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Microplastic ingestion in invertebrates within rockpool communities

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By Abigail Outred

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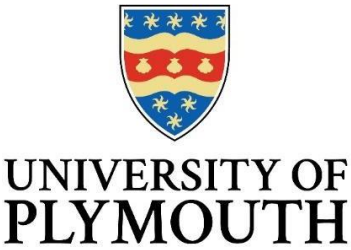

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Author's Declaration

At no time during the registration for the degree of Research Masters has the author been registered for any other University award without prior agreement ofthe Doctoral College Quality Sub-Committee.

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Microplastic ingestion in invertebrates within rock pool communities

By ABIGAIL OUTRED

ABSTRACT

Microplastics (<5 mm) are abundant across the world in the marine environment and so it is vital that we gain further understanding of their fate and their possible impacts on marine life. Due to their size, microplastics can interact with small marine organisms which are part of the lower trophic levels and the main interaction with these plastics is ingestion. Chemical characteristics and changes to the plastic properties, due to, for example, adsorbed chemicals and colonisation of biofilms, may affect how readily plastics are ingested. Research into the interactions of a range of organisms with microplastics enables for a better understanding of how they could be taken in, impact the organism as well as predict potential trophic transfer. This in turn could aid in predicting bigger impacts in the marine environment and on humans themselves. Rockpools are a key environment and nursery for many important marine and intertidal species, particularly those that we rely on commercially, such as crab species. This study exposed three key rockpool species of three feeding types - Beadlet anemone (*Actinia equina*), common prawn *(Palaemon serratus),* Thick top shell (*Phorcus lineatus)* to nylon fibres within *ex-situ* mesocosms. These species represent three feeding types found in a rockpool community – Suspension feeding, Filter feeding of the whole water column and deposit feeding. The organisms were exposed to either biofouled or non-biofouled, blue, black, red, or white in colour and 0.5 mm or 2 mm microfibres for six hours. This was undertaken when individuals were individually housed as single species, as well as a mixed community with a representative of all three species. Once biofouling was complete, dissection to observe the digestive tract was undertaken and then an alkaline digest was completed to obtain evidence of retention other than in the digestive tract. Beadlet anemones ingested the most microfibres and thick top shell the least. This study shows that overall, biofouled fibres are significantly more likely to be ingested than

that of non-biofouled (H(1)= 16.780 , p<0.001). Some ingestion and interaction colour patterns were found – black in anemones and shrimp ((H(1)= 6.224 , p=0.013 and (H(1)= 6.008 , p=0.014) and black (H(1)= 12.270 , p=0.007) and white in shrimp (H(1)= 8.143 , p=0.043). This could possibly be to do with the dye chemicals on the plastics rather than visual cues. The 0.5mm fibres were ingested and retained more than 2mm (H(1)= 20.924 , p<0.001). Thick top shells were the only organism with a difference between housing with more microfibres ingested/retained when housed individually than when housed in a mixed community. This study provides further evidence of the potential ingestion and retention of microplastics in a rockpool setting and therefore highlights the potential impact on these organisms and predator species. This may likely cause negative impacts within that rockpool as well as present a route for microfibres to expose other intertidal organisms to microfibres, particularly as the three study organisms are prey animals to many other species.

Abbreviations

MP - Microplastic

MF - Microfibre

KOH – Potassium hydroxide

PE - Polyethylene

PP – Polypropylenes

PS – Polystyrene

PVC – Polyvinyl chloride

PA/Nylon – Polyamide

PET – Polyethylene terephthalate

NBF – Non-biofouled

BF – Biofouled

BPA – Bisphenol A

RO – Reverse osmosis water

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1 Introduction

1.1 Plastics in the marine environment

Plastics have various ways in which they can enter the marine environment: improper disposal of waste, enter via waterways, runoff from the land or even deliberate mismanaged waste (Law *et al.,* 2020). Plastic comprises a substantial amount of marine litter and global production of plastic is quadrupled in the past 30 years, reaching 460 million tonnes produced from 2000-2019 alone (Geyer *et al.,* 2017). Of that, 352 million tonnes are considered to be waste but only 9% is recycled. In 2019 1.7 million tonnes of plastic leaked into the aquatic environment, adding an estimated 30 million tonnes to that in the oceans already (Lebreton *et al.*, 2019; Rees *et al.*, 2022). It is estimated that in 2015, 5.25 trillion particles were floating within the oceans which was mapped using oceanography modelling of debris (Eriksen *et al.*, 2014). *Eriksen et al.*, 2014 also presented other studies that also found movement of plastics via winds and currents, moved plastics from their original entry point which then accumulated on shorelines or gyres (Thushari and Senevirathna, 2020), within coastal sediments (Bagaev *et al.*, 2021), and even reached areas where humans have not inhabited, such as the Antarctic (Caruso *et al.*, 2022) or some of the most remote island of the world, such as, Azores archipelago and the Henderson Islands (Martins *et al.*, 2020; Nichols *et al.*, 2021). As well as being found on the ocean surface, plastics break down and sink when they become denser than the seawater (Amaral-Zettler *et al.*, 2021). The sinking of plastics in the deep-sea marine environment is currently being monitored under a 30-year recording scheme which has already found plastics at >6000m at a maximum rate of 3.35 items per square metre (Chiba et al., 2018; Table 1). A more recent study off the coast of Corsica has further supported the issue, with 190 pieces per 50 grams of sediment on the seafloor, and 1.9 million pieces per square metre. Terrestrial plastic waste contributes to 80% of marine litter (Andrady, 2011). This can be wastewater, inland rivers, stormwater and sewage outflow and industry outputs (Coyle *et al.*, 2020). Accidental spillages, fragmentation and shipping discard of plastic are additional examples of at-sea plastic inputs (Cole *et al.*, 2011).

Plastics may have negative impacts on biota and understanding transport and uptake can help us predict where these effects may occur. Of particular importance is the effect of biotic factors of transport, uptake, and trophic transfer plastics that reach the marine environment and are subject to fragmentation which then break down into microplastics (Cole *et al.*, 2011). Once in the marine environment, horizontal and vertical distribution can move these plastics to become accessible to a range of biota. Horizontal distribution occurs from hydrodynamics such as currents, drift, and river inflow. Inshore currents bring in plastics from further out in the sea, particularly gyres, causing a high concentration of plastics into the intertidal zone. Vertical distribution occurs largely due to the density of the polymer of the plastics itself as well as biotic transfer (Courtene-Jones *et al.*, 2017).

1.2 Plastic Properties and their implication in the marine environment

The wide use and properties of plastics result in varying pathways of plastic into the environment and their subsequent impacts. MPs can also come in two forms – primary and secondary. Primary MPs are plastic that are created by a manufacturer to be a specific small size and are typically in the form of pellets, beads, fibres and powders (Guo and Wang, 2019). Secondary plastics are formed by the fragmentation of larger plastics into smaller pieces from factors such as weathering or ageing and be defined as fibres, fragments, films and foam (Cole *et al.*, 2011). Size, shape, chemical composition of the plastic, density, colour of the plastic, and susceptibility to adhere chemicals used in manufacturing can increase or lessen its impacts (Wang *et al.,* 2022; Thompson, *et al.,* 2013; Zhu *et al.,* 2019) .

The break down of plastics can also affect how an MP can interact with the marine environment. Weathering is common in the marine environment due to exposure to UV, fluctuations in temperature, mechanical abrasions and biodegradation. Changes in a plastic's physicochemical properties from the UV photodegradation or hydrolysis can affect the surface area, oxygen groups and crystallinity effect on the sorption of chemicals. Both primary and secondary plastics when subject to degradation will see changes in both physical and chemical properties which would include colour, size, surface and density changes from their original state (Guo & Wang, 2019). Thermal degradation is the break down due to elevated temperature – thermos-oxidative reactions. Every plastic has a

different thermal degradation point with varying points of rigidity, flexibility and melting which in turn will affect how quickly a plastic will break down. Physical changes in MPs such as embrittlement and cracks from reduced tensile strength are more common in MPs that lack additives (Zhang, K. *et al.*, 2021). Furthermore, weathering affects the plastic's chemical and physical properties, which in turn, impact how it behaves (Vroom *et al.,* 2017).

The density of plastic can greatly affects where the plastic may be accessible within the water column and can impact on which organisms can interact with plastic of different properties due to where the plastic will sit within the water column. Surface seawater has an average density ranging from 1.02 – 1.03 g/cm^3, however, the average density also varies in different marine locations due to varying freshwater inputs or temperatures, such as, hypersaline waters, equatorial and polar inputs. The density of the plastics in the water varies on the type of plastics (Table 1). Less dense waters, for example, of the Arctic that floats above the Atlantic contained less plastic pollution (Lusher *et al.*, 2015). In the Black Sea, the sinking rate of microplastics varied between brackish water, surface water and seawater, with a halocline at 100m found to limit exchanges between surface and deep water (Aytan *et al.*, 2016).

Within the marine environment, Polyethylene (PE), Polypropylenes (PP), Polystyrene (PS), Polyvinyl chloride (PVC), Polyamide (PA/Nylon), and Polyethylene terephthalate (PET) are the most frequently found polymers of plastics, these are also the most commonly produced and have different densities (Guo & Wang, 2019; Table 1). Dense plastics such as PVC will sink quicker than PE (Table 1)(Harris *et al.*, 2021; Sanz-Lázaro *et al.*, 2021). This in turn presents different marine organisms with different types of plastics, however, density will vary depending on the length of time in the environment due to adsorption and biofouling. With benthic organisms more likely to encounter denser plastics found first, pelagic organisms are more likely to encounter less dense plastics first (Dai *et al.*, 2018). As PE has been found to be the most common microplastic type overall in the marine environment (de Haan *et al.*, 2019; Gedik *et al.*, 2022), this could suggest organisms that interact with the ocean's surface will be much more susceptible to plastic interactions than those on the ocean floor. This is, however, subject to various other factors, such as the biofouling of plastics or the area which has been most studied over others. Surface waters

are the most studied areas of MPs and so other areas in the water column may have high concentrations but, they have not been studied thoroughly yet.

Table 1 Types and properties of microplastics. Surface seawater density ranges from 1.02 – 1.03 g/cm^3 (Barboza *et al.,* 2018; Eriksen *et al*., 2014; Horton *et al*., 2018)

Microplastic	Commonly used for	Density (g/cm^3)
PE	Bottles, containers, shopping bags, food wrappers	0.92-0.97
PP	Bottle caps, straws, rope	0.88-1.23
PVC	Pipes, electrical cable, shower curtains	1.15-1.70
PS	Food packaging, disposable cutlery and plates	1.04-1.50
PA/Nylon	Fabric, fishing line, fishing nets	1.25-1.60
PET	Bottles, microwave trays, fabric	1.30-1.50

Similar to density, size also plays a factor in the potential impact of plastics encountered by organisms. Marine plastics are categorised as mega (>1 m diameter), macro (between 2.5 cm and <1 m), meso (between 5 mm and <2.5 cm), micro (between 0.1 µm and <5 mm) and nano (<0.1 µm) (GESAMP Working Group, 2016). The impacts can be variable for different marine organisms. Larger plastics, such as fishing nets, were mostly seen to have impacts on turtles, fish, marine mammals and birds (Gall and Thompson, 2015) whereas nano and MPs were seen in smaller organisms, and also have numerous evidence from studies of ingestion (Gall & Thompson, 2015; Mihai *et al.*, 2022; Uzun *et al.*, 2022). However, this could be due to sample bias with smaller organisms that are easier to study as well as less contentious compared to larger organisms such as birds, sea turtles, cetaceans and larger fish (Crump, 2022). Crump (2022) illustrates the importance of UK Animal Welfare (Sentience) Act 2022 and indicated the importance of using washed-up marine animals for autopsy. MPs are of concern due to their ability to interact with small organisms and lead to trophic transfer.

1.3 Microplastic fibres

There are various types of MPs found in the marine environment: microbeads, fragments, nurdles, foam and fibres (Guo & Wang, 2019). Microplastic fibres (MFs) are the most abundant type of microplastic in the marine environment and have been documented in every corner of the world (Barnes *et al.,* 2010; Amelia *et al.,* 2021; Perumal and Muthuramalingam, 2022). Within the marine environment, MFs have polluted almost all areas from the sea ice, surface waters, beaches and all the way down to the sea floor sediment (Kelly *et al.*, 2020; Mishra *et al.*, 2019; Suaria *et al.*, 2020). A main contributor of MFs is from domestic and industrial wastewater in which fibres are released during the wear and washing of synthetic clothes. These fibres enter sewage treatment works and can then be applied to land via contaminated slurry. Wastewater systems are limited in filtering out MP or directly entering the ocean via untreated water or lack of treatment facilities altogether. It has also come to light in recent years, the extent of untreated wastewater dumping occurring which would contribute large numbers of MPs as well as chemicals (Singh *et al.*, 2004; Tariq and Mushtaq, 2023; Woodward *et al.*, 2021). However, some machines have been made compliant and are able to filter out MFs before they reach the sewage system. Cristaldi *et al.,* (2020) reviewed 15 papers on wastewater treatment plants (WWTPs) for MPs removal and found overall, a 90% efficiency or more. With the introduction of laws requiring filters for MFs by 2025 in France and a 90-105% recovery rate of MFs from real samples (Gaylarde *et al.,* 2017), there will be a reduction of MFs in the environment. Napper *et al.*, (2020) study looked at the efficiency of devices in reducing MFs and found that the XFiltra was able to prevent 78% of MF from going into wastewater. Liu, W. *et al.*, (2021) looked at the implementation of filtering within the wastewater treatment process, finding that filter-based treatment exhibited the best results, however, larger plastics were easily removed when primary settling was used. Smaller particles were also found to become trapped by bacteria in activated sludge in a bioreactor system. Napper & Thompson, 2016 also investigated MF composition from washing machines, with acrylic, polyester and cotton garments being high and saw the release of fibres during a 6kg cycle found that between 130,000 to 700,000 fibres, with acrylic fabric releasing the most. Garments are not the only source of MF, cigarette butts and fishing gear which are discarded, also contribute to the problem (Belzagui et al., 2021; L. S. Wright et al., 2021).

Considering the amount of discarded or waste fishing gear, in Norway alone, 4000 tonnes of waste fishing gear was produced between 2007-2016, and the contribution of fishing gear to MP pollution remains relatively understudied. Surface sediments of Beibu Gulf found that 61.6% of the fibres recovered originated from abrasion of fishing gear (Xue *et al.*, 2020). The most common types of polymer for fishing gear in the UK are nylon and PE which are used for the ropes for nets and pots and fishing line (Kim et al., 2016; Perumal & Muthuramalingam, 2022; Plastic Soup, 2011). Nelms *et al.,* 2021 study in the Ganges River found that 27.6% of all plastics were fishing gear, followed by PE (22.4%), poly(1,4-cyclohexanedimethylene terephthalate (PCT) (15.3%), high-density polyethylene (14.1%), PS (1.2%) and PET (0.6%). While *in-situ* studies observe a high abundance of MFs (Kelly e*t al*., 2020; Mishra *et al*., 2019; Suaria *et al.,* 2020), few laboratory studies have experimentally tested this. Many of these studies focus on polyethylene MFs *in-situ* and in a laboratory setting and many use chemicals during digestion which would dissolve nylon fibres which will bias the results of *in-situ* MFs counts. In a range of studies (Cole *et al.*, 2014; Thiele *et al.*, 2019, 2021) sodium hydroxide (NaOH), hydrochloric acid (HCl) and nitric acid (HNO3) and have been found to dissolve nylon fibres. Whilst this is less of a problem when just looking at PE, PP, PA and PS singularly, when looking at unknown mixes of MPs types, it would create biases (Pfeiffer and Fischer, 2020).

1.4 Plastic Biofouling

As previously stated plastic properties change as it persists and reacts with the environment. These changes in its properties impact how organisms interact with the MP and its impact on the environment in which it is found (Galloway *et al.*, 2017). Once MP enter the environment, microorganisms accumulate on the plastic surface forming biofilms, known as biofouling (Kooi *et al.*, 2017) The biofilm, made from the accumulated organisms can affect the hydrophobicity and buoyancy of the plastic and when the density of the biofilm outweighs the density of the seawater, the plastic begins to sink (Kooi et al., 2017; Ye & Andrady, 1991). The sinking of plastics can lead to vertical transport and so open up plastic interaction to more organisms. Bacteria, algae, protozoans, and fungi are all found as part of biofilms but the composition can vary depending on various spatial and temporal factors (Rummel *et al.*, 2017). Biofouling (BF) can occur quickly in some settings with strong

attachments found within a week of exposure (Kaiser *et al.*, 2017). Kaiser *et al*., 2017 found specific plastic types or water types affected BF of the plastics differently, with PE <5mm pellets in estuary water did not sink after 14 weeks of exposure however, they sank in six weeks when exposed to coastal water which was primarily due to blue mussels (*Mytilus edulis*) attachment. It has also been found that biofilms can also slow the degradation of MP due to the blocking of UV exposure which is a significant cause of plastic degradation (Qiongjie *et al.*, 2022). Some evidence (Vroom et al., 2017) has been found that biofouled plastics may be selected over un-colonised plastic which may be accredited to chemicals emitted by the biofilm which act as a feeding indicator to various organisms.

1.5 Availability of MFs within the marine environment

Due to the size of MFs, many organisms can easily interact with them, particularly in coastal environments which see high rates of MPs overall (Kim et al., 2015; Van der Hal et al., 2017; Zhang, 2017). Various physical factors, such as plastic-type, colour, chemicals used and size of MPs can increase ingestion rates via prey misidentification or due to their size leading to the MPs being passively ingested (Zhang, 2017; Savage *et al.,* 2022). Interactions with microplastic, dependant on plastic size, can lead to varying impacts on the organism that encounter them: entanglement, chemical intake, chemical toxicity, physical damage from interactions or ingestion, false satiation, changes in natural behaviours from toxicological effects, transfer to the circulatory system, tissue or the intestine causing blockages (Huang, *et al.,* 2021; Savage *et al*., 2022). Ingestion and respiration are the main pathways in which MPs are uptaken, with many studies looking into this (Coyle *et al*., 2020; Pinheiro *et al.,* 2020).

MFs have been found in a range of organisms, from small to large and shows the extent of the MF issue in the marine environment. Microplastics in beluga whales (*Delphinapterus leucas*) from the eastern Beaufort Sea were found, of all the MPs ingested, fibres accounted for 49% in the intestines and stomach (Moore *et al.*, 2020). In South America, of 51 scats of female fur scat (*Arctocephalus australis*) MFs were found in high abundance - 2.7 to 13.35 items g−1 (wet weight) of which, 67% of scat contained MFs (Perez-Venegas *et al.*, 2018). In North Peninsular Malaysia, 72 fish species' digestive tracts were examined, with 100%

having MPs, of which 41.9% were MFs (Foo *et al.*, 2022). In a collective look at marine mammals, 72% of articles identified MFs as the most prominent MPs that were ingested (Ugwu *et al.*, 2021). In Sea turtles, 54.4% of all MPs were MFs (López-Martínez *et al.*, 2021). Suaria *et al.*, (2020) found of the 916 global surface water samples taken, 99.7% of all samples contained MPs, totalling 23,593 fibres (median 18 fibres), showing surface water vulnerability to all organisms that interact with it, particularly to surface invertebrates. All the above studies show the extent of MFs in the marine environment and how MFs account for the largest portions of MPs within it.

1.6 Microplastic ingestion and effects on marine life

It is known that marine plastics can cause damage to organisms externally which is largely documented across many organisms in the marine environment, from the largest mammals (Moore *et al.*, 2022; Zantis *et al.*, 2021) to some of the smallest (Cole *et al.*, 2013; Hitchcock, 2022). However, it is only recently that the internal impacts of plastics have been reported. Of the external and internal impacts of plastic, 2248 species have been studied or observed and identified to be negatively impacted by plastics (Marine Litter, 2022). MPs can be ingested through the gills, through filter feeding, or ingested as prey. MFs taken through the gills can cause breathing issues for the organism due to the impact on the function of the gills (Watts *et al.*, 2016; Zhang *et al.*, 2021) however, this is greatly understudied and mostly undertaken in mussels or crab species. Ingestion as prey has been shown to interfere with false satiation and impede the digestive system which in turn can impact energy, growth and the immune system (Wright *et al.,* 2013). There are also possible implications of toxic effects due to the various chemicals and additives used during processing, as well as those adsorbed to the plastic surface. Gray and Weinstein (2017) found that size and shape affects the plastic ingested by grass shrimp (*Palaemonetes pugio*) focusing on microspheres, fibres and fragments of various sized plastics. It was found that MF resulted in a significantly higher mortality rate than that of the other forms. Although ingestion can cause issues, organisms have been seen to egest MF without obvious negative impacts. There can also be bioaccumulation - accumulation of contamination in or on an organism, which may lead to trophic transfer, although there is still limited impact information due to a lack of understanding of specific species interactions of MF (Carbery *et al.*, 2018; Nelms *et al.*, 2018).

1.7 Microplastic interactions within the food web

MPs have many characteristics that can affect their interactions within the food web. As previously mentioned, size, density, abundance, adhered chemicals, biofouling and age of the plastic can all influence its availability to organisms as well as different marine habitats. Ingestion is the main factor in MP entering the food web (Huang *et al.,*2021), particularly when the MPs look or potentially smells, like natural prey (*Debroy et al*., 2021; Chavarry *et al.,* 2022). As well as natural predation, passive ingestion also occurs through filter feeding (He *et al.*, 2022). When ingested, MPs can cause negative effects on the bodily functions of an organism, such as internal blockages. These negative impacts can lead to a reduction in energy uptake which would leave the organism vulnerable to predation, however, more studies looking at retention time and egestion of MPs and MFs are needed to fully assess impacts. MPs have the potential to stay longer within organisms and so this increases the likelihood that when predated on, the MPs within the prey will pass these onto the predator leading to trophic transfer and bioaccumulation (Huang et al., 2021; Kim et al., 2021). With MFs being retained longer than MPs in some organisms (Gray & Weinstein, 2017; Rillig *et al.,* 2017), the likelihood of trophic transfer and bioaccumulation is further increased, however, much like trophic transfer and bioaccumulation studies, more studies are needed looking at retention in a range of marine organisms. Some studies have shown evidence of trophic transfer (Carbery *et al.*, 2018; Nelms *et al.*, 2018; Welden and Cowie, 2016), but there is still very little evidence. Although a large range of marine organisms has been found to ingest MPs, without more research it is unclear of the full potential ecological impacts of MPs and more specifically, MFs.

1.8 Beadlet anemone (Actinia equina), Thick top shell (Phorcus lineatus) and Common prawn (Palaemon serratus)

Rockpool fauna vary with respect to feeding guilds, for example, suspension, filter, and deposit feeding. Feeding guild could influence the level of exposure to microplastic contamination. The organisms used for this study were chosen to represent different feeding guilds within a rockpool environment. The Beadlet anemone (*Actinia equina*) represents suspension feeding, common prawn *(Palaemon serratus)* filter feeding and thick top shell (*Phorcus lineatus)* represents deposit feeders. This is intended to give a better

understanding of the influence of feeding guild on the access of MPs within rockpool communities.

Beadlet anemones are common within UK rockpools and live at all levels of a rocky shore. Using its tentacles, it traps passing food and draws it into its mouth when the tentacle senses it. These anemones eat what their tentacles can catch and appear not to be selective in what they feed on (Ager, 2008). Sea anemones typically feed on mussels, shrimp, dead fish and other prey however, they can obtain sugar from the photosynthesizing algae that live inside the although this has not been studied in beadlet anemones (Bedgood *et al.*, 2020). Due to the nonselective nature of their feeding, MPs contamination could be high as they cannot selectively choose to not ingest the MPs.

The common prawn, like beadlet anemones, can be found at all heights of the rocky shore but moves offshore during the winter in the UK. They are filter feeders that will graze on algae, living and decaying plant material, decaying organisms and some living organisms when found (Persson *et al.*, 2008). The common prawn has shown selective feeding habits within its feeding type and so looking at filter feeding can highlight ways in which MPs can enter into the food web from this feeding type. If the common prawn also selectively filter feeds on MPs, it further highlights issues with MPs pollution being chosen to be ingested, rather than through accidental ingestion.

Thick top shells are found up to the mid-shore of the rocky shoreline and typically graze on microalgae. They do this by crawling along rocks and moving their head from side to side to follow algae growth on the rocks. There are suggestions that whilst doing this, the snail leaves a mucous trail to trap food from the water column, and attract a mate or a marking to follow to home (Sousa *et al.*, 2017).

1.9 Rockpools and their vulnerability to MPs

The intertidal zone is the most abundant for MPs and so is an area most likely to be impacted by plastic pollution and also sees an overlap between humans, marine life and MPs (Enders *et al.*, 2015). MPs in the intertidal zone originate mostly from land sources through rainwater runoff, sewage input and via rivers (Enders *et al.*, 2015). MPs are also

introduced to the intertidal zone through the currents and tides as MPs float on the surface waters (Peng *et al.*, 2022). The way in which MPs transport within and from the intertidal zone is greatly affected by the physical characteristics of the plastics, such as size and density as well as the coastal conditions, such as wind and tide (Kangas *et al.*, 2023).

Rockpools are a complex and diverse ecosystem that supports diverse communities and provides important nurseries and shelter for many species as well as provides a source of food within the intertidal zone (Brendonck *et al.*, 2016). When the tide in the intertidal zone goes out, rockpools are isolated until the tide comes back in and so any MPs trapped in the rockpool during this time are locked in and will now be available to the organisms which inhabit the rockpool. Rockpools are potentially at risk of higher impact and interaction with rockpool fauna while isolated from coastal water and flowing within the rockpools during periods of low tide.

As previously mentioned, rockpools are extreme environments and are subject to stormy weather, temperature changes both diurnally and seasonally, high salinity, lower oxygen concentration, acidic or alkaline pH and lower or higher nutrient concentration (Legrand *et al.*, 2018). Furthermore, they are subject to extreme UV exposure and temperature fluctuation that can directly impact the fragmentation and embrittlement of the plastics. Many of these factors can cause degradation to MPs such as heat, pH and storms which can speed up the breaking down of the MPs or release chemicals added to the plastics during manufacturing. This can potentially expose rockpool organisms to more risks of MPs, either from the MPs themselves or the chemicals it releases, than in other areas of the marine environment. Although various studies have looked at the intertidal zone in relation to MPs (Blumenröder *et al.,* 2017; Bendel *et al*., 2020; Wu *et al.,* 2022), there are no studies look at MPs within a rockpool environment and so the potential impacts are unclear.

1.10 Human health and microplastics

The impacts of MPs and their presence in the human diet are little known (Toussaint *et al*.,2019). A major point in the ingestion of MPs by humans is via contaminated food with evidence documented in 201 edible animal species, 5 food products, as well as water and

beer (Toussaint *et al.,* 2019). A study conducted by Cox *et al.,* 2019 found evidence of nano and MPs in sugar (0.44MPs/g), salt (0.11 MPs/g), alcohol (0.03 MPs/g) and bottled water (0.009 MPs/g). Through diet, it is estimated that humans intake as much as 5g of MPs per week (Belzagui *et al.*, 2019). Although it is speculated that MPs bigger than 150 µm are unlikely to be absorbed by the human body, it is suggested that MPs smaller than 150 µm may cross over into lymph and the circulatory system (Yuan *et al.*, 2022). For transfer into organs to occur, ≤ 20 µm whereas 0.1>10 µm have shown evidence to access all organs, cross-cell membranes, the blood-brain barrier and the placenta (Barboza *et al.*, 2018). Zhu, L *et al.,* 2023 found within 17 placenta samples, an average of 2.70 ± 2.65 particles/g and a range of 0.28 to 9.55 particles/g of which 11 polymers were identified, PVC being the most common (43%) and ranged from 20.34 to 307.29 µm.

The ingestion of MPs in species that humans readily eat has been well documented (Alexandre *et al.*, 2016; Santonicola *et al.*, 2023). The common ditch shrimp (*Palaemon varians)*, in the same genus as the study species common prawn (*Palaemon serratus),* were found to have ingested microbeads of 0.1-99µm ad were found to break down the plastic when in the digestive system. It is estimated in the EU that 24kg (live weight) of fish or seafood is ingested per year, shrimps accounted for 1.47kg per capita and mussels 1.23kg per capita per person (EAA, 2022). Furthermore, it is estimated that within the EU, 11,000 MPs are ingested from shellfish (Smith *et al.*, 2018). Further understanding routes and uptake of MPs within marine species will further help us understand the possible MPs impacts on the human diet.

1.11 Objectives and hypothesis of this study

By using rockpool organisms that represent different feeding types, we can gain a greater understanding of MFs ingestion and interactions via the retention of MFs. By looking at biofouling, colour, size and interspecies interactions with MFs, will help further theories of how they may lead to bioaccumulation, trophic transfer, and biomagnification as well as impact key species on the rocky shore as well as ecological impacts from this. This study exposes three rockpool organisms that represent different feeding types, to MFs. By Exposing the beadlet anemone (*Actinia equina*) to MFs, we can observe how a non-selective

filter feeding can impact the amount of MFs it may ingest. Thick top shell (*Phorcus lineatus*) are grazers and by looking at this feeding type, we can susceptibility to MFs when they are found on the sediment or adhered to rocks or seaweed. Looking at filter feeding in the Common prawn *(Palaemon serratus)* will allow for a better insight into how selective feeding by be at play in MFs ingestion as well as how filter and detritivore behaviours can possibly impact trophic transfer. The aim of the study is to look for species association with BF and NBF MFs, different size MFs, different colour MFs, as well as the effects of individual or mixed communities of the organism.

Aim: To investigate whether there is a difference in the number of nylon microfibres, ingested or adsorbed by *Actinia equina*, *Phorcus lineatus*, and *Palaemon serratus* when tested *ex-situ* experimentally, dependant on colour, size and whether the species is housed independently or collectively as a mixed community of members from each species.

Hypotheses:

- There will be a statistical difference in the number of 0.5mm and 2mm nylon microfibres, ingested or adsorbed by *Actinia equina*, *Phorcus lineatus*, and *Palaemon serratus* when housed individually.
- There will be a statistical difference in the number of 0.5mm and 2mm nylon microfibres, ingested or adsorbed by *Actinia equina*, *Phorcus lineatus*, and *Palaemon serratus* when housed as a mixed community.
- There will be a statistical difference in the number of blue, black, red and white nylon microfibres, ingested or adsorbed by *Actinia equina*, *Phorcus lineatus*, and *Palaemon serratus* when housed individually.
- There will be a statistical difference in the number of blue, black, red and white nylon microfibres, ingested or adsorbed by *Actinia equina*, *Phorcus lineatus*, and *Palaemon serratus* when housed as a mixed community.
- There will be a statistical difference in the number of biofouled and not biofouled nylon microfibres, ingested or adsorbed by *Actinia equina*, *Phorcus lineatus*, and *Palaemon serratus* when housed individually.

- There will be a statistical difference in the number of biofouled and non biofouled nylon microfibres, ingested or adsorbed by *Actinia equina*, *Phorcus lineatus*, and *Palaemon serratus* when housed as a mixed community.
- There will be a statistical difference in the number 0.5 and 2mm ingested or adsorbed by *Actinia equina*, *Phorcus lineatus*, and *Palaemon serratus* overall.
- There will be a statistical difference in the number of blue, black, red and white ingested or adsorbed by *Actinia equina*, *Phorcus lineatus*, and *Palaemon serratus* overall.
- There will be a statistical difference in the number of biofouled and non biofouled ingested or adsorbed by *Actinia equina*, *Phorcus lineatus*, and *Palaemon serratus* overall.
- There will be a statistical difference in the number of fibres ingested or adsorbed by *Actinia equina*, *Phorcus lineatus*, and *Palaemon serratus* overall in an individual or mixed community setting.
- There will be a statistical difference in the number of fibres ingested or adsorbed by *Actinia equina*, *Phorcus lineatus*, and *Palaemon serratus* overall.

2 Method

2.1 Pilot study - Rockpool microplastic presence

Due to microplastic being under-studied within rockpool environments, there is little data which could be referenced or used to create a method and so a pilot was conducted to assess this. The aim was to determine the concentration and spatial distribution of microplastics within rockpools in order to determine concentrations of fibres as well as colour selection.

2.2 Method

2.2.1 Pilot Study area – In-situ presence of MPs

Water samples were collected on the rocky shore of Fistral Bay, Newquay (Cornwall, United Kingdom). Fistral Bay (50°41275'N, 5°10404'W) is an exposed west-facing bay. The bay has a border of cliffs the length of half of the rock pool site (Fig. 1) and is subject to Atlantic swell and subject to high-energy waves (Tumung *et al.*, 2012). There are two sources of input of MFs in this area are from surface runoff and sewage and storm overflows (Bathing water profile, 2023). Selected rockpools were approximately 1.5m in length and 400cm – 600cm in depth from the upper, middle, and lower shore (Davis *et al.*, 2018). This length and depth allowed for the prevention of hitting obstacles as well as avoiding hitting or running along the bottom of the rockpool.

2.2.2

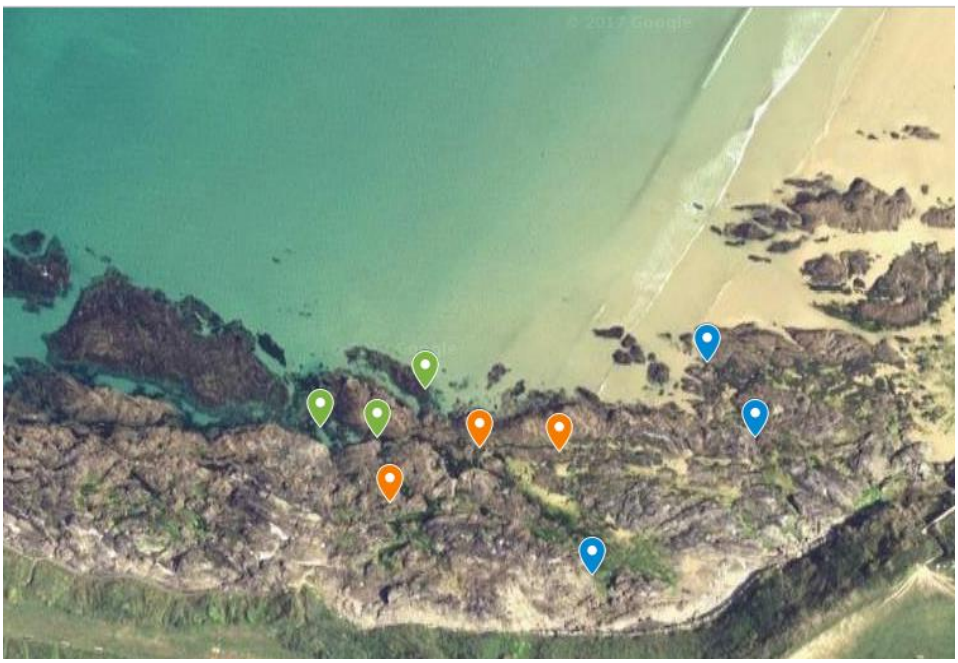

Figure 1 Location of sampling sites at Fistral Bay 50°41275'N, 5°10404'W. Image adapted from Google Map (2019)

Method

The use of one of the standardised mesh sizes of 200 µm, a trawl net of 1.9m length with a plastic sample bottle attached to a 300mm diameter ring was used (Pasquier *et al.*, 2022). Samples sites were horizontally towed at a distance of 1.5 m through the centre of the rockpool, trawling 106 litres of water per site. To assess the intertidal zone, three replicate pools at three different tidal heights (upper, middle and lower shore were towed, totalling nine rockpools sampled (Davidson *et al.*, 2004). To avoid cross-contamination during sampling, the net was cleaned in between sampling in order to lessen microplastic cross-over between each rockpool. The trawled water was held in 500 ml labelled bottles within a cool bag and transported to a refrigerator at Cornwall College Newquay (Cornwall, UK).

2.2.3 Sample analysis

Under a fume hood, each sample was individually filtered prior to analysis through a 20µm, 6cm diameter plastic mesh filter as there was a low level of marine-based organic debris. Using this finer mesh size allows for better and more accurate at retrieving microplastics (Kang *et al.*, 2015). After each sample was individually filtered, distilled water was used to rinse the mesh filter three times into the corresponding labelled glass 500ml beaker to assure all plastics are rinsed into the beaker. The sample, including the water, was stirred to

prevent settling and then further divided into two Petri dishes to allow for easier analysis. Each beaker was once again rinsed three times into a grid-marked Petri dish to prevent any plastics or zooplankton from being recorded and all beakers were covered with foil to avoid contamination.

Prior to counting, a Petri dish containing filtered distilled water was always placed next to the work area to assess any contamination in the samples. Petri dishes with the sample water were treated with 7% $MgCl_2$ to anesthetise any plankton, for ethical reasons before processing, without damaging the plastics themselves. Using a dissection microscope (35 x), all microplastics were counted in sections via the marked grids on the Petri dish. Microplastics were categorised into four: fibres, beads, film and fragments. Identifying any plastics was undertaken through the use of a seeker as the use of Transformed- Infra Red (FT-IR) or Raman Spectroscopy was not available for plastic-type identification. The seeker was able to manipulate and damage the item of interest. Plastics would crush and not tear however, organic materials would tear apart, tear, release liquid or disintegrate when manipulated with the seeker (Mariano *et al.*, 2021). The identification was into basic categories of fibres, beads, fragments, and films due to limited access to more specific identification tools. Microplastics were further categorised into colour as well as size: small 0.5mm – 1mm, medium 1.1mm -1.9mm and large 2mm – 5mm.

2.3 Results

Of the nine rockpools filtered, a total of 1487 microplastics were found. Fibres were found at a higher average in the lower sure (238.33) with the least in the upper shore (114)(Figure 2). Per litre, 1.56 plastics were found. Figures 3 and 4 show an example of the type of plastics that were found during the sampling, with Figure 3 showing signs of bioaccumulation.

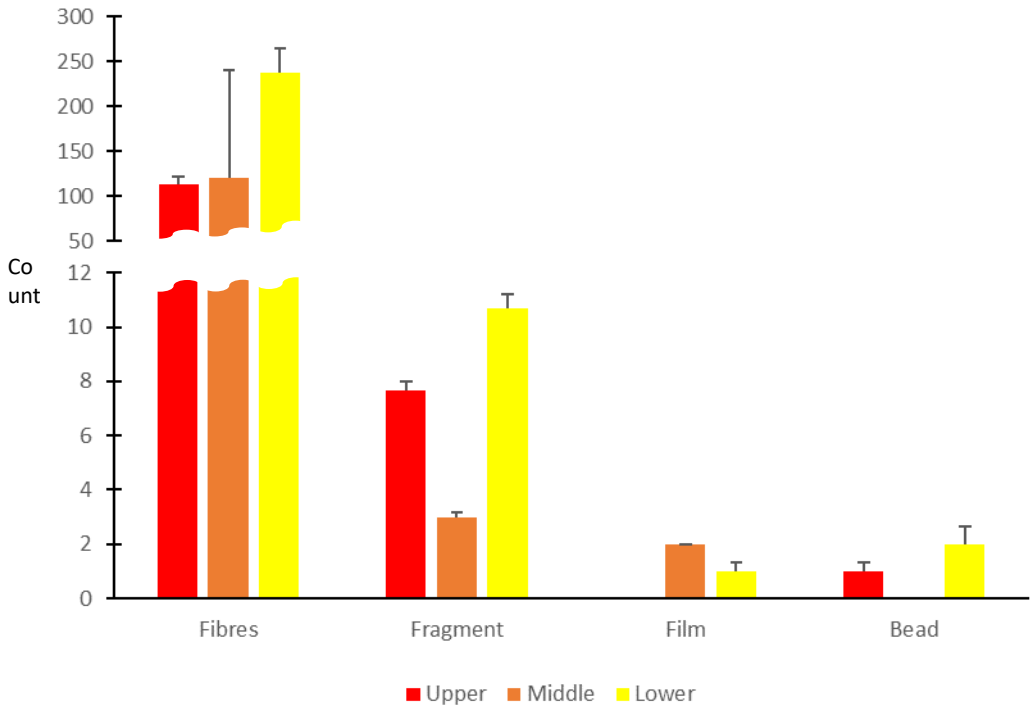

Figure 2 Average of each MP type found in the upper, middle and lower shore.

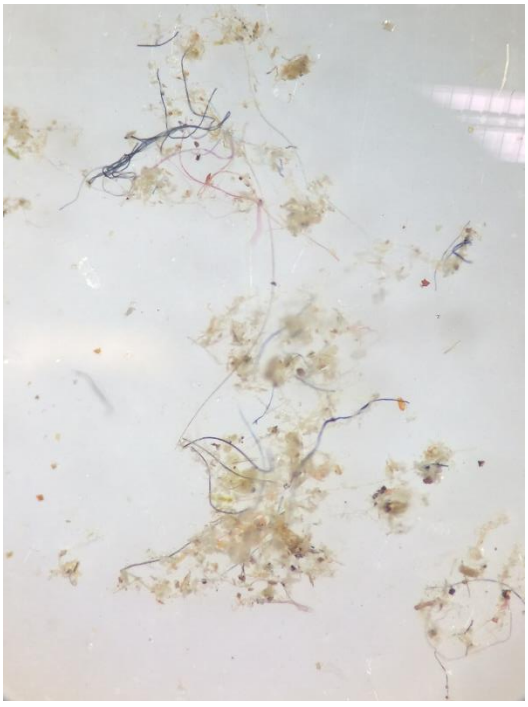

Figure 3 Example of MFs found in the rockpools showing entanglement with organic matter as well as with other MFs.

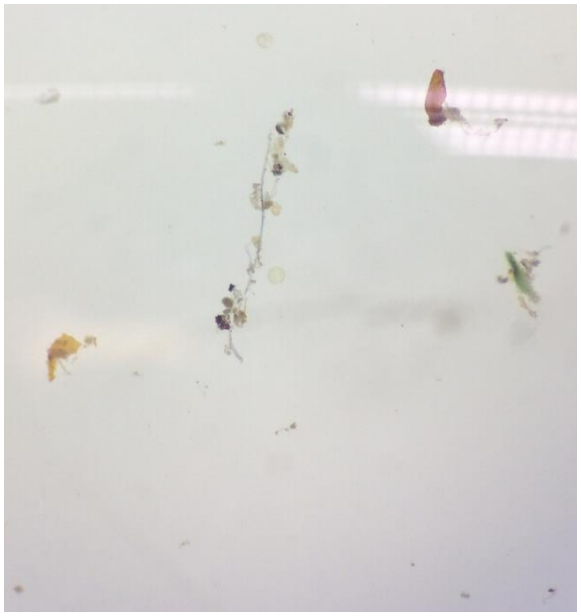

Figure 4 MFs found with evidence in rockpool with evidence of BF as well as an arthropod species interacting with the BF on the MF

2.3.1 MF colour in relation to shore height upper, middle and lower shore rockpools.

As MFs are the primary interest of the study, a further look into colour was needed to assess which colours were to be used. For ease of categorisation and the difficulty of identification, white fibre category includes any clear or grey-appearing fibres as well as white. Of the fibres, 152 of blue found, 649 black, 80 red, 117 white, 11 orange, 5 purple and 1 green. The most predominant colours found were blue, black, red and white which accounted for 1399 of the 1417 total fibres found.

2.4 Main method

2.4.1 Sample organism collection and preparation

Organisms were collected from the same location as the pilot study (Figure 1). Figure 4 shows a clearer view of the intertidal zone selected for the study.

For the study conducted, three organisms were selected to represent different feeding guilds within a rockpool environment. Individuals were selected of roughly the same size where possible. Shrimp were caught using a Flashmer Seiche Tele Landing Net (200 cm with a 50cmx50cm net and a mesh size of 20mm). Once caught, all shrimp were placed into a bucket (Figure 5) and those that did not match in size overall were released back into the rockpool in which they were found. Beadlet anemones and thick top shells were collected by hand. Extra organisms were collected in case of death however, all organisms left after the experiment were released.

Figure 5 Bucket in which organisms (in this case the common shrimp) were held to make sure organisms were of similar size and correct species.

Organisms used for the study were held in a 60-gallon holding tank which replicated a rockpool environment to allow for egestion of any matter ingestion prior to the study. They were held in this tank for at least a week to allow for settling into a new environment, settling onto rocks in the case of the anemones as well as said egestion. When tested as individuals, the holding tank consisted of just one species at one time. When a mixed community setting was to be tested, all three organisms (five of each) were housed together. To reduce egestion ingestion contamination, siphoning of the tank took place every day as well as two water filters constantly in places filtering waste out of the water. Reverse osmosis (RO) water with a pore size of approximately 0.0001 micron was used to reduce non-study organisms as well as microplastic contamination (Cyrus & Blabe, 1987). The salinity used for the study was set to 3.5% and the pH set to 8.2 which aligned with UK pH levels of coastal water (Birchenough *et al.*, 2017). The tank was run empty for one month to allow for bacteria build-up which made the water liveable to the organisms which will be placed into it. Water changes were also done with RO water.

The tank was filled with rocks from their *in-situ* environment which were dried and cleaned to remove organic materials. These were placed in a way to replicate different heights within a rockpool and also allow for the beadlet anemones to individually attach to a single rock to allow for easy moving for the study. No sediment was used at the bottom of the tank. A glass cover was added to the tank to prevent the thick top shells from coming out of the tank as well as the light hood on top. The glass cover and hood also acted as further protection from contamination from outside sources. To transfer organisms from *in situ* collection to the holding tank, organisms were caught using a mesh net (0.4 x 15.5 x 45.1 cm) or picked up by hand and placed into handpicked from the housing tank. This took 96 hours and allowed for settling into a new environment and allowed for feeding behaviour to return. Each organism was fed according to their specific feeding needs: Common shrimp were fed shrimp-specific pellets (API Bottom Feeder Shrimp Pellets), algae flakes (Aquarain complete nutrition flakes) and feed on a natural build-up of algae in the holding tank once a day. Beadlet anemones were spot-fed 10ml of freshly grown artemia as well as natural capture algae flakes which were fed to the rockpool shrimp. Thick top shells were fed algae pellets, 3cm of cucumber weighted down with a metal spoon as well as leftover flakes, pellets and natural algae build up in the holding tank.

2.4.2 MF set-up

To prepare MFs for the BF factor of the study, a lidded container of filtered seawater from *in-situ* was used and MFs added. To create the MP mix, 1g of each colour was added to the seawater and then the MFs were exposed for six hours prior to the study in order to gain some biofouling (Cho *et al.*, 2008). The use of six hours replicates the maximum time that plastic would be isolated in a rockpool to gain biofouling. For ease of counting, NBF MFs were added to a RO water in a lidded container (Figure 6). The nylon MFs were obtained from www.flock-king.co.uk with the colours red 22dtex, navy blue 22dtex, black 22dtex, and white 22dtex nylon flock in pack sizes 0.5mm and 2mm.

For the individually housed organisms (100 plastics per L) (NBF or BF), they were manually removed with tweezers under the dissection microscope and placed into individual 15ml falcon tube for transport. One tube was then poured into one beaker. For MF spiking of mixed community testing, a solution needed to be made which was done by using a Sedgewick rafting cell 1ml at a time to get a total per 10 ml. It was found that 0.002g of MFs made 477 fibres per 10 ml concentration. For 0.5mm it was found that 0.001g in 10ml made a concentration of 482 fibres per 10ml. This was undertaken for both NBF and BF MF. After mixing the solution, 10ml of the solution was pipette out and placed into a 15ml falcon which was then poured into the mixed community tank.

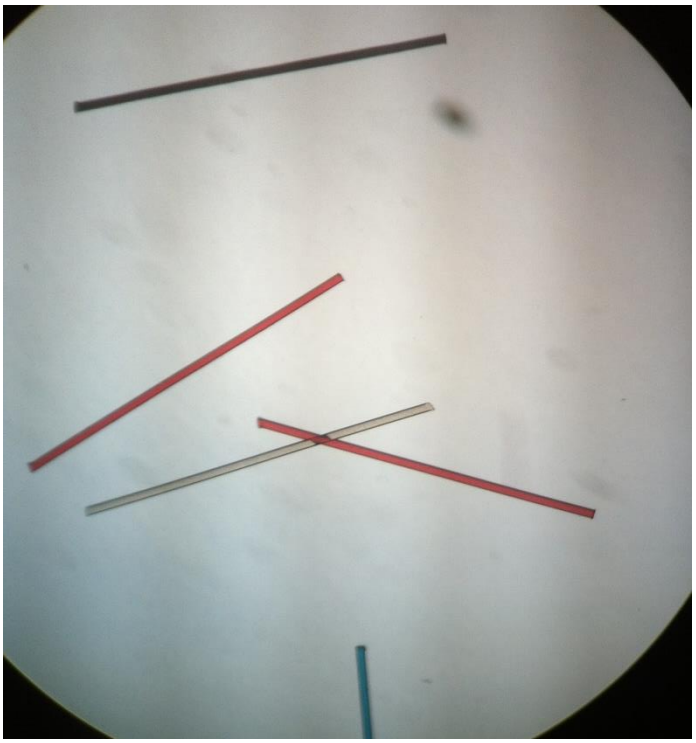

Figure 6 Imagine of MFs without BF. Imagine shows the colour of fibres and the identifiable shape of them under the dissection microscope compared to contamination plastics (Figure 14)

2.4.3 Set up of individual and community testing

Organisms used for the study were held in a 60-gallon holding tank which replicated a rockpool environment to allow for egestion of any matter ingestion prior to the study. Once ready for study, organisms were either caught using a mesh net (0.4 x 15.5 x 45.1 cm) or handpicked from the housing tank. Anemones were taken with the individual rock which they had attached. Organisms were rinsed with freshly made RO water of 3.5% salt and then put into a bucket of filtered holding tank water. They were then placed into either beakers or the testing tank using the net or taken by hand which was already filled with filtered holding tank water. The use of holding tank water was needed in order to maintain the natural bacteria to be present in the water which is vital to keeping water liveable for the organisms. The holding tank was then topped up with freshly made RO water.

Individuals testing organisms were subject to 40 plastics in 500 ml beakers filled with 400 ml of holding tank water was used to allow sufficient space for organisms to feed naturally and were sealed with tin foil (Saborowski *et al.*, 2018). The water had been filtered to remove

contaminants. From this, either a 10ml stock was taken or fibres handpicked under a dissection microscope which was also then used to check for contamination (Richter optica S2-SPS 10x/30x stereo microscope). Each beaker was subject to slow movement during this time via a shaker arm at 100rpm (Stuart Flask Shaker with 2 Side-Arms; 8 Clamps and Allen Key; 80 - 800 rpm Min/Max Speed) to prevent potential settling on the bottom, which would bias thick top shells as they are grazers. The density of nylon fibres is between 1.25-1.60 and so does not readily float in seawater as seawater is 1.02 – 1.03 g/cm3 (Table 1).

Mixed community testing was undertaken with five of each organism housed together (a total of 15 in each mix community tank) exposing them to the solution from the MF set-up was used 477 fibres per 10 ml concentration solution. This was undertaken in a 25-litre glass tank. The filter was turned off and a bubbler was used to keep water moving (Hidom HD-601 Single Outlet Aquarium Air), allowing microplastics to keep moving around the tank. The bubbler consisted of a plastic level hose (40cm) with holes drilled 0.5cm apart, an airflow regulator, rocks to hold the tubing in place and a Hidom HD air pump kit. The tank was dismantled and cleaned thoroughly and rebuilt to undertake the next MF size testing.

2.4.4 Main study testing

Prior to the experiment, beakers were filled with filtered housing tank water using a 200 µm, plastic mesh. A 6cm diameter filter was used for water for individual housing and 15cm was used for mixing due to the amount of water needing to be filtered. This process was undertaken under a fume hood to reduce the risk of contamination. The water was then distributed into the beakers or housing tank for the experiment and then sealed with foil to further reduce contamination risk. After the beakers or tank were set in place, the equipment previously mentioned was added and then organism/s were added. MFs were then added after all set-up was completed and then the shaker or bubbler was turned on for the experiment.

Data collection for each individual organism went as follows (Figure 7): five beakers, each containing one organism, four colours type – ten of each, totalling 40 fibres and NBF plastics. Five beakers contained five organisms, four microplastic colours of which are BF. Five beakers contained five organisms with no microplastics at all as a control. One beaker

only had RO water. This process was repeated for two sizes of microplastic – 0.5mm and 2mm and was undertaken on a total of 30 beadlet anemone (*Actinia equin*a), 30 common prawn *(Palaemon serratus)* and 30 thick top shells (*Osilinus lineatus*) (Figure 7). Each beaker had a foil lid and the premade solution containing the 40 fibres was added to the beaker and then sealed back over to reduce contamination

As with the individual exposure process, it went as follows: Four microplastic colours, NBF plastics. No microplastic exposure at all as a control. Four microplastic colours of which are BF. One beaker next to the tank was kept empty, except for water, as a control (Figure 7).

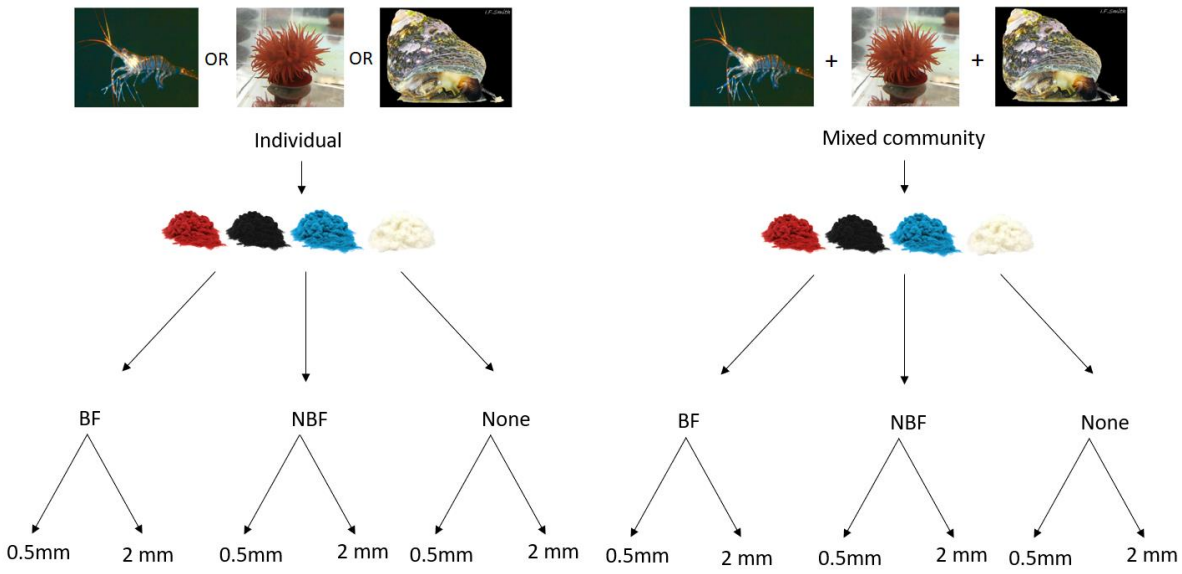

Figure 7 Diagram of the process of the method. Images adapted from Smith, 2015; Maskrey *et al.,* 2021; Bianchi *et al.,* 2022.

2.4.5 Digestive tract dissection

After six hours of exposure to the MP was completed. Organisms were anaesthetized using to 7% MgCl pipette into the beakers and then were frozen euthanised and preserved for future analysis (Figure 8,9 and 10) (Al-Badran *et al.*, 2018; Murray, M. J., 2006). A new pipette tip was used for each beaker to reduce cross-contamination. The first analysis was undertaken under a dissection microscope (Richter optica S2-SPS 10x/30x stereo microscope) and was used to observe the digestive tract content to see the ingestion of microplastics (Figure 11. This was undertaken in a Petri dish with a scalpel, surgical scissors and a dissection needle. Water was also observed under the dissection microscope to retrieve, counted and remove MFs to allow for all MF to be accounted for after tissue digest.

Figure 8 Imagine of common prawn after euthanising and defrosting, ready for digestive tract dissection. 2.4cm coin used for size reference. Organism had already been taken out of the water in which it was frozen.

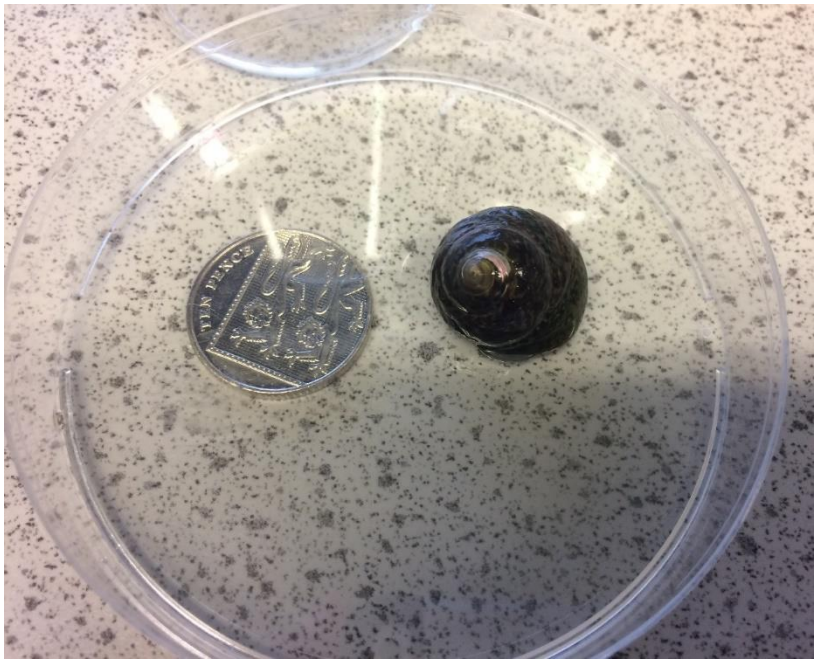

Figure 9 Imagine of thick top shell after euthanising and defrosting, ready for digestive tract dissection. 2.4cm coin used for size reference. Organism had already been taken out of the water in which it was frozen.

Figure 10 Imagine of beadlet anemone after euthanising and defrosting, ready for digestive tract dissection. 2.8cm coin used for size reference. Organism had already been taken out of the water in which it was frozen.

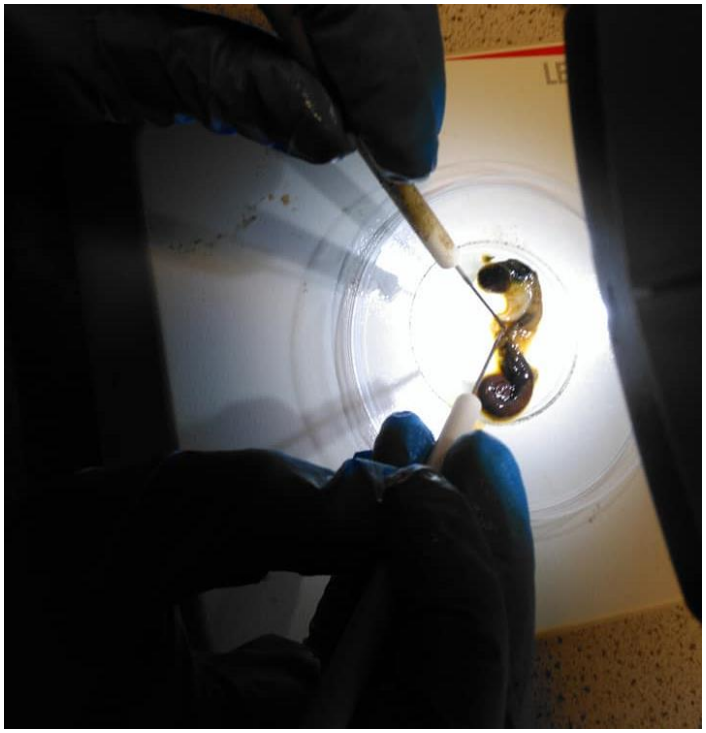

Figure 11 Beginning of dissection of thick top shell digestive trac under dissection microscope.

2.4.6 Alkaline Tissue Digestion

All contents for all organisms of the study were then subject to alkaline tissue digestion using 2M solution (112.22g in 1L distilled water) potassium hydroxide (KOH) for two hours whilst boiling on a hot plate (Cole-Parmer™ Stuart™ Hot Plate SD500), under a fume hood (Alexandre *et al.*, 2016). Organisms, after dissection, were held with tweezers and then rinsed into the dissection dish and then placed into a new 250ml beakers, lidded with foil, and the KOH 2M solution was added to cover the whole organisms. After the alkaline tissue digest was completed, the contents were rinsed into a Petri dish using distilled water from a wash bottle, lidded and then counting took place under a dissection microscope. A final count of study MFs, as well as contamination was taken to ensure all MFs were accounted for at the end of the study.

2.4.7 Contamination

Ways to avoid contamination were considered during the study. Whenever possible, covering samples was undertaken to stop MFs from falling into the sample from the surroundings. Regular cleaning was undertaken of the holding tank to reduce egestion contamination to other organisms however this could not eliminate possible contamination. Interaction with the holding tank was kept to a minimum to avoid MF introduction from the outside environment. Cleaning and water changes of the holding tank were undertaken through the enclosed lid and were only opened as far as necessary. The RO water was made in a cleaned 220L water barrel and the was lid was kept on with only a small pipe placed into a top fill hole. Salts used were stored in a plastic bucket and kept sealed when not being used for the experiment. To identify contamination during open-lid dissection and alkaline tissue digestion, a Petri dish with water was used to capture any contamination presence. After each experiment in the mixed community housing tank, the tank was fully broken down and cleaned to remove any possible contamination from the previous study. The water filter for the tank however was kept but cleaned after each experiment also. Equipment was cleaned or new was used to reduce cross-contamination. Although steps were taken to reduce contamination, contamination was found (Figure 12, 13) however, contamination was easily identifiable to those of the study fibres.

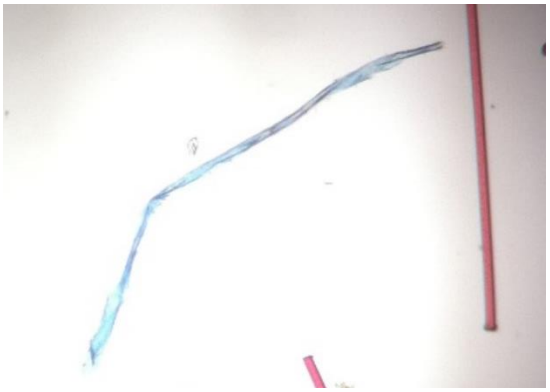

Figure 12 Blue MF contamination found during water observations of a common prawn subject to individual testing. In the presence of the study MFs (red) it is easily identifiable as contamination

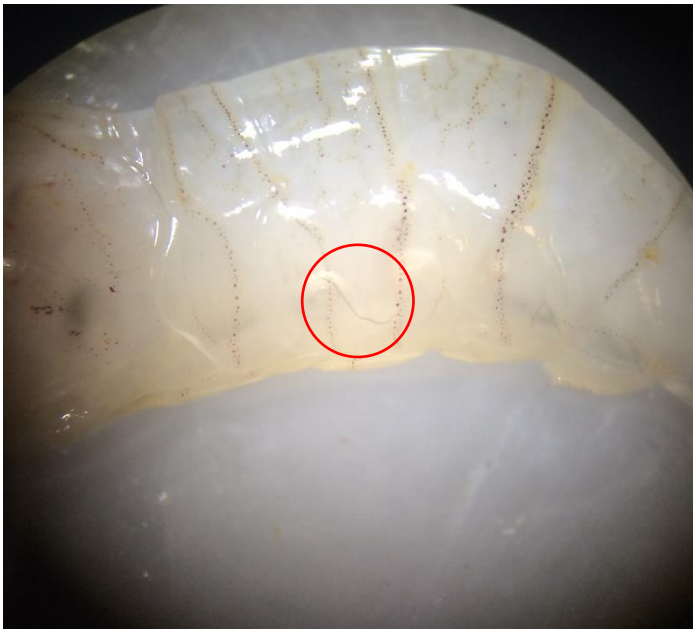

Figure 13 Contamination MF under the shell of a common prawn before digestive tract dissection and alkaline tissue digest

2.4.8 Statistical analysis

Microsoft Excel (Version 2022 for Microsoft) was used for raw data and graph composing. IMS SPSS (Versions 28.0.1) was used to carry out Freidman two-way ANOVA to find general differences and then non-parametric Kruskal–Wallis one-way analysis of variance was used to obtain further detail of significances. To avoid the chance of a Type I error when conducting multiple tests, a Bonferroni adjustment was applied. For contamination data, a mix of Chi-squared was used with a Bonferroni adjustment as well as a one-sample Binominal test. The significance level was set to $p < 0.05$.

3 Results

3.1 Ingestion

One of the main aims of the study was to look for evidence of ingestion of spiked MP fibres within selected organisms. Observations and dissection of the digestive tract were undertaken with fibres counted and noted to which sample the fibres were from. For ease of writing and space in diagrams, the study organisms will be referred to as Beadlet anemone *(Actinia equina)* - anemones, Thick top shell *(Phorcus lineatus)* – snail and Common prawn *(Palaemon serratus)* – shrimp.

3.1.1 Individually housed organism MF ingestion

Anemones exposed independently to 0.5mm MF had the highest ingestion rate following dissection with white BF fibres (H(1)= 3.888 , *p*=0.049) found significantly more than other colours. On average, anemones ingested 1 BF, 1.15 NBF fibre. Shrimp were found to ingest BF and NBF but no significant difference was found (H(1)=12.742 *p*=0.218). On average, shrimp ingested 0.3 BF and 0.6 NBF fibres. Snails were found to ingest BFs most (H(1)= 7.813 , *p*=0.005) with blue being the highest, however, no significant colour differences were found (Figure 14). Snails on average ingested 1.05 BF and 0 NBF fibres.

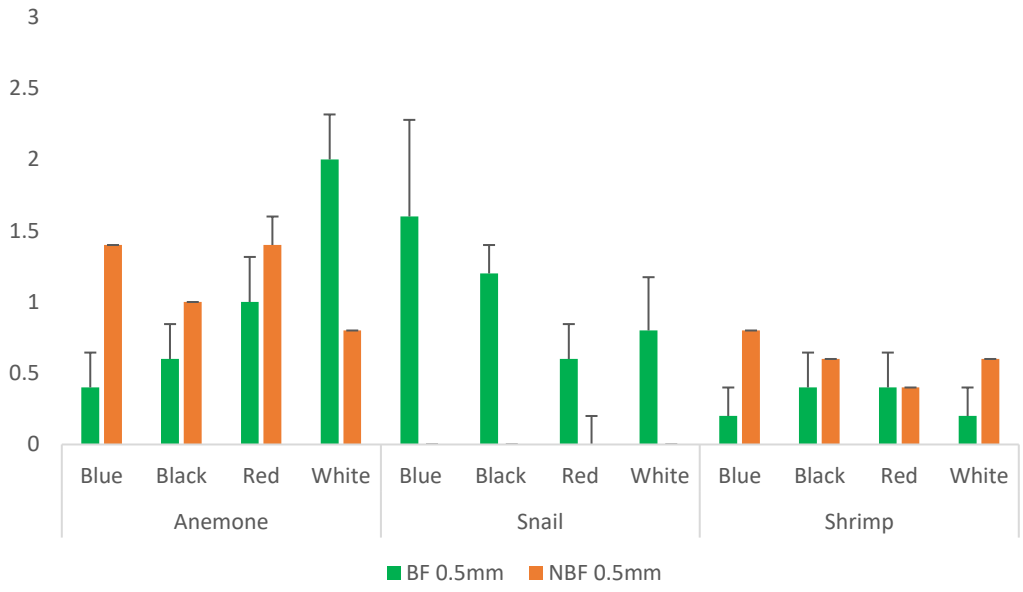

Figure 14 Mean (± se) number of ingested 0.5mm MFs found to be ingested of BF and NBF MFs per organism

per colour when individually housed.

3.1.2 Individually housed organism MF ingestion 2mm MFs

Within the 2mm spiked MF, anemones had the highest rate of ingestion in BF fibres compared to snails and shrimps (Figure 15) and only red NBF fibres were found within anemones and snail digestive tracts. Anemones on average ingested 1 BF and 0.05 NBF fibres. Snails had a lower rate of ingestion. Snails on average ingested 0 BF and 0.05 NBF fibres. There was a significant difference between the exposure and coloured fibres ingested by Shrimp which showed a pattern towards red fibres that were BF - $H(1)= 3.857$, $p=0.050$ and black BF fibres – $H(1)= 75.714$, $p=0.017$ being associated with more than red and black NBF fibres. Shrimp also had an overall association with BF fibres $H(1)= 5.538$, $p=0.019$). Shrimp saw an average 0.65 BF and 0 NBF fibre ingestion. Anemones did not have colour significance however, they were found to have a higher uptake of BF fibres than NBF $H(1)= 6.776$, $p=0.009$) (Figure 15)

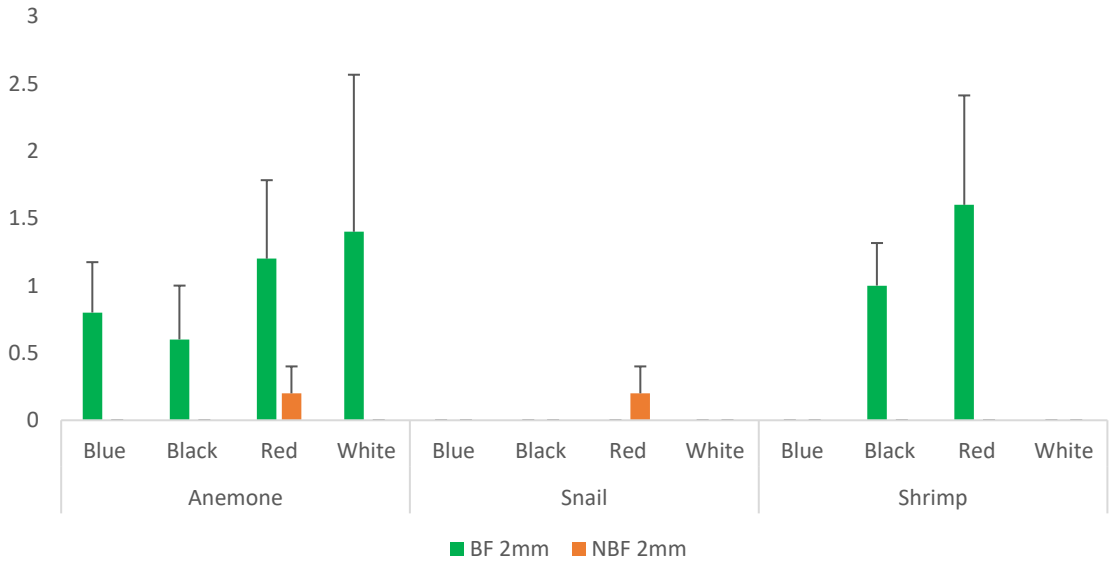

Figure 15 Mean (± se) number of coloured 2mm MFs ingested by the three test species for BF and non-biofouled (NBF) trials.

3.1.3 Individually housed organism MF ingestion 0.5 and 2mm MF

When comparing 0.5mm ingestion to 2mm ingested, anemones were found to ingest the most plastic of all the organisms with a higher number of 0.5mm ($H(1)= 12.258$, $p=0.007$)(Figure 16). Overall, anemones had ingested more white 0.5mm fibres ($H(1)= 14.698$, $p=0.002$). Shrimp ingested significantly more BF 2mm fibres compared to 2mm NBF fibres $H(1)= 9.318$, $p=0.025$) as well as significantly more 0.5mm NBF fibres than 2mm NBF fibres $H(1)= 9.318$, $p=0.025$). Overall, 0.5mm was ingested more than 2mm ($H(1)=7.877$, $p<0.001$)in anemones and snails, and BF fibres more than NBF ($H(1)= 11.667$, $p=0.005$) in anemones and shrimp.

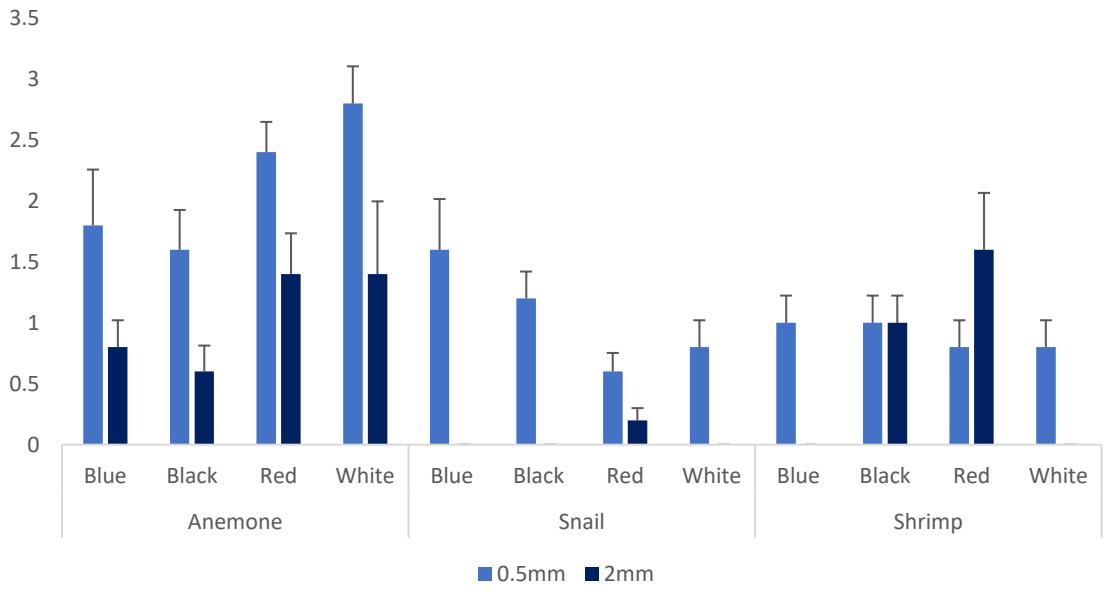

Figure 16 All plastics ingested when individually housed showing colour and size interactions per organism.

3.2 Community housed organism MF ingestion

Unlike individual ingestion testing, mixed testing consisted of all the organisms tested within one tank exposured to the spiked MF. This allowed for the assessment of the community effect on MF ingestion and interaction.

3.2.1 Mixed housed organism MF ingestion 0.5mm MFs

Ingestion of 0.5mm fibres was seen in all three organisms, however, the lowest intake was seen in snails which only ingested BF fibres. Overall, no other significant differences were found for all species (Figure 17). On Average, anemones ingested 1.45 BF ad 1.5 BF fibres, Snails 0.25 BF and 0 NBF and shrimp 0.95 BF and 0.95 NBF fibres.

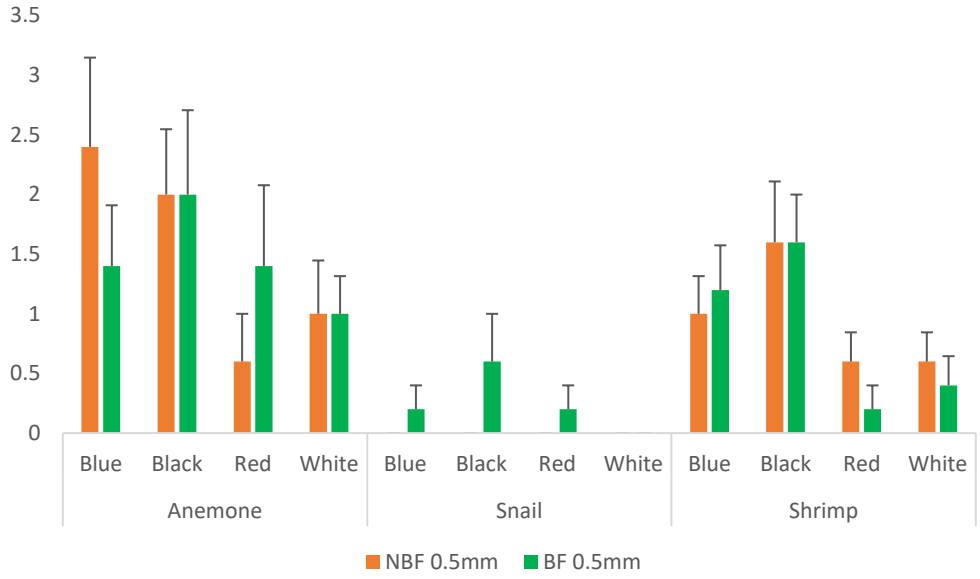

Figure 17 Average number of ingested 0.5mm MFs found to be ingested when housed in a mixed setting of BF and NBF MFs per organism per colour.

3.2.2 Mixed housed organism MF ingestion 2mm MFs

Observation of 2mm found that snails did not ingest any fibres and so are excluded from the analysis (Figure 18). Both anemones and shrimp had higher mean (± se) number of BF MF ingested compared to NBF ($H(1)= 6.944$, $p=0.008$ and $H(1)= 7.212$, $p=0.007$) with anemones ingesting the most plastics of all organisms. Anemones, on average ingested 13 BF fibres with a lot less for NBF fibres on average (0.25 NBF fibres). Overall black fibres were ingested in higher numbers than other colours and were found to be associated with both anemones and shrimp ($H(1)= 6.224$, $p=0.013$ and $H(1)= 6.008$, $p=0.014$) . Further to this, anemones also saw more black BF ingestion $H(1)= 4.078$, $p=0.043$) and shrimp a red NBF association

H(1)= 3.857 , *p*=0.050). Shrimp ingested, on average 0.75 BF and 0.15 BF fibres.

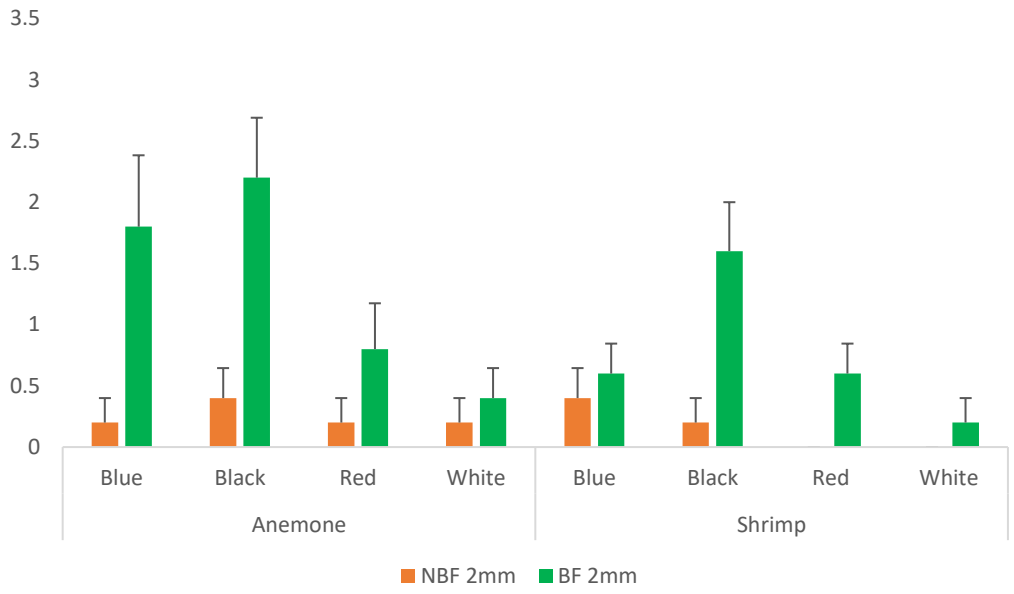

Figure 18 Mean (± se) number of ingested 2mm MFs s found to be ingested when housed in a mixed setting of BF and NBF MFs per organism per colour.

3.2.3 Mixed community organism ingestion of 0.5 and 2mm MFs

Comparing 0.5mm with 2mm, anemones were not found to have any difference in the MF colour ingested however, there was a significant difference in the ingestion of BF and NBF with BF being more common (H(1)= 11.012 , *p*=0.012)(Figure 19). Snails were found to ingest 0.5mm fibres during mixed testing which was also found seen when in individual housing. Shrimp were found to ingest more black (H(1)= 12.270 , *p*=0.007) and white fibres (H(1)= 8.143 , *p*=0.043) and significantly more 0.5mm fibres (H(1)= 15.909, *p*<0.001). Collectively, colour patterns were found with black (H(1)= 3,882, *p*=0.049) and blue (H(1)= 4.777, *p*=0.029) for ingestion compared as well as BF and 0.5mm associations (H(1)= 4.841, *p*=0.028 and H(1)= 7.837, *p*<0.005)

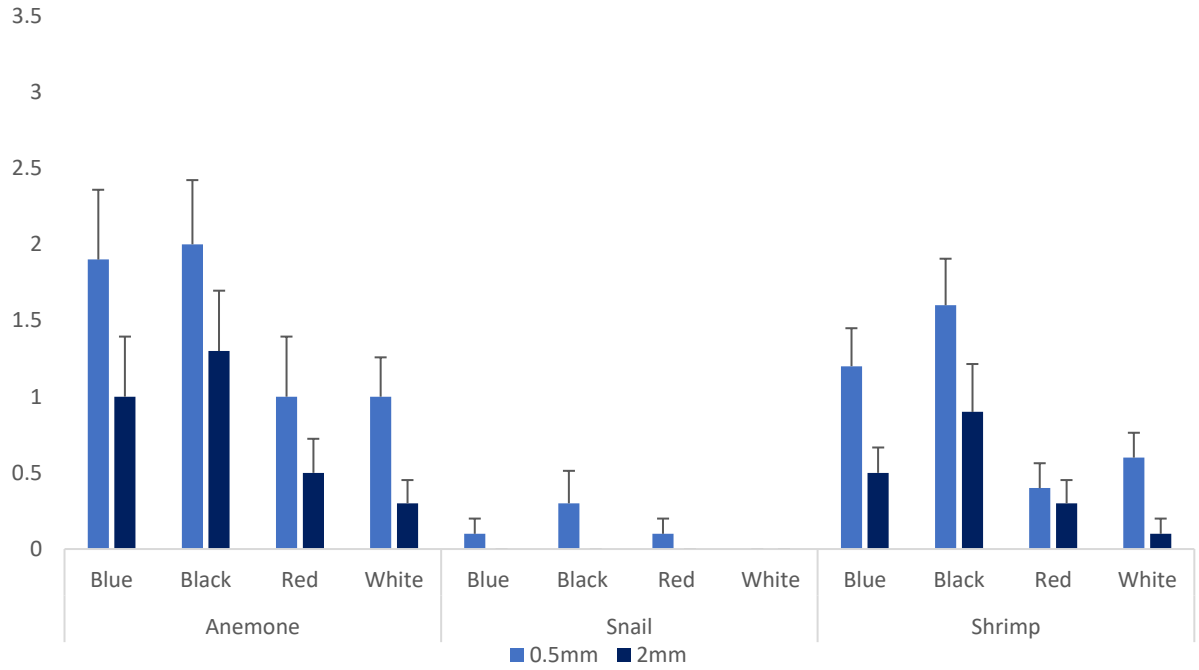

Figure 19 All plastics ingested when within a mixed housing, showing colour and size interactions per organism.

3.3 Individual vs mixed ingestion of MFs

Individual and mixed organism spiked ingestion was compared to find any difference specific to these factors (Figure 20, table 2). Shrimp ingested significantly more MF in mixed testing ($H(1)= 6.209$, $p=0.013$), with more BF fibres ingested than NBF fibres overall ($H(1)= 24.024$, $p<0.001$). On average, anemones ingested 1.48 fibres when individually housed and slightly less when housed communally – 1.43 fibres. Shrimp saw an overall, mixed communities that resulted in more MFs ingested than when housed individually ($H(1)= 8.073$, $p=0.018$) and more 0.5mm fibres than 2mm ($H(1)= 17.837$, $p<0.001$) overall. Shrimp, on average, ingested more fibres communally (0.81) compared to 0.5 when housed individually. Snails saw a large difference between individual housing (0.52) and communal (0.06).

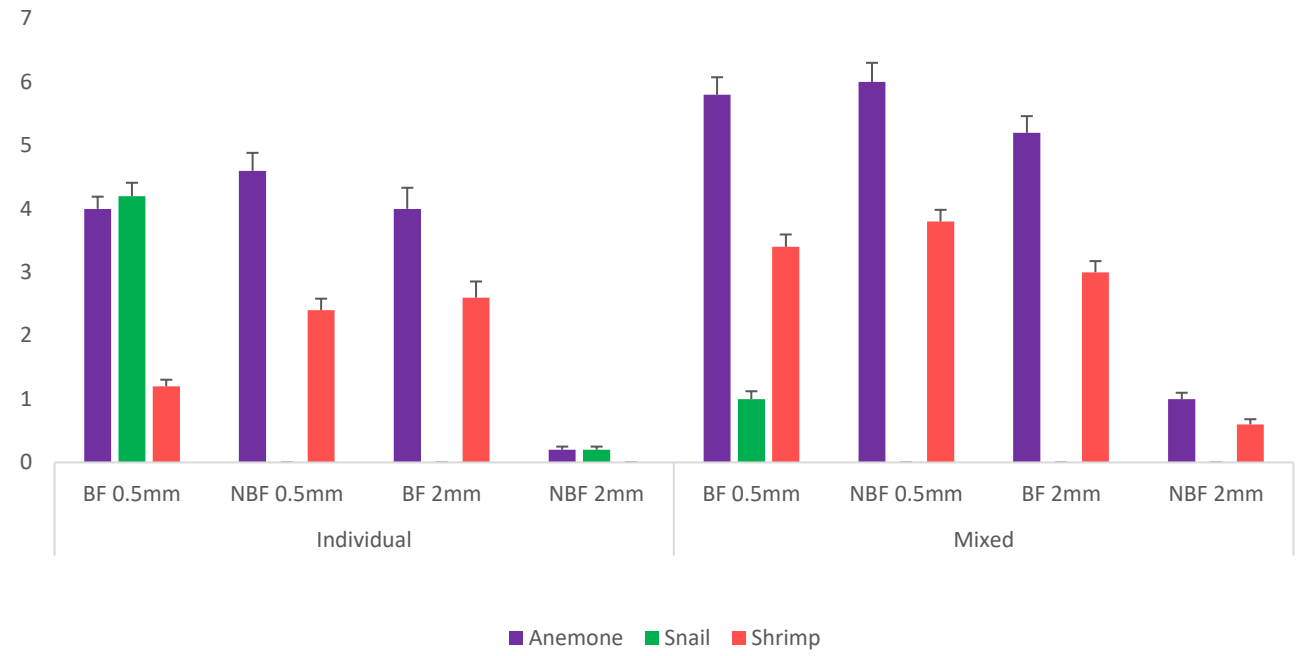

Figure 20 All plastics ingested when within an individual setting compared to a mixed setting, showing colour and size interactions per organism.

Table 2 p values obtained with Kruskal-Wallis tests and averages presenting organism's initial ingestion of study MFs.

Shrimp				
Treatment	Factors	H-value	*P*-value	Average
Housing (1, N = 160)	Individual: Mixed	7.575	0.005	Individual (0.4) Mixed (0.7)
Treatment (1, N = 160)	BF:NBF	5.1501	0.023	BF (0.7) NBF (0.4)
Size (1, N = 160)	0.5mm:2mm	7.1765	0.007	0.5mm (0.7) 2mm (0.4)
Colour (3, N = 160)	Blue,Black,Red,White	2.0023	0.571	Blue (0.8) Black (0.9) Red (0.7) White (0.3)

Snail				
Treatment	Factors	H-value	*P*-value	Average
Housing (1, N = 160)	Individual: Mixed	4.2181	0.4	Individual (0.7) Mixed (0.06)
Treatment (1, N = 160)	BF:NBF	6.0793	0.013	BF (0.3) NBF (0.01)
Size (1, N = 160)	0.5mm:2mm	6.0793	0.013	0.5mm (0.3) 2mm (0.01)
Colour (3, N = 160)	Blue,Black,Red,White	0.5972	0.897	Blue (0.2) Black (0.2) Red (0.13) White (0.1)

Anemone				
Treatment	Factors	H-value	*P*-value	Average
Housing (1, N = 160)	Individual: Mixed	3.555	0.6	Individual (0.8) Mixed (1.25)
Treatment (1, N = 160)	BF:NBF	7.7166	0.005	BF (1.2) NBF (0.7)
Size (1, N = 160)	0.5mm:2mm	13.8874	0.001	0.5mm (1.3) 2mm (0.7)
Colour (3, N = 160)	Blue,Black,Red,White	0.9341	0.817	Blue (1) Black (1.1) Red (0.9) White (0.9)

Overall				
Treatment	Factors	H-value	*P*-value	Average
Housing (1, N = 480)	Individual: Mixed	3.0164	0.082	Individual (0.5) Mixed (0.6)
Treatment (1, N = 480)	BF:NBF	16.135	0.0006	BF (0.7) NBF (0.4)
Size8 (1, N = 480)	0.5mm:2mm	25.122	<0.0001	0.5mm (0.8) 2mm (0.4)
Colour (3, N = 480)	Blue,Black,Red,White	6.5039	0.089	Blue (0.6) Black (0.7) Red (0.5) White (0.4)

3.4 Spiked plastic retention

After all organisms were subject to KOH treatment, fibres were counted to account for all spiked fibres added during the experiment. If all the fibres were accounted for after the alkaline tissue digest but were not all present prior to the digest, then it can be assumed that the spiked plastics were retained within the organism in some form. Elimination of fibres being attached to the shell or container before analysis allows for the assumption that any fibre can be assumed to have been found under the organism's shell or within the organism. This retention could be from within mucus, strongly entangled on appendages (organisms were rinsed into the sample water thoroughly before gut observation) or stored within the tissue.

3.4.1 Individual retention after alkaline digest

Red fibres were the most retained in anemones after alkaline digest ($H(1)= 27.728$, $p<0.001$) however, due to the nature of the organism, this could be due to extraction difficulty, particularly the red base colour of the anemones digestive tract when undergoing dissection (Figure 21). This could also be the opposite case with white fibres, with white fibres being very easy to recover. Both anemones and shrimp saw retention more in BF fibres than NBF fibres ($H(1)= 5.951$, $p=0.015$ and $H(1)= 5.024$, $p=0.025$). Anemones saw an average of 0.7 BF and 0.8 NBF fibres retained. Shrimp saw 0.4 BF and 0 NBF fibres. Snails were found to retain more fibres from BF than NBF with a significance found in 0.5mm fibres ($H(1)= 7.813$, $p=0.005$). On average, snails retained 0.1 BF and 0 NBF fibres.

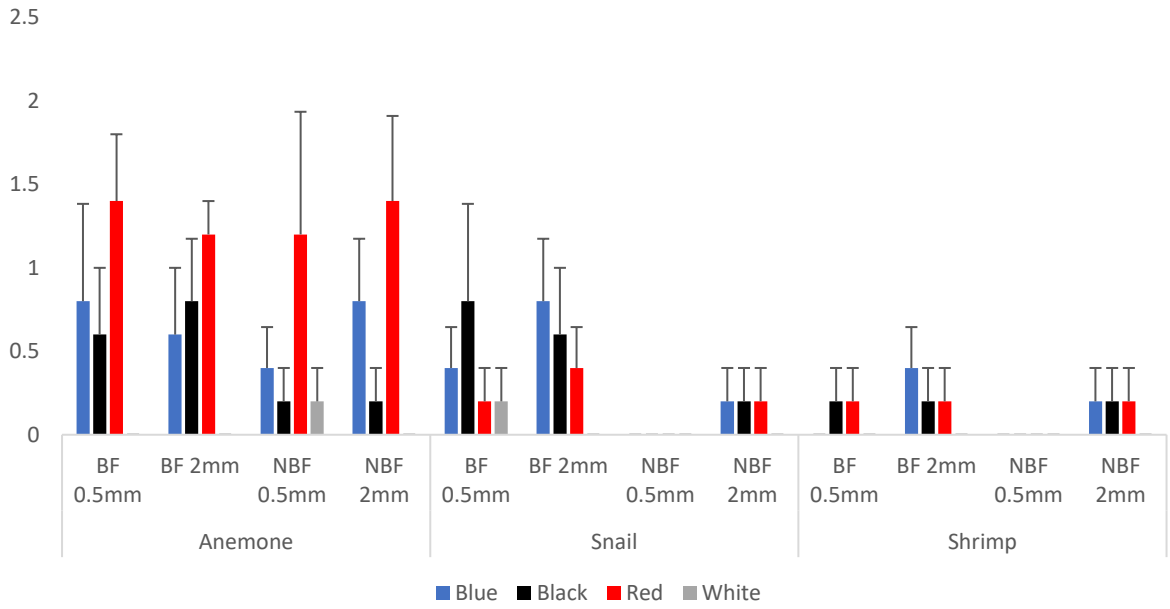

Figure 21 MFs retained when within an individual setting showing colour, MF exposure and size interactions per organism.

3.4.2 Mixed community organism retention after alkaline digest

After alkaline digestion snails were found to not retain any fibres and so are excluded from mixed testing analysis, this also corroborates with the very low ingested found during dissection observation of the mixed snails (Figure 20). Snails saw an average of 0.2 BF and 0.15 NBF fibres. Overall, red was the most frequently retained ($H(1)= 5.470$, $p=0.019$) which was seen to be found in both anemones and shrimp. Anemones were also found to have an association with black ($H(1)= 8.235$, $p=0.004$)(Figure 22). Anemones, on average, retained 0.65 BF and 0.6 NBF fibres and shrimp retained 0.45 BF and 0.15 NBF fibres.

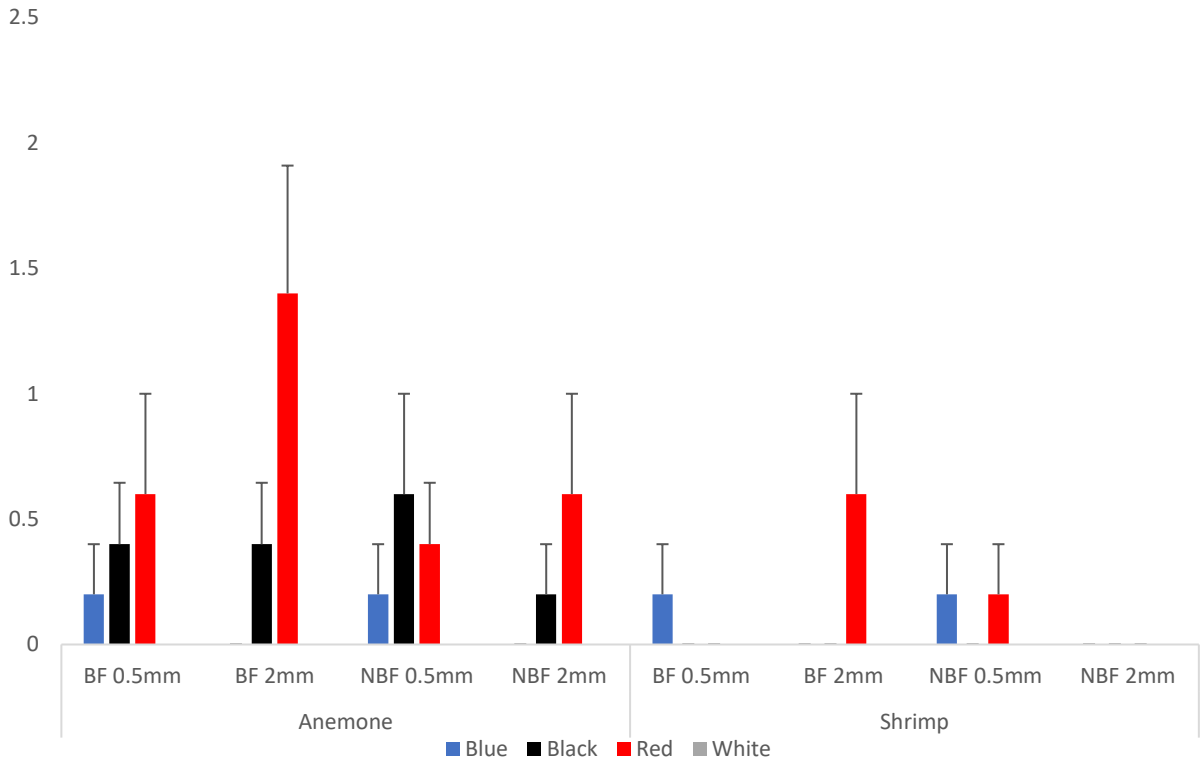

Figure 22 MFs retained when within a mixed setting showing colour, MF exposure and size interactions per organism.

3.4.3 Individual compared to mixed community organism retention after alkaline digest

When comparing individual testing to mixed testing overall there was not a patten of increased retention in individual testing ($H(1)= 2.282$, $p=0.131$) compared to mixed (Figure 23,table 3). Snails were seen to only retain MF in an individual setting. Anemones associated with blue MF in a individual setting over mixed, in terms of retention ($H(1)= 15.284$, $p<0.001$) as well as red $H(1)= 16.526$, $p<0.001$). Anemones on average retained more MFs when individually housed (0.68) compared to 0.31 in a community setting. There was on overall association to 0.5mm fibres over 2mm ($H(1)= 18.290$, $p<0.001$) and BF to NBF ($H(1)= 11.974$, $p<0.001$). Snails on average saw a slightly higher average during individual housing (0.11) than that of a community setting (0). Shrimp saw a slight increase in average in an individual setting (025) than that of a community setting (0.07).

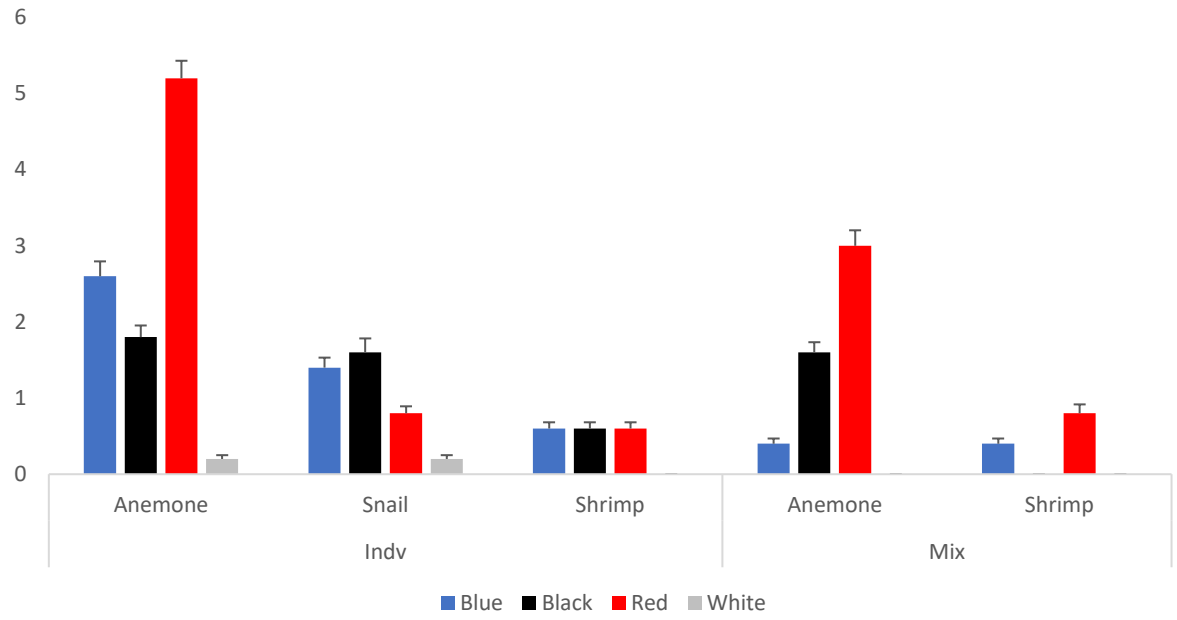

Figure 23 MFs retained when within an individual compared to a mixed housing, showing colour interactions per organism.

Table 3 p values obtained with Kruskal-Wallis tests and averages presenting organisms retention of study MFs.

Shrimp				
Treatment	Factors	H-value	*P*-value	Average
Housing (1, N = 160)	Individual: Mixed	0.281	0.59	Individual (0.1) Mixed (0.07)
Treatment (1, N = 160)	BF:NBF	0.592	0.44	BF (0.15) NBF (0.05)
Size (1, N = 160)	0.5mm:2mm	0.307	0.57	0.5mm (0.06) 2mm (0.1)
Colour (3, N = 160)	Blue,Black,Red,White	1.578	0.66	Blue (0.13) Black (0.07) Red (0.18) White (0)

Snail				
Treatment	Factors	H-value	*P*-value	Average
Housing (1, N = 160)	Individual: Mixed	4.77	0.02	Individual (0.25) Mixed (0)
Treatment (1, N = 160)	BF:NBF	1.905	0.16	BF (0.2) NBF (0.03)
Size (1, N = 160)	0.5mm:2mm	0.29	0.58	0.5mm (0.1) 2mm (0.15)
Colour (3, N = 160)	Blue,Black,Red,White	1.06	0.78	Blue (0.18) Black (0.2) Red (0.1) White (0.03)

Anemone				
Treatment	Factors	H-value	*P*-value	Average
Housing (1, N = 160)	Individual: Mixed	4.815	0.02	Individual (0.9) Mixed (0.3)
Treatment (1, N = 160)	BF:NBF	0.55	0.45	BF (0.5) NBF (0.5)
Size (1, N = 160)	0.5mm:2mm	0.0098	0.92	0.5mm (0.5) 2mm (0.5)
Colour (3, N = 160)	Blue,Black,Red,White	23.025	0.00004	Blue (0.4) Black (0.4) Red (1) White (0.2)

Overall				
Treatment	Factors	H-value	*P*-value	Average
Housing (1, N = 480)	Individual: Mixed	8.35	0.003	Individual (0.4) Mixed (0.12)
Treatment (1, N = 480)	BF:NBF	2.344	0.12	BF (0.3) NBF (0.2)
Size8 (1, N = 480)	0.5mm:2mm	0.483	0.48	0.5mm (0.23) 2mm (0.25)
Colour (3, N = 480)	Blue,Black,Red,White	13.95	0.002	Blue (0.22) Black (0.23) Red (0.43) White (0.06)

3.5 Cumulative uptake of study MFs

In order to see a spiked MF interaction, both ingestion and retention data were analysed together to observe any difference overall (Figure 25, table 4).

Anemones saw the highest rate of overall interaction of MF with no specific observations to spiked fibres exposure or size but, did find a stronger association to mixed setting over individual (H(1)= 8.922 , p=0.003)(Figure 24). Although overall there was an increased uptake compared to a mixed setting (H(1)= 4.461 , p=0.035), snails were found to interact more with MFs when in individual housing (H(1)= 4.281 , p=0.039) with BF fibres significantly more (H(1)= 7.407 , p=0.006). Shrimp, like anemones, were not found to have a specific size or exposure association, however, did see an association to interact more with MF in a mixed setting (H(1)= 7.042 , p=0.008). Overall, there was an association to more MF interaction in a mixed setting than not (H(1)= 10.118 , p=0.006). There was an overall association to 0.5mm fibres over 2mm (H(1)= 20.924 , p<0.001) and BF to NBF (H(1)= 16.780 , p<0.001).

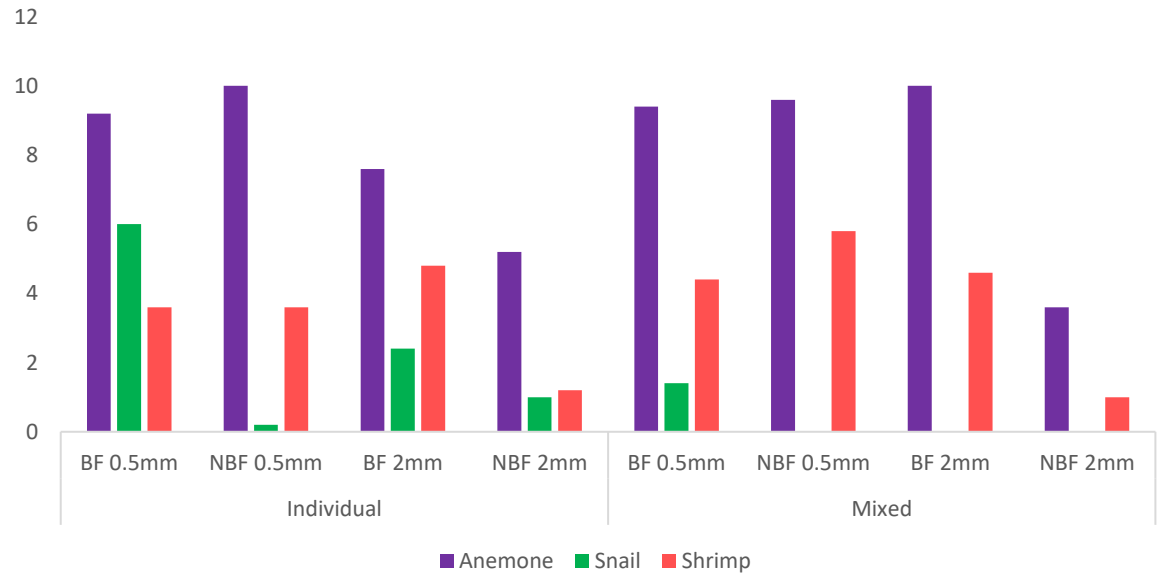

Figure 24 Total MF interactions in individual and mixed housing per MF exposure and size per organism.

Table 4 p values obtained with Kruskal-Wallis tests and averages presenting organisms overall interactions (ingestion and retention) of study MFs .

Shrimp				
Treatment	Factors	H-value	*P* -value	Average
Housing (1, N = 160)	Individual: Mixed	5.44	0.01	Individual (0.5) Mixed (0.8)
Treatment (1, N = 160)	BF:NBF	4.614	0.03	BF (0.8) NBF (0.5)
Size (1, N = 160)	0.5mm:2mm	4.234	0.04	0.5mm (0.8) 2mm (0.5)
Colour (3, N = 160)	Blue,Black,Red,White	11.276	0.01	Blue (0.7) Black (1) Red (0.7) White (0.3)

Snail				
Treatment	Factors	H-value	*P* -value	Average
Housing (1, N = 160)	Individual: Mixed	9.193	0.002	Individual (0.5) Mixed (0.06)
Treatment (1, N = 160)	BF:NBF	9.475	0.002	BF (0.5) NBF (0.05)
Size (1, N = 160)	0.5mm:2mm	1.455	0.22	0.5mm (0.4) 2mm (0.2)
Colour (3, N = 160)	Blue,Black,Red,White	2.132	0.54	Blue (0.4) Black (0.4) Red (0.2) White (0.1)

Anemone				
Treatment	Factors	H-value	*P* -value	Average
Housing (1, N = 160)	Individual: Mixed	0.044	0.833	Individual (1.5) Mixed (1.4)
Treatment (1, N = 160)	BF:NBF	8.855	0.002	BF (1.7) NBF (1.2)
Size (1, N = 160)	0.5mm:2mm	10.991	0.0009	0.5mm (1.1) 2mm (1.8)
Colour (3, N = 160)	Blue,Black,Red,White	14.532	0.002	Blue (1.5) Black (1.5) Red (1.9) White (1)

Overall				
Treatment	Factors	H-value	*P* -value	Average
Housing (1, N = 480)	Individual: Mixed	0.049	0.82	Individual (0.8) Mixed (0.7)
Treatment (1, N = 480)	BF:NBF	21.91	<0.0001	BF (1) NBF (0.6)
Size8 (1, N = 480)	0.5mm:2mm	13.57	0.0002	0.5mm (1) 2mm (0.6)
Colour (3, N = 480)	Blue,Black,Red,White	17.568	0.0005	Blue (0.8) Black (1) Red (0.9) White (0.4)

3.6 Microplastic Contamination

Whilst as many measures as possible were implemented to reduce microplastic contamination during the study, there were observations of non-spiked plastics which were also found to be ingested, however, determining how the organism came across them was difficult. Any contamination fibres found were easily identifiable from the study fibres due to length, thickness, colour and shape differences. Study fibres were new, not damaged and consistent in size and colour. All 40 fibres used in the individually house organisms study fibres were also retrieved however, with mixed community housing, this accuracy could not be achieved due to weight per ml being used, rather than a count. These contamination observations were noted with colour and size recorded which enabled data analysis to be

undertaken. Contamination recording was undertaken on ingested, sample water and the housing tank before testing. Contamination fibres found in the study were blue, black and white fibres however, white fibres were found in the control and so discounted from the recording as it was considered fallout during analysis. For further information on contamination, refer to the appendix (Section 6 – Appendix).

3.6.1 Individual Ingested contamination

During observations only blue and black fibres were found to be contaminates from the environment of which black was the most common. The contaminates were identified due to size and structure which vary greatly from the spiked plastics of the experiment. Anemones were found to ingest over black over blue contaminate fibres (n=29 p<0.001) and consumed the most of the three organisms. Shrimp were only found to ingest small MF of which black was again the highest consumer of these. Snails were not found to ingest any contaminated MF during the individual testing and so were excluded from analysis (Figure 25).

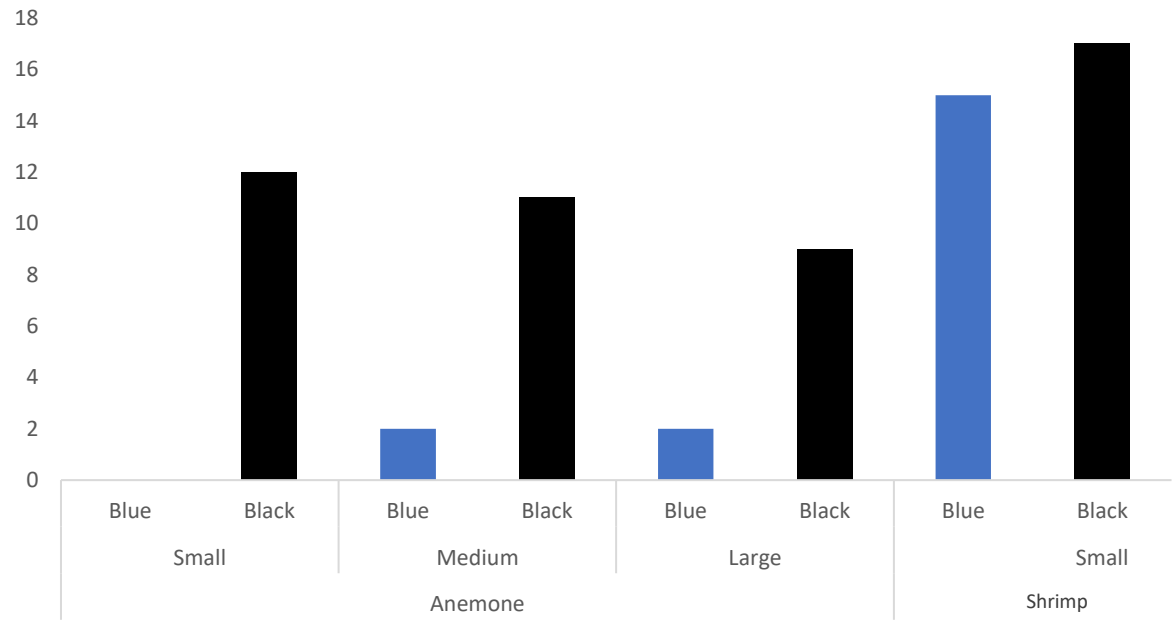

Figure 25 Microplastic ingestion contamination per organism per size category per colour

3.6.2 Mixed Ingested contamination

Ingestion of contaminated MF was found across all three species with small being found most commonly (Figure 26). Anemones were found to ingest MF of all sizes however, shrimp and snails only ingested small MF of blue and black colour. Shrimp and snails were not found to ingest contamination of medium and large and so are not included in this analysis. Anemones were found to significantly ingest black contaminate fibres more than blue (n=54 p<0.001) (Figure 26).

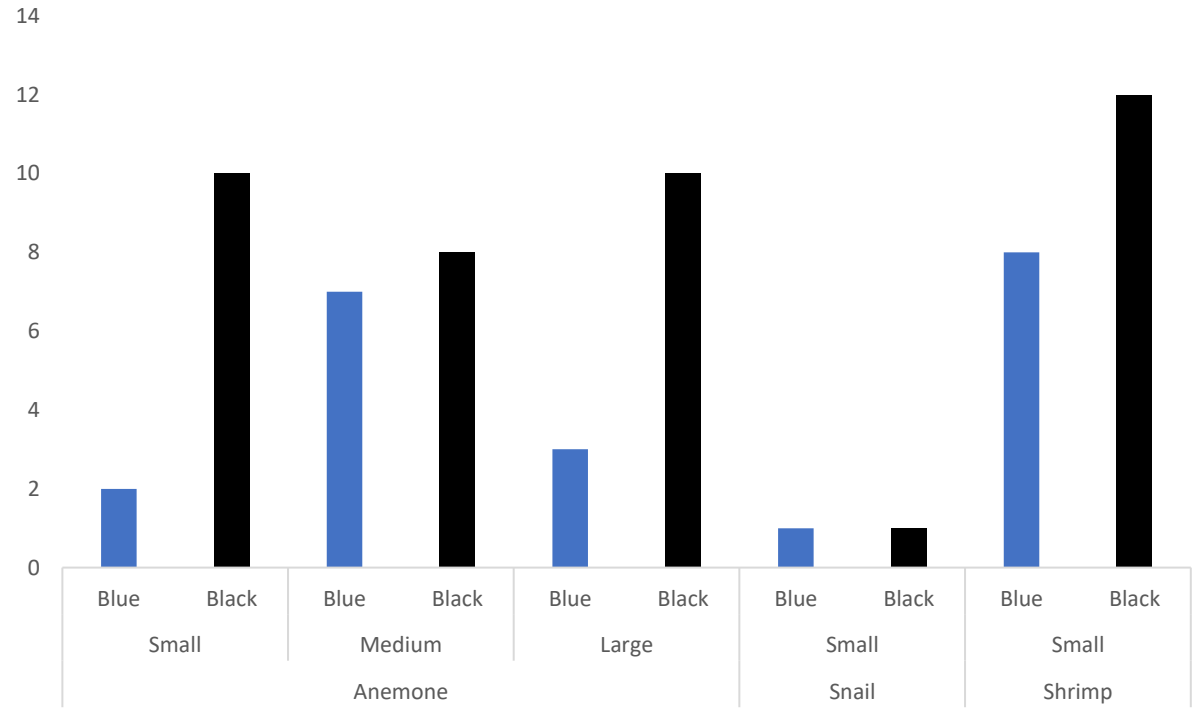

Figure 26 Microplastic ingestion within organism in a mixed community setting per size and colour per organism

3.6.3 Individual vs mixed Ingested contamination

All organisms ingested contamination fibres whilst in a mixed housing setting however, snails did not ingest any contamination when in an individual setting however, this was at a very low rate (one of each colour). Anemones ingested more contaminate fibres with black found significantly more to be ingested (n=77 p<0.001) with black being present more than

blue total. Black fibres were found more than blue (n=106 p<0.001). Whereas shrimp and snails were only found to ingest small fibres. Anemones, when contamination was considered, saw an interaction average of 1.5 fibres compared to 1.3 when housed communally. This is an increased average from prior contamination, with 0.6 on average in individual housing and 0.3 in community housing. Shrimp also saw a similar increase with 0.6 when individually housed, compared to 0.25 without contamination consideration. During community housing, an average of 0.07 was seen, however, with contamination, this increases to 0.8 MFs.

4 Discussion

4.1 Organism ingestion and retention

All three study organisms were found to ingest study MFs, with beadlet anemones ingesting 154, thick top shells snails 27, and rockpool shrimp 85 of the study plastics. After an alkaline digest, more study plastics were retrieved, showing that the organisms were retaining the study plastics in some form on or within their body. Beadlet anemones were found to have 74 spike plastics present after the alkaline digestion, thick top shells 20 and rockpool shrimp 15. Due to the nature of anemone's sticky tentacles and mucous protection, it is likely that this is where most retention occurred. This may be a similar case for snails where the mucous they produce could stick to the study plastics. Rockpool shrimp were de-shelled and did not have a thick mucus layer like beadlet anemones and thick top shells and so may indicate retention within its tissues in some way.

Both ingestion and retention can present issues to the organisms such as reduced appetite, blockages and leaching of chemicals. The longer the organism is exposed to the MFs internally, the higher the risk this poses, such as internal damage due to abrasion and false satiation or blockage which can prevent feeding and therefore reduced energy levels (Galloway *et al*., 2013; Egbeocha *et al.,* 2018). Further to this, the break down of microplastic to nano plastics can occur, which could lead to even greater biological transfer. Antarctic Krill, which are in the same superorder as rockpool shrimp, have been found to biologically fragment MPs during digestion by cutting and grinding using its mandible (Dawson *et al.*, 2018). With rockpool shrimp observed to retain MFs, fragmentation via their mandible during ingestion may increase thier MP exposure within thier natural habitat. Susceptibility to MFs has also been found to be in relation to sea anemone health. Caldeira *et al*., (2019) found that due to unfit conditions causing bleaching, sea anemones that were bleached were found to be more susceptible to MFs than healthy ones, as well as had a much higher retention of MFs than non-bleached anemones. This is likely due to reduced energy to allow for natural maintenance such as self-cleaning or the lack of energy to select food however, it is also possible that they are simply easier to identify as seen in this study with beadlet anemones and red fibres.

Studies on similar species of shrimp have found comparative or stronger results of ingestion. Gutlow *et.al,* 2018 found that with when presented with 2.5mg of food*, Palaemon varians* ingested a mean fibre count of 16.5 and 17 when presented without food. This is greater than the highest rate within this study, which was 6 MFs, however, Gutlow *et.al*, 2018 study exposed their organisms to vastly more MFs – 0.5mg. Further to this, (Santonicola *et al.*, 2023) presented data of retention in *Pleoticus muelleri*. It was found that 1.31 fibres/g wet weight was retained in the abdominal muscle. Similarly, *Metapenaeus monocerus* and *Penaeus monodon* were observed to ingest 3.40-3.87 fibres, comparable to this study's results also with the most report being 6 on average.

Few studies have looked into cnidaria in reference to MPs (Devereux *et al.*, 2021; Duis and Coors, 2016; Lengar *et al.*, 2021). Janssens and Garcia-Vazquez, 2021 looked at beadlet anemones on the north coast of Spain. The most common MPs were MFs and comprised of blue, white/transparent and black colours which correlate to the contamination data from this study (Figure 25,26, appendix) as well as the significances found in spiked MFs: white $H(1)= 8.143$, $p=0.043$, blue n ($H(1)= 15.284$, $p<0.001$) or black ($H(1)= 8.235$, $p=0.004$. Although very few studies have looked specifically at beadlet anemones and MFs ingestion and few on MPs, there are a few studies on other species of anemone that live in the study area. Snakelock anemones (*Anemonia virdis*) were found to uptake MPs readily with a mean of 142.1 ± 83.4 per gram of tissue but found no preference in size or shape. Closer examination also found that uptake involved both ingestion and external tissue adhesion to the mucus (Savage *et al.*, 2022). Of the MPs uptake by the snakelock anemone, 91% were MFs of which were blue, black and white in colour. Savage *et al.,* (2022) study shows similar results to that of this study. Beadet anemones were found to be unselective in MF size (Figure 20). There was some significance towards the colour of which were white $H(1)= 8.143$, $p=0.043$, blue n ($H(1)= 15.284$, $p<0.001$) or black ($H(1)= 8.235$, $p=0.004$, further supporting what was found by (Savage *et al.*, 2022). Furthermore, the only colours found in contamination were blue, black and white, however, all white fibre data had to be discounted due to white fibre fallout (Fig 25, 26, appendix).

Much like the beadlet anemone, there are few studies of thick top shells in reference to MPs. Janssens and Garcia-Vazquez, (2021) found uptake of MFs with compounds that can be irritant, toxic, mutagenic, carcinogenetic, and environmental hazard however, it found a higher uptake of MFs than that of beadlet anemones (0.56-148.28 per gram of tissue). This result contradicts that of this study and shows the need for further studies within this field. Within other marine snail species, a novel study found a mean of 2.8-6.86 MFs in *tramonita haemastoma* and *Melongena corona* species (Kleinschmidt and Janosik, 2021). Furthermore, 7 ± 2 items/kg-53 ± 6 items/kg MPs were found within snails in the mangroves of the Beibu Gulf (Li *et al.*, 2020). This further shows that there is a discrepancy between other studies and this study, requiring further, more specific investigation.

4.2 Plastic exposure

Study organisms were found to interact with BF plastics more than that of NBF. There was also a significance towards BF plastics found amongst all organisms for ingestion. Various organisms were also found to have patterns towards BF when paired with another factor such as colour or size. There were no NBF significance found in relation to ingestion or in retention however there was with BF which further supports that BF MFs were more likely to be interacted with than that of NBF MFs. Within their natural habitat, it is far more likely that they will encounter plastics that will have at least the beginnings of biofouling and so this study suggests that all three organisms will likely uptake BF fibres more due to the biofouling itself, as suggested by the study, rather than solely the plastic presence in the rockpool. This is likely due to the MP becoming more attractive to those that consume it, through taste or smell. Vroom *et al.,* (2017) further provides evidence that biofouling promotes the ingestion of MPs. Various copepod species were exposed to biofouled and non biofouled 15 and 30 µm beads and <30 µm fragments and saw multiple preferences to plastic that was BF although they found no negative impacts on the organism. This can be further supported by (Fabra *et al.*, 2021) in which European native oysters were found to uptake *e.coli* coated MPs significantly higher than virgin plastics with average concentrations of 42.3 ± 23.5 no. g^{-1} and 11.4 ± 0.6 no. g^{-1} microbeads. This study's organism, beadlet anemone, was seen to have a pattern toward BF plastics, although it is not known to selectively feed and so further studies into this would provide clearer evidence of its feeding behaviours around BF plastics.

4.3 Colour

Various patterns were found with MF colour in the study although the three study species may not be able to distinguish colour through vision. This may suggest that different colour dyes that are used in the MFs dying may increase or decrease interaction and retention rates within organisms. For example, white fibres were ingested and retained fewer than black MF. Depending on the organism, this could associate taste with the fibre itself, either from the dye or from more biofouling on specific colour fibres. Rockpool shrimp do have vision via compound eyes and so may be able to distinguish between different fibres colour which was seen in total with black and white being significantly more interacted with than other colours. This could be from an association with a natural prey or food source, however, further studies would need to be undertaken to provide this evidence. Blue, black, red and white were the most common fibres found in the study organism's natural habitat and this study provides some suggestion that there are more interactions with some colours than others. This may show vulnerabilities in areas that use more-colour specific equipment near the rocky shore than others. For example, various fishing gear typically comes in the colour blue and black, such as crab pots and ropes, and so if an increased interaction was seen in these colours, it could increase the possible uptake of these fibres in the study species in areas that have increased fishing activity. There have been a few studies that have found some colour preference in wild-caught organisms. Steer *et al.*, (2017) reported that 66% of all plastics found within the digestive tracts of fish larvae were the colour blue which supports the high amounts of blue found as contaminate plastics in this study however, this matches the high concentrations of blue MPs typically (Montoto-Martínez *et al.*, 2020). Desforges *et al.*, (2015) also supports the findings of (Montoto-Martínez *et al.*, 2020) in that copepods and euphausiid species were found to predominantly have black, blue and red MPs within their digestive tracts.

4.4 Size

Likely due to organism size, 0.5mm was ingested more often than 2mm ($H(1)= 18.290$, $p<0.001$). Common shrimp and thick top shells interacted with 2mm fibres however, less than 0.5mm, likely due to the limitations of mouth size. Anemones, due to size and ability to capture, were able to interact significantly with both sizes (Janssens and Garcia-Vazquez, 2021; Morais *et al.*, 2020a; Savage *et al.*, 2022). The interaction with small MFs was also seen in contamination ingestion and retention (Figures 25 and 26). During the examination of the holding tank that organisms were in prior to the study, smaller plastics were also seen in larger numbers than medium or larger plastics (Appendix). These plastics were either from egestion from the organisms or were strongly stuck or within the organism in some form as the organism was rinsed before moving into the holding tank. This further supports higher rates of smaller fibres *in-situ* to rockpool species. A study by Wu *et al.,* (2022) investigated the abundance and distribution of MPs in intertidal zones across the world and found almost all locations had a high abundance of smaller MPs (>10 μm 5mm). This may show that intertidal organisms are more susceptible to smaller MFs (less than 1mm fibres) (Lagos *et al.*, 2023; Wu *et al.*, 2022) and this study shows they are more susceptible to interacting with 0.5mm fibres, presenting smaller MFs as a concern in the intertidal one.

4.5 Housing

In order to assess organism interaction with MFs, organisms were tested individually before being put in a mixed community setting. Only snails saw a difference between housing types. When housed in a mixed community, like its natural habitat, the snail did not interact with MFs as much as when individually housed. This is likely due to the beadlet anemones and rockpool shrimp interacting with the MFs before they can reach a place in which the snail can then interact with the MFs. When housed individually, snails were found to interact significantly with MFs, specifically BF MFs. This could impact thick top shells that prefer rockpools that are in the more extreme areas of the intertidal zone. Very few other species will be able to tolerate the conditions. With fewer species present, this study highlights that they may be more susceptible to MFs interaction as snails only ingested MFs in an individual setting in this study.

4.6 Contamination

Contamination was found during the experiment. These contaminate sources could be from *in-situ* or from laboratory exposure and consisted of only fibres of blue and black when found in relation to the organism ingestion or retention and were of various sizes (Figure 25 and 26). During the inspection of housing prior to the experiment, contaminates were a mix of MFs, fragments and films and were found in a variety of colours and sizes (Appendix). Blue and black fibres were the only fibres which were found to be ingested with a pattern found towards black colour overall (n=77 p<0.001). This could suggest that colour associations may occur, however, further studies would need to be undertaken, particularly for the selective feeding of anemones. Colour association could also be at play, due to the various colours found in the housing tank prior to the study (Appendix). Much like the main study, thick top shells ingested very little which further supports the findings of the main study. Shrimp were only found to ingest small fibres contamination with no significant to colour, further supporting that smaller fibres have more of a pattern than larger ones in terms of ingestion.

4.7 Feeding Types

The use of the anemone, snail and shrimp species within the study represent different feeding guilds within a rockpool. Thick top shells were observed to interact the least with MFs indicating that snails that graze may be of least concern when investigating MP vulnerability, however, they were not exempt from eating MFs and so can still access and interact with MFs. Although snails were observed to interact the least, their feeding type can be damaging to MPs and potentially create further microplastics. They were recorded to ingest more MFs during individual housing as well as BF fibres, which may suggest that when in a rockpool with lower diversity and fewer organisms, they could be prone to interact and ingest more. When in a mixed setting, other species were likely to interact with MFs before reaching the snails, lowering the likelihood that they will interact with MFs. Although the focus of the study was on the thick top shell it is likely that similar gastropod species found to utilise rockpools that are predominantly grazers would also see similar risks, particularly to BF fibres which were found to be ingested in the study.
Beadlet anemones are an opportunistic omnivorous suspension feeders (Chintiroglou &

Koukouras, 1992) which makes them susceptible to MP contamination, particularly MFs, which can easily be non-selectively or unintentionally caught by the anemone's tentacles due to its size and shape. Beadlet anemones feed constantly when they are submerged in water (Carling *et al.*, 2019) and so within a rockpools, they are exposed to MFs at all times. During low tide after tidal flushing is the likeliest time to encounter MFs as new, high quantities of MFs, are brought in by sea and are mixed into the water column of the rockpool. Beadlet anemones ingested both the spiked and contaminate fibres, indicating that suspension-feeding organisms are at high risk within rockpools. Anemones were also observed to ingest BF fibres more than NBF which may suggest that they are capable of selectively feeding however, further research would need to be undertaken. Due to the persistence of plastic in the environment and structural integrity, it is likely the majority of MFs in the ocean will be exposed to biofouling. This would show a vulnerability to beadlet anemones as well as other sessile opportunistic suspension feeders.

Rockpool shrimp are filter-feeding detritivores (Janas *et al.*, 2008), opportunistic and also known to predate, however, around 80% of the shrimp's diet is detritus (Janas *et al.*, 2008). Due to the range of feeding methods the shrimp undertakes, it is able to intake MFs through various routes such as indirectly through contaminated prey items that have ingested MFs directly when filtering water, sediment and organic matter in addition to the risk of ingestion while cleaning other organisms. Within the study, shrimps ingested a high number of MFs and this increased when they were housed within a community setting over an induvial one. This could be due to the egestion of fibres from other organisms, the feeding off of other organisms or its change in use of the habitat when in the presence of other organisms. This would likely increase the likelihood that they will ingest MFs when within rockpools, either from direct ingestion or from detritivore feeding. Due to the various routes in which the shrimp has been shown to feed and the high numbers of MFs ingested and interacted with in the study, it can be suggested that rockpool organisms that are predominantly detritivores are highly likely to ingest MFs.

Thick top shells are grazers on intertidal zone and so are influenced by tidal cycles. They forage when they are submerged at high tide and avoid exposure during low tide. Grazing on biofilms and seaweeds, a possible vector for ingestion may be plastics that are adhered

to these in some way (Gutow *et al.*, 2016). They may also graze on a larger piece of plastic itself resulting in the uptake of microplastic in which they have created. Biofilms and seaweeds have been found to bioadhere MPs as o may be a source of MPs uptake (Kalčíková, 2023).

The study species are a small look into the feeding mechanism of some rockpool organisms. There are a variety of feeding types in the intertidal zone, some that feed similarly to the study species. Some that feed similarly but in a different habitat and some that feed differently all of which present different vulnerabilities to MPs are varied. Setälä *et al.*, (2016) looked at various organisms that have different types of feeding within a rockpool. It found that feeding type played a large role in MPs uptake, mainly, how many MPs the organisms ingested. *Marenzelleria spp*, a deposit feeder polychaete mudworm, lives in burrows and tends to feed on the surface or within the subsurface of sediment which can transfer MPs deeper into the sediment however, ingestion in this species was low and selective to smaller particles when in the presence of food. *Monoporeia affinis*, was also found to have low ingestion. The benthic amphipod feeds nocturnally and on phytoplankton, predation on bivalve larvae or decomposing material (Ecol *et al.*, 1998). *M. affinis* is able to access MPs via interaction with the water column when feeding as well as within the sediment. The study also found both *Gammarus spp.* and *Littoral mysids* ingested the most microbeads and both had feeding activity that is on the sediment or *Fucus* surface and in the water column. Mysid shrimps feed on detritus and plankton and *Gammarus* graze on algae and biofilms on algae or rocks (Viherluoto and Viitasalo, 2001). With many MPs being in high concentrations in or on the sediment surface, these feeding types are more susceptible to MPs interaction (Wu *et al.*, 2022). *Macoma balthica and Mytilus trossulus,* two bivalves species, were found to also ingest high amounts of microbeads. *Macoma balthica* feeds by siphoning whilst being buried in the surface. *Mytilus trossulus* however, siphons organics suspended in the water as well as on the sediment surrounding it (Skilleter and Peterson, 1994). These feeding types open up the vulnerabilities though both the water column as well as the high concentration in the sediment surface and how different feeding types can impact how much each organism could uptake MPs.

4.8 Primary producers

Algae are often overlooked when considering microplastic impacts. Although significantly understudied in terms of uptake of MFs, it has been found that algae (*Scenedesmus spp*.) can adsorb microbeads which resulted in obstruction of photosynthesis (Bhattacharya *et al.*, 2010). Growth inhibition was found by Gorokhova *et al*. (2020) with a rate of 50% less growth compared to those that were not exposed. Larger particle sizes were found to block light transport, affecting photosynthesis, and smaller particles were found to destroy the cell wall. Various other studies have also found growth impact among primary producers (Liu, G. *et al.*, 2020; Sjollema *et al.*, 2016; Yokota *et al.*, 2017) which highlights a possible bottom-up vulnerability within the food web. Although these consisted of microbead uses, it is likely that MFs will also cause similar impacts on organisms. Small MFs were found to be the most interacted with in this study and so plankton and algae are also likely to interact with smaller fibres. As primary producers are the beginning of the food web, indirect and direct feeding on these organisms will likely introduce MFs into food webs, creating an additional source of MFs interactions on top of direct feeding on MFs, as found in the study.

4.9 Invertebrates

All three organisms of the experiment were invertebrates, with the study finding ingestion amongst all three which highlights the risk to invertebrates within an intertidal pool and small fibres were the most ingested. Many rockpool invertebrates are small, with the largest resident being 30cm (Humphreys and Hall, 2022). This means that many may not have the capability to interact with larger MPs when in a rockpool. With small MFs being most abundant in the intertidal zone (Wu *et al*., 2022) this makes many invertebrates in the intertidal zone vulnerable to small MFs.

Invertebrates within a rockpool may be particularly susceptible to MF which is also supported by various other studies that specifically looked at intertidal species (Manríquez *et al*., 2006; Ivar Do Sul & Costa, 2014; Sharma & Chatterjee, 2017). Beadlet anemones and rockpool shrimp that were used in this study are also known to ingest various small invertebrates. Although this study looks at the uptake from seawater, it could highlight potential trophic transfer within the study organisms. Due to the range of feeding types of ,

three of which are represented in this study, interactions with MFs can enter the food web in various avenues, from direct ingestion as well prey ingestion that has ingested MFs themselves (Haegerbaeumer *et al.*, 2019) and multiple studies have found MP negative effects (Joyce *et al.*, 2022). Although only present in rockpools for a short time, heterotrophic zooplankton such as crab and barnacle larvae are food sources to many larger organisms living within a rockpool and so MP interaction and ingestion at this low level on the food web could see a cascade.

Although beadlet anemones, rockpool shrimp and thick top shells were use in this study, they were used to represent various factors of invertebrates, to further understand possible interactions within a rockpool. Zooplankton represent a range of species of different life stages as well as present different feeding types. In a study by Cole *et al.*, (2013) using fluorescence and coherent anti-Stokes Raman scattering (CARS) microscopy, 13 zooplankton were observed to ingest polystyrene beads (0.4-30.6 µ). This ingestion varied based on taxa, life stage and bead size. As well as ingestion, there were observations of beads within faecal pellets and evidence of adhering to the external carapace and appendages. The study also found that the copepod *Centropages typicus* significantly decreased its algal feeding and therefore implies a negative impact on zooplankton and microbead ingestion (Cole *et al.*, 2013). Setälä and Lehtiniemi (2014) also found that zooplankton including shrimps, copepods, worms, cladocerans, ciliates and polychaeta in the Baltic Sea, ingested microplastic, specifically microbeads. Leads *et al.*, (2019) found that the ingestion of study of weathered MFs saw a 35-55% mortality in glass shrimp. Part of the study found that zooplankton labelled with ingested microbeads were ingested by mysid shrimp when presented to them, showing how plankton can play a part in microplastic transfer from one trophic level to another. Moffat and Russell, 2014 further found acute toxicity that the commonly used plasticiser Di(2-ethylhexyl) phthalate (DEHP) within beadlet anemones when ingestion occurred.

Marine worms are a keystone species and are an important food source for coastal fish and wading birds (Wright *et al.,* 2013) with lugworm commonly used as an indicator and ecosystem species. Observation of microplastic uptake has been observed with negative

impacts on growth, reproduction, and ageing (Wright *et al.,* 2013) which was attributed to energy depletion. Although Besseling *et al.,* 2017 found some evidence of chemical impacts, ingestion was observed which present the possibility of microplastics entering the food web as well as egesting microplastic into the sediment. However, Teuten *et al.*, (2007) highlights that deposit feeds, like many marine worms, can excrete these MPs without necessarily taking up organic contaminants from the MPs.

Within a rockpool, there are a variety of crustaceans from crabs, shrimp, barnacles, lobster and crayfish. Various crustaceans make up part of the human diet, contamination at this level will have an unknown impact on humans as well as various organisms that feed on crustaceans. Farrell and Nelson, 2013, observed 0.5mm microspheres uptake from spiked mussels to crab species *Carcinus maenas*. Over intervals of up to 21 days, tissue samples were taken which found microspheres in the stomach, hepatopancreases, ovary and gills which declined over the trial period. This study is one of few that shows the possibility of trophic transfer of microplastics representing the potential risks to the food web and well as human consumption. Although this study was based on microspheres (beads), it shows the potential for other types of MPs to be transferred, especially if some organisms are able to retain MPs in some form, as is suggested in Figure 21,22 and 23. Tropic transfer can also be supported by the findings of Mateos-Cárdenas *et al.,* (2020) in which *Gammarus duebeni*, a freshwater amphipod crustacean, was found to rapidly break down microplastic beads into nano fragments, particularly when presented along with food, which became small enough to cross into cell membranes. Although the study was found in fresh water Gammarus species, there are many marine Gammarus species and so would implicate it in the marine food web as they are a keystone species (Chaumot *et al.*, 2015). Even in deep-sea species, plastic contamination was also found in high numbers in wild *Nephrops norvegicus* with 83% containing plastic, predominantly consisting of MFs, in their stomachs (Murray, F. and Cowie, 2011). Although crustaceans readily uptake plastics (Enyoh *et al.*, 2020; Gouin, 2020; Sanchez-Vidal *et al.*, 2018) there is evidence that some shrimp may prevent microplastic retention. Saborowski *et al.,* (2019) found that *Palaemon varians* were able to pass fibres and beads of various sizes via egestion and ecdysis, as well as regurgitation within 12-14 hours. Although not presented in Saborowski *et al.,* (2019) study, this can also occur during ecdysis. While this highlights the ability to negate plastic harm, it does show how plastic introduction to the sediment can also occur.

Molluscs are the biggest marine phylum and the intertidal zone contains a variety of inhabits, many of which are used as bioindicators. Some species found in rockpools are also of commercial importance such as mussels, oysters, octopus, whelks, clams and young scallops. Of these commercially important species, *Mytilus edulis* has been subject to various microplastic studies (Bråte *et al.,* 2018; Toussaint *et al*., 2019; Bendell *et al*., 2020) and is an important food source for many animals and so contamination of this species could then implicate the rest of the food web. Evidence of this has been found by Farrell and Nelson, (2013) finding that microspheres within *Mytilus edulis* meat were transferred to *Carcinus maenas*, which feeds readily on *M. edulis* meat, and microspheres were found within the tissue up to 21 days later. Although the study species is not a commercially important species, many molluscs species being eaten by animals as well as humans, are likely to introduce microplastics into the food web at various levels. While Farrell and Nelson, (2013) focus on microbeads, this study has suggested a possibility of MPs retention and so with observations of the transfer of beads, there is also a possibility of the transfer of other types of retained MPs. This evidence further supports (Browne *et al.*, 2008) that found MPs accumulated in the organs of mussels. The MPs were able to translocate from the digestive tract to the circulatory system which caused blockages and satiation. With the accumulation evidence and *M. edulis* a commercially important species (Picoche *et al.*, 2014), the likelihood of this species and those similar to it, passing MPs into those that eat them is high, although, impacts of this are not known. As this species is a food source in the human diet also, the impacts are of concern in human health, as well as impacts on the marine food web.

Although anthozoan species are not as commercially important, they still play a role in the food web and environmental health. Various studies have found links between plastic contact and disease risk, for example, Lamb *et al.,* 2018 found an increase from 4% to 89% increase in disease in 124,000 coral species and Seeley *et al.*, (2023) found increased virus-induced mortality in salmon when exposed to the virus and MPs concurrently. Ingestion of MFs may be dependent on the type of plastic as well as the food presence when they come into contact with them as was found by Caldeira *et al.,* 2019. The results suggested that nylon was ingested at a higher rate compared to other fibres when offered without food

and observations of 80% ingestion of all fibres when food was present. It was also seen that bleached anemone;s egestion of MFs was decreased and so highlights coral vulnerability when combined with bleaching events. Although ingestion of microplastic is never a good thing, anemones could be a bioindicator to help monitor microplastic pollution. A recent finding by Morais *et al.,* (2020) found weak evidence of the weight and number of particles ingested and also between prey items. Of the 90 individuals studied, 68 were found to ingest plastics with 84% of those plastics being MFs. The high intake found in the study as well as this study suggests anemone species, particularly in coastal areas, may prove to be a good choice to monitor microplastic frequency.

4.10 Vertebrates

Although the study did not include vertebrates, the three study species can represent a hazard to rockpool vertebrate users. Sea birds will predate on organisms such as small fish, crabs and shrimp. Small fish will predate on organisms such as sea anemones, shrimp and whelks. Fish that eat shrimp, anemone tentacle, whelks which in turn eat plankton or seaweed. These are just a few examples of prey which, if it ingested MFs, could trophic transfer to vertebrates.

Sea bird's studies mainly focus on diet, dead cadavers, regurgitated samples and faeces (Lusher, A., 2015). Although many studies focus on birds that gather food in the open ocean, their susceptibility is still relevant to the intertidal zone. This study has found MFs ingestion does take place in all the organisms to a certain degree, indicating that this could also be the case in a rockpool environment. Lusher, 2015 presented data showing over 50 species of birds, mainly fulmars, petrels, shearwaters and albatross, were found to have MPs in their digestive system. These species feed on the sea surface, as many birds do, and so, birds that interact with the intertidal zone are also susceptible. The intertidal zone, as mentioned previously, has a high concentration of MPs and many young fish that use the rockpools as nurseries will become prey to sea birds when they live as adults interacting with the intertidal one. A positive however, is that there is evidence that sea birds are able to regurgitate MPs to protect themselves (Lindborg *et al.*, 2012).

Within the UK, harbour (*Phoca vitulina*) and greys seals (*Halichoerus grypus*) are the only mammals to potentially interact with rockpools. A study released by Sarker *et al.,* 2022 also provides evidence of biomagnification of MPs in successive trophic levels within Sundarbans mangrove forest in Bangladesh. A further study by Nelms *et al.,* 2018 studied MPs from captive grey seals *(Halichoerus grypus)* and whole digestive tracts of the wild-caught Atlantic mackerel (*Scomber scombrus*) that was fed to the seals. The results found that half of all the samples of scat had MPs and a third of all fish had 1-4 MPs. There are strong suggestions that bioaccumulation and trophic transfer does occur however, studies on vertebrate impacts are few, largely due to ethical constraints of testing on vertebrates as well as logistical difficulties working with larger vertebrates in a laboratory setting, due to their housing and husbandry requirements. (Bravo Rebolledo *et al.*, 2013) found that of 100 harbour seals in the Netherlands, 11% of stomachs had plastic, 1% found in the intestines and 0% in scat. This low level is promising in seals that live in a relatively polluted area in the Netherlands, however, MPs were not considered in the observations.

Fish have been subject to many MPs studies and some of the earliest (Healing, 1973). Not only fish are an important part of the food web, they are an important source of food for humans also. Makhdoumi *et al.*, (2023) found 26 fish species were able to retain MPs in muscle tissue, equating to 56.5% of samples of the study. Another study conducted by (Lusher *et al.,* 2013) found 37% of 10 English Channel species ingested MPs. Boerger *et al.*, (2010) found 35% of planktivorous fish were found to have MPs in their stomach. These studies show the extent of MPs studies in fish species and the extent of how much is interacted with. These studies, however, were looking at the presence of MPs rather than the impacts of these on the organism itself. This extent of uptake does however show how fish species are vulnerable to MPs and how fish in the intertidal zone, where MPs numbers are high, may be at greater risk of MPs interaction.

4.11 Plastic Additives

Degradation of the additives that protect plastics from ozone, temperature, light radiation and bacteria (Campanale *et al.*, 2020) is likely to have an impact on rockpools, whether is from interaction with the plastics or the degradation and release occurring in the rockpool itself. Many plastics have additives that are not usually chemically bound to the plastic such as bisphenol A (BPA) - used to produce polycarbonate and as a hardener, heavy metals- used for colorants, flame-retardants, fillers, and stabilizers, phthalates – used as a plasticiser and flame retardants – used for raising the flashpoint of the material (Campanale *et al.*, 2020). Many of these chemicals are classed as harmful under EU regulations (Nordlander *et al.*, 2010) and many are linked to cancer, mutations, reproductive toxic effects, toxic environment impacts, build-up in the body and hormone disruption (Muncke *et al.*, 2020). As ingestion and retention was observed in the study, it is plausible that these organisms are also subject to the additives applied to the MPs. Rockpools come in various sizes and depths and so the amount of heat, UV and salinity MPs are subject do will vary across the intertidal zone. They are also subject to some extreme weather and tidal conditions which also has their own UV and strong temperature differences. Organism exposure to plastic additives would also vary. For example, rockpool shrimp are known to feed in all areas of the rockpool and so feeding on or near the surface where MPs are being exposed to extreme heat, could leave to high likelihood of leaching of chemicals compare to a beadlet anemone which will ingest plastic once it has sunk to where it can capture it. The most common additives - polybrominated diphenyl ethers, phthalates and the constituent monomer bisphenol A (Campanale *et al.*, 2020) are known to cause endocrine disruption, hormone imbalances and genotoxic damage (Cole *et al.*, 2011). All the study species were found to ingest and retain MFs and so prolonged exposure to MFs that could leach additives could lead to negative effects on the organism. As well as negative impacts on the organism itself, they are also all prey species which in turn could lead to bioaccumulation or new exposure routes to predators that feed on them.

As well as MP colour potentially playing a role in ingestion, the colour itself can pose a risk to organisms that ingest it. Plastics are dyed using soluble or insoluble dyes that are organic or inorganic in substance (Campanale *et al.*, 2020). Many of these dyes contain harmful

chemicals or metals that are known risks to humans as well as animals (Chung, 2016). Colouring is added via fine powders which gives the plastic the chosen colour with soluble substances maintaining the transparency of the plastics and insoluble give the plastics an opaque colour. Inorganic pigments can contain various heavy metals whereas organic pigments can include various chromophoric families such as azo pigments (typically red, orange and yellow pigments), phthalocyanine pigments (blue pigments), anthraquinone chromophores (typically naturally derived pigment), and various other chromophores (Sastri, 2014). Marine macro-alga of *Euchema Spinosum* has been investigated as a biosorption of azo-dye (Mokhtar *et al.*, 2017). It was found that all red, brown and green algae subject to testing had great potential to remove methylene blue dye from aqueous solutions at 27^{o}c or above, with red *Euchema Spinosum* exhibiting outstanding results in biosorption and affinity. Although UK rockpools may not reach 27^{o}c, they do see highs of 23^{o}c (Carling *et al.*, 2019) and so likely would see some sorption within a rockpool setting of azo-dyes as well as other chemicals. This would then make the chemicals biologically available to any rockpool organisms. This would present a way for chemicals to enter the food web, such as through grazer organisms, like that of this study, which are then predated on.

4.12 Microplastics implications in rockpools and humans

Rockpools play a vital role in the intertidal marine environment. Although this study focused on just three organisms within a rockpool, MPs interaction is likely to occur with many other rockpool-dwelling organisms. Zooplankton have been found to grow slower and reproduce less in the presence of MPs (Botterell *et al.*, 2019) for example and so as a food source to many rockpool organisms, the reduction or absence of zooplankton would negatively impact those that predate on them, such as the common prawn or beadlet anemones. This will then have a great impact on the organism that utilise rockpools as part of their diet and likely see a shift in how they interact with them. As already shown, many negative implications can come from MPs interactions, whether this has a greater impact is still yet to be fully explored thought. Various organisms that rely on rockpools are also a food source for humans. Crabs, various fish species and various molluscs that humans eat start life or live in the intertidal zone. Both the rockpool shrimp and thick top shell are subject to human

foraging and so a negative impact on these species will likely see an impact on humans as well. When people consume shrimp and thick top shells, they typically remove the shell but not all people remove the digestive tract of shrimp, and it is not possible to do so for snails. As seen in this study, both organisms ingested MFs and so provides a route for MFs to be ingested by humans and possibly a large scale due to the amount of organisms consumed in one sitting. This also could suggest a route for plastic additives to also find a route into the human diet.

4.13 Further research

As rockpools are understudied as well as hard to quantify, very little data is known in regard to MPs. The *in-situ* study looking at water trawls of the rockpools only gives a glimpse of the total MPs within the rockpool and so a future study of sediment and the water column would give a more inclusive look at the exposure a rockpool could face. Another avenue for investigation would be a seasonal look at MPs within rockpools. An investigation would observe differences during the summer when coastal areas receive more visitors and in the winter months, which would expose any seasonal risks to rockpool communities. In terms of the main study, a further look at spiked plastic exposure time would allow for further observation of ingestion and interaction amongst the study organism as well as various other organisms. A 12-hour exposure time would observe the maximum amount of time an organism would be isolated within a rockpool, and a three-hour study would show ingestion amongst those that have shorter digestive times, this is particularly the case with the shrimp species of the study. This would allow for a better understanding of MP ingestion and how long the exposure to these MPs was and in turn, allow for investigation into possible implications from this. Furthermore, running the study on a larger scale would also present greater findings. This study suggests that beadlet anemones may show selective feed also and so would be another avenue to research. The plastic exposures and whether there is an observed difference from food intake in the presence of NBF and BF fibres or a difference between plastic fibres and organics fibres or food type fibres. This further study would allow for observation of any plastic ingestion observation in preference to food and further implicate MPs within the food web if significance is found. Finally, an investigation to further look at other organisms, such as fish species, and feeding types would prove useful to fully identify vulnerabilities within a rockpool environment.

5 Reference list

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6 Appendix

6.1 Sample contamination

Whilst being subject to spiked microplastics during individual and mixed testing, water that the organisms were in was also examined for return of spiked microplastics and contaminate microplastics. Due to the nature of mixed tank testing, organism specific data in relation to water was not able to be collected. As previously stated, white fibres were excluded due to presence in the control dish during observations.

6.2 Individual Ingested and water contamination

During spiked testing organism where house water and during testing organism likely released some fibres into the water and retained some in the digestive tract. Black fibres were seen to be the most found in both ingestion and within water samples. Anemones saw ingestion and presence in water in both colour of contaminate fibres however, black was ingested and found more than the colour blue. Although snails did not ingest any contaminant fibres, within the water fibres were found and only in the small size category with an observation significance to black fibres over blue. Shrimp interacted with small contaminate fibres over medium and large, with observations almost equal in ingestion and water presence. Shrimp saw the single highest observation of contamination of 20 with a total interaction of 61 fibres, however, anemones so greater observations across small, medium and large out from 68 fibres.

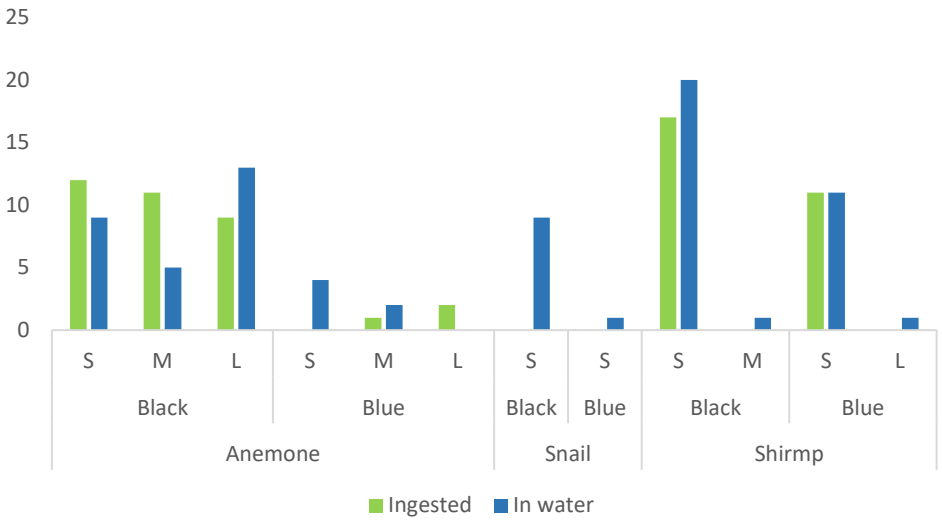

Figure 27 Count of contaminated fibres of individually housed organism either via ingested or presence in housing water.

6.3 Mixed housing water contamination

During mixed tank testing white, red, black, blue fibres were found, however, due to red and white fibres being found in controls petri dishes during mixed dissection, they were excluded from the data set. Both black and blue small fibres were found in the largest quantity and medium and large fibres were found in much small numbers.

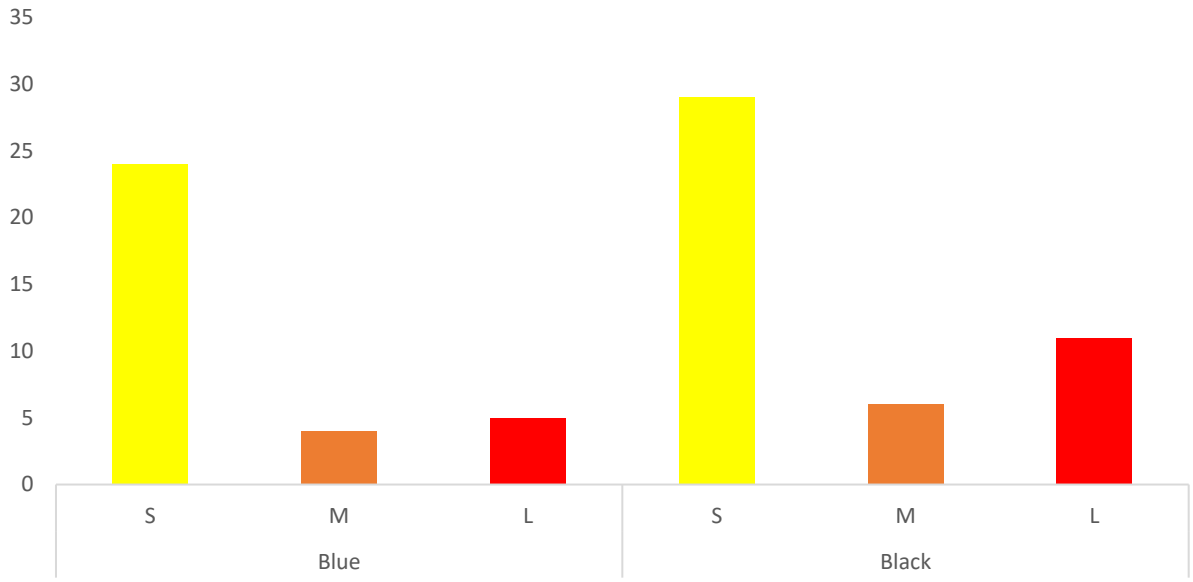

Figure 28 Size of contamination plastics found in mixed community testing. Small 0.5mm to 0.9mm, medium 1mm to 1.9mm, large 2mm to 2.5mm..

6.4 Pre-testing housing contamination

When organisms were collected and placed in a housing tank precautions were taken to prevent introduction of MP into the housing tank however, this did not eliminate contamination. Housing contamination was bought in by the organism into the housing tank of which they lived prior to testing. Microfibres, microfilms and micro fragments were all found in the housing analysis with a range of colours and sizes three size categories recorded. No microbeads were found during the analysis. These plastics were retrieved from both the tank as well as the water filter.

*6.*4.1 Pretesting *housing contamination size*

The most common size class of contamination was the small category, 0.5 mm to 0.9mm which represented over half of the accounted MP (68.4%), followed by large, 2mm-2.5mm (22.6%) and medium, 1mm – 1.9mm (8.9%) .

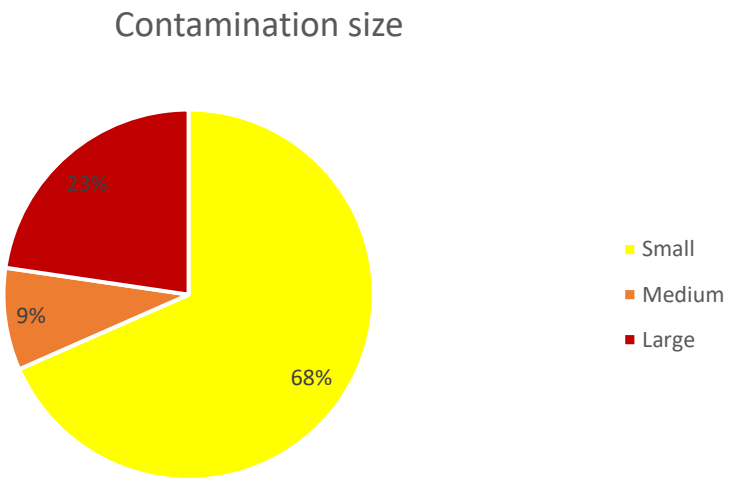

Figure 29 The range of sizes of contamination MPs found in pre-testing housing.

6.4.2 Pre-testing housing contamination colour

Contamination can be further broken down into three types: fibres, fragments and films. MF accounted the most found with 238 out of 269, fragments 24 and film the lowest with the lowest (7). Within the category of micro fibres, small accounted for 167 out of 238 of the fibres found, large 48 and medium 23.

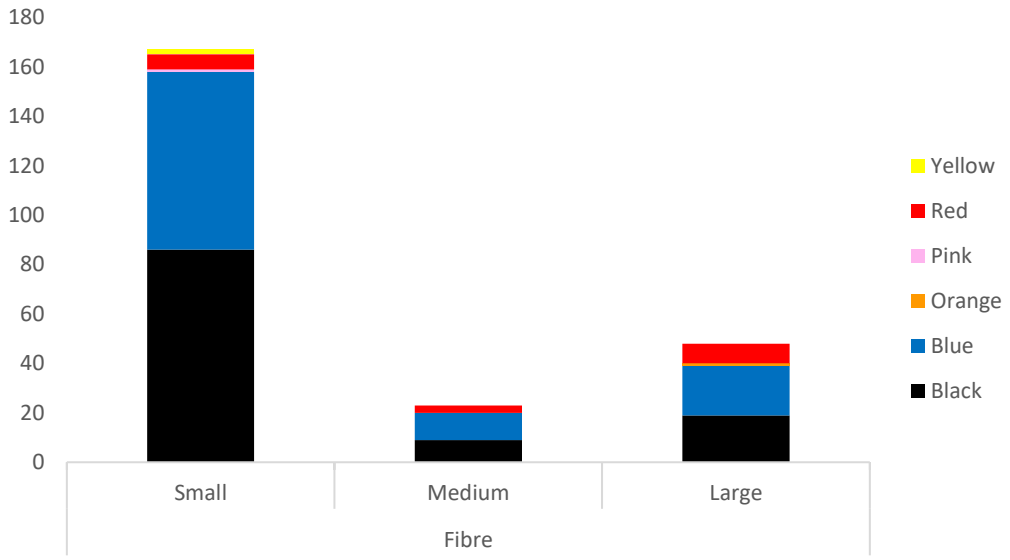

Figure 30 Colours of contamination MFs of pre-testing housing.

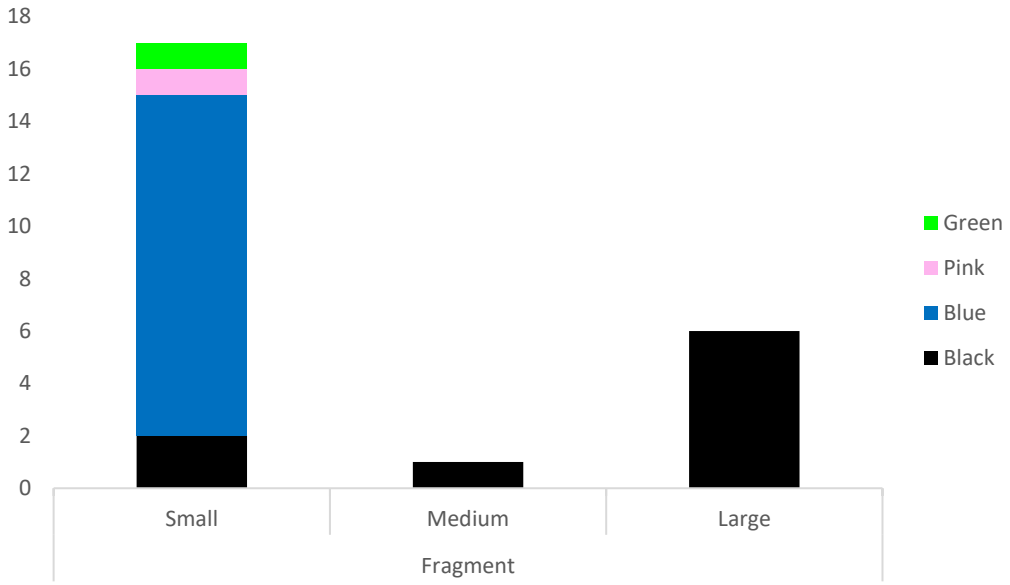

Figure 31 Colour of contamination fragments found in pre-testing housing.