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Microplastic and metal contamination in Bermuda fishes at different trophic levels

UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia

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Mestrado em Biologia Marinha

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Declaração de autoria de trabalho

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

_______________________________________ Justine Nathan

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Abstract

Marine pollution has attracted increasing attention from scientists and the public in recent years due to its impacts on marine life. Studies have focussed on presence of plastics and metal contaminants in both the water and biota. Links between microplastics and metals in different ares of the environment are often overlooked. Analyses have focussed on many areas of coastal waters and the open ocean but, despite being located within the North Atlantic Gyre, Bermuda has never been studied for plastic presence. Therefore a sequence of impacts of marine pollution were analysed in the water and fishes of Bermuda to provide an overview of the country's marine pollution situation. Surface trawls were used to determine plastic density in the ocean. This ocean plastic was then evaluated for its bioavailability of metal contaminants. Eight species of fish across three trophic levels were analysed for microplastics present in their guts. Two of these species were selected to investigate the level of metal contaminants in fish tissue. All species and 32% of individual fish contained plastic in their guts. Evidence of biomagnification of microplastics by trophic levels and length was found. Microplastics were confirmed as a vector for metals into fish as they are biovailable on injested microplastic particles. Illegal levels of Cd were found in all fish as well as some containing dangerous Pb and Cr levels. Cd showed a positive correlation with fish length whereas Pb showed a negative one. The presence of plastic and metals across species and trophic levels may pose a threat to their health and consumption of these fish and their predators causes concern of potential impacts on humans. These risks should be evaluated in future studies, focusing on higher trophic levels to improve understanding of the affects of marine pollution on ecosystems and humans.

Keywords: Microplastics, Bermuda, fish, ingestion, contaminants

Resumo

A poluição marinha tem atraído uma atenção crescente de cientistas e do público nos últimos anos devido ao seu impacto ambiental cada vez mais percetível. Um dos exemplos é o plástico é omnipresente no ambiente marinho, onde a sua introduçãotem vindo a aumentar continuamente nos últimos anos. Isso significa que a quantidade de plástico que chega ao oceano e, portanto, a incidência do impacto na vida marinha também está a aumentar, na forma de macro como de microplásticos levando à ingestão de plástico. Os estudos têm-se concentrado sobre a quantificação da presença de plásticos e contaminantes dissolvidos, incluindo metais na água e em organismos, incluindo peixes. O plástico encontrado e quantificado em vários locais do mundo tem sido ingerido por vários organismos marinhos. A ingestão de plástico pode causar problemas na saúde dos organismos, como danos internos e bloqueio gástrico, mas também impactos secundários associados aos metais e poluentes orgânicos que são absorvidos na sua superfície. As propriedades dos microplásticos encontrados no oceano, nomeadamente a sua grande superfície em relação ao volume e a degradação que sofrem devido às condições ambientais, tornam provável a adsorção de metais na sua superfície. Estes metais podem então ser acumulados nos tecidos dos peixes. Se acumulados em grandes quantidades, metais não essenciais podem interferir nos processos metabólicos, reduzindo a saúde do animal. A ligação entre o modo pelo qual a poluição afeta diferentes aspetos do oceano são frequentemente negligenciados. O plástico presente na água do mar que foi ingerido pelo peixe, e os contaminantes existentes na superfície desse plástico podem ser acumulados nos seus tecidos dependem um do outro, mas poucos estudos os vinculam. A deteção e quantificação dos plásticos têm-se concentrado em muitas áreas da zona costeira e do oceano aberto, mas, apesar de estarem localizadas no Gyre do Atlântico Norte, o plástico presente nas Bermudas nunca foi estudado na água ou em peixes da zona. Portanto, uma abordagem multifacetada para analisar a poluição marinha provocada pelos plásticos foi projetada para avaliar o impacto na água e nos peixes das águas das Bermudas para fornecer uma visão geral da situação da poluição marinha no país.

As redes de arrasto de superfície foram usadas para determinar a densidade do plástico na superfície do oceano, tanto em locais costeiros como no exterior da ilha das Bermudas. Foram amostrados três locais onde foi avaliado quanto a biodisponibilidade de seis contaminantes metálicos (Zn, Cu, Cr, Cd, Pb e Ni), digerindo-o numa solução projetada para imitar as condições intestinais de um peixe. A solução foi então analisada usando Espectrofotometria de Absorção Atômica (EAA). Oito espécies de peixes em três níveis tróficos, variando de pequenos peixes planctivoros a predadores pelágicos maiores, foram recolhidos e dissecados quanto à presença de plástico no seu trato intestinal. O conteúdo intestinal foi digerido em hipoclorito de sódio e analisado ao microscópio para identificar a presença de plástico. Duas dessas espécies, *Decapterus macarellus* e *Harengula humeralis*, comercialmente mais valiosas e frequentemente consumidas por seres humanos nas Bermudas e no mundo todo, foram selecionadas para investigar o nível de contaminantes metálicos no tecido muscular dos peixes. Essa é a parte do peixe que é consumido pelos seres humanos, representando, portanto, o maior risco. Amostras de tecido muscular de 17 peixes foram digeridas em ácido nítrico e os níveis de metais analisados através do uso de EAA.

O plástico encontrado na superfície do oceano variou entre 30.000 a 130.000 partículas / km2, 89% das quais eram microplásticas. Não foi encontrada diferença significativa entre os locais offshore e costeiros. Todas as oito espécies de peixes e 32% dos peixes individuais continham plástico, 92% dos quais eram microplásticos. A espécie que ingeriu mais plástico foi *D. macarellus*, uma espécie de alimentação superficial e de um grupo trófico de nível médio. As partículas encontradas foram menores que 14 mm, portanto é pouco provável que causem bloqueio gástrico, mas ainda podem causar lesões no intestino e efeitos secundários de contaminantes associados. O número de peças microplásticas encontradas no intestino dos peixes aumentou com o comprimento e o nível trófico. Essa bioacumulação e causa preocupação para espécies maiores e potencialmente para os seres humanos que frequentemente consomem esses peixes. A análise da EAA mostrou que contaminantes metálicos aderidos a partículas microplásticas ou adicionados durante a fabricação estão biodisponíveis para os peixes após digestão no intestino, permitindo que eles se acumulem nos tecidos do peixe. Os metais foram encontrados nos tecidos musculares de todos os peixes analisados. Níveis acima do valor legal de Cd foram encontrados em todos os peixes, e alguns que continham níveis perigosos de Pb e Cr.

O Cd mostrou uma correlação positiva com o comprimento dos peixes, enquanto o Pb mostrou uma correlação negativa. Todos os outros metais não tiveram relação significativa com o comprimento do peixe. Os metais não essenciais, como Cd e Pb, têm a capacidade de afetar os processos metabólicos em peixes. Se os níveis de poluição continuarem a aumentar, a acumulação de metais nos peixes pode ter efeitos nocivos para a saúde e ter impacto no meio ambiente em geral. O consumo desses peixes em grandes quantidades pelos seres humanos pode potencialmente colocar as pessoas em risco de terem problemas de saúde associados a altos níveis de Pb e Cd, incluindo funções cerebrais e renais reduzidas.

Os padrões na densidade microplástica na superfície do oceano foram difíceis de determinar, portanto considera-se que uma amostragem mais frequente ajudaria a revelar padrões espaciais ou temporais que poderiam ajudar a compreender o impacto do plástico ao redor das Bermudas. Tendo comprovado a presença de detritos plásticos e contaminantes metálicos no ambiente e na biota das Bermudas, pode dizer –se que esses aspetos devem ser analisados em maior profundidade no futuro para determinar em que medida estão a afectar o ambiente marinho como um todo. A presença de microplásticos e metais nas espécies e nos níveis tróficos pode representar uma ameaça à sua saúde humana devido ao consumo de peixes e seus predadores, causando preocupação de possíveis impactos sobre os seres humanos. Estudos adicionais sobre peixes comercialmente valiosos de níveis tróficos mais elevados apresentariam uma visão mais clara da ameaça a que os seres humanos estão expostos pelo consumo de peixes contaminados. Uma compreensão mais ampla dessas questões permitiria aos governos implementar leis apropriadas que visem proteger a saúde humana e a saúde dos oceanos como um todo.

Palavras-chave: Microplásticos, Bermudas, peixe, ingestão, contaminantes

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Abbreviations

AAS	Atomic absorption spectroscopy
DNA	Deoxyribonucleic acid
D. m	*Decapterus macarellus*
EAA	Espectroscopia de absorção atômica
E. a	*Euthynnus alletteratus*
E. b	*Elagatis bipinnulata*
FAAS	Flame atomic absorption spectroscopy
FTIR	Fourier-transport infrared
GESAMP	Group of Experts on the Scientific Aspects of Marine Environmental Protection
GFAAS	Graphite furnace atomic absorption spectroscopy
HCl	Hydrochloric acid
HNO_3	Nirtic acid
H. ha	*Hypoatherina harringtonensis*
H. hu	*Harengula humeralis*
H. b	*Hemiramphus balao*
N/A	Not applicable
NaCl	Sodium chloride
NaClO	Sodium hypochlorite
NaOH	Sodium hydroxide
n.d.	Not detected
NOAA	National Oceanic and Atmospheric Administration
O. o	*Opisthonema oglinum*
PCB	Polychlorinated biphenyl
POP	Persistent organic pollutant
S. c	*Selar crumenophthalmus*
UV	Ultra violet

1. Introduction

1.1 Plastic Pollution

Marine plastic debris has become a common worldwide problem in recent decades, affecting marine species from zooplankton (Steer *et al.*, 2017), to sharks (Bernardini *et al.*, 2018), as well as turtles (Pham *et al.*, 2017) and whales (Unger *et al.*, 2016). Plastic is now produced at the rate of 348 million tonnes per year as of 2017 (PlasticsEurope, 2018). It has been estimated that 4.8-12.7 million tonnes of plastic enter the ocean every year (Jambeck *et al.*, 2015). Despite the point of entry, plastic has made its way into almost every habitat in the ocean (Lusher *et al.*, 2015). The impact of this plastic on marine species has been extensively documented and proven to affect over 690 marine species (Gall and Thompson, 2015). The manner in which plastic comes into contact with species ranges from entanglement to ingestion as debris in the ocean exists in many forms and across a wide size spectrum. Entanglement is the biggest threat to larger marine species, as it is more likely to be lethal than other risks such as ingestion and contamination (Wilcox *et al.*, 2016). The high frequency of entanglement in the ocean is unsurprising, especially since recent data has shown that 46% of plastic in the Great Pacific Garbage Patch is fishing gear (Lebreton *et al.*, 2018). These sources of plastic, when reduced in size over time by fragmentation caused by physical characteristics of the ocean, will account for ingestion across a wide range of body sizes.

Microplastics constitute an important proportion of marine debris that has received increasing attention in recent years. Despite no universal definition of microplastics existing, they are generally referred to as pieces measuring less than 5 mm in one dimension (NOAA, 2018). They can originate from primary forms including manufactured microplastics such as microbeads, which are used in cosmetics and as industrial abrasives (Lassen *et al.*, 2015), or raw material plastic in the form of preproduction pellets such as nurdles (Sundt *et al.*, 2014). Larger plastic can also break up and degrade into microplastics once it is exposed to conditions in the ocean such as UV-B radiation, saltwater and wave power, known as secondary microplastics (GESAMP, 2015). Fragmentation may be facilitated and accelerated by bacterial communities creating nanoplastics from microplastics (Zettler *et al*., 2013). The small size of these particles means that they are more readily ingested by a variety of organisms including zooplankton such as fish larvae (Steer *et al*., 2017), seabirds (Amélineau *et al*., 2016) and filter feeding whales (Besseling *et al*., 2015).

The consequences of plastic ingestion include, but are not limited to, inflammation of the gut, starvation and death (Rochman *et al*., 2016). Though it is difficult to prove inflammation in the gut of a wild living animal, post mortem examinations on various species have proven that plastic can be the cause of lesions in the gut lining. In more elevated cases of continued accumulation of plastic, such as that seen in seabirds, stomachs can become full of plastic and it is unable to be passed through the digestive system, causing gastric blockage (Azarello & Van Vleet, 1987). The plastic occupies important space in an animal's stomach, causing a feeling of false fullness and preventing them from absorbing enough nutrients to maintain growth. This state reduces body condition and overall health of the animal (Ryan, 1987). In extreme cases, it can cause starvation and death. Gastric blockage from ingested plastic is thought to have caused three Mediterranean sperm whale deaths (Alexiadou *et al*., 2019) as well as multiple other cases of large amounts of plastic in the stomachs of beached whales globally. The majority of plastic ingested by ocean creatures is not large enough to completely block the stomach, instead it is small and therefore more easily and frequently ingested.

Demonstrating a variety of feeding habits, fishes have been shown to be susceptible to plastic ingestion (Boerger *et al*., 2010), particularly filter feeders. Due to the specific gravity of seawater (~1.025), only polypropylene, polyethylene and some types of polystyrene float in seawater (Andrady, 2011). The remaining materials including nylon and polyester, which have a higher specific gravity than seawater, sink to the bottom. The proportion of buoyant plastic to non-buoyant plastic is roughly 50:50

(Association of Plastic Manufacturers, 2011). Fish that feed either on the surface of the water or on the benthic environment are more likely to come into contact with plastic due to its density. Microplastics that have a lower density than saltwater will float and can accumulate at the surface of the ocean. In 2014, 15-51 trillion plastic particles entered the ocean (van Sebille, 2015). A 2014 estimate of the amount of plastic in the surface layer of the ocean was calculated at 7000-35000 tonnes globally and 1000-7000 tonnes in the North Atlantic alone (Cozár *et al.*, 2014). The presence of plastic in fishes has been extensively studied in recent years and documented in multiple species and locations (Li *et al.*, 2016) (Table 1.1). The presence of plastic in fish stomachs varies dependent on location. Areas of ocean with relatively low levels of plastic, such as the Baltic Sea (<10 particles/m^3) (Setälä *et al.*, 2016), show low occurrences of plastic (0-1.8% of samples per species) (Budimir *et al.*, 2018). Whereas, accumulation zones include protected seas such as the North Adriatic, where up to 100% of individuals for some species of fish sampled contain plastic (Anastasopoulou *et al.*, 2018) and ocean gyres, where ingestion rates can be high (Davison and Asch, 2011) and average plastic particles per individual can be as high as 7.2 ± 8.4 (Boerger *et al.*, 2010). Considering that the mass of plastic in the North Pacific Central Gyre is six times that of the mass of plankton in the area (Moore *et al.*, 2001), it is no wonder that fish ingest microplastic after confusing them for a food source.

Table 1.1 Evidence of plastic ingestion in fish species

Species	**Location**	**Type of plastic**	**% with plastic**	**Mean particles ± SD**	***n***	**Reference**
Atlantic mackerel (Scomber scombrus)	Eastern Atlantic	Ethylene propylene	32	0.6 ± 1.1	31	Nelms et al., 2018
Easter Island flying fish *(Cheilopogon rapanouiensis*)	Easter Island (Pacific)	Microplastic	16	-	43	Chagnon *et al.*, 2018

Yellowfin tuna (*Thunnus albacares*)	Easter Island (Pacific)	Mesoplastic	2	-	50	Chagnon *et al.*, 2018
Baltic herring (*Clupea harengus membras*)	Baltic Sea	Microplastics	1.8	-	164	Budimir *et al.*, 2018
European Sprat (*Sprattus sprattus*)	Baltic Sea	Microplastics	0.9	-	154	Budimir *et al.*, 2018
Golden grey mullet (*Chelon auratus*)	Slovenian Sea (North Adriatic)	Micro-litter	95	9.5 ± 8.4	20	Anastasopoulou *et al.*, 2018
Gilthead seabream (*Sparus aurata*)	Slovenian Sea (North Adriatic)	Micro-litter	100	7.3 ± 6.6	20	Anastasopoulou *et al.*, 2018
Common sole (*Solea solea*)	Slovenian Sea (North Adriatic)	Micro-litter	65	1.9 ± 2.7	20	Anastasopoulou *et al.*, 2018
Striped red mullet (*Mullus surmuletus*)	Croatian Sea (Adriatic)	Micro-litter	70	1.8 ± 1.9	30	Anastasopoulou *et al.*, 2018
Common pandora (*Pagellus erythrinus*)	Croatian Sea (Adriatic)	Micro-litter	50	1 ± 1.6	30	Anastasopoulou *et al.*, 2018
European pilchard (*Sardina pilchardus*)	Croatian Sea (Adriatic)	Micro-litter	37	0.9 ± 1.4	30	Anastasopoulou *et al.*, 2018
Red mullet (*Mullus barbatus*)	Ionian Sea	Micro-litter	32	0.5 ± 0.8	25	Anastasopoulou *et al.*, 2018
Common Pandora (*Pagellus erythrinus)*	Ionian Sea	Micro-litter	42	0.8 ± 1.0	19	Anastasopoulou *et al.*, 2018
European pilchard (*Sardina pilchardus*)	Ionian Sea	Micro-litter	47	0.8 ± 1.1	36	Anastasopoulou *et al.*, 2018
Atlantic cod (*Gadus morhua*)	North Sea	Microplastics 0.4-4.8mm	13	-	80	Foekema *et al.*, 2013
Whiting (*Merlangius merlangus*)	North Sea	Microplastics 0.4-4.8mm	5.7	-	105	Foekema *et al.*, 2013
Haddock (*Melanogrammus aeglefinus*)	North Sea	Microplastics 0.4-4.8mm	6.2	-	97	Foekema *et al.*, 2013

Atlantic herring (*Clupea harengus*)	North Sea	Microplastics 0.4-4.8mm	1.4	-	566	Foekema *et al.*, 2013
Atlantic horse mackerel (*Trachurus trachurus*)	North Sea	Microplastics 0.4-4.8mm	1	-	100	Foekema *et al.*, 2013
Grey gurnard (*Eutrigla gurnardus*)	North Sea	Microplastics 0.4-4.8mm	0	-	171	Foekema *et al.*, 2013
Atlantic mackerel (*Scomber scombrus*)	North Sea	Microplastics 0.4-4.8mm	0	-	84	Foekema *et al.*, 2013
King mackerel (*Scomberomorus cavalla*)	Brazil, South West Atlantic	Plastic pellets (1-5mm)	8	-	63	(Miranda and de Carvalho-Souza, 2016)
Brazilian sharpnose shark (*Rhizoprionodon lalandii*)	Brazil, South West Atlantic	Plastic pellets (1-5mm)	6	-	33	Miranda and de Carvalho-Souza, 2016
Snaggletooth (*Astronesthes indopacifica*)	North Pacific Central Gyre	Small plastic fragments (~1-2.79mm)	-	1	-	Boerger *et al.*, 2010
Pacific saury (*Cololabis saira*)	North Pacific Central Gyre	Small plastic fragments (~1-2.79mm)	-	3.2 ± 3.1	-	Boerger *et al.*, 2010
Reinhardt's lantern fish (*Hygophum reinhardtii*)	North Pacific Central Gyre	Small plastic fragments (~1-2.79mm)	-	1.3 ± 0.7	-	Boerger *et al.*, 2010
Lanternfish *sp.* (*Loweina interrupta*)	North Pacific Central Gyre	Small plastic fragments (~1-2.79mm)	-	1	-	Boerger *et al.*, 2010
Lanternfish *sp.* (*Myctophum aurolanternatum*)	North Pacific Central Gyre	Small plastic fragments (~1-2.79mm)	-	6.0 ± 9.0	-	Boerger *et al.*, 2010
Bigfin lanternfish (*Symbolophorus californiensis*)	North Pacific Central Gyre	Small plastic fragments (~1-2.79mm)	-	7.2 ± 8.4	-	Boerger *et al.*, 2010

Atlantic herring (*Clupea harengus*), European pilchard (*Sardina pilchardus*) and European anchovy (*Engraulis encrasicolus*)	European Seas	Plastics identified by Raman Spectroscopy	-	1.2	9	Collard *et al.*, 2015
Blackfin barracuda (*Sphyraena qenie*)	South Pacific	Microplastics	14	0.1 ± 0.4	7	Forrest and Hindell, 2018
Daisy parrotfish (*Chlorurus sordidus*)	South Pacific	Microplastics	25	0.3 ± 0.5	4	Forrest and Hindell, 2018
Christmas wrasse (*Thalassoma trilobatum*)	South Pacific	Microplastics	25	0.3 ± 0.5	4	Forrest and Hindell, 2018
Coronation grouper (*Variola louti*)	South Pacific	Microplastics	20	0.2 ± 0.5	15	Forrest and Hindell, 2018
Reticulated flagtail (*Kuhlia sandvicensis*)	South Pacific	Microplastics	20	0.3 ± 0.6	15	Forrest and Hindell, 2018
Surge wrasse (*Thalassoma purpureum*)	South Pacific	Microplastics	50	0.5 ± 0.7	2	Forrest and Hindell, 2018
Diaphanous hatchetfish (*Sternoptyx diaphana*)	North Pacific Subtropical Gyre	Microplastics (mean 2.2mm)	25	-	4	Davison and Asch, 2011
Highlight hatchetfish (*Sternoptyx pseudobscura*)	North Pacific Subtropical Gyre	Microplastics (mean 2.2mm)	16	-	6	Davison and Asch, 2011
Pacific blackdragon (*Idiacanthus antrostomus*)	North Pacific Subtropical Gyre	Microplastics (mean 2.2mm)	25	-	4	Davison and Asch, 2011
Andersen's lanternfish (*Diaphus anderseni*)	North Pacific Subtropical Gyre	Microplastics (mean 2.2mm)	15	-	13	Davison and Asch, 2011

Lanternfish sp. (*Diaphus fulgens*)	North Pacific Subtropical Gyre	Microplastics (mean 2.2mm)	29	-	7	Davison and Asch, 2011
Bolin's lanternfish (*Diaphus phillipsi*)	North Pacific Subtropical Gyre	Microplastics (mean 2.2mm)	100	-	1	Davison and Asch, 2011
Cocco's lanternfish (*Lobianchia gemellarii*)	North Pacific Subtropical Gyre	Microplastics (mean 2.2mm)	33	-	3	Davison and Asch, 2011
Pearly lanternfish (*Myctophum nitidulum*)	North Pacific Subtropical Gyre	Microplastics (mean 2.2mm)	16	-	25	Davison and Asch, 2011
Slender snipe eel (*Nemichthys scolopaceus*)	North Atlantic mesopelagic zone	Microplastics	100	1	1	Lusher *et al.*, 2016
Spotted barracudina (*Arctozenus risso*)	North Atlantic mesopelagic zone	Microplastics	21	0.3	14	Lusher *et al.*, 2016
Bluntsnout smooth-head (*Xenodermichthys copei*)	North Atlantic mesopelagic zone	Microplastics	60	1.2	5	Lusher *et al.*, 2016
Glacier lanternfish (*Benthosema glaciale*)	North Atlantic mesopelagic zone	Microplastics	22	0.3	27	Lusher *et al.*, 2016
Lancet fish (*Notoscopelus kroyeri*)	North Atlantic mesopelagic zone	Microplastics	14.6	0.2	417	Lusher *et al.*, 2016
Silvery lightfish (*Maurolicus muelleri*)	North Atlantic mesopelagic zone	Microplastics	2.8	0.03	282	Lusher *et al.*, 2016
Scaly dragonfish (*Stomias boa boa*)	North Atlantic mesopelagic zone	Microplastics	40	0.8	5	Lusher *et al.*, 2016

As the frequency of studies focusing on plastic ingestion in fishes has increased, there has been no standard method followed by researchers to extract and identify plastics from fishes. The methods used to collect fish samples include directly using nets, acquiring fish from trawling surveys and collection from fish markets, where the origin may be unknown. Plastic, depending on the size, can make its way into multiple organs of a fish but most studies have focussed either on the digestive tract or the organism as a whole (Lusher *et al*., 2017). Extraction methods often depend on the size of the fish. Large species are usually dissected under sterile conditions to prevent contamination, whereas some fish are digested as a whole. Protocols used to digest fish include the use of acids such as nitric acid (HNO_3) and hydrochloric acid (HCl), alkalis such as sodium hydroxide (NaOH), enzymes and sodium hypochlorite (NaClO) (Lusher *et al*., 2017). A combination of NaClO and HCl has previously been used to isolate plastic from fishes in Bermuda and has shown no degradation of plastic particles (Antonition, 2017). In some studies, plastic is identified as any anthropogenic material that is not digested by the applied methods. Other studies have used methods of Raman Spectroscopy (Collard *et al*., 2015) or Fourier-transform Infrared Spectroscopy (FTIR) analysis (Nelms *et al*., 2018) to identify type of plastic. This information can be important in the determination of the effects plastic ingestion can have on fish.

1.2 Dissolved Contaminants

As previously mentioned, plastic can have physical effects on the gastrointestinal tract of marine animals including fish. Plastics are readily present in the water column waiting to be ingested but they are not the only threat to animals. Plastics contain contaminants that are added during manufacturing that can leach out of the plastic once broken up, degraded and ingested by an organism, which can be hazardous to their health (Lithner *et al*., 2009). Pollution can also come in the form of contaminants in the ocean such as organic dissolved pollutants and metals. These contaminants mainly enter the ocean from land-based sources as they are utilised on land and are transported as waste by rivers to the ocean (Windom, 1992). Persistent Organic Pollutants (POPs), which can be divided into three categories (industrial chemicals, pesticides and by-products), originate from anthropogenic land based activities (Breivik *et al*., 2004). This group of chemical compounds have an ability to cause harmful health effects to wildlife and humans if they are allowed to accumulate within an organism. Polychlorinated biphenyls (PCBs) are industrial chemicals used in heat exchangers and as a plasticiser among other functions. The exposure of fish to PCBs can disrupt endocrine function and oestrogen production causing reduced fecundity, reproduction and sex differentiation (Oberdörster & Cheek, 2001). Pesticides such as Aldrin, Chlordane and DDT, generally used to kill insects, are often strong enough to affect other organisms such as marine birds and fish by reducing production and growth rate (Khan & Law, 2005).

1.2.2 Metal Contaminants

Metal contaminants can enter the ocean via numerous pathways. Metals usually originate from land sources and can be transported to the ocean via rivers, surface runoff or the atmosphere (Church *et al*., 1984). Land based anthropogenic sources have been confirmed as the source of metal pollutants in oceanic environments including in the Indian Ocean from river outlets on the coast of Kenya (Ochieyi *et al*., 2009). Each metal may have originated from a different source. Batteries are a source of multiple metals including Cd, Ni and Zn leaching into the environment (Almeida *et al*., 2006). The burning of fossil fuels also plays a major contributing role in the release of Cd to the environment (Nriagu & Pacyna, 1988). Cu in the ocean originates from herbicides such as copper sulphate, used mainly on land but reaches the ocean from surface runoff and river discharge. Zn is a by-product of domestic and industrial waste but can also come from geological weathering of rocks. Cu and Zn are used in ship paints and for corrosion protection and could be the origin of some metal contaminants in the ocean.

Metals are available in the environment to be readily taken up by organisms. Some of these metals are essential for biological functions such as Zn, which is used in the production of enzymes, proteins and DNA transcription (Wu & Wu, 1987). Cu is used in enzyme production, protein transport and gene regulation (Linder & Hazegh-Azam, 1996). Other trace metals also contribute to essential functions. These metals in low quantities are beneficial to the organism. The issues arise when levels become too high and accumulated metals begin to have negative health effects such as interfering with biological processes and lesions on tissues, where there is a build-up of metal (Amundsen *et al.*, 1997). For some metals it does not take a large amount to negatively affect biological processes. In chronic cases, metals can reduce fish growth, inhibit reproduction and cause behavioural changes (Amundsen *et al.*, 1997).

Each metal can affect fishes in different ways. High levels of Cu in fish can reduce the function of gills to transport salts as well as affect the olfactory system having an affect of fish behaviour (Baldwin *et al.*, 2003). High Zn levels can trigger behavioural changes in fish as well as haematological ones, inducing anaemia (Kori-Siakpere & Ubogu, 2008). Cd, Cr and Pb are non-essential metals that are used as indicators of pollution. Cr can alter the blood composition of a fish, causing anaemia and eosinophilia as well as causing lesions in the kidneys (Afshan *et al.*, 2014). Cd can reduce the function of the kidneys and inhibit reproduction (Mansour & Sidky, 2002). Traditionally this would be monitored in water quality surveys but this does not show how bioavailable a metal is to the organisms in a habitat. Monitoring the levels of pollution within living organisms, termed biomonitoring, has been more frequently used to infer the effect that contaminants have on the biotic environment. Fish tissues have been used along with water samples to assess aquatic metal pollution (Birungi *et al.*, 2007, Ashan *et al.*, 2018). Therefore organisms including fish can be used as indicators of pollution levels in water and infer impact to an ecosystem, although this is complicated as the species are migratory and shift habitat. Assessing levels of metals in fish in Bermuda will give an idea of the wider impact to the environment as if fish are being affected; it is likely that other organisms across various trophic levels may be impacted. This includes bivalves, in which trace metal uptake has been noted in Bermuda (Burns *et al*., 1990; Gunther, 1999).

1.2.3 Contaminants in Bermuda

Waters around Bermuda receive metal inputs from the land and maritime sources. There are no rivers or streams but pollutants can find their way into drainage courses and be washed into the ocean with this discharge primarily in the inshore bays and sounds (Jickells *et al*., 1986). Contaminant levels in some areas of Bermuda, specifically the bulk waste dumpsite, have been shown to exceed the legal guidelines of Florida and Australia (Jones, 2010). Levels of Zn and Hg here are so high that they are categorised as biological effects being probable (Jones, 2010).

1.3 Trace Metals and Organic Contaminants Adhering to Microplastics

1.3.1 Adsorption

Chemical contaminants present in the water column adsorb to plastics due to their non-polar surface (Rochman *et al*., 2013, 2014). The high surface area to volume ratio of microplastics means that organic contaminants and metals are more likely to adsorb to them. Studies have demonstrated the ability of these contaminants to adhere to plastic using both microplastics from the ocean with adhered POPs (Rios *et al*., 2007) and trace metals on virgin plastic in laboratory studies (Brennecke *et al*., 2016).

Beached microplastics that have undergone ocean processes of degradation and photo-oxidation are more likely to accumulate metal contaminants than virgin plastics (Holmes *et al*., 2012). The presence of trace metals (Ni, Cu, Cd, Pb, Fe and Ba) on beached microplastics has already been proven on various beaches in Bermuda (Antonition, 2017). The type of plastic also plays a role in explaining how much contaminants can adhere to its surface. Polyethylene is the plastic that accumulates the most organic contaminants (Teutan *et al*., 2009). The accumulation of contaminants has been proven in shearwater chicks after ingestion of plastic (Teutan *et al*., 2009).

1.3.2 Bioavailability

The presence of contaminants on plastic is not necessarily synonymous to the amount of chemicals that is bioavailable to a fish by ingesting the material. Bioavailble metals are those that can be dissociated from the plastic during digestion, therefore enabling them to accumulate in the organism's tissue. One study looking at bioavailability of marine pollutants proved that phenanthrene sorbed to microplastics can be digested by polychaete worms after ingestion (Teutan *et al*., 2007). However, information on the bioavailability of metals on microplastic after ingestion by fish lacking.

1.4 Contamination in Fish Tissues

Metals can be freely dissolved in water and can be directly ingested by an animal. Fish accumulate metals in their gills as contaminated water passes through them as they absorb dissolved oxygen. As the characteristics of microplastic facilitates the adherence of metals to their surfaces, it is likely that the consumption of microplastic contributes to the amount of metals found in a fish. This has already been proven in laboratory studies (Bennecke *et al*., 2016) but confirming the relationship in wild fish is more complicated.

After ingestion, the conditions in the gut facilitate the absorption of chemicals allowing them to accumulate in the tissues of animals (Batel, 2016). Typically, contaminants are known to accumulate in the liver and kidneys as these organs act as filters for the body (Jezierska & Witeska, 2006). Studies have focused on these tissues to demonstrate the accumulation of contaminants in fish. Muscle tissue is also affected and is more significant in determining the possible threats that the human population may face from consuming contaminated fish. These chemicals can include POPs and metals, as mentioned above. The extent of how efficiently metal contaminants are transferred from the surface of plastics into the tissues of animals, especially fish, after ingestion has not been studied extensively. Many studies have mimicked the gastrointestinal conditions of a fish in order to determine uptake efficiency of nutrients from different feed sources in aquaculture practices (Moyano *et al.*, 2014) but none of these have been applied to plastics and metals. However, the transfer of chemicals and metal contaminants is often assumed, allowing the subsequent impacts of these substances to be examined.

1.4.1 Impacts of Contaminants on Fish

Physiological impacts of plastic ingestion have been studied in the laboratory to determine its effect on fish health (Table 1.2). The accumulation of contaminants in fish tissue can lead to various consequences including behavioural changes (Barboza *et al.*, 2018a) and physiological impacts such as hepatic stress (Rochman *et al.*, 2013). The metabolism of fish can also be affected by nanoparticles (<1µm) as they move up trophic levels after being ingested by primary consumers on the surface of algae (Mattsson *et al.*, 2014). Many more studies on plastic ingestion in fish have hinted towards potential physiological changes but have not been tested substantially (Dantas *et al.*, 2012).

Table 1.2 Evidence of impacts of microplastics and adsorbed chemicals on fish health

Species	**Test**	**Result**	**Reference**
European seabass, (*Dicentrarchus labrax)* (juveniles)	Microplastic (1-5µm) and mercury ingestion	Reduced swimming velocity and resistance time, lethargic and erratic swimming behaviour	Barboza *et al.*, 2018a
Spiny chromis (*Acanthochromis polyacanthus*)	Ingested <300µm plastic particles	Negative effect on growth	Critchell and Hoogenboom, 2018

Japanese medaka (*Oryzias latipes*)	Polyethylene (<500µm) with sorbed chemical (PCBs, PBDEs and PAHs)	Glycogen depletion, fatty vacuolation, cellular necrosis, lesions in the gut and hepatic stress.	Rochman *et al*, 2013
Rainbow fish (*Melanotaenia fluviatilis*)	Exposure to microbeads (10-700µm) with sorbed polybrominated diphenyl ethers for up to 63 days	Contaminants are affectively accumulated in fish tissues	Wardrop *et al.*, 2016
Crucian Carp (*Carassius carassius*)	Exposed to nanoparticles (24-27nm) through the food chain from algae to Daphnia	Changes in metabolism and swimming activity when hunting	Mattsson *et al.*, 2014

1.4.2 Trophic Transfer of Contaminants

Transfer of chemicals up the food web has been demonstrated in laboratory experiments. *Artemia* that accumulate chemicals from microplastics transfer them to their predators such as zebrafish (Batel, 2016). Trophic transfers have also been shown to exist in the wild through fish species such as from flying fish to yellowfin tuna (Chagnon *et al.*, 2018). Marine mammals are also exposed to plastic transfer through trophic interactions as shown by the accumulation of microplastics in the scat of captive fur seals that were fed wild caught fish (Nelms *et al.*, 2018). Trophic interactions may cause accelerated accumulation of chemicals in larger predators. These larger predators (e.g. tunas) are often species of high commercial value that are directly consumed by humans. Eating fish, therefore, may have the potential to adversely affect human health through the contaminants associated with marine plastics.

1.5 Problems to Human Health

Humans have consumed fishes as part of their diet since we first learned to fish. Fish meat has been historically viewed as a healthy source of protein, containing numerous beneficial components to human health such as omega-3, minerals and high protein content (Sidhu, 2003). These nutritional facts may still stand but due to the aforementioned problems of pollution in the oceans today, fish meat now may also contain contaminants that adversely affect human health.

Accumulation of metals in human tissues has been linked to multiple health problems. Pb and Cd are two common contaminants that are present in polluted waters. In high levels, Pb can cause brain damage as it affects the nervous system and impede kidney function (Järup, 2003). This could be from consuming large amounts of highly polluted seafood. In low level, which can occur more easily, Pb has the greatest effect on young children, as they are less able to eliminate it from their systems causing a build up (Järup, 2003). This increased concentration can impede their intellectual development. It is considered very dangerous for pregnant females as Pb has the ability to pass the foetal membrane. Cd is not so easily absorbed into the human body but once it is absorbed, it is also very difficult to eliminate so short term exposure may have prolonged affects. Once absorbed, it accumulates mostly in the kidneys, where it causes renal damage (Järup *et al.*, 1998). Cd contamination can also significantly impact human health by causing skeletal damage. An inverse relationship has been found between Cd concentration and bone density, which has led to many cases of bone fractures and breaks (Frery *et al.*, 1993). A more severe skeletal disease, known as Itai-Itai, was first noted in humans in Japan after rice fields were contaminated with Cd, leading to osteoporosis and osteomalacia (Kazantzis, 1979).

Many Governments have implemented restrictions and monitoring procedures to ensure that fish sold for human consumption does not contain high levels of metal contaminants (NOAA, 2019; European Commission, 2006). These standards are sporadically examined in samples of caught fish to maintain reduced risk to human health and identify fishing locations or species of concern to human health. Increasing pollution of global waters and unmonitored fisheries make it possible for fish with illegal levels of contamination to be consumed by humans.

1.6 Study Site

Mid-ocean gyres are accumulation zones for floating plastic on the ocean. 60% of plankton net trawls in the North Atlantic Subtropical Gyre contained plastic pieces (Law *et al.*, 2010). 80% of marine plastic originates from land-based sources (Andrady, 2011). Bermuda is situated within the North Atlantic Gyre (Figure 1.1), which transports water, and therefore waste from the coastlines of Europe and Africa from the East and the US and Caribbean from the South and West. Previous research has indicated that fishes around Bermuda frequently ingest microplastics whilst feeding, both plankton-feeders and their carnivores (Antonition, 2017; Sacco, 2017; Lorimer-Turner, 2017).

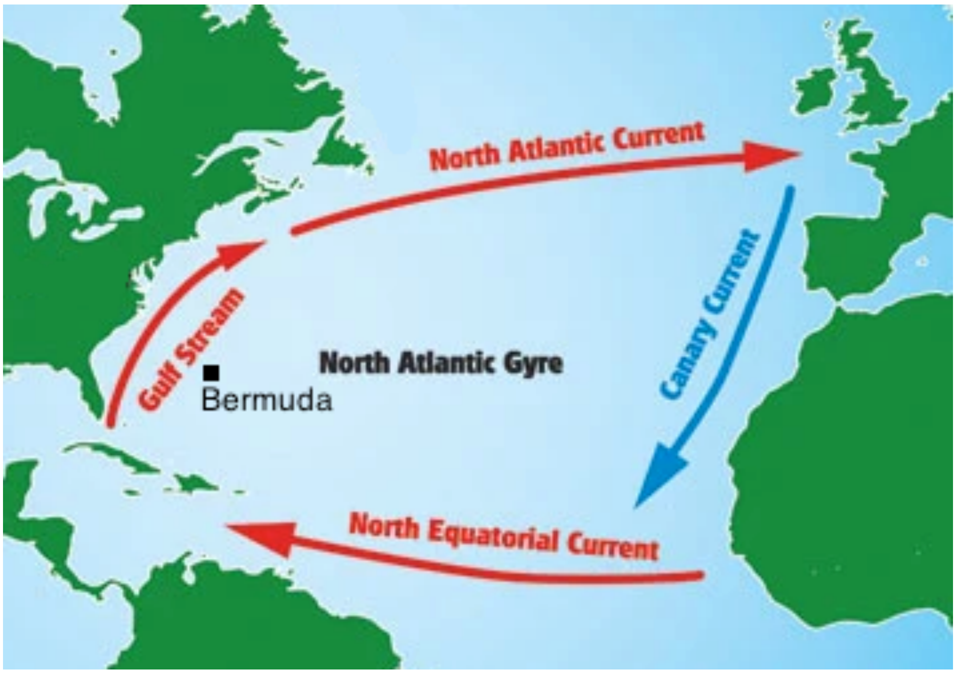

Figure 1.1 Map showing the location of Bermuda within the North Atlantic Gyre (Image adapted from Carey (2017).

This study specifically looks at commercially and ecologically important fishes within Bermuda, used either as bait or for direct consumption. These fishes provide information on the trophic transfers up the food web and may be important in highlighting the impacts of consumption of fish on human health. The human body is able to absorb plastic particles as large as 150 µm through the lymphatic capillaries and plastic measuring ≤20 µm has the ability to enter most organs (Barboza *et al.*, 2018b). Nanoplastics (<1 µm) are most likely to end up in the ocean after being removed from the land via run off or in freshwater systems (da Costa *et al.*, 2016). Here they can easily enter the lowest levels of the food web via plankton and have a high capability for bioaccumulation. The level of exposure from fish consumption increases the likelihood of humans accumulating plastics. Implications of bioaccumulation of contaminants adsorbed to these plastics on human health are also becoming increasingly explored. Recent studies have highlighted the importance of understanding the consequences of marine plastics on human health (Carbery, 2018).

1.7 Objectives

This study aims to assess the level of plastic ingestion by fishes in Bermuda across three trophic levels and relate this to levels of contaminants in their tissues in order to determine any evidence of plastics as a vector of bioaccumulation of pollutants. Methods were designed in order to answer the following questions:

- Are microplastics present in Bermudian waters? If so, how much?
- What is the level of microplastic ingestion in Bermudian fishes? Is there a difference across three trophic levels?
- Is there any relationship between the amount of microplastic ingested and length of a fish?
- Are common marine pollutants bioavailable on microplastic found in Bermuda?
- Is there any evidence of bioaccumulation of pollutant metals in fishes caught?

In order to understand the dynamics of microplastics and contaminants in fishes in Bermuda, multiple aspects of the topic were investigated. To conclude a relationship between aspects of the study, the aim was to firstly determine if there is microplastic present in the waters of Bermuda. Then investigations determined if fishes eat the plastic, if there are any bioavailable metals on the surface of plastic found in Bermudian waters and finally if the metal contaminants accumulate in their tissues.

2. Materials and Methods

2.1 Microplastics in the Ocean

2.1.1Neuston Trawls for Microplastics

Between 11th October 2018 and 24th October 2018, four manta trawl samples were taken both inshore and offshore in Bermuda to collect plastic floating at the surface of the ocean. Inshore trawls were conducted on 11th, 12th and 24th October whereas only one offshore sample was taken on 16th October. The manta trawl net was suspended behind a boat moving at roughly 2 nm for around 30 minutes for each sample. The net and codpiece were thoroughly rinsed to ensure that all plastic particles were collected. The contents were suspended in water to remove all plastic pieces and they were categorized by type and colour. Plastic pieces were stored in glass viles separately for each trawl sample to be used at a later date. Trawls were conducted on days with very calm water conditions in order to maximize the likelihood of plastic particles that are present in the water column to be present at the surface and able to be collected. A trawl was attempted on a day with less than favourable conditions (wind 10-15 knots, seas ~1m). It was found to contain a total of 5 pieces of plastic. This was deemed possibly unrepresentative of the actual abundance and perhaps due to increased mixing of the water forcing plastic deeper below the surface (Reisser *et al*., 2015).

For comparison to other studies, a standardised density of number of plastic pieces per km^2 was calculated using the formula:

$$\frac{Number\ of\ plastic\ pieces}{\left(\frac{Trawl\ width\ (m)}{Distance\ travelled\ (km)}\right)}$$

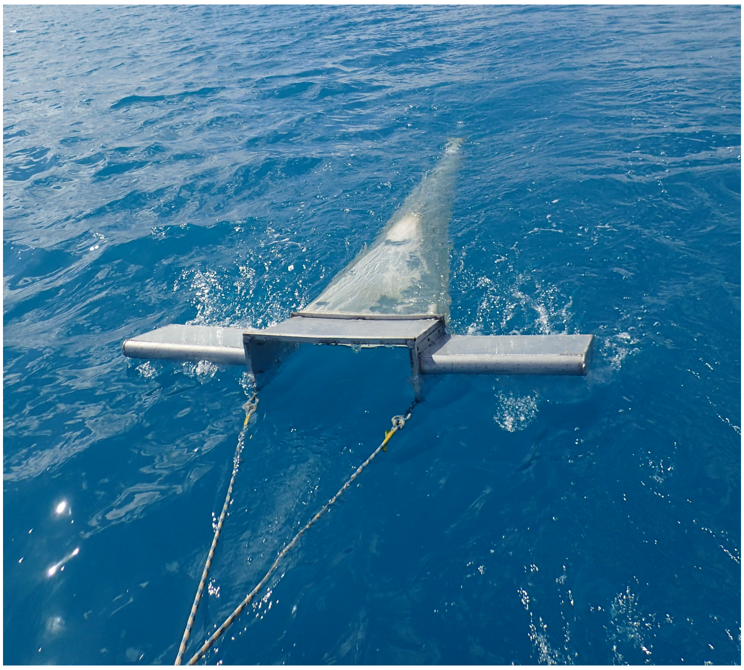

Figure 2.1 Neuston trawl in the water to collect microplastics from the surface.

2.2 Microplastics in Fishes

2.2.1 Fish Collection

137 fish from eight species were collected from areas around Bermuda (Figure 2.1) and analysed for plastic ingestion between November 2017 and October 2018. Four of these are commercial baitfish in Bermuda, *Harengula humeralis* (Redear herring), *Opisthonema oglinum* (Threadfin herring), *Hypoatherina harringtonensis* (Reef silverside) and *Selar crumenophthalmus* (Bigeye scad). These species are generally known as forage fishes, meaning they are either small fish, measuring less than 20 cm at their maximum length or are juveniles of pelagic species that use inshore areas as a nursery. These fish only occasionally directly ingested by humans, are more commonly used as bait to directly catch larger fish or as chum on deep sea fishing operations. These fish are natural prey to larger fish such as the others caught in the study. Two species, *Decapterus macarellus* (Mackerel scad) and *Hemiramphus balao*

(Balao halfbeak) are used as live bait by recreational fishermen in Bermuda to catch larger pelagic species such as the last two species in this study, *Euthynnus alletteratus* (Little tunny) and *Elagatis bipinnulata* (Rainbow runner). The latter two are commonly eaten by humans not only in Bermuda but worldwide. A large proportion of individuals caught were of the species *D. macarellus*, which feed opportunistically near the surface of the ocean. This strategy exposes them to a variety of prey including forage fish, such as those analysed in this study, planktonic crustaceans and other organisms living in the *Sargassum spp.* that is frequently found around the island of Bermuda in the Sargasso Sea. Forage fishes were caught either by throw nets from shore or were acquired from local fishermen who catch them using nets from boats. Larger species were caught using pole and line or acquired from local fishermen who used the same technique. After the fishes were caught they were stored in the freezer until further analysis. Several *H. harringtonensis* specimens were stored in alcohol until further analysis to preserve them for future reference by the Bermuda Natural History Museum.

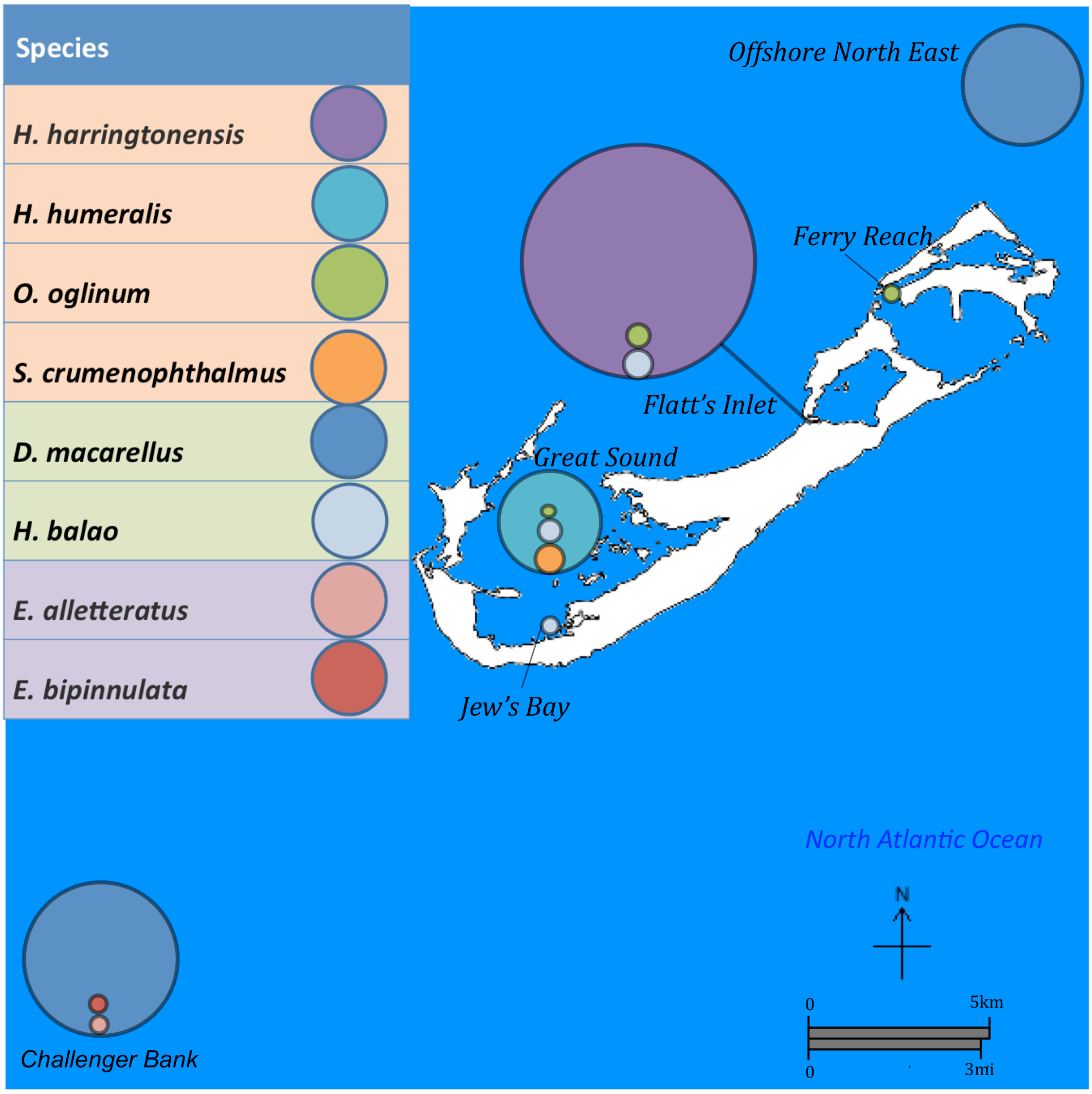

Figure 2.2 Locations of fish samples where colour of circle refers to the species collected and size of circle indicates number of samples collected at each location. Offshore North East refers to the deeper reef edge (20-60m), ~1-3 nm East of Bermuda. Challenger bank is a shallow seamount, about 50m deep and ~15 nm South West of Bermuda. The species list is arranged by trophic level of fish, where orange is the 1st trophic level studied, green is the 2nd and purple is the 3rd.

2.2.2 Fish Dissection

Once defrosted, total length measurements were taken (±0.1cm). Fishes were dissected to remove the stomach contents, which were placed into sodium hypochlorite for two days to digest organic matter. The entire stomachs of forage fishes were placed into sodium hypochlorite for two days. This was then vacuum filtered to reveal small plastics using a filter size of 5 µm. 10% hydrochloric acid was run through the filter and used to dissolve any carbonate sand grains, to prevent misidentification as microplastic. Contents were viewed under a microscope to look for plastic pieces. Larger fish stomach contents were viewed under a microscope in a glass dish. Any potential plastic that was found whilst under the microscope was placed into a separate dish and exposed to a couple of drops of HCl to eliminate carbonate material. According to these methods, the remaining material was classified as plastic. Each piece was photographed and colour, number and approximate size recorded.

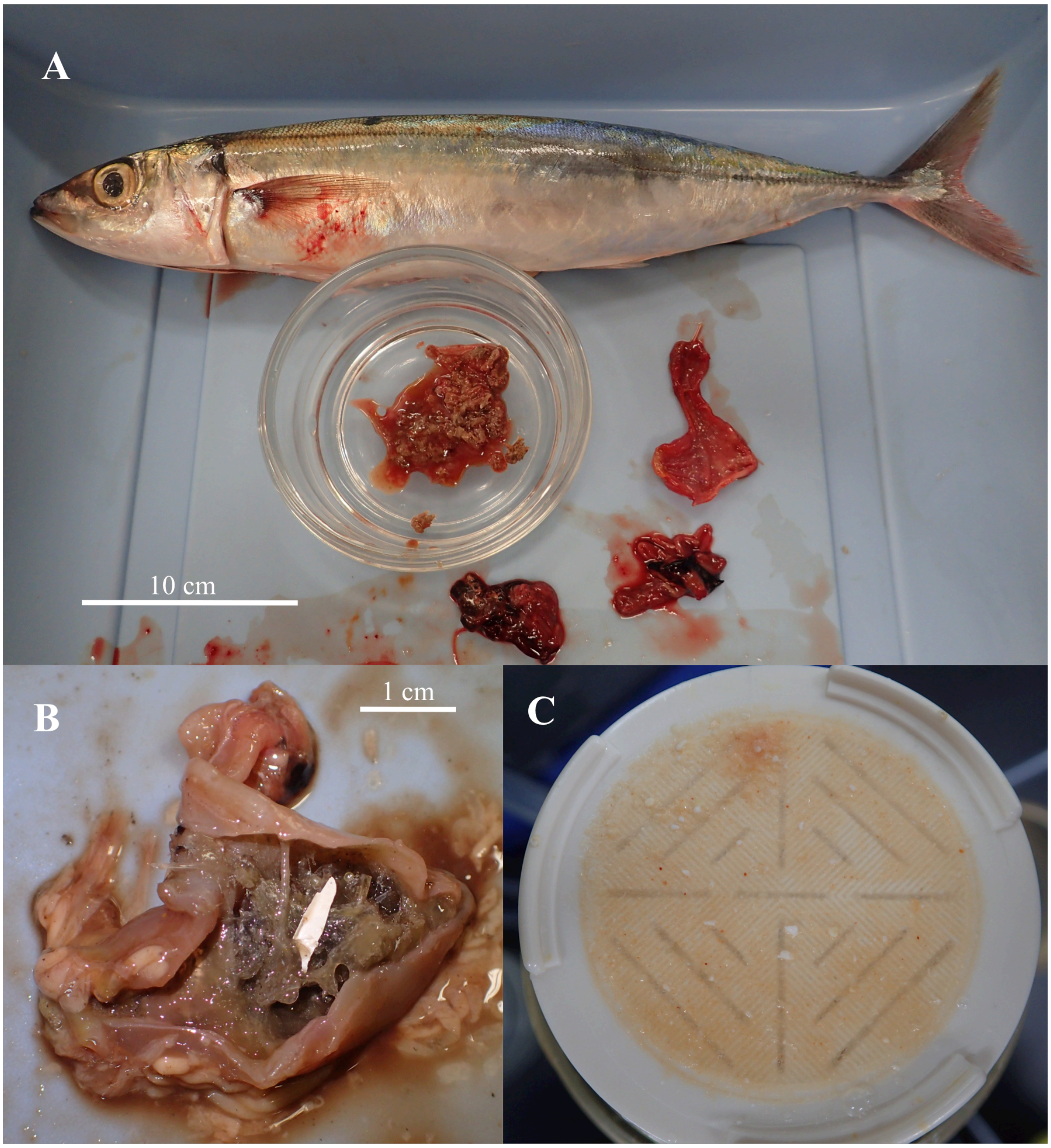

Figure 2.3 Extraction process of plastics from fish. **A**: Disection of *D. macarellus* with stomach contents in a glass dish for further inspection and other organs removed. **B**: Plastic particle visible inside a partially dissected stomach. **C**: Remnants of *H. harringtonensis* gut contents after digestion with sodium hypochlorite and filtration.

2.3 Bioavailability of Metal on Microplastics

Plastic particles that were collected from surface trawls of Bermudian water, both inshore and offshore, were analysed by atomic absorption spectrophotometry (AAS) to look for evidence of metals adsorbing to the surface of the plastic. Common metal contaminants found in Bermuda's waters include Cu, Cd, Pb and Zn (Jones, 2010). Ni and Cr were also included as they are common contaminants in other areas.

2.3.1 Method Justification

Bioavailability of metal contaminants is the amount that can be taken up into the organs of a fish due to its digestive capacity. Conditions in the digestive system of a fish vary by species dependent on their prey. The most predominant enzyme in the gut of teleost fish is pepsin. Opportunistic species that have a varied diet, such as *D. macarellus*, will also have chitinases in their guts, which enables them to break down chitin that is found in exoskeletons of arthropods. Optimal pH for gastric activity also varies by species. Although little work has been done on the digestive activity of species in this study, there have been many studies conducted on species that have similar feeding strategies. Many studies have aimed to mimic the gastric activity of Rainbow trout (Gomes *et al.*, 1998) by creating a solution of pepsin and adjusting the pH (Moyano *et al.*, 2014). These fish occupy a similar trophic level and feeding habits to *D. macarellus*, therefore methods were adapted from such studies.

2.3.2 Microplastic Digestion

The trawl samples of plastic collected on the surface of the ocean in Bermuda were used to determine the bioavailability of metal contaminants on plastic. 500 mg of plastic was taken from each site sampled, two inshore and one offshore. A solution mimicking the gut conditions of a carnivorous fish was prepared. 2.92 g of NaCl were dissolved into 500 ml of deionised water to create a 0.1M solution. 5 g of pepsin porcine was dissolved into this at an activity of 1:10000. The pH was adjusted to 4.5 using 20% NaOH. 40 ml of this solution was added to Erlenmeyer flasks along with the 500mg samples of plastic. A blank was also prepared containing only the solution. All Erlenmeyer flasks were covered with aluminium foil and placed in a P Selecta Rotabit lateral shaker at 175U/min at 20°C for 24 hours. After this time the flasks were removed from the lateral shaker and the contents filtered through a 0.45 μm filter using a suction pump to ensure no plastic contaminants remained in the sample. Samples were acidified using 500 µl HNO_3 to ensure a low pH. The sample was then digested to remove pepsin that may interact with the AAS. 5 ml HNO_3 was added to 20 ml of each sample in plastic tubes, covered with reflux covers and incubated at 80°C for 2 hours in a hotplate bath. Reflux covers were removed and left to evaporate overnight and filled to 20 ml solution using acidified water.

2.4 Metal Contaminants in Fish Tissues

After removing the gut contents of fish, ten *H. humeralis* and seven *D. macarellus* were frozen again to be analysed for metal contaminants at a later date. Fish tissue samples were analysed using AAS in order to determine the amount of metal contaminants accumulation within them.

2.4.1 Fish Dissection

All 17 specimens were defrosted and dissected in sterile conditions using a scalpel, taking care to prevent contamination of the samples. Around 5 g of tissue was removed from above the lateral line and placed into sterile plastic vials. Vials were weighed with and without samples to calculate the exact wet weight of the tissue samples. Samples were dried overnight in a hotplate. They were then weighed again to find the dry weight of each sample. 5 ml of nitric acid was added to each sample to digest the tissue. The tubes were placed in a hotplate bath at 80°C for 2 hours in a fume cupboard. The tops were left uncovered to prevent the reaction from bubbling over. Once reaction is stable, cover with reflux cover and left until completely dried overnight. Once all nitric acid had evaporated, tubes were filled to the 50 ml mark with acidified water using nitric acid to keep the pH low.

2.4.2 Atomic Absorption Spectroscopy

An Atomic Absorption Spectrophotometer was prepared by creating calibration curves using standards for six metals, Cd, Cu, Cr, Zn, Pb and Ni. There was no difference found between the certified value and the values obtained using the prepared standards. Due to its sensitivity to low levels of metal, a Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS) was used for all metals except Zn. As Zn was present in high amounts a Flame Atomic Absorption Spectrophotometer (FAAS) was used. Samples were run and the level of absorbance was inferred from the transmittance of each liquid metal. Samples from mimicked gut digestion and dissolved tissue samples were both analysed by AAS. The conditions for metal analysis in AAS can be found in Appendix A.

2.5 Contamination Prevention

Surfaces, instruments and containers were cleaned before use. Stomach contents were processed in glass containers. These were covered immediately to prevent contamination from airborne particles. Uncovered control dishes were placed in the laboratory during the processing of samples. These were analysed under the microscope to look for plastic particles. Only thin, coloured fibres were found in the controls, which probably originated from clothing, these were therefore excluded from the analysis of fish stomachs. Replicate control filters were processed in the vacuum pump filter with just NaClO and water. These were analysed under the microscope but no contamination was found. Plastics from surface trawls were stored in glass vials to prevent contamination from other plastic. Whilst dissecting muscle tissue for AAS analysis, care was taken to prevent any contamination coming into contact with the fish by storing them in the fridge in covered containers and using sterile plastic dissection trays and stainless steel scalpels.

2.6 Statistics

All statistical tests were conducted using R software.

For comparison between species, the number of pieces found in each stomach was tested for normality using the Shapiro Wilk normality test. The same data set was tested for homogeneity using a Chi squared test for given probabilities. A *p* value greater than 0.05 allows one to assume data is normally distributed and can use a parametric ANOVA test to determine if there is a difference between species. A *p* value less than 0.05 means it can be assumed that the data is not normally distributed and a non-parametric Kruskall Wallace was used to determine any difference between species. A *p* value of less than 0.05 shows there is a significant difference and a pairwise comparison Wilcoxon test was used to determine between which species the significant difference lies.

For determining any difference between the trophic levels, fish species were divided into planktivores (*H. humeralis*, *O. oglinum, H. harringtonensis* and *S. crumenophthalmus*) and predators (*D. macarellus, H. balao*, *E. alletteratus* and *E. bipinnulata*). For normally distributed and homogenous data a two sample T-test was used to determine any significant difference between these two groups and to determine if the consumption of plastic is significantly higher in predators compared to planktivores. For non-normal data, a Wilcoxon test can be used.

Plastic data from surface trawls was compared to what was found in fish stomachs. The contribution of each type of plastic (fragment, fibre and film) was compared between the two groups using a two-proportions Z test. The same test was used to determine the equality of proportions for different colours of plastic to determine the percentage contribution. This enabled determination of any selective feeding of fishes by colour or type of plastic.

Amounts of bioavailable metals were compared to the blank using a one way T-test to determine if the mean of the results was significantly higher than the blank.

Levels of metal contamination were tested for normality within each species. A two-sample T test was used to determine any difference between species for normally distributed samples. Variance was tested between species for each non-normal metal and those with equal variances were tested for difference between species using a Mann-Whitney U-Test. Samples with unequal variances were tested for any difference using a Kolmogorov-Smirnov Test.

A Pearson's test was used to create a correlation matrix between all samples to determine any correlation of metal accumulation between individual fish. The same test was used to include the length of each fish to determine any relationship between the size of a fish and the amount of metal in its tissues.

3. Results

3.1 Microplastic in the Ocean

A total of 425 pieces of plastic were collected from the surface of the ocean during four trawls. One trawl was considered an anomalous data due to unfavourable weather conditions causing elevated mixing of the water surface. The maximum number of pieces in one sample was 263, found close to shore at high tide. When results were standardised by distance and time to obtain density values for pieces of plastic per km^2, there does not appear to be a relationship between density and distance from the shore as Inshore trawls account for the highest (130,000 pieces/km^2) and lowest (30,000 pieces/km^2) observed densities (Figure 3.1). 89% of pieces measured <5mm, categorising them as microplastics. The largest piece was from a plastic bottle top measuring 34mm.

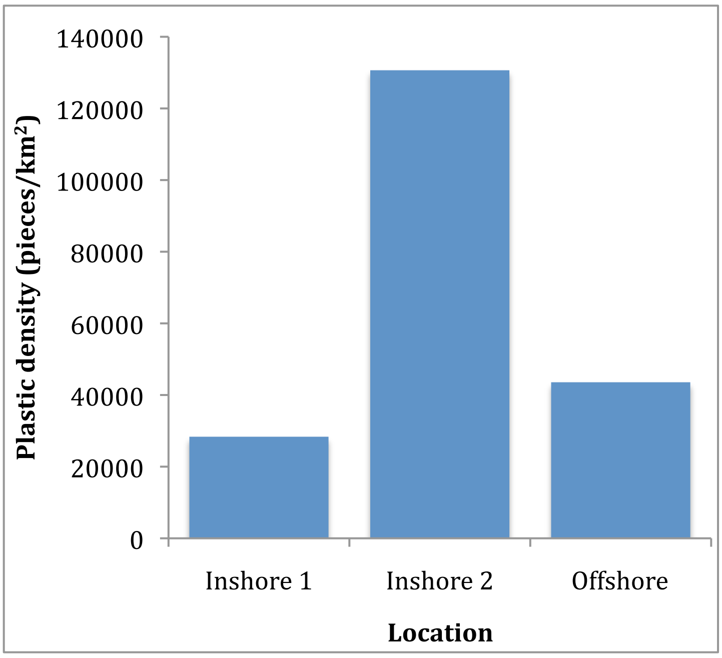

Figure 3.1 Density of plastic by pieces/km^2 for each trawl sample site.

Surface manta trawls of the water indicated that fragments are the most common type of microplastic found both inshore and offshore (Figure 3.2), accounting for 76% of all pieces found. Fibres, film and polystyrene foam accounted for 17%, 5% and 1% respectively (Figure 3.3).

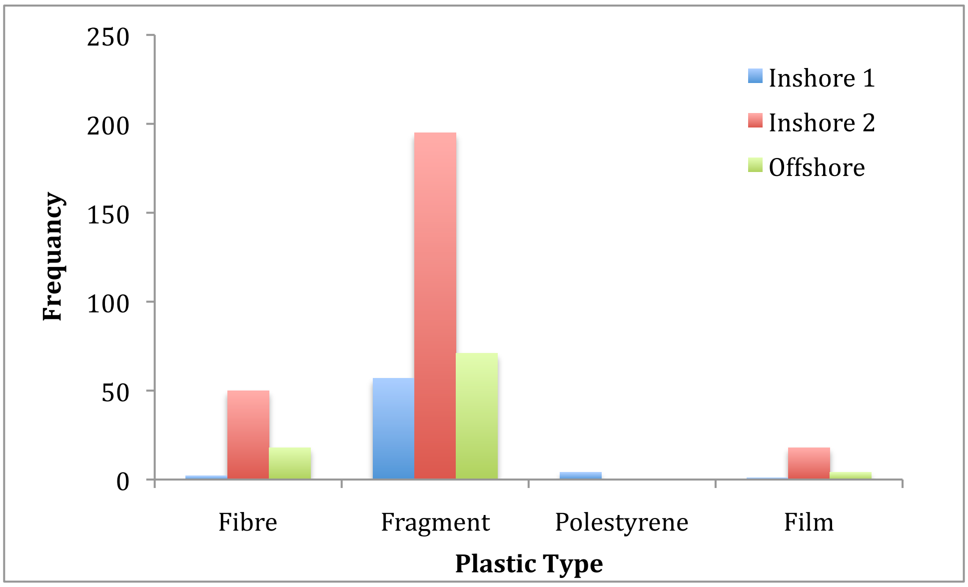

Figure 3.2 Frequency of each type of plastic collected by inshore and offshore trawls.

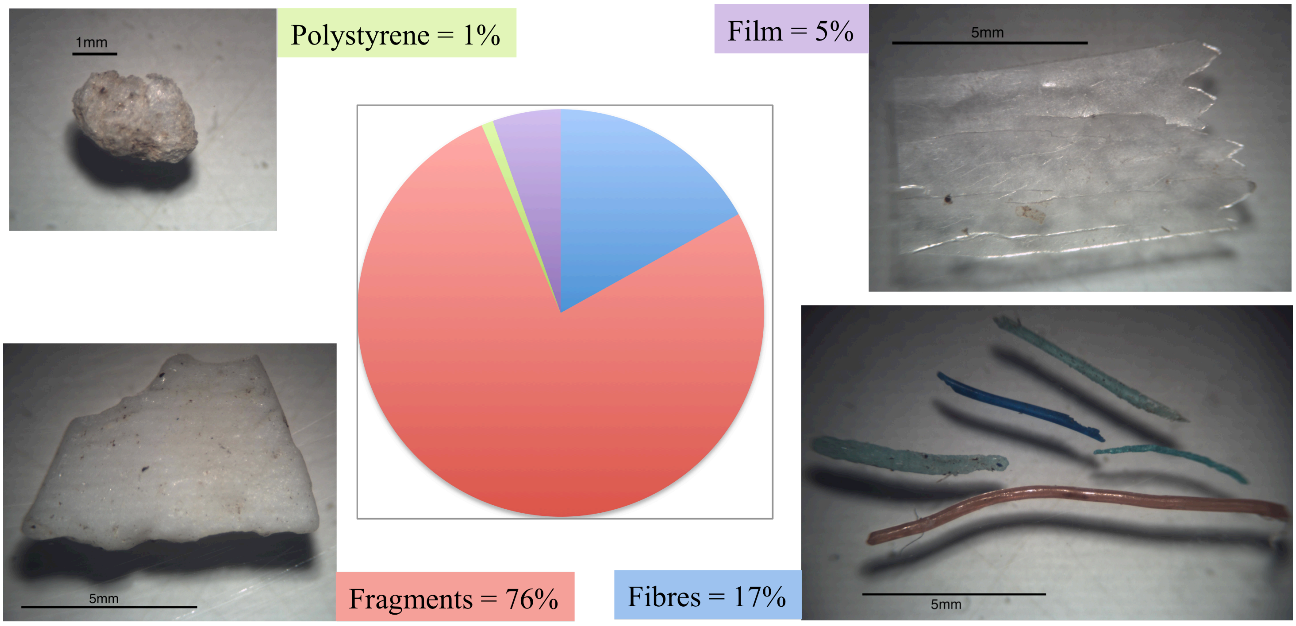

Figure 3.3 Contribution of each type of plastic collected in surface trawls including example images of each type.

A range of different colours of plastic were found in Bermudian waters, the most common being white (69%) (Table 3.1).

Table. 3.1 Occurrence of plastic by colour and type.

	Fibre	**Film**	**Fragment**	**Polystyrene**	**Total**	**%**
White	11	4	273	4	292	**69**
Clear	15	14	11	0	40	**9**
Blue	18	1	16	0	35	**8**
Black	5	1	12	0	18	**4**
Green	10	2	4	0	16	**4**
Orange	8	1	1	0	10	**2**
Grey	1	0	6	0	7	**2**
Yellow	2	0	3	0	5	**1**
Pink	0	0	2	0	2	**<1**
Total	70	23	328	4	425	
%	**17**	**5**	**77**	**1**		

3.2 Microplastics in Fish

A total of 85 microplastics were found in 137 fish. Microplastic was found in all eight species sampled (Appendix B). 32% of fishes had plastic in their stomachs with an average of 0.6 pieces per fish (Table 3.2). The number of pieces of plastic found in the stomachs ranged from zero to nine. *D. macarellus* was found to be most likely to accumulate plastic both by number of individuals that had plastic in their stomachs and by the number of pieces. Adversely, *H. harringtonensis* was the species less likely to accumulate plastic as only 12% of stomachs contained plastic. 92% of particles were microplastics (<5mm) with only seven pieces measuring more than this. The largest piece was clear film measuring 14mm, found inside the stomach of a *H. humaralis* individual.

Table 3.2. Percentage of fish with plastic and average number of pieces by species

Species	n	Pieces per individual mean ± standard error	% Individuals with plastic in stomachs	Trophic level
H. harringtonensis	41	0.15 ± 0.07	12	1
H. humeralis	18	0.39 ± 0.12	39	1
O. oglinum	8	0.50 ± 0.19	50	1
S. crumenophthalmus	5	0.40 ± 0.4	20	1
D. macarellus	48	1.27 ± 0.28	52	2
H. balao	11	0.18 ± 0.12	18	2
E. alletteratus	3	0.33 ± 0.33	33	3
E. bipinnulata	3	0.67 ± 0.33	67	3

3.1.1 Relationship of Plastic with Fish Length

The number of plastic pieces found inside fishes was plotted against length by species to determine if there is a relationship between the size of a fish and the amount of plastic it ingests.

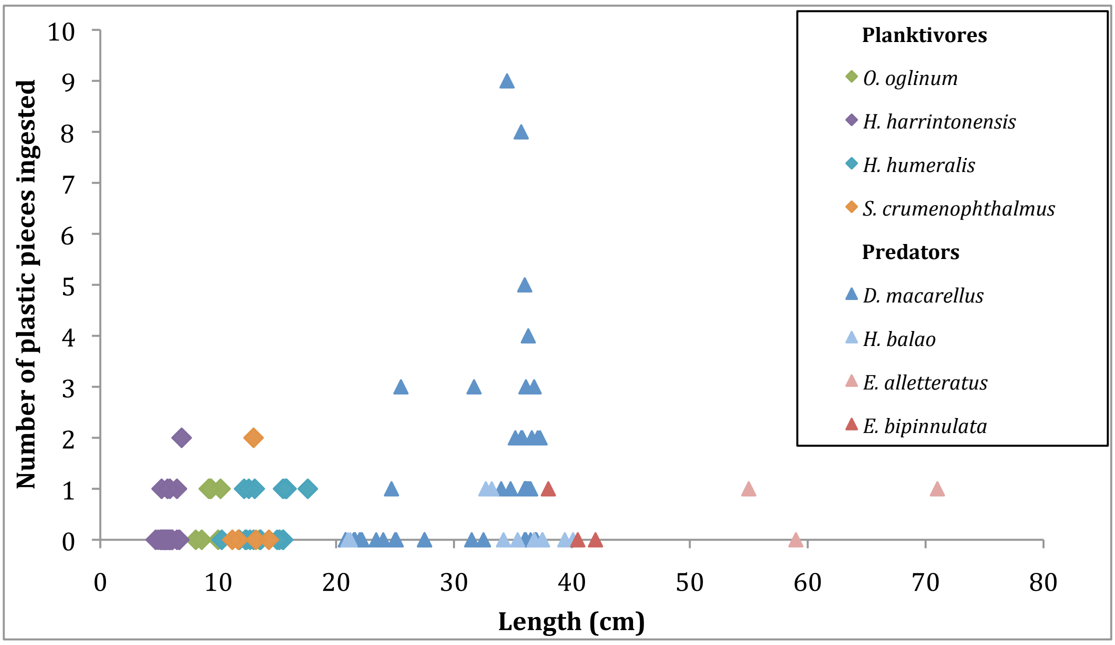

Figure 3.4 Relationship between length of fish and number of pieces of plastic ingested between planktivores (diamonds) and predators (triangles).

R^2 values indicate that the number of plastic particles ingested cannot be explained by fish length, either for all species (Figure 3.4) or each species individually, except *E. alletteratus* (R^2 = 0.862,), which had a sample size of only 3 individuals, therefore not reliable. When tested for correlation, both *D. macarellus* and *H. harringtonenesis* show a significant relationship between length and number of plastics (p <0.05). A significant correlation was found between length and number of plastic particles ingested for all species collectively (p <0.05). Species form two distinct clusters by length (Figure 3.4), the planktivores (shown as diamonds) and the predators (triangles). The number of pieces of plastic is higher in the predators but all values above one are from just one species (*D. macarellus*).

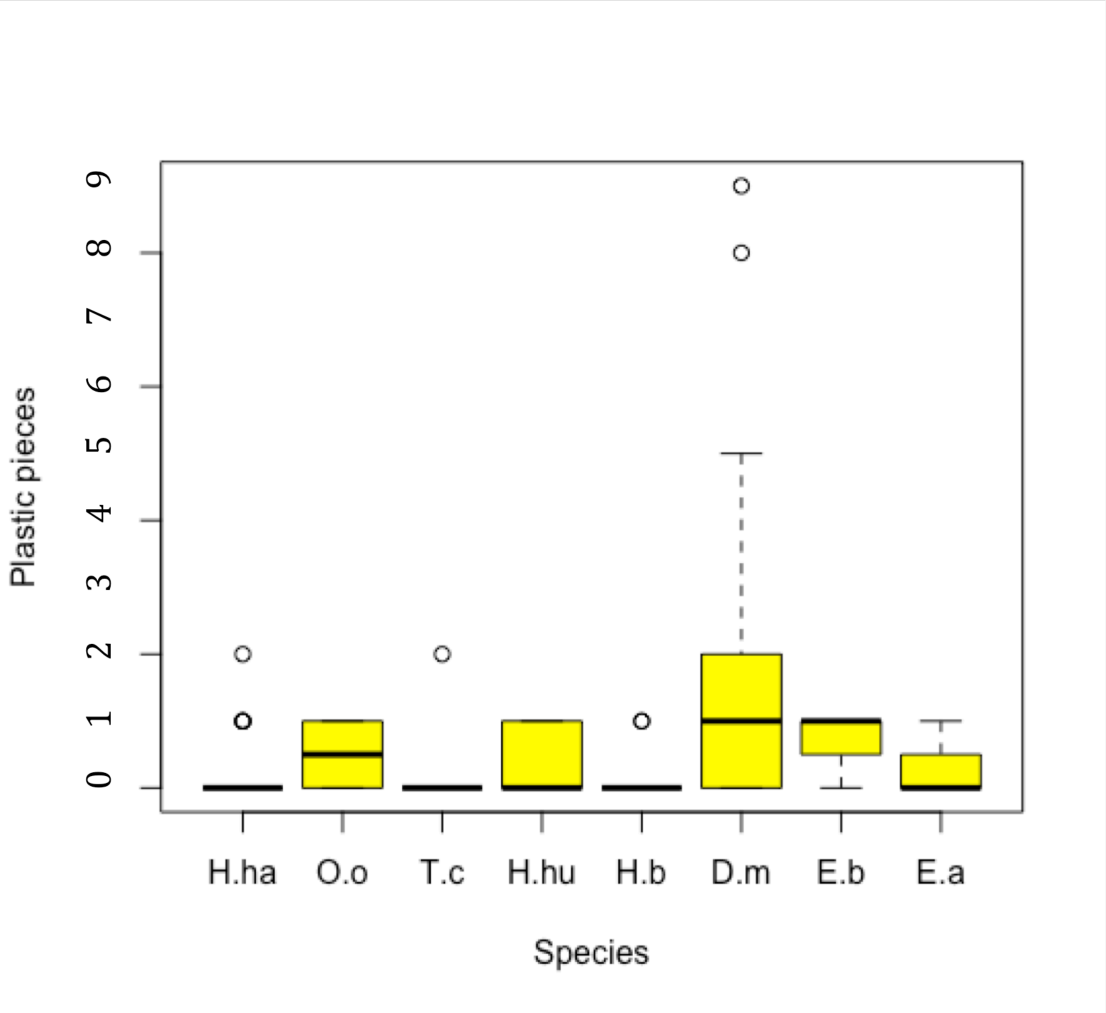

Figure 3.5 Boxplots of microplastic pieces for each species. Ordered by trophic level: H.ha (*H. harringtonensis*), O.o (*O. oglinum*), S.c (*S. crumenophthalmus*) and H.hu (*H. humeralis*) belonging to the 1st trophic level, H.b (*H. balao*) and D.m (*D. macarellus*) belonging to the 2nd trophic level and E.b (*E. bipinnulata*) and E.a (*E. alletteratus)* belongong to the 3rd trophic level.

Figure 3.5 also shows that the increased plastic ingestion for predatory fish accounted for by one species (*D. macarellus*). Except for this species, all other species do not seem to show much variation and only ingest a small number of microplastics.

The Shapiro-Wilk normality test showed that the amount of plastic found in the stomachs of fish is non parametric. The Chi-squared test showed that the data is not homogenous. Therefore, a Kruskal-Wallis test was used to determine any difference between species by testing the null hypothesis of no significant difference in the amount of plastic ingested by different species. The test gave a p value <0.05, allowing the null hypothesis to be rejected and conclude that there is a significant difference between the amounts of plastic ingested dependent on species. A Pairwise Comparison was made using a Wilcoxon test to identify between which groups the significant difference lies. The only significant difference was found between *D. macarellus* and *H. harringtonensis* ($p<0.05$).

When planktivores (*H. humeralis*, *O. oglinum, H. harringtonensis* and *S. crumenophthalmus*) and predators (*D. macarellus*, *H. balao*, *E. alletteratus*, and *E. bipinnulata*) are grouped separately and tested to see if predators consume a significantly higher amount of plastic using a Wilcoxon test, the result confirms that predators consume significantly more plastic than planktivores ($p<0.05$).

3.1.2 Comparison of Ocean Plastic and Ingested Plastic

The plastic samples from surface trawls were compared to the microplastic collected from fish stomachs to determine if there was a difference between what was present in the water column and what fish actually ingested.

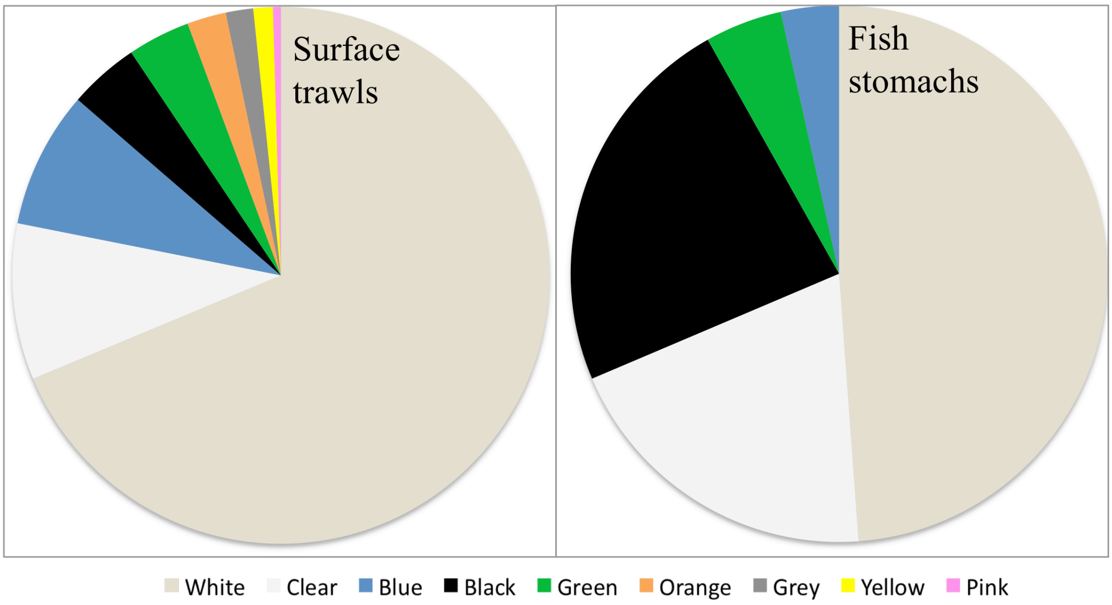

Figure 3.6 Occurrence of plastic colours in the ocean compared to colours found in fish stomachs

The most common colours of plastic found in the ocean around Bermuda according to the three surface trawls conducted are white, clear, blue, black and green (Figure 3.6). These colours account for 94.4% of all plastic found in the water. The same colours account for 100% of the plastic found in the gastrointestinal tract of fishes. Figure 3.6 suggests that despite the majority contribution of white plastics to what is available in the water column, fish may be consuming more black plastic in proportion to what is readily available. Statistical tests for percentage contribution of each colour reveal inequalities in contributions of white, clear and black plastic. These tests and the percentages contributions show that fish stomachs contained proportionally more black and clear plastic but less white plastic compared to what was available on the surface of the ocean (Table 3.3).

Table 3.3 Results of Two Proportions-Z test for equality of contribution by colour of plastic in the water surface and in the stomach of fishes.

Colour	White	Clear	Black	Blue	Green	Orange	Grey	Yellow	Pink
p value	0.001	0.000	0.000	0.192	0.221	0.490	1.000	0.313	0.682

Statistical tests to determine any difference between the percentage contribution of plastic types between the water column and those ingested by fish showed no evidence of selective feeding for fragments or fibre but a *p* value <0.05 and a higher percentage contribution to stomach contents for film suggests that fish may be choosing to consume plastic in the form of film (Table 3.4).

Table 3.4 Results of Two Proportions-Z test for equality of contribution by type of plastic in the water surface and in the stomach of fishes.

Plastic type	Fibre	Film	Fragments
p value	0.137	0.001	0.640

The majority of the fibres found in both fish stomach and in the trawls of the water were most likely derived from fishing nets or line. Only one piece of textile fibre was found inside the stomach of an *E. alletteratus* specimen.

Many samples contained whole or partially digested prey items such as smaller fish. The gut contents of these prey items were not examined for presence of plastic, although it is possible that they may contain them. Although other studies have incorporated this into their research, it was not the aim of this study.

3.3 Bioavailability of Metals

All samples contained higher levels of metals than the blank sample suggesting that through the digestive process, metals in plastics or adhered to their surface are bioavailable to animals that consume them (Table 3.5). The one sided T-tests showed that the amount of metal in the digested samples was significantly different to the blank sample for Cr, Cu and Ni ($p < 0.05$). No significant difference was seen for Cd, Pb and Zn even though all digested samples are higher than the blank. No obvious relationship can be noted between the offshore and inshore locations of the samples.

Table 3.5 Level of bioavailable metals found at each site around Bermuda.

Sample	**Location**	**Date**	**Cd (mg/kg dw)**	**Cr (mg/kg dw)**	**Cu (mg/kg dw)**	**Ni (mg/kg dw)**	**Pb (mg/kg dw)**	**Zn (mg/kg dw)**
Blank	-	-	0.02	0.41	1.54	0.44	1.04	35.7
1	Inshore	24-Oct	0.61	0.73	1.94	0.86	1.14	38.8
2	Offsore	16-Oct	0.14	1.26	2.28	1.08	1.44	43.9
3	Inshore	11-Oct	0.03	1.07	2.30	0.81	1.10	38.0

3.4 Metal in Fish Tissues

Zn was the most highly concentrated metal for all fish. Ni levels were very low, often below the detection limit (Table 3.6). EU regulations set the lowest legal levels for Cd and Pb contamination in fish products, 0.005 and 0.3 mg/kg dry wt respectively (European Commission, 2006). Contamination levels of Cd are consistently higher than the legal limits stated by the European Commission regulations for both fish species (Table 3.6). On average, detected limits of Cd in fish tissue are 14 times higher than the legal limit. Pb also shows two specimens having contamination higher than the legal level, equating to 11% of the specimens tested. Five fish (29%) contained Cr in their tissues higher than the lowest legal amount, as set by regulations for fish exportation in Hong Kong (NOAA, 2019). Four specimens of *H. humeralis* contained illegal levels of Cr, compared to only one *D. macarellus* specimen. The other essential metals do not show illegally high values in the fishes examined.

Table 3.6 Levels of metals in tissue of fish species. Values are averages of three smaples from AAS. Values in red are those that fall above the legal maximum limit of metal contamination in fish set by EU standards or those stated for specific countries by NOAA (European Commission, 2006; NOAA, 2019). Values classified as n.d contained levels below the detection limits for that metal.

Species	**Cd (mg/kgdw)**	**Cr (mg/kgdw)**	**Cu (mg/kg dw)**	**Ni (mg/kg dw)**	**Pb (mg/kg dw)**	**Zn (mg/kg dw)**
Lowest legal limit	**0.05**	**1**	**10**	**N/A**	**0.3**	**100**
H. humeralis	1.55	0.60	3.00	0.01	0.12	29.2
H. humeralis	0.33	0.57	1.93	n.d.	0.17	29.2
H. humeralis	1.48	2.71	2.93	n.d.	0.30	30.6
H. humeralis	0.56	2.44	1.49	n.d.	0.13	24.1
H. humeralis	1.20	0.56	2.18	n.d.	0.12	30.7
H. humeralis	0.60	0.47	2.15	n.d.	0.11	15.0
H. humeralis	0.55	2.66	1.67	1.62	0.13	29.5
H. humeralis	0.79	0.96	3.15	0.01	0.09	33.9
H. humeralis	1.05	0.63	3.81	0.11	0.15	28.2
H. humeralis	0.23	3.67	2.04	0.12	0.14	27.3
D. macarellus	1.05	0.43	1.66	0.01	0.28	34.1
D. macarellus	0.31	1.14	1.02	0.01	0.24	25.3
D. macarellus	0.25	0.36	1.21	0.02	0.24	23.9
D. macarellus	0.24	0.58	3.54	n.d.	0.27	21.7
D. macarellus	0.59	0.98	1.05	0.01	0.25	23.0
D. macarellus	0.24	0.59	1.51	0.01	0.31	31.7
D. macarellus	0.49	0.54	6.91	n.d.	0.12	30.7

3.4.1 Relationship between Metal and Length

Figure 3.7 suggests that there is little relationship between the length of fish and the level of contaminants in its muscle tissue for any of most metals as shown by weak slopes and low R-values, with all falling below 0.4. The two highest R^2 values are for Cd and Pb, which when tested for correlation indicate that there is a significant relationship between fish length and the two metals as well as between length of a fish and the amount on Cd and Pb in fish muscle tissue ($p < 0.05$). Cd shows a negative correlation, meaning that as the length of a fish increases, the amount on Cd in its tissues decreases. Pb shows a positive correlation, meaning that as the size of a fish increases, the more Pb is accumulated in its tissues but still remained within legal acceptance.

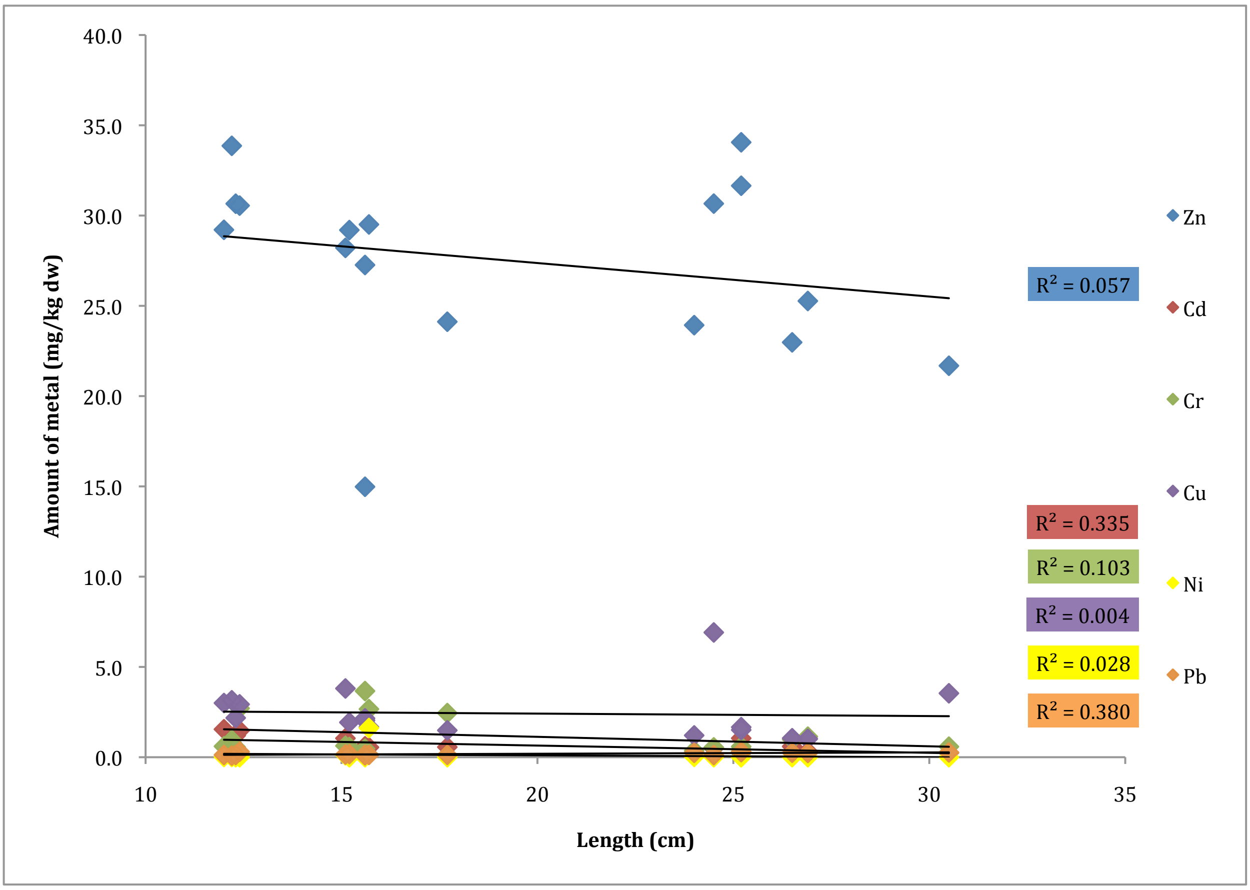

Figure 3.7 Relation ship between the length of a fish and the amount of meal contaminants in its tissues.

When Cd was plotted against Zn, there was no relationship to suggest that Cd is replacing Zn in biological processes because the amount of Cd did not increase as the amount of Zn decreased (Figure 3.8). A slight positive relationship is seen, but the low R^2 value of 0.13 and $p > 0.05$ concludes it is not a strong relationship.

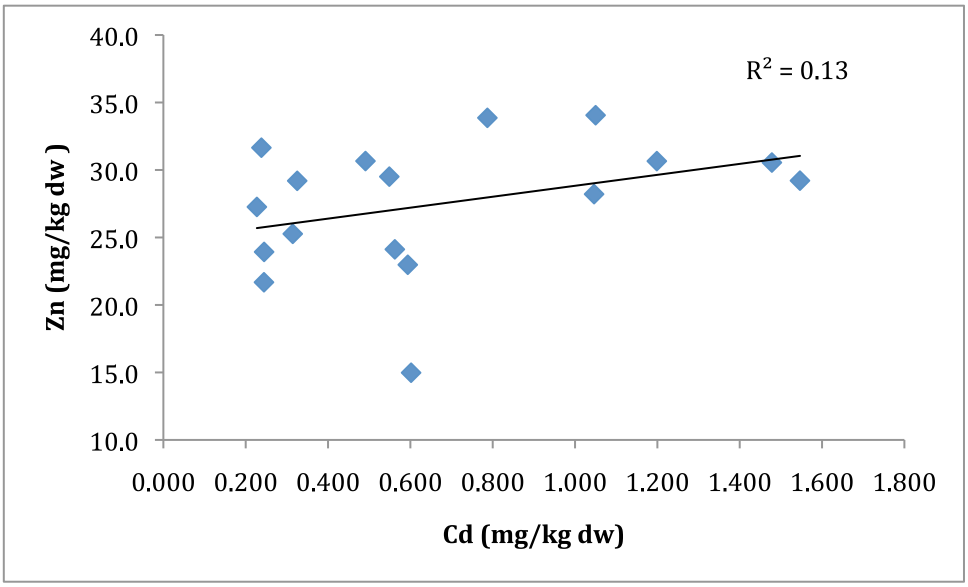

Figure 3.8 Correlation between amount of Zn and Cd in fish tissues.

3.4.2 Differences between Species

The distribution of data for metal contaminants was shown to be non-normal for at least one species for all metals. When tested for variance, Cd, Pb and Zn were shown to have equal variances, therefore a Wilcoxon rank-sum test was used to look for significant differences between the amounts of metals in each species. Cr, Cu and Ni were shown to have unequal variances; therefore a Kolmogorov-Smirnov Test was used. Cd, Cr, Ni and Zn showed no sign of differences in concentration of metal between the two species. However, a significant relationship for Pb between species was found (p <0.05). A one-sided Wilcoxon rank-sum test showed that the amount of Pb is significantly higher in *D. macarellus* compared to *H. humeralis* (p <0.05). A one-sided Kolmogorov-Smirnov test was used and determined that there was a significantly higher amount of Cu in *D. macarellus* compared to *H. humeralis* (p <0.05).

4. Discussion

4.1 Microplastic in the Ocean

Manta trawls on the surface of the ocean confirmed the presence of microplastics in both offshore and inshore environments. The difference in the density of plastics across locations may be due to a number of factors. Each trawl was conducted on different days over a period of two weeks. During this time, variations in the tidal cycles and prevailing wind direction and strength have the possibility to affect the movement and concentration of microplastics in the water. This is especially likely to affect the inshore areas as high tides and higher surf conditions can wash particles from the land into the ocean. Wind has the potential to affect the distribution of microplastics as they may aggregate during calm conditions, or when there is a distinct wind event inshore. Conversely, stormy conditions could disperse microplastics that have previously aggregated. Rainfall may be another influencing force on plastic density as it has the potential to wash particles from the land into the ocean. All these factors make microplastic distribution very dynamic and therefore it is often difficult to find patterns in results. Inshore aggregations have been observed in other locations as highest concentrations of plastic in near shore areas are witnessed after increased flows from rivers (Hajbane & Pattiaratchi, 2017). A higher density of plastic in inshore areas compared to offshore areas in Bermuda corresponds with findings of other, more extensive studies in the same region that found the inshore density to be 2.25 times higher (Lorimer-Turner, 2018). Comparisons of nearshore and offshore microplastic concentrations in other regions of the world have often found similar patterns including in Chile (Thiel *et al.*, 2003) and California (Moore *et al.*, 2005). The lack of correlation between density and location is undoubtedly linked to the small sample size. A larger number of trawls in both onshore and offshore areas would help to expose any true density patterns and the affects of prevailing weather conditions from day to day.

4.2 Microplastics in Fishes

Through the dissection of the stomachs of fishes, this study has successfully identified evidence of microplastic ingestion in fishes across three different trophic levels within Bermudian waters. Statistical tests also confirmed differences between the species and trophic levels. Although a relationship was proven between ingested microplastic and fish length, it is likely that the results are skewed by the high occurrence of microplastic in *D. macarellus*. There is evidence to say that a larger individual can consume more plastic than a smaller individual of the same species as shown for *D. macarellus* and *H. harringtonensis*. However, these species had the largest sample sizes and so may be more accurately representative of the relationship. Larger sample sizes for all other species would be useful in determining any relationship with length and ingested plastic. The difference seen between trophic levels could also be seen as a proxy for length. However, trophic differences are probably more attributed to feeding strategy than length. The two species with the largest samples sizes (*D. macarellus* and *H. harringtonensis*) were responsible for the differences between species. This is probably because a large number of the former contained microplastic and few of the latter contained microplastic. *H. harringtonensis* is a planktivore and *D. macarellus* a predator, and thus these two species probably contribute the majority of the difference detected between these two feeding strategies. *D. macarellus* feeds opportunistically at the surface and could possibly target floating plastic pieces. Pieces of *Sargassum* were also found in the stomachs of multiple *D. macarellus* specimens, proving that they graze on the organisms living here, increasing their risk of ingesting plastic as microplastic pieces were often found attached to *Sargassum* whilst catching these fish from the boats. A larger sample size of all other species could help determine any more distinct differences between species and trophic levels.

4.2.1 Selective Feeding

Selective feeding may be present in the fishes studied. As a whole, the group of fishes demonstrated a difference between the colours of plastic they ingested and what was readily available on the surface of the water. It seems as if they are selectively feeding more on black and clear pieces and avoiding white pieces. This may be because these colours resemble their prey more accurately. White particles may be more visible in the water but it is possible fish are actively disregarding these as their natural prey try to avoid being seen. Therefore muted colours may evoke a stronger behavioural response when detected by the fish.

4.2.2 Effect of Microplastic

The small size of the particles found inside fish guts suggests that these pieces are not retained in the gut for extended periods of time. Rather, they would be passed through the digestive system and excreted back into the environment. The fact that there is little retention of particles in the gut, as 92% of pieces were microplastics and all were less than 14 mm, means that fish may not be greatly affected by false fullness and therefore is unlikely to reduce their will to feed and condition factor as can be seen in other cases (Derraik, 2002). However, the plastics still have the ability to cause lesions in the digestive tract and the fish may still absorb chemicals on or in the microplastic. As it is likely that fish eventually pass the particles out of their system through elimination, as seen by other animals during plastic ingestion studies (Andrady, 2011), it can be assumed that all individuals studied may have ingested plastic throughout their lifetime, even if at time of dissection, no plastic was present. Even the smallest fishes contained microplastic. It can therefore be concluded that larger species can also ingest plastic during early life stages as small fish have a diet entirely made up of plankton and plastic. The results here only relate to a snapshot in time where 32% of all fish happened to have microplastic in their stomachs but the real value of fishes that have ingested plastic at some point may be much higher. The largest particle found was a piece of clear film measuring 14 mm inside a *H.*

humeralis measuring 15.7 cm. This piece constituted a large proportion of the stomach, therefore may not have been able to be passed and could have affected the condition factor but this was the only case of this. As a ram-feeding planktivore it may have been just poor luck to encounter a large piece of flexible plastic that it swallowed in error. But the frequency of this type of error will only increase as microplastic density increases on the surface of the ocean.

Estimates for percentages of fish containing plastic vary by study and region. In Australia 43% of 93 fish sampled contained microplastics (Halstead *et al.*, 2018). Studies in the UK and the North Pacific found results similar to this study where 32% and 35% of fish ingested plastic respectively (McGoran *et al.*, 2018; Boerger *et al.*, 2010). The percentage found in this study is higher than the value of 11% found by another study in the North Atlantic (Lusher *et al.*, 2016). The higher instance in our study could be due to Bermuda's location in the North Atlantic Gyre and the high microplastic concentrations available for consumption (Law *et al.*, 2010).

The high frequency of fibres from fishing gear found in both the water and fish stomachs is unsurprising considering net and trawl fishing is very common in the North Atlantic and perhaps from local Bermuda sources. Studies in other geographic areas have shown synthetic fibres to be more common, including off the Portuguese coast where they accounted for 68.5% of ingested plastic, the majority of which were from textile sources (Neves *et al.*, 2015) and in Australia where 83% of plastics in fish were fibrous (Halstead *et al.*, 2018). However, for this investigation, textile derived fibres were not witnessed in the surface trawls at all and only once in the stomach of a fish.

4.3 Bioavailable Metals

The methods used to assess the biovailability of metal contaminants on microplastics have never been used before in this context. Mimicked conditions of a fish gut may not be completely accurate. However, as conditions differ between all species, it would be difficult to assess the exact bioavailability to each species. *H. balao* for example is a stomach-less species and would therefore contain other enzymes including α-amylase and tripsin in its gut, which work at a higher pH (7-11) (Abidin *et al.*, 2016). The pH often differs throughout the digestive system from the mouth, stomach and intestines. Tests at multiple pH levels would have enabled a more representative view of availability of contaminants to their consumer. It is reasonable to assume that the applied methods would correspond to fish of a similar niche as those examined in this study.

The presence of metals in a solution after mimicking the digestive process of a fish shows that microplastic particles act as vectors for contaminants into the food web. This transfer from microplastics to an organism may be via metal that has adhered to the surface of the microplastic or from the original metal components of the plastic, added during manufacture, leaching out. Contaminants of other forms will also be transferred by this process including POPs. POPs themselves are up to 30 times more likely to detach from an object in the gut than in seawater (Bakir *et al.*, 2014). Further study on desorption rates of metals and POPs from plastic in the gut would improve the understanding of sorbed metal contaminant impacts to marine life. With the current research it can be concluded that microplastic particles in the ocean facilitate the transfer of contaminants into fish species.

Fishes are constantly exposed to metals present in the water. Commonly, as water passes through the gills of a fish, metals accumulate in these tissues. Metal contaminants can also be transferred to the bloodstream directly and transported around the body to different tissues. This pathway will be responsible for a significant amount of the metals found in muscle tissues of fish. Fishes will also be exposed to contaminants in their food. It is difficult to accurately estimate the contribution of each factor to the overall contamination levels in fish tissues. Despite this, the proven bioavailability of metals from plastics means that some fraction of this contamination is due to plastic ingestion.

Although all tests showed signs of bioavailable contaminants on plastics, the low sample size may not be representative. Further studies looking at bioavailability of contaminants on ocean plastic should consider a larger sample size and a variety of digestive techniques to mimic the various stages of digestion within a digestive tract from the mouth to the intestines.

4.4 Metals in Fish Tissues

As both *D. macarellus* and *H. humeralis* are commercially valuable species and often sold for human consumption any metal contamination can easily be passed on to humans. Cd, a non-essential metal, was found in illegal quantities in all fish tested and should be cause for concern. Illegal quantities of Pb and Cr in some fish also pose a threat to human health.

4.4.1 Trophic Bioaccumulation

The differences between levels of Pb and Cu in *D. macarellus* and *H. humeralis* provide evidence for trophic bioaccumulation of metals as the larger species contained a higher concentration of metal. The larger size of *D. macarellus* suggests that they are older; therefore, metal levels may also be higher as they have had more time to accumulate in the body. All other metals presented no significant difference between the species but physiological variation may play a role in the way metals are taken up by each species. *H. humeralis* may be more likely to consume metals as they feed on phytoplankton and zooplankton, which have been suggested to contain higher levels of metals than other organisms (El-Moselhy *et al*., 2014). Other studies have reported similar findings of small forage fish, especially sardine species, containing high levels of contamination and accounted it to plankton feeding (Chen & Chen, 2001). Biomagnification of Zn has been proven over three trophic levels culminating highest metal levels in dolphins in Argentina (Vilches *et al*., 2019). Assessing metal levels in large predatory fish such as tunas and wahoo in Bermuda would provide further information on bioaccumulation of metals in this area an the risk for human consumption.

As it can be assumed that due to the small size of plastic particles found, all fish in the study were exposed to plastic ingestion at some point in their lives, all individuals are equally as likely to accumulate metals in their tissues. Therefore, no comparison was conducted between amount of plastic in the stomach and metal in the tissue of individuals.

4.4.2 Exceeding Legal Limits

Bermuda does not have its own legal limits for contamination in fish, therefore international levels were used. For Cd, multiple limits were found for various regions including Australia, China and EU, which are 0.2, 0.1 and 0.1/0.05 mg/kg dry wt respectively (NOAA, 2019; European Commission, 2006) Even for the least conservative value, found in Australia, all fish sampled contained illegal amounts of Cd. Legal limits of Pb varied more globally with levels from Australia, New Zealand, China, Ecuador, EU being 1.5, 2, 0.5, 5, 0.3 mg/kg dry wt respectively. Using the most conservative of these values, from EU (0.3), two individuals (12%) contained illegal levels of Pb. Hong Kong states legal Cr levels in exported fish to be 1mg/kg dry wt. Four specimens of *H. humeralis* contained illegal levels of Cr, compared to only one *D. macarellus* specimen.

4.4.3 Tissue Type

For this study muscle tissues were chosen for analysis in order to assess the likelihood of metal contaminants being available for human consumption. A study suggested that Pb was the most easily accumulated in fish muscle tissues, followed by Cd, Cr and Ni in order (Afshan *et al*., 2014). Gills are likely to collect high levels of contaminants directly from the water as it passes through them. This is not relevant to this study as these metals will not have been a consequence of metals adhered to ingested plastic and humans do not consume gill tissues. Though metal absorbed through the gills will contribute to concentrations in muscle tissues. Previous studies into metal contaminants in fish have shown that although metals can accumulate in muscles, other organs are more likely to accumulate higher levels after exposure (Jezierska & Witeska, 2006). The livers affinity to attract metals is probably due to their use in metabolic processes in this organ. In larger fish species, the guts are removed before consumption; therefore, this is not relevant either. However, in smaller forage fish such as anchovy and sardine species (e.g. *H. humeralis* and *O. oglinum*), the body may be consumed whole, exposing humans to the high levels of contaminants in all organs. Accumulation sites vary by metal, including Cu, which in some fish can accumulate in high concentrations in all tissues examined except muscle tissue (Kalay & Canli, 2000). Further studies should take this into consideration and regard the liver as a potential contributor to bioaccumulation and impacts on humans.

4.4.4 Length and Metal Elimination

Although easily ingested, fish do have physiological defence mechanisms that allow them to recognise foreign, potentially dangerous substances in their bodies and eliminate them from their tissues. The size or age of a fish is often related to levels of metals in its tissues (Yi & Zhang, 2012). The relationship varies with species, tissue and metals analysed. Metal accumulation is organ specific, and can become concentrated in specific tissues in the body, usually where the metal is used in metabolic processes such as Pb, which has a high affinity to the brain, where it causes the most damage (Allen, 1994). In some cases, there is a positive correlation between size and contamination. As Zn is an essential metal, it is more easily eliminated from tissues when it is present in surplus amounts as a result of efficient regulation (Amiard *et al.*, 1987). Despite this, there have been cases of positive correlations of Zn with fish size (Karadede *et al.*, 2004). In previous studies, Cd and Cu levels have been shown to have a positive correlation with fish length (Karadede *et al.*, 2004) whereas studies on Pb concentrations have found a negative relationship between the two (Widianarko *et al.*, 2000). The negative correlation found between Cd and length in this study may be because Cd is already in high levels so it is possible that the fish is constantly removing Cd from its tissues using physiological processes but becomes more effective at this as they get larger in size. The positive correlation found between Pb and length may mean that it is possible that the species analysed are not as efficient and eliminating Pb from their tissues with a growth in size. Once fishes reach a certain age and size, the concentrations of metals tend to be maintained at a stable level for easily eliminated metals such as Zn (Douben, 1989). This may be due to physiological regulations within the body that are able to effectively eliminate some metals (Zn, Cu, Pb) from various tissues (Kalay & Canli, 2000) and also help explain negative trends with size. Cd is not usually easily eliminated but some laboratory studies show evidence of fast elimination from tissues after a period of exposure to low pollution (De Conto Cinier, 1999). Therefore if fish found in Bermuda's waters were to be exposed to a low Cd environment, Cd in their tissues may fall to a level that is safe for human consumption. However, nature is unlikely to award such a recovery period to allow levels to decrease. More likely, global pollution levels will increase and metals will persist within tissues until they are passed up trophic levels, eventually to humans. Levels of metals in different fish species have very complex relationships with biotic and abotic factors, making it difficult to conclude specific reasons for any variations and trends from this study.

Water quality samples were not collected in this study so levels of metals in fish cannot be directly related to conditions of their environment. Previous studies on water quality in Bermuda show the presence of metals (Pb, Zn and Cu) and POPs in the water, sediment and bivalve species (Burns *et al*., 1990; Jickells *et al*., 1986) but more recent levels are needed to form an accurate comparison. Using fish tissues as a proxy for wider ecosystem pollution is a form of biomonitoring. As all fish displayed levels of Cd in their tissues that can be a threat for human consumption, and some displayed illegal levels of Pb, this gives evidence for a wider environmental issue that may be affecting various species, especially commercial ones that are consumed by humans. Some studies assessing metal levels in tissues of commercial fish have concluded no risk to the environment or human health (Prudente *et al*., 1997), whereas more recent studies have begun to uncover dangerous levels of metals in fish. Fish destined for human consumption have previously been confirmed to have higher than legal levels of metal contaminants in a Turkish lake (Dural *et al*., 2007). A large study of commercially important fish in Oman showed evidence of a few fish containing illegal levels of Pb, Cd and Hg (Al-Busaidi *et al*., 2011). A study by Olmedo *et al.* (2013) in Spain concluded that frequent fish consumers of specific species were at risk of high metal intake due to illegal levels in the fish.

Metal accumulation in fish tissues is accelerated by factors including water temperature and acidification, which cause higher metabolic rates and change the solubility and bioavailability of metals respectively (Jezierska & Witeska, 2006). Future warmer ocean temperatures and predicted lower pH levels may also indirectly cause more accumulation of metal pollutants in fish.

A more comprehensive understanding of spatial and temporal patterns in microplastic density would allow conclusions to be drawn on its behaviour in Bermuda's waters. Bioavailability studies that encorporate a broader spectrum of mimicked gut conditions would help determine the extent to which microplastics act as vectors for metals into fish tissues. Future studies should also focus on unravelling the risks posed by potential plastic and metal contamination in larger fish including pelagic predators that are of commercial value to humans, understanding trophic linkage and the extent of the risk to humans with fish consumption. A broader understanding of these issues would allow governments to make sensible recommendations of consumer choices, enforce mitigation programs for contaminated sites and act as sound proof to promote the reduction of plastic production and use.

4.5 Conclusion

Marine pollution in the form of both plastic debris and metal contaminants plagues the waters and biota of Bermuda. The presence of plastic has been proven to be ubiquitous in Bermuda's marine environment from the water surface, to fish stomachs in the form of plastic particles. These plastic particles have aided the demonstration of the presence of metal contaminants adhering to plastic. This in turn contributes to the accumulation of metals in fish tissues, adding to what may be absorbed directly from the water. The ephemeral nature of aspects of the marine environment make it difficult to attribute patterns witnessed to specific factors such as the distribution of microplastics. Although microplastics have been confirmed as a vector for metals into fish tissues, bioavailability to a wider number of organisms should be proven to create an ecosystem approach to the affects of metal contaminants in the marine environent. Biotic differences in fish species also make conclusions on patterns of metal uptake and elimination troublesome. It can be concluded that plastic and metal contaminants are having an impact on fish, which is only likely to be exacerbated in the future by the increasing about of marine contaminants in all forms. Correlations between the amount of plastic and length and trophic level are evidence of bioaccumulation and biomagnification, which cannot be ignored when relating contaminant levels to consumer choices of fish species. Large, commercial species may be posing the greatest risk to humans but more studies on specific species are needed. The risk to humans is unclear, but a precautionary approach to fish consumption would reduce any risk of consumption of potentially hazardous materials. Evidence such as this should be taken into account when setting legislation for health risks concerning seafood and a need for future fish tissue assessments in Bermuda.

5. Appendices

Appendix A: Conditions for Metal Analysis by AAS

Metal	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
Method	GFAAS	GFAAS	GFAAS	GFAAS	GFAAS	FAAS
Units	mg/kg dw	mg/kg dw	mg/kg dw	mg/kg dw	mg/kg dw	mg/kg dw
Quantification Limit (mg/kg dw)	0.008	0.08	0.4	0.4	0.4	4
Wavelength (nm)	228.8	357.9	324.8	232.0	283.3	213.9
Slit (nm)	0.7	0.7	0.7	0.2	0.7	0.7
Matrix modifier	NH4H2PO4+ Mg(NO3)2	Mg(NO3)2	Pd+ Mg(NO3)2	not used	NH4H2PO4+ Mg(NO3)2	not used
Atomization temperature °C	1400	2300	1900	2300	1500	air/acetilen flame

Appendix B: Plastic Found in Gut Contents of Fishes

Fish number	**Fish Code**	**Type of Plastic**	**Colour**	**Number of Pieces**	**Total plastic**
1	D.m 1				0
2	D.m 2				0
3	D.m 3				0
4	D.m 4				0
5	D.m 5				0
6	D.m 6				0
7	D.m 7				0
8	D.m 8				0
9	D.m 9				0
10	D.m 10				0
11	D.m 11	Fragment	White	2	2
12	D.m 12	Fragment	Green	1	1
13	D.m 13				0
14	D.m 14	Fragment	White	1	1
15	D.m 15	Film	Clear	1	
		Fragment	Clear	1	2
16	D.m 16	Fragment	White	1	1
17	D.m 17	Fragment	White	1	
		Fragment	White	1	2
18	D.m 18	Film	White	1	1
19	D.m 19	Fragment	Clear	1	

		Fragment	White	2	
		Film	Clear	1	4
20	D.m 20	Fragment	Green	1	
		Fragment	White	1	2
21	D.m 21				0
22	D.m 22	Fragment	White	2	2
23	D.m 23	Fibre	Green	1	
		Fibre	Black	1	
		Fragment	White	1	3
24	D.m 24	Film	Black	1	
		Fragment	Black	1	
		Fragment	White	3	5
25	D.m 25	Fragment	White	1	
		Fragment	White	8	9
26	D.m 26	Fragment	White	1	1
27	D.m 27	Film	White	1	1
28	D.m 28	Fragment	White	1	
		Fragment	White	2	3
29	D.m 29				0
30	D.m 30	Fragment	White	1	
		Film	Clear	1	2
31	D.m 31	Film	Clear	1	1
32	D.m 32				0
33	D.m 33				0
34	D.m 34	Fragment	White	1	1
35	D.m 35	Fragment	Green	1	
		Film	Clear	2	
		Fragment	White	1	
		Fragment	Clear	4	8
36	D.m 36	Fibre	Black	1	1
37	D.m 37				0
38	D.m 38	Fragment	White	1	1
39	D.m 39				0
40	D.m 40				0
41	D.m 41				0
42	D.m 42				0
43	D.m 43	Film	Black	3	3
44	D.m 44				0
45	D.m 45				0
46	D.m 46				0
47	D.m 47	Fragment	White	1	1
48	D.m 48	Fragment	White	3	3
49	O.o 1	Fragment	Black	1	1
50	O.o 2	Fibre	Black	1	1
51	O.o 3	Fragment	Black	1	1
52	O.o 4	Fragment	Black	1	1
53	O.o 5				0
54	O.o 6				0
55	O.o 7				0
56	O.o 8				0
57	H.ha 1				0
58	H.ha 2				0
59	H.ha 3				0
60	H.ha 4				0
61	H.ha 5				0
62	H.ha 6				0
63	H.ha 7				0
64	H.ha 8				0
65	H.ha 9				0

66	H.ha 10				0
67	H.ha 11				0
68	H.ha 12				0
69	H.ha 13				0
70	H.ha 14				0
71	H.ha 15	Fragment	Black	1	1
72	H.ha 16				0
73	H.ha 17				0
74	H.ha 18				0
75	H.ha 19				0
76	H.ha 20				0
77	H.ha 21				0
78	H.ha 22				0
79	H.ha 23				0
80	H.ha 24				0
81	H.ha 25				0
82	H.ha 26				0
83	H.ha 27				0
84	H.ha 28				0
85	H.ha 29				0
86	H.ha 30				0
87	H.ha 31				0
88	H.ha 32	Fragment	Blue	1	1
89	H.ha 33	Fibre	Blue	1	
		Fibre	Black	1	2
90	H.ha 34				0
91	H.ha 35	Film	Clear	1	1
92	H.ha 36				0
93	H.ha 37				0
94	H.ha 38				0
95	H.ha 39				0
96	H.ha 40				0
97	H.ha 41	Fibre	Clear	1	1
98	H.hu 1				0
99	H.hu 2				0
100	H.hu 3				0
101	H.hu 4	Fragment	Black	1	1
102	H.hu 5				0
103	H.hu 6				0
104	H.hu 7	Fragment	Black	1	1
105	H.hu 8				0
106	H.hu 9				0
107	H.hu 10	Fragment	Black	1	1
108	H.hu 11	Fragment	Green	1	1
109	H.hu 12	Fragment	Clear	1	1
110	H.hu 13				0
111	H.hu 14				0
112	H.hu 15	Film	Clear	1	1
113	H.hu 16				0
114	H.hu 17				0
115	H.hu 18	Fragment	Black	1	1
116	S.c 1				0
117	S.c 2				0
118	S.c 3	Fragment	Black	2	2
119	S.c 4				0
120	S.c 5				0
121	H.b 1				0
122	H.b 2				0
123	H.b 3				0

124	H.b 4				0
125	H.b 5	Fragment	Black	1	1
126	H.b 6	Fragment	Blue	1	1
127	H.b 7				0
128	H.b 8				0
129	H.b 9				0
130	H.b 10				0
131	H.b 11				0
132	E.b 1	Fragment	White	1	1
133	E.b 2	Fragment	White	1	1
134	E.b 3				0
135	E.a 1				0
136	E.a 2				0
137	E.a 3	Fibre	White	1	1

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