there are reports of plastic ingestion in fish from as early as 1972 (Carpenter *et al.* 1972). Research has been undertaken, on a slew of species, to determine the presence of microplastic in fish. I synthesised this constantly growing dataset and found that 49% of all fish sampled globally had ingested microplastic, with an average of 3.5 pieces per fish (Chapter 2). These results are higher than other reviews, for example Markic *et al*. (2019) where 38% of fish had microplastic and Savoca *et al.* (2021) where 26% had microplastic. My study found an overall higher abundance of fish ingesting plastic due to a variety of factors, including broader range of environments (other reviews focus exclusively on marine fish while ours also includes freshwater and estuarine), methodological restraints ensuring only studies with comparable methodologies were included, and most recent datasets.

Synthesising the existing literature emphasised certain regions and species that are still comparatively under-represented. Filling these knowledge gaps with high quality information is essential in broadening the microplastic baseline data set needed to guide future waste management and inform potential human and fish health risks (Lusher *et al.* 2017a; Barboza *et al.* 2018).

6.2 Plastic ingestion in fish from Oceania

My work aimed to fill the large gap in published literature looking at microplastic ingestion in fish from the Southern Hemisphere, particularly the Oceania region, as well as fish caught and sold through seafood markets (Mercogliano *et al.* 2020; Savoca *et al.* 2021). I chose to focus on this region to collect data for Chapters 3 and 4, sampling throughout the Oceania region by purchasing commercially important fish from fishmongers across Fiji and Australia. Broadly, we found microplastic in between 35% and 61% of fish, and an average of 0.9 to 1.6 pieces from all samples, with differences depending on location (Figure 6.2). It is difficult to identify the specific drivers of these differing levels of microplastic. However, population size, waste management strategies, ocean currents, water retention and effluent discharges in each country and/or state where fish were caught are likely to contribute (Lebreton *et al.*

2012; Jambeck *et al.* 2015; Siegfried *et al.* 2017; Ziajahromi *et al.* 2021). Fish in South Australia, for example, had the highest microplastic levels compared to other southern Australia states, likely driven by greater abundances of microplastic in the water and sediment (Ling *et al.* 2017) caused by high water retention (Nunes Vaz *et al.* 1990) and ocean currents which wrap around southern Australia (Lebreton *et al.* 2012; van Sebille *et al.* 2015). Moreover, microplastic were present in all 14 fish species investigated, with varying abundances depending on species habitat occurrence, feeding strategy and trophic level. This is consistent with studies that suggest microplastic accumulates in higher trophic level species with carnivorous diets (Batel *et al.* 2016; Carbery *et al.* 2018). Additionally, the literature suggests that as more microplastic settles in the sediment and ocean floor (Woodall *et al.* 2014; Barrett *et al.* 2020), benthic fish have a higher chance of encountering plastic, which is also consistent with my findings.

Generally, there was less microplastic in fish across Oceania than what was found in the global dataset, both from a plastic load and frequency of occurrence perspective (excluding the frequency of occurrence of microplastic in fish from Queensland) (Figure 6.2). Notably the average amount of microplastic per fish globally was considerably higher than any Oceania locations, with a global average of 3.5 pieces per fish compared to 0.9 – 1.6 pieces. Although these global values represent fish from all aquatic environments (marine, freshwater and estuarine), when we compared just the marine data, the global averages were still significantly higher, with 2.7 pieces per fish. Population size is likely key, as both Australia and Fiji have small populations in contrast to some larger countries such as China and USA. Larger populations produce more waste, and there is an increased likelihood of plastic ending up in the environment, and eventually the ocean (Lebreton *et al.* 2012; Jambeck *et al.* 2015). Legislation and education to limit both plastic production and disposal may also be playing a role, as Australia has relatively advanced recycling and rubbish collection facilities compared to other regions (Commonwealth of Australia 2018). Despite this, ocean plastics are transnational and, once leaked into waterways, can be transported via currents, winds and waves into other nations' waters (Lavers and Bond 2017; Lebreton *et al.* 2017; Galaiduk *et al.* 2020). Therefore, without knowing the exact origin of microplastic identified in the fish species sampled throughout this thesis, it is intrinsically difficult to ascertain why microplastic levels differ between Oceania regions and the global average.

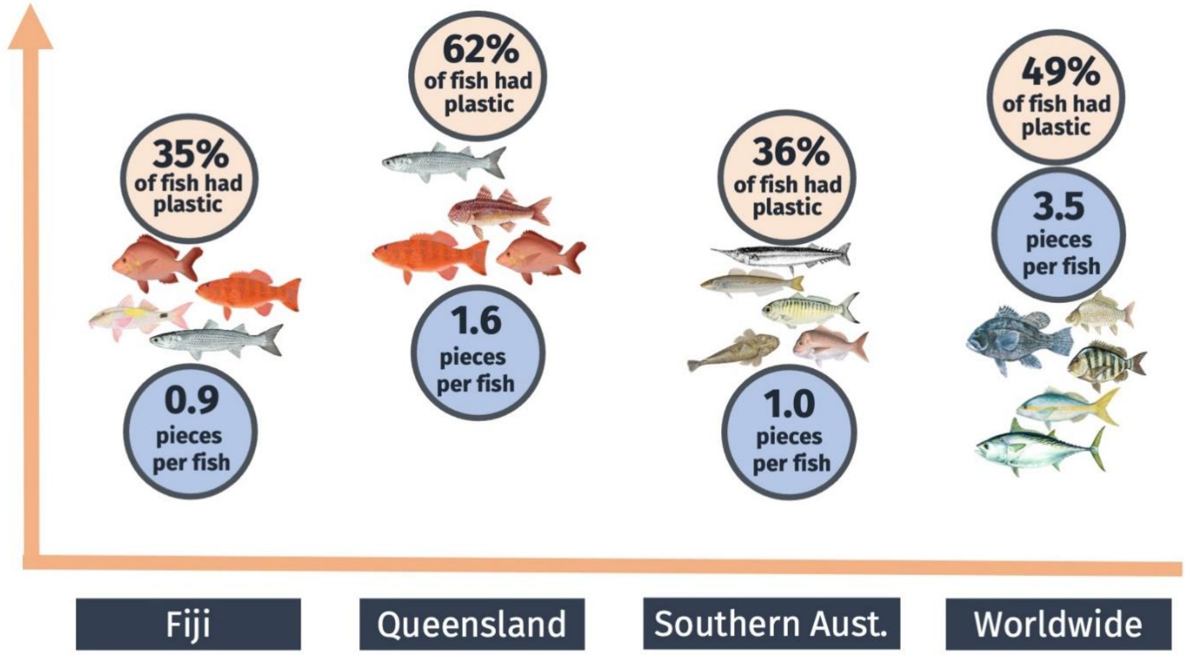


Figure 6.2: Graphical summary of microplastic frequency of occurrence and load in all species sampled across Fiji and Queensland (Chapter 3), Southern Australia (Chapter 4) and worldwide (Chapter 2)

6.3 Advances in microplastic research methodology

Microplastic laboratory and sampling methods have shown steady improvements in microplastic detection in recent years (Savoca *et al.* 2021). As discussed in Chapter 2, it is essential that microplastic research follows strict and standardised protocols to remove ambiguity and allow future comparisons between studies (Lusher *et al.* 2017b; Provencher *et al.* 2019b). The systematic review (Chapter 2) underpinned the choice of methodologies used in Chapters 3 and 4, ensuring reliable, accurate and comparable data. Research focused on methodological development recommended abundant sample sizes; using chemical digestion and heat to break-down organic matter (Dehaut *et al.* 2016; Karami *et al.* 2017; Kuhn *et al.* 2017); the use of filter paper, or fine sieves to isolate microplastic; adequate contamination controls and a clean laboratory environment; and chemical verification of polymer type (Figure 6.3) (Lusher *et al.* 2017b; Kroon *et al.* 2018; Markic *et al.* 2020). Throughout this thesis, I implemented these high standard methods and provided examples where studies using different approaches yield disparate or irreconcilable results, thereby further highlighting the importance of the strict and standardised criteria used throughout Chapters

2, 3 and 4. The detection of microplastic may pose a high risk to seafood business and livelihoods, therefore strict diligence and accuracy in methodology is essential to boost confidence and stakeholder engagement in scientific research and partnerships.

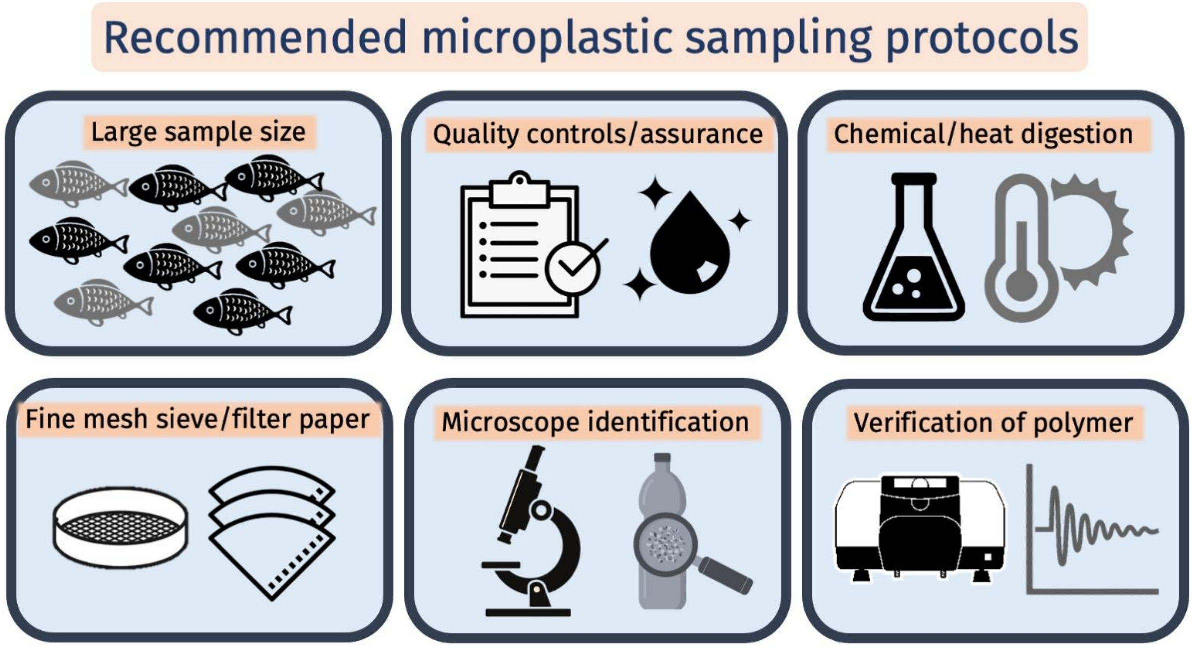


Figure 6.3: Recommended microplastic sampling protocols in biota

6.4 Significance to the seafood industry

The presence of plastic in fish species unsurprisingly has the potential to negatively affect the seafood industry which catches and sells such fish (Lusher *et al.* 2017a; Barboza *et al.* 2018; Hantoro *et al.* 2019). First, and what should be of great concern to fishers particularly, is the potential that fish health will decline if microplastic continues to infiltrate their ecosystems (Wang *et al.* 2020)(Figure 6.4). This could transpire by increased numbers of fish dying from suffocation or obstruction, or suffering from population-wide health effects as their diminishing health renders them more susceptible to disease and predators (Carbery *et al.* 2018; Provencher *et al.* 2019a). In turn, this could negatively affect fish stocks and hence fishers' catch, which is alarming to stakeholders across fisheries globally. Additionally, there is the potential for microplastic in fish to affect the demand and popularity of fish products with consumers, particularly if microplastic, or the chemicals associated with them, are documented to be translocating to muscle tissue (i.e., the fillets of fish, which are commonly eaten) (Smith *et al.* 2018; Vethaak and Legler 2021). If consumers become anxious about the presence of microplastic in seafood, particularly through media avenues, the cascading effect onto the seafood industry could hurt the livelihoods of fishers and fishmongers alike.

It is critical that protections are put in place to lessen the likely negative effects of microplastic on the seafood industry due to the presence of microplastic. Key stakeholders such as fishers and fishmongers must be involved in this process, as messaging and change are unlikely to be supported if they lack approval within the industry (Walker-Springett *et al.* 2016). For these reasons, I chose to interview fishers (both commercial and recreational) and fishmongers to explore levels of concern and knowledge about microplastic, allowing insights for potential awareness and avenues for improvements to lower plastic use and disposal within the seafood industry.

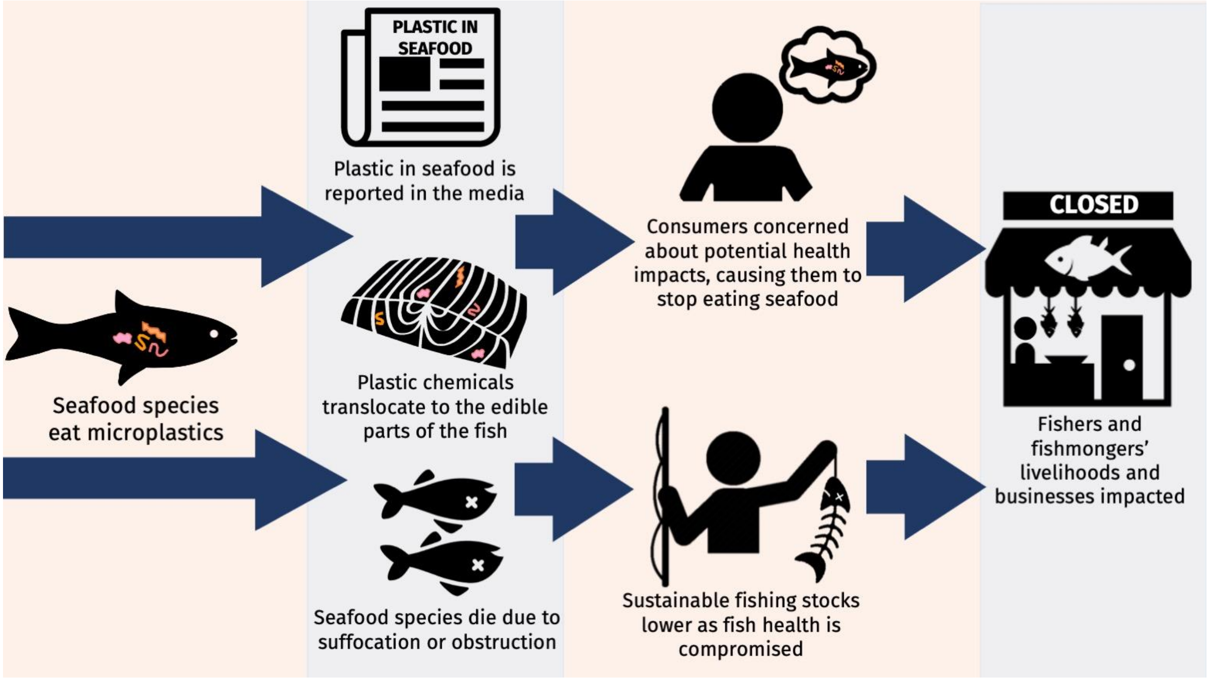


Figure 6.4: Pathway of potential impacts on the seafood industry if plastic pollution continues to impact fish

6.5 Solutions within the seafood industry to lower plastic pollution

Stakeholders within the South Australian Marine Scalefish Fishery (MSF) held differing views regarding plastic use and disposal. This was not surprising, as the MSF is renowned for being diverse in people and attitudes (Nursey-Bray *et al.* 2018). Despite the broad spectrum of responses in interviews, there was a clear set of overarching themes, which I synthesised into a set of recommendations that have the potential to be incorporated into fisheries and waste management protocols. Fisheries management is intricate and there can be scepticism towards certain issues, particularly with an environmental focus (Bailey *et al.* 2017). For this reason, the first recommendation I put forward, which is supported by key seafood industry stakeholders, is to provide education materials about marine plastic pollution and share information with those within the seafood industry. To effectively build trust and confidence within local stakeholders this information must be scientifically sound, trusted and understood by all parties (Failing *et al.* 2007; Reed 2008). Consequently, this idea can envelop the earlier chapters of my thesis, where I have knowledge showing that fish locally are ingesting microplastic (Chapters 3 and 4). This evidence could be utilised in educational materials. Incorporating scientific information allows these educational messages to create localised understanding towards the topic, as ocean users realise that plastic is indeed a local issue with local impacts. In time, this will hopefully encourage better opportunities and attitudes towards the disposal of plastic waste and, importantly, reduction of plastic use. The implementation of these recommendations, alongside the potential development of plastic-free fishing equipment, will benefit the seafood industry as a whole (Figure 6.5).

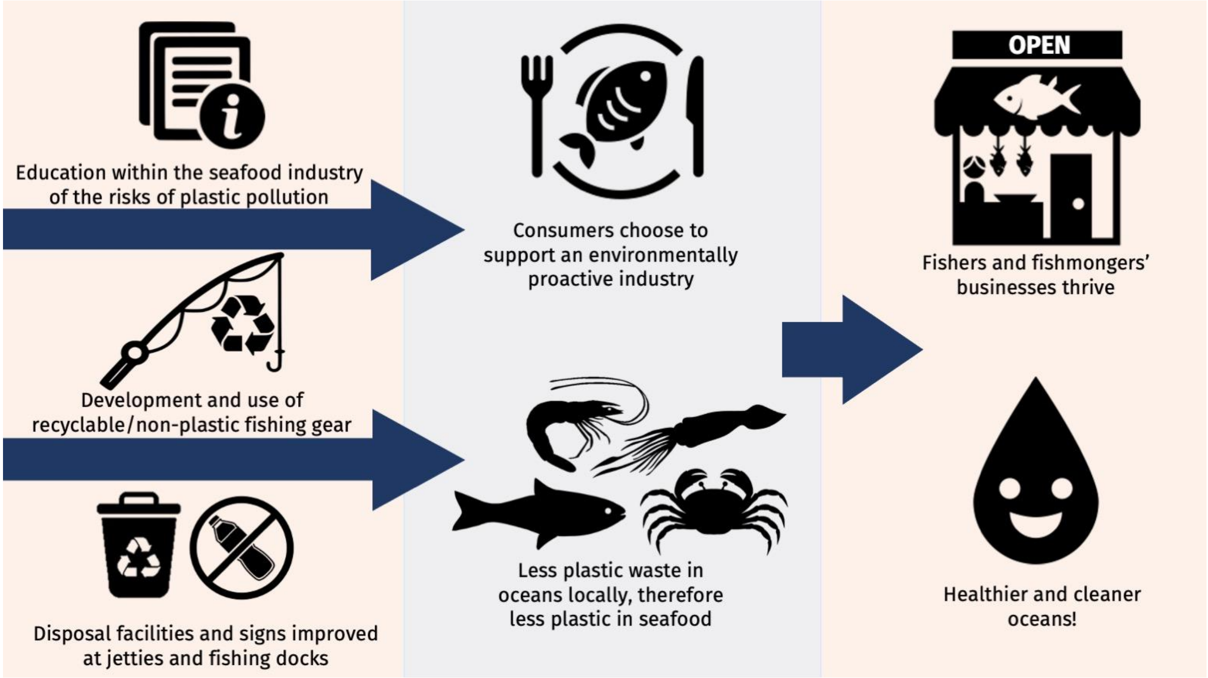


Figure 6.5: Pathway of potential effects on the seafood industry if modifications are implemented to lower plastic use

6.6 Future directions

Research into microplastic has grown swiftly and rapidly in the past decade, triggering concerns about the reliability and applicability of some research outputs, particularly regarding applied sampling and methodological approaches (Hermsen *et al.* 2018; Provencher *et al.* 2020; Miller *et al.* 2021). In this regard, my main recommendation for future microplastic researchers is to report microplastic presence with high standard methodological criteria, and assurance/quality controls (Dehaut *et al.* 2019; Cowger *et al.* 2020). This will ensure both reproducibility and comparability of microplastic data going forward (Cowger *et al.* 2020). Additionally, I suggest expanding plastic detection to include smaller size micro and nano plastics, by including a finer sieve or filter paper in the filtration stage of the methodology. Further sampling of additional organs and tissue types (e.g. gills, liver and fillet) for micro and nano plastics would assist in understanding how pervasive plastic presence is in seafood species. As we continue to sample and test microplastic presence and polymer type in fish and diverse groups of marine biota, I would envisage the implementation of a long term strategy based on consistent methods and routine sampling of seafood from markets, this should develop a broad-scale dataset with high statistical power (Wesch *et al.* 2016; Lusher *et al.* 2020). This is an easy-to-implement approach that can provide key insights on the temporal and spatial variations in microplastic contamination.

More microplastic presence studies are still needed globally, to fill known research gaps or provide knowledge on the effect and implications of microplastic (Lusher *et al.* 2017a). While I have added to the under-studied Oceania database of microplastic in fish, Africa and surprisingly North America are still under-documented, as are freshwater and estuarine environments, for both fish and other aquatic biota (Sequeira *et al.* 2020; Savoca *et al.* 2021). Furthermore, while Chapter 2 has identified that fish caught from aquaculture have a higher presence and abundance of microplastic, it would be of great benefit to investigate the reasons behind this in more detail (FAO 2017; Garcia *et al.* 2021). Fish raised in mariculture and aquaculture sources could be exposed to higher levels of plastic through contaminated food sources and plastic aquaculture equipment, such as sea pens. (Law and Thompson 2014; Wu *et al.* 2020). This has the scope to influence the seafood industry in years to come, as we have moved into a space where aquaculture production had surpassed wild-caught fisheries, with 52% of fish and fish products consumed by humans being sourced from aquaculture (FAO 2020).

We still have limited information on how microplastic and their associated chemicals may be negatively affecting fish (Wang *et al.* 2020) or human health (Smith *et al.* 2018; Vethaak and Legler 2021), and this should become a research priority (Campanale *et al.* 2020; Katyal *et al.* 2020). We still do not know if microplastic are having indirect or direct effects on fish from a cellular, organism or population level (Katyal *et al.* 2020). Microplastic contamination in fish has the potential to negatively impact seafood safety and the longevity of the seafood industry, so providing knowledge on health effects in fish on both an individual and ecosystem level is not just desirable, but necessary. Microplastic presence in fish gastro-intestinal tracts may not have a negative effect on fish health if fish are able to excrete microplastic, but we still have limited information regarding gut retention of plastic (Grigorakis *et al.* 2017; Sun *et al.* 2019). Furthermore, from a human consumption perspective, the extent to which microplastic and their associated chemicals are translocating into the fillets of fish is still unknown (Akoueson *et al.* 2020; Vethaak and Legler 2021). Therefore, a better understanding of associated chemical contamination, toxicological effects and impacts on fish is needed and will highly benefit the messaging behind seafood consumption.

Plastic pollution is a complex, multi-faceted environmental problem with equally intricate solutions (Dauvergne 2018). One pathway to cleaner oceans includes education programs and encouragement of pro-environmental behaviours in certain groups – including school children, fishers and the general public (Cheang *et al.* 2019; Dalu *et al.* 2020). The social aspects behind plastic use and disposal should continue to be investigated, as should investigation of what messaging and tools are needed to encourage the general public to limit their plastic use (Soares *et al.* 2021).

6.7 Concluding remarks

Throughout this thesis I show that microplastic are present in fish species sold in seafood markets throughout Oceania. Although the abundance is less than what I found when reviewing microplastic in fish globally, microplastic presence is still extensive across species and has the potential to negatively affect the seafood industry. Fisheries provide an important food source for populations globally, and I engaged key stakeholders and outlined potential solutions to help lower plastic use and disposal within fisheries. Ultimately, knowledge on the presence of microplastic will be key to support actions towards lowering plastic use within both the seafood industry and the general population.

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Appendix One:

Outreach, Presentations and Awards

Appendix Table 1: List of presentations and outreach activities conducted during PhD

Year	Style	Organisation	Audience demographic
2018	Seminar	School of Biological Sciences, University of Adelaide	Academics
2018	Invited speaker	British Sub-Aqua Club	General public
2019	Seminar	University of South Pacific	Academics and general public
2019	Invited speaker	Prince Alfred College Year One class	Children
2019	Invited speaker	Bright Sparks Science Club	Children
2019	Invited speaker	St Ann's College	Undergraduate students and general public
2019	Poster presentation	School of Biological Sciences, University of Adelaide	Academics
2019	Conference presentation	Australian Society of Fish Biology	Academics/industry/government
2019	Graphical abstract presentation	Australian Society of Fish Biology	Academics/industry/government
2019	Seminar	University of Adelaide Marine Biology class	Undergraduate students
2019	Invited speaker	Australian Marine Science Association – international collaboration event	Academics/industry/government
2020	Seminar	University of Adelaide Zoology class	Undergraduate students
2020	3 minute thesis competition	University of Adelaide	Academics
2020	Visualise Your Thesis video competition	University of Adelaide	Academics and general public
2020	Conference presentation	Australian Society of Fish Biology	Academics/industry/government
2020	Graphical abstract presentation	Australian Society of Fish Biology	Academics/industry/government
2021	Invited speaker	St Ann's College Environmental Science night	Undergraduate students
2021	Conference presentation	World Fisheries Congress	Academics/industry/government
2021	Video competition	World Fisheries Congress	Academics/industry/government
2021	Invited speaker	Clean Ocean Art	General public
2021	Press release	Fresh Science and the University of Adelaide	General public
2021	Radio interview	ABC Adelaide Rural Report	General public

2021	Radio interview	ABC Adelaide Country Hour	General public
2021	Radio interview	FiveAA	General public
2021	TV interview	Nine News	General public
2021	TV interview	Ten News	General public

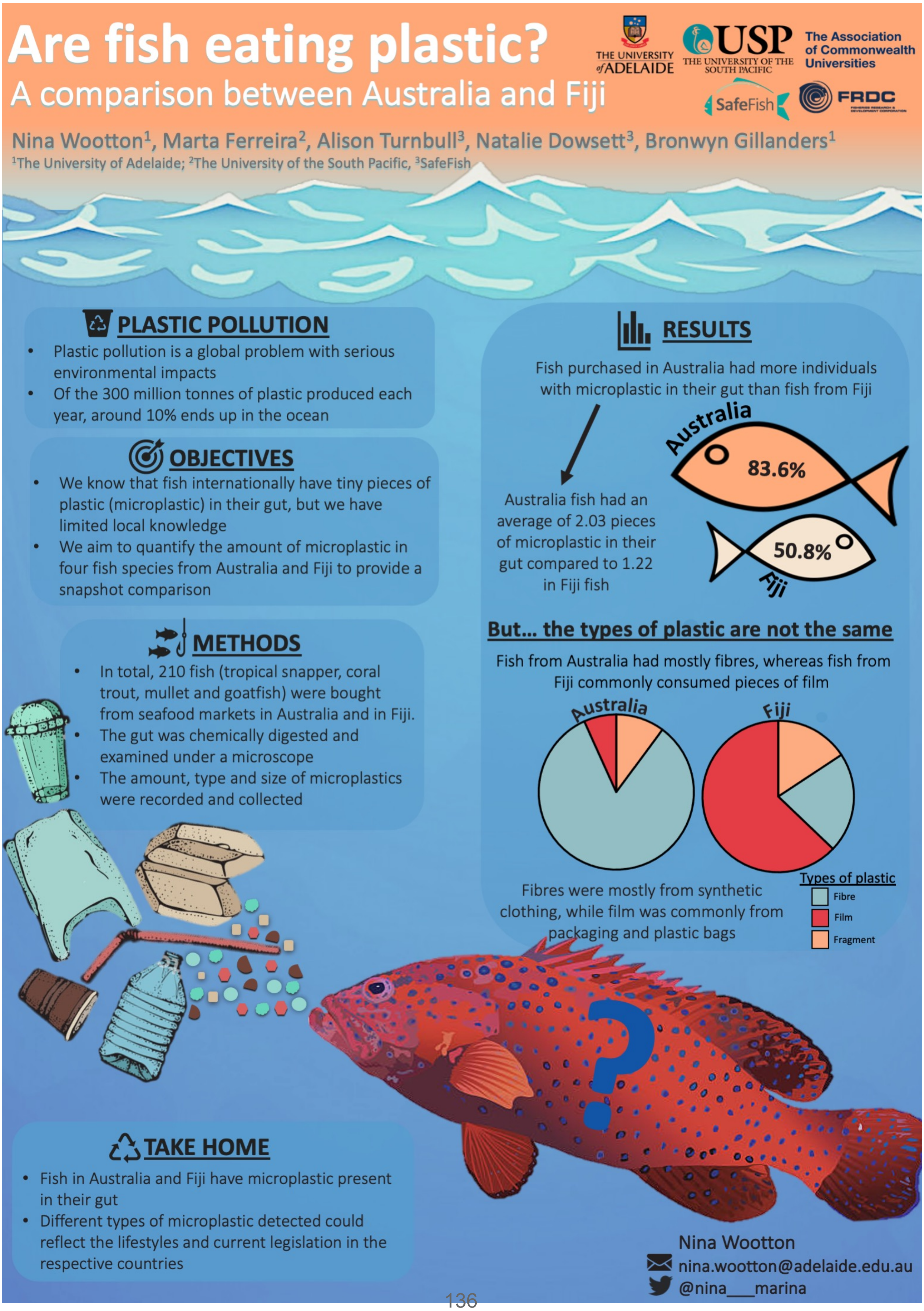
Appendix Table 2: List of awards received during PhD

Year	Award
2019	Association of Commonwealth Universities Blue Charter Fellow
2019	Best Poster Award for School of Biological Sciences, University of Adelaide
2020	3 Minute Thesis Winner for School of Biological Sciences, University of Adelaide
2020	Visualise Your Thesis video competition 2nd place in the University of Adelaide
2021	South Australian Fresh Scientist

Appendix Table 3: List of blogs written during PhD

Year	Title	Link
2018	Time to tide up – Fishing debris in Australian oceans	https://www.gillanderslab.org/single-post/2018/11/06/time-to-tide-up-fishing-debris-in-australian-oceans
2019	Beating plastic pollution – Collaboration is key	https://www.acu.ac.uk/news/beating-plastic-pollution-collaboration-is-key/

Appendix Figure 1: Scientific poster awarded 'Best Poster' at the University of Adelaide School of Biological Sciences Post-graduate research day



Are fish eating plastic?

A comparison between Australia and Fiji

Nina Wootton[1], Marta Ferreira[2], Alison Turnbull[3], Natalie Dowsett[3], Bronwyn Gillanders[1]
[1]The University of Adelaide; [2]The University of the South Pacific, [3]SafeFish

PLASTIC POLLUTION

- Plastic pollution is a global problem with serious environmental impacts
- Of the 300 million tonnes of plastic produced each year, around 10% ends up in the ocean

OBJECTIVES

- We know that fish internationally have tiny pieces of plastic (microplastic) in their gut, but we have limited local knowledge
- We aim to quantify the amount of microplastic in four fish species from Australia and Fiji to provide a snapshot comparison

METHODS

- In total, 210 fish (tropical snapper, coral trout, mullet and goatfish) were bought from seafood markets in Australia and in Fiji.
- The gut was chemically digested and examined under a microscope
- The amount, type and size of microplastics were recorded and collected

RESULTS

Fish purchased in Australia had more individuals with microplastic in their gut than fish from Fiji

Australia fish had an average of 2.03 pieces of microplastic in their gut compared to 1.22 in Fiji fish

But… the types of plastic are not the same

Fish from Australia had mostly fibres, whereas fish from Fiji commonly consumed pieces of film

Fibres were mostly from synthetic clothing, while film was commonly from packaging and plastic bags

TAKE HOME

- Fish in Australia and Fiji have microplastic present in their gut
- Different types of microplastic detected could reflect the lifestyles and current legislation in the respective countries

Nina Wootton
nina.wootton@adelaide.edu.au
@nina___marina

Appendix Figure 2: Adelaide Advertiser news article published in 2019 – ‘Probing the scale of marine plastic pollution

IN-DEPTH: Adelaide University PhD candidate Nina Wootton with King George whiting she used to research microplastics in fish. Picture: TOM HUNTLEY

Probing the scale of marine plastic pollution

CLARE PEDDIE
SCIENCE REPORTER

MICROPLASTICS are everywhere, the World Health Organisation says in a report released this week, and Adelaide research confirms we are not immune to the pervasive pollutant.

When University of Adelaide PhD candidate Nina Wootton studied fish sold at seafood markets in Australia and Fiji, she was surprised to find more plastic in our fish.

"From a food safety point of view I think fish is still fine to eat," she said. "I think there is more plastic coming through our drinking water and apparently dust particles than from seafood. For me it's more a worry about fish health than human health."

More of the Aussie fish in the study had eaten microplastic (83.6 per cent of Australian fish compared to 50.8 per cent in Fiji) and on average, they had twice as many pieces of plastic in their gut (2.03 pieces compared to 1.01). However Australian fish contained mostly filaments from synthetic clothing, while film from plastic bags and packaging was more common in fish from Fiji. Now the project, funded by the Fisheries Research and Development Corporation, is comparing fish from different Australian states.

Ms Wootton's supervisor, Professor Bronwyn Gillanders, is exploring multiple avenues with several research students.

One is looking at prawns, crabs, mussels and oysters, another is searching for microplastics in sediments and the team is developing methods to detect whether contaminants have leached into the body of an animal.

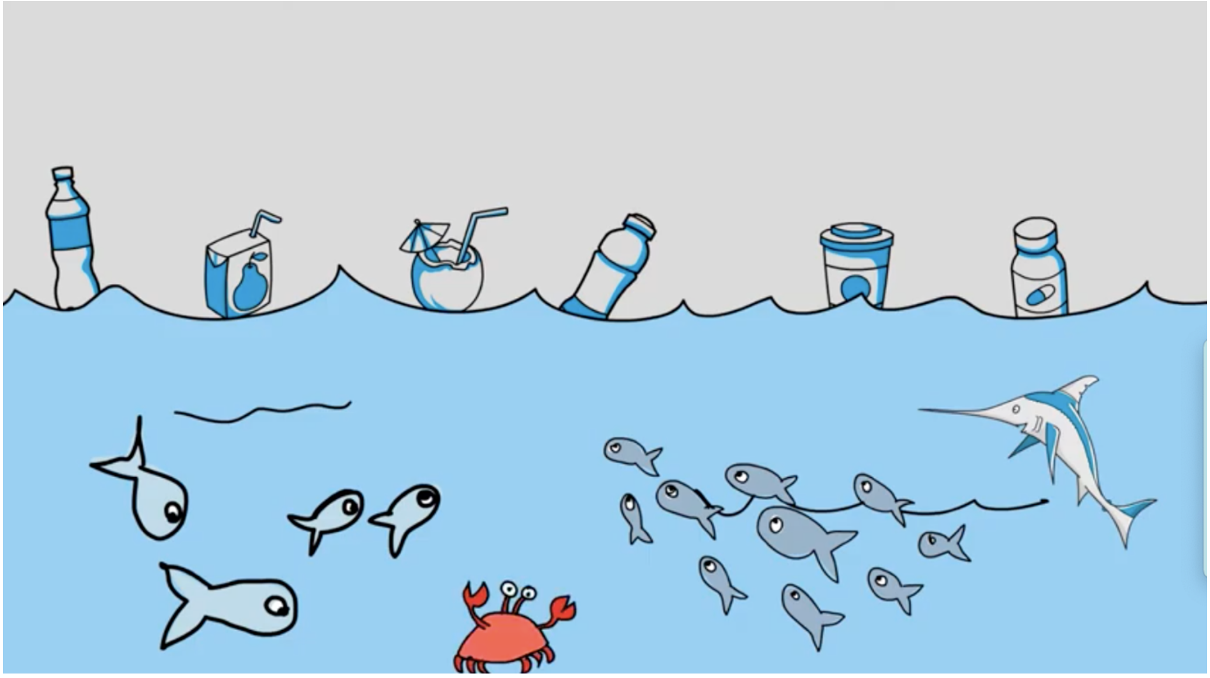
The WHO report calls for further assessment of microplastics in the environment and their impacts on human health. WHO public health department director of environment and social determinants of health, Dr Maria Neira, says we need to stop the rise in plastic pollution worldwide.

"We urgently need to know more about the health impact of microplastics because they are everywhere – including in our drinking-water," she said.

SA Water senior manager of water expertise and research Dr Daniel Hoefel says the report provides no evidence for human health concerns. "WHO advises that water utilities and regulators continue prioritising the removal of microbial pathogens and chemicals to ensure public health," he said.

PAGE 19: VALDMAN'S VIEW

Appendix Figure 3: Screen grab of video, which won 2nd place in the University of Adelaide Visualise Your Thesis video competition. Full video can be found here: https://www.youtube.com/watch?v=PLEf0s6BvZI



Appendix Figure 4: Photograph from the press conference held at Seafood Works, Adelaide where multiple news stations reported on my findings.