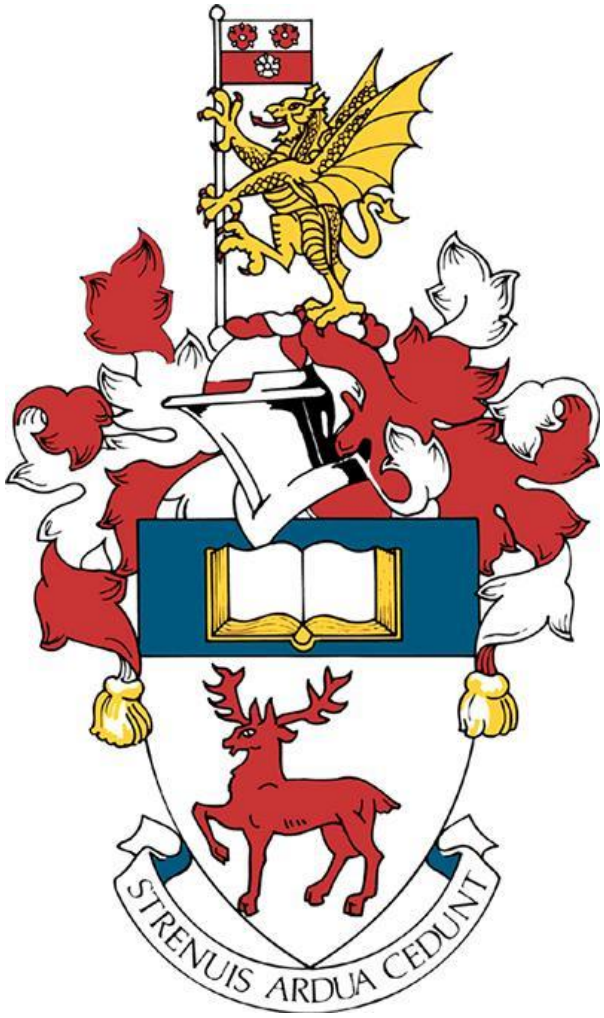
Quantifying microplastics in Southampton Water estuary and investigating microplastic ingestion in the local cnidarian polyp, *Aurelia aurita*.

M. McKee | 30180597

SOES3046 Independent Research Project



BSc Biology and Marine Biology

Supervisor: Dr Cathy Lucas

Word count: 7919

Abstract

Southampton Water (SW) is an industrialised estuarine system in southern England and at risk of microplastic (MP) contamination, yet only one existing study has quantified MPs within the SW catchment. Using three different capture methods (plankton net trawling, CTD bottle capture, glass plate method) at offshore and inshore sites, a total of 799 MPs were recorded; 39.8% fibres and 49% fragments, 7.6 beads and 3.6% film. However, laboratory studies typically expose zooplankton to microbeads, which are not representative of the MPs predominately found in the natural environment. This study aimed to investigate whether a cnidarian polyp, local to SW, (*Aurelia aurita*) showed evidence of ingesting fibrous or fragmented MP material in a series of 24 hr laboratory exposure experiments. The polyps ingested significantly more fibres than fragments with (p-value = 0.005213) and without (p-value = 0.01669) the presence of prey (1–2-day old *Artemia* nauplii). The occurrence rate of MPs in polyps was not significantly different between the exposure experiments (p-value = 0.2933), nor was the quantity of MPs ingested by *Artemia* with (p-value = 0.3261) and without (0.2003) a predator, suggesting MP ingestion was not induced by trophic transfer. By using more environmentally relevant MPs, this study highlights how cnidarian zooplankton may be more susceptible to ingesting specific, highly abundant MPs fibres in SW estuary.

Table of Contents

Acknowledgements

Firstly, I would like to thank my supervisor Dr Cathy Lucas for her assistance with my fieldwork, laboratory experiments and for her helpful insights. I would also like to thank the Solent Forum and Prof. Damon Teagle for the bursaries granted to this project, which aided in the collection of data. Further, I would like to thank Matt O'shaughnessy, George Clark and Robbie Robinson for their help in the laboratory and research aquarium. Thank you to Laura Fantuzzi (MSci alumni) for her advice early on. Finally, I would like to acknowledge the University of Southampton and National Oceanography Centre for the use of their facilities and equipment throughout the project.

1 Introduction

1.1 Microplastics

Plastic is an omnipresent, versatile material in modern society (Worm et al., 2017). However, the mismanagement of plastic waste has resulted in 4.8 - 12.7 million tons of plastic accumulating in marine environments in 2015 alone (Jambeck et al., 2015). Plastic debris has been reported in all oceans, impacting a plethora of marine life. The risk that larger plastic debris presents to marine organisms are well recorded (Nelms et al., 2016; Eerkes-Medrano et al., 2015; Li et al., 2016; Jacobsen et al., 2010). Yet, plastic debris isn't always visible. Since the identification of microplastics (MPs) (Thompson et al., 2004), their presence in marine systems has become of large concern (Persson et al., 2022; Small and Nicholls, 2003). Unlike large plastic debris, the microscopic size of MPs limits removal options from ecosystems (Alabi et al., 2019) and increases their availability to smaller organisms, such as zooplankton, detritivores, and filter feeders (Kowalski et al., 2016; Kooi et al., 2017). Further investigation is necessary to understand how these microscopic novel pollutants are affecting marine organisms previously thought to be unaffected by plastic debris.

The widely cited definition of MPs states size ranges from 1 μm – 5 mm, comprised of primary or secondary MPs (GESAMP, 2015; Frias and Nash, 2018). Primary MPs are particles manufactured at a microscale and are spherical in shape, such as scrubbing agents in cosmetic products (Boucher and Friot, 2017). Secondary MPs originate from the degradation of a large piece of plastic litter into smaller particles once in the marine system (Boucher and Friot, 2017). MPs occur in different shapes, categorised into fibres, fragments, microbeads or film. Fibres and fragments are recorded in the highest abundance in marine environments (Frias and Nash, 2019). Approximately 50% of

global plastic belongs to the polyolefin family, including polyethene (PE), polypropylene (PP) and polystyrene (PS), (Zheng and Shu, 2019; PlasticsEurope, 2011). Plastics of all sizes age and fragment in marine environments. Degradation typically occurs from thermos-oxidation, ultraviolet (UV) radiation and hydrolytic cleaving (Gewert et al., 2015; Wayman and Niemann, 2021).

MPs in the oceans originate from a multitude of sources; the fishing and textile industries are thought to contribute heavily to the fibrous MPs in our seas (Claessens et al., 2011; Browne et al., 2011). Plastic enters coastal systems via urban and agricultural runoff, river discharge and beach littering. Due to the harbours, marinas, industrial sites, and wastewater inputs that surround coastal zones, MPs are found in high concentrations in these areas (Cole et al., 2011; Desforges et al., 2014; Auta et al., 2017; Browne et al., 2011; Zhao et al., 2015; Yonkos et al., 2014 Murphy et al., 2016). Moreover, estuarine environments are known to contain high concentrations of MPs as they face intensive urban, industrial, agricultural, and recreational activity (Ivar do Sul and Costa, 2013; Claessens et al., 2011).

Particle density and size influence the vertical positioning of MPs in marine systems. Low-density MP particles float on or near the ocean surface; trawling data has concluded there to be 5 - 50 trillion MPs on the ocean surface (Van Sebille et al., 2015). MPs have also been found in deep-sea sediments (Van Cauwenberghe et al., 2013). Higher densities and biofouling (aggregation of extracellular biofilm) are thought to contribute to MPs sinking in the water column (Michels et al., 2018; Kooi et al., 2017). MPs accumulate along coastal zones, as high anthropogenic activity and rain, wind, and wave regimes significantly influence their distribution along shorelines and in

oceans (Eerkes-Medrano et al., 2015). Sub-tropical gyres and enclosed bodies of water are hot spots for MP accumulation (Lebreton et al., 2012), whilst sunken MPs can be transported deep into the ocean with sediment due to tidal action. Organisms can also ingest, retain, and transport MPs great distances in the ocean (GESAMP, 2015). Currently, little is known about the factors influencing the uptake of MPs in marine organisms.

1.2 Zooplankton and Microplastics

Zooplankton are important grazers in ocean food webs, transferring energy from primary producers to higher trophic levels (Richardson, 2008). Zooplankton are abundant in coastal zones, typically feeding near the water surface and therefore at risk of MP ingestion, where MPs are plentiful and of similar size to prey (Botterell et al., 2019; Sun et al., 2017). Evidence suggests zooplankton ingest MPs in laboratory experiments (Cole et al., 2013; Nobre et al., 2015; Kapsoi et al., 2014; Sucharitakul et al., 2020), but these studies are limited by the routine methodology of exposing zooplankton to MP bead/spheres. New, unaged microbeads are a poor representation of the types of MPs marine organisms are primarily exposed to; they are low in abundance and have not developed the biofilm that contributes to the ingestion of MPs by zooplankton (Anderson et al., 2018; Aytan et al., 2016; Desforges et al., 2015; Rotjan et al., 2019; Vroom et al., 2017). Botterell et al. (2020) have shown shapes other than beads to influence MP ingestion rates in certain zooplankton species. Studies that have collected zooplankton *in situ* have found > 70% of their samples to be contaminated with fibres (Rotjan et al., 2019; Desforges et al., 2014). Thus, feeding MP beads to zooplankton that are unlikely to encounter this MP shape in their natural

environment is not a holistic approach to understanding the ingestion of MPs in zooplankton.

Arguably, filter feeding zooplankton are at heightened risk of ingesting MPs due to the likelihood of exposure to surface MPs (Kosore et al., 2017), yet little research exists to show evidence for MP ingestion in polyps. Multiple laboratory studies and field observations have recorded the ingestion of MPs in zooplankton copepods (Desforges et al., 2015), euphausiids (Cole et al., 2011) amphipods (Sun et al., 2017) and one coral polyp species (Hierl et al., 2021), but no available studies have researched MP ingestion in cnidarian zooplankton polyps. The health of polyp colonies during strobilation determines the population size of blooming medusae (Lucas et al., 2012). Jellyfish medusa are an integral component of marine food webs, consumed by a diverse range of fish, birds, turtles and some invertebrates (Hays et al., 2018). Ergo, understanding factors that influence polyp health is of great importance. Nonetheless, there is little to no information regarding the potential for cnidarian polyps to ingest MPs or how ingestion may affect polyp health.

1.3 Southampton Water estuary

Southampton Water (SW) estuary is a shallow, partially mixed, meso-tidal estuary located on the south coast of England (Townend, 2008), receiving freshwater inputs from the surrounding river catchments (River Test, River Itchen and Hamble River) with tidal saltwater fluxes from the English Channel (Dyer, 1982). SW is ~ 10 km long and 1.9 - 2.5 km wide (Dyer, 1982), but has a river catchment area of 1800 km^2 (Gallagher et al., 2016). The central channel reaches depths of 15 m and tidal ranges vary from 1.5 – 5 m (Soulsby et al., 1985). The system features double high water,

each 2.5 h apart with a 15 cm water level drop between, with flood flushing times varying between 4.5 – 20 d depending on river flow (Lucas et al., 1995). Sediment in SW is predominantly mud or sandy mud, with salinities ranging between 25 – 31% (Croudace and Cundy, 1995; Lucas et al., 1995). The River Test, Itchen and Hamble River all contain active marinas and ports, so are likely to be at risk from MP pollution (Gallagher et al., 2016).

SW has been a major port for hundreds of years, undergoing significant industrial, recreational, and urban development. Well known for the trace metal and metalloid pollution from the Exxon Fawley oil refinery (Celis-Hernandez et al., 2022), SW has a history of anthropogenic pollution. Yet, there has been a lack of investigation into plastic pollution from the surrounding marinas and ports within SW catchment. Only one study has quantified MPs in the estuary (Gallagher et al., 2016), reporting a total of 2759 MPs in SW, which were predominantly fibres (54%) but no other studies have yet verified these findings. Although not intended as a quantification study, Anderson et al. (2018) tested new rapid MP collection methods in the SW catchment and reported finding MPs.

This study used polyps of *Aurelia aurita* as a model scyphozoan polyp and a species thought to be endemic to SW (Lucas and Williams, 1994). Polyps of *A. aurita* display low selectivity in their natural diets, eating a variety of zooplankton prey such as copepods, molluscs, and fish larvae. If prey is scarce, larger polyps may ingest smaller ones at a near distance (Lucas et al., 2012). Polyp colonies grown in inter- and subtidal zones between 0.1 – 3 m depths, settling on hard substratum in industrialised coastal settings to filter feed (Lucas, 2001; Rekstad et al., 2021). Strobilation of *A. aurita* occurs

in SW estuary between January and March annually, reaching peak blooms as temperatures increase from May to the end of June (Lucas and Williams, 1994). Most laboratory experiments in polyp ecology use 1 – 2-day old *Artemia* nauplii as food (Lucas et al., 2012). There is evidence to suggest *Artemia* nauplii ingest microbeads (Sucharitkal et al., 2020) in a laboratory setting, which could consequently mean polyps are ingesting MPs via trophic transfer, but further investigation is necessary.

1.4 Aims and Objectives

The first aim of this study is to quantify MPs in the River Test, Hamble River, and Beaulieu River, from inshore and offshore sites using different collection methods. Minimal data currently exists regarding assessing MP pollution within Southampton Water estuary. This study predicts that fibrous material will be the most abundant type of MP in the estuary, based on evidence from the existing SW estuary MP quantification study (Gallagher et al., 2016) and MP quantification studies in coastal areas (De Sá et al., 2018; Aytan et al., 2016; Desforges et al., 2015; Rotjan et al., 2019). Specific aims for MP quantification in this study are:

1. To quantify MP abundance, shape, colour, and size at offshore (River Test) and inshore (River Hamble, Beaulieu River) sites within Southampton Water estuary.
2. Assess the usability of common MP collection methods (plankton net trawling) and less frequently used or new MP collection methods (CTD bottle capture; glass plate method (Anderson et al., 2018)).

The second aim of this study is to investigate whether *A. aurita* polyps show evidence of ingesting MP types that are found in the highest abundance within the estuary. Evidence suggests zooplankton ingest MP beads (Cole et al., 2013; Nobre et al. 2015), but no studies have assessed whether filter feeding cnidarian polyps ingest any type of MP material. MP fibres are currently the most abundant MP in SW (Gallagher et al., 2016), so it is likely that *A. aurita* polyps in the estuary will be exposed to MP material that is not spherical. Therefore, this study aims to conclude whether *A. aurita* polyps ingest commonly occurring MP types in SW estuary, through laboratory exposure experiments. This study will also aim to determine whether *A. aurita* polyps ingest MPs via trophic transfer. Specific aims include:

1. To investigate whether *A. aurita* polyps show evidence of ingesting the most commonly occurring MP types in Southampton Water, whether this be fibres, fragments, beads or other, without the presence of natural prey.

   H01: polyps will not ingest of any MP material without the presence of prey

2. To investigate whether *A. aurita* polyps show evidence of ingesting the most commonly occurring MP types in Southampton Water, with the presence of natural prey.

   H02: polyps will not ingest any MP material in the presence of prey

3. To investigate whether *Artemia.* nauplii show evidence of ingesting and tropically transferring MPs to *A. aurita* polyps.

   H03: Artemia will not tropically transfer MPs to A. aurita polyps

2 Methodology

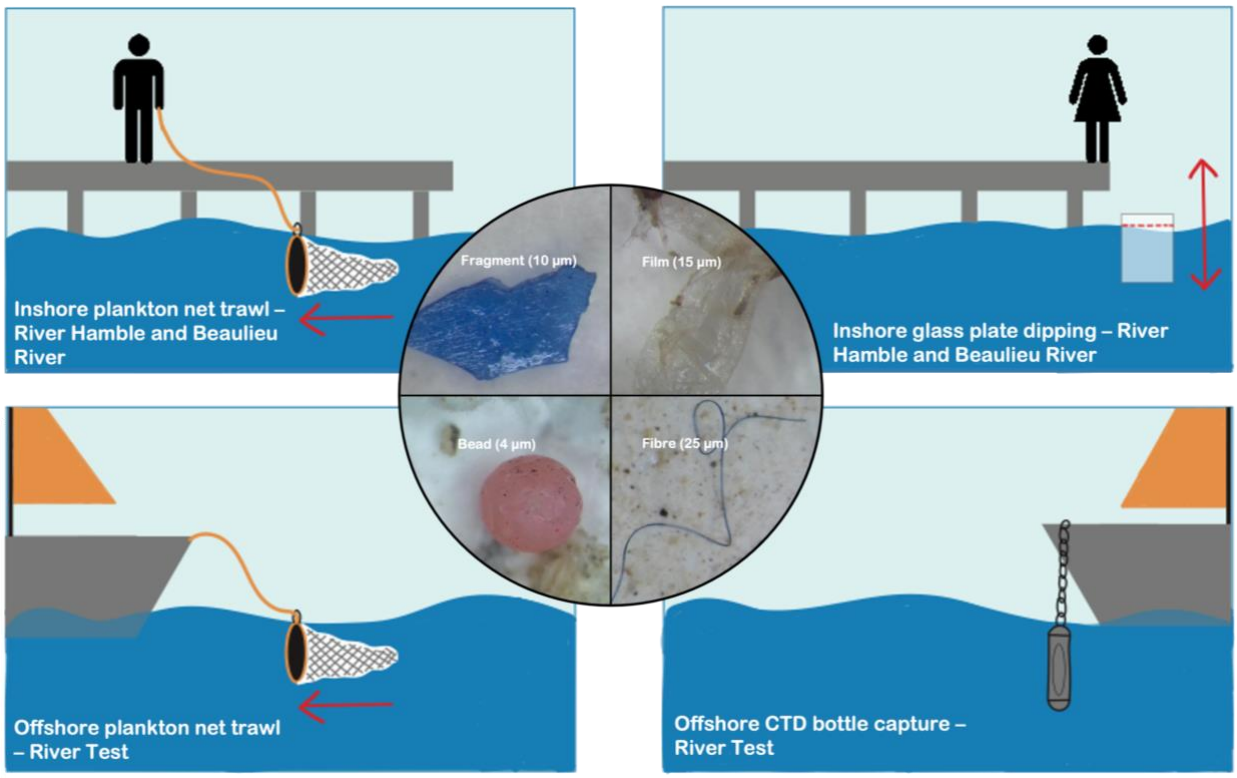


Figure 1. Infographic summarising the four MP sampling methods. Top left and top right represent inshore collection methods conducted by hand. Bottom left and bottom right represent offshore collection methods conduced on the R.V Callista. Centre images are of examples of MPs collected from the sample sites, images captured using a camera under a light microscope (GT Vision GXCAM Camera). Top left is a blue fragment, recorded from the River Test. Top right is a piece of film, recorded from the River Hamble. Bottom left is a bead recorded from the River Test. Bottom right is a fibre from Beaulieu River.

2.1 Offshore sampling

On the 20th of July 2022, the University of Southampton Research Vessel Callista was taken into Southampton Water to collect surface water samples at high tide. Water samples were collected at four locations using two different capture methods.

A 200 µm mesh cod-end plankton net, 50cm in diameter a flow meter attached, was towed horizontally in the upper 50 cm of the water column at 2 knots in sample locations for 5 minutes. The procedure was based on commonly used MP sampling

protocols (Aytan et al., 2016; Steer et al., 2017; Schmidt et al., 2018). The net was rinsed with seawater to disperse any MPs to the bottom. Approximately 500 mL of the water sample was transferred into a glass bottle and ~ 50 mL of 4% formaldehyde was added to kill any organic substances (e.g. plankton, algae). The bottle was labelled with the date, location, and collection method (as with all samples collected). Changes in flowmeter revolutions were recorded to then calculate the total volume of water sampled. The equation M x 0.3 = L was used, (M = number of flow meter revolutions, L = towing distance (m)), followed by π x r^2 x L = V, (r^2 = radius of the plankton net opening (0.25 m), L = towing distance previously calculated, V = volume of seawater sampled).

A single CTD rosette bottle (6 L) was deployed into the upper 50cm of the water column for an instant water sample capture. A 500 mL sample was released into a glass bottle and formaldehyde added.

Table 1: Sample site summary of offshore plankton net tows and CTD bottle captures

Location in River Test	Date	Time	Lat/Long
Upper Swinging Ground (S.G)	20/07/2022	09:20	50°54.169 N, 01°.26.840 W
Marchwood Quay	20/07/2022	09:48	50.54.250 N, 01°25.956 W
Mayflower Park	20/07/2022	10:10	50°53.630 N, 01°24.645 W
Dock Head	20/07/2022	10:28	50°52.813 N, 01°23.550 W

2.2 Inshore Sampling

On the 21st and 22nd of July 2022, inshore MP collection was conducted in Beaulieu River and Hamble Marine, respectively, to collect surface water samples at high tide. Samples were collected at using two different capture methods.

A 200 μm mesh cod-end plankton net, 25cm in diameter with an added flow meter, was hand-towed at a near-constant speed (~ 10 m / 60 s) for 2 minutes over 100 m. The net was towed horizontally in the upper 25 cm of the water column. Towing was conducted in the nearshore along jetties in Buckler's Hard (Beaulieu River) and Hamble Marina. The net was rinsed with distilled water to collect all MPs. A 500 mL sample was transferred into a glass bottle and formaldehyde was added. The process was repeated twice at each sample site. Due to pulling the net by hand, the flow meter did not record any revelations. The volume of water sampled was calculated using the equation $\pi \times r^2 \times L = V$.

Following the rapid MP sampling method, outlined by Anderson et al. (2018), a glass plate 30 cm x 19 cm was retrieved from a laboratory at the NOCS. A line was drawn horizontally across the glass plate, measuring the suggested 27.5 cm dipping length. At Beaulieu River and Hamble Marina, the plate was dipped into the water surface 25 times at a depth of 27.5 cm within the nearshore. The plate was submerged and retracted into the water column steadily at an approximate rate of 5.5 cm / 1 s. After each dip, the plate was rinsed on either side with distilled water and drained into a 500 mL glass bottle. After the dipping process, formaldehyde was added. This method was repeated twice at each sample site.

Table 2: Sample site summary of inshore plankton net tow and glass plate sampling

Location	Date	Time (plankton net tow)	Time (glass plate sampling)	Lat/Long
Buckler's Hard (Beaulieu River)	21/07/2022	10:31	11:06	50°47.593 N, 1°25.167 W
Hamble Marina (River Hamble)	22/07/2022	10:29	10:52	50°51.063 N, 1°18.395 W

2.3 Filtering

Filtering water samples is a common MP quantification practice (Anderson et al., 2018), replicated in this study to prepare for light microscopy. Water samples were filtered onto 47 mm microfibre paper using a pump (Welch; model no.2534C-02) and Buchner flask. Multiple filters were used per water sample to avoid build-up of sediment and organic materials. The Buchner funnel was rinsed thoroughly with distilled water between filters. Filters were placed into individual labelled petri dishes and left to dry for 24 hr.

2.4 Counting Microplastics

Multiple papers were used as visual aids to identify MPs (Steer et al., 2017; Sun et al., 2017; Hall et al., 2015). Before light microscopy (Nikon SMZ-10) analysis, each filter was marked into 4 equal pie-shaped areas to avoid miscounting. Markings were made very delicately with a pencil and ruler so as not to disturb the filter surface. For every MP identified, the type (fragment, fibre, bead, film, other), size (length/diameter μm) colour (blue, black, red, white, other) and overall abundance was recorded.

2.5 Polyp Acquisition and Culture

Polyps of *A. aurita* were collected from a culture tank at the National Oceanography Centre (NOC), Southampton. The polyps were removed from the tank and placed into individual wells in clear plastic well plates with water from the original tank. One well plate contained 6 wells and 12 plates were used, thus 60 polyps in total. These plates were incubated at 16 °C and left until the polyps attached. The polyps were fed twice a week for 10 weeks with 1 mL of *Artemia* nauplii per well from a copepod culture grown in the NOC research aquarium. Polyps were left to feed for 3 hours before ¾ of the well water was removed and replaced with clean filtered seawater (32 PSU). A 5 L bucket of seawater was kept in the fridge to maintain water temperature during water change. Feeding and water changes were performed using a disposable plastic 3 mL pipette. If a polyp died, a budding polyp from a different well was gently removed using a scapula and placed into the empty well. If there was a surplus of polyps, they were placed in a 'spare polyp' well plate to ensure there was only 1 polyp / well at any time. Polyps remained incubated except for feeding and cleaning. A polyp logbook was kept over the 10-week period to record polyp mortalities, budding and general health.

2.6 Experimental Design

2.6.1 Laboratory experiments

The experiments aimed to assess 1) whether *A. aurita* polyps ingest MP fibres and fragments found in SW, 2) whether the presence of *Artemia* influences polyp MP ingestion and 3) whether *Artemia* ingest MP fibres and fragments found in SW. Fibres and fragments were found in the highest abundance in SW estuary (see results), so *A. aurita* polyps were exposed to these MPs to reflect the natural environment. In total, 7 experiments were run (table 3). Each well (experiments 1 – 5) contained one polyp. To

each well requiring *Artemia* (experiments 1, 4, 5, 6, 7), 10 ± 2 *Artemia* were added. To each well requiring MPs (experiment 2 – 7), a total of 5 fibres or fragment dosages were added; MPs ranged from 5 - 50 μm in length (fibre) or diameter (fragment). Each experiment used two well plates (12 wells) in total. Experiment 1 acted as a control group, to confirm that *A. aurita* polyps ingest *Artemia* as a natural prey.

Table 3: Summary of organism and MP shape used in all exposure experiments.

Experiment	Polyp	*Artemia*	Fibres	Fragments
1	✓	✓		
2	✓		✓	
3	✓			✓
4	✓	✓	✓	
5	✓	✓		✓
6		✓	✓	
7		✓		✓

Experiments were conducted simultaneously and ran for 24 hr. All well plates were kept at 13 °C in a ventilated, temperature-controlled lab in NOC. All well plates were placed on a shaking table (Infors AG) to imitate moving water during the experiments, at 75 RPM.

After 24 hr, each individual polyp was placed into a vile with a lid using a pipette. The remaining *Artemia* in each well plate were pipetted into their own vial. The remaining well plate water was then pipetted into individual vials. All vials were labelled. Ethanol was added to all vials to preserve organic matter before acid digestion.

2.6.2 Artemia dosages

The 10 ± 2 / mL *Artemia* dosage (Barros and Valenti, 2003) was created the day of the experiments. A 50 mL beaker of *Artemia* in seawater was obtained from the NOC research aquarium. A volumetric pipette was used to put 1 mL of *Artemia* from the 50 mL beaker into a bogof chamber. To avoid miscounting under a light microscope, 1 mL of 90% ethanol was added to kill the *Artemia*. The number of *Artemia* was counted and the process was repeated twice more in a cleaned chamber to produce an average count of *Artemia* / mL. The beaker of *Artemia* was diluted and the process was restarted until 1 mL of *Artemia* solution contained 10 ± 2 *Artemia*. When the experiments began, 1 mL of this solution was added to every well requiring *Artemia*.

2.6.3 Microplastic fibre and fragment dosages

For each well plate requiring fibres or fragments, a MP dosage was created. The length or diameter of the fibres and fragments ranged between 1 - 60μm, collected directly from the filtered SW water samples. The size range was decided upon based on those seen in other studies (Sucharitakul et al. 2020, Desforges et al., 2015). Fine tweezers and the light microscope were used to identify, measure, and remove each MP, which were placed into snap vials. A total of 5 fragments or fibres were added into an individual snap vile until there were enough dosages for each respective well plate. The MP dosages were added to the respective well plates at the same time as the *Artemia*. The quantity of MPs added to each well (1 MP / mL) is not reflective of the number of MPs which would be found in 5 mL of water in the estuary (table 4, 6). Common laboratory concentrations are much higher than environmentally relevant values (Cole et al., 2013).

2.6.4 Acid digestion

This procedure was replicated to follow the protocol proposed by Desforges et al. (2015). All vials were left open in a fume hood for 48 hours to evaporate. Following this, 1 mL of 30% hydrogen peroxide was added to each vial. The vials were then left for 48 hr at 40°C in an incubator (GENLAB Prime). The digestate of each sample was then removed from each vial with a treatment-specific Pasteur pipette and filtered onto 47mm glass fibre filter paper using a vacuum pump. Each vial was rinsed with distilled water onto the filter three times.

2.6.5 Ingestion analysis

Under a light microscope, the filter papers from the experiment were examined. The remaining *Artemia* in experiment 1 were counted to estimate how many were ingested by polyps. For the remaining experiments, the final distribution of all MPs was observed and recorded. For example, the number of individual fibres found on the polyp digestate filters, *Artemia* digestate filters and well water digestate filters were recorded for experiment 4 to determine how many fibres were ingested by polyps, *Artemia* or remained in the well water.

2.6.6 Contamination control

Every filtered sample was placed in a petri dish, with the petri dish lid left on top but slightly ajar on the dish. This was to avoid other particulates gathering on the filter papers when drying. After drying, the petri dish lids were fully closed and not opened again until light microscopy.

3 Results

3.1 Offshore Microplastic Quantification

In total, 737 individual MPs were recorded over four sites offshore in the River Test, Southampton Water via two different collection methods (fig.1).

Table 4: Summary of the volume of water sampled and the shape and abundance of MPs recorded from the offshore sample sites.

Site	Method	Vol of water sampled (m^3)	Fibre	Bead	Fragment	Film	Total MPs
Upper S.G.	Plankton net	163.5	51	7	86	0	144
Dock Head	Plankton net	150.1	27	26	111	7	171
Mayflower	Plankton net	107.7	66	16	105	7	194
Marchwood	Plankton net	131.0	44	8	73	4	129
Upper S.G.	Bottle capture	0.0005	34	0	1	0	35
Dock Head	Bottle capture	0.0005	12	0	2	2	16
Mayflower	Bottle capture	0.0005	6	1	1	0	8
Marchwood	Bottle capture	0.0005	8	1	5	0	14

During the plankton net trawling, 663 MPs were recorded: 56.86% fragments, 31.52% fibres, 8.75% beads and 2.87% film. The sample site with the most recorded MPs was Mayflower (1.8 MP m^{-3}), compared with Dock Head (1.14 MP m^{-3}), Marchwood (0.98 MP m^{-3}) and Upper Swinging Ground (0.88 MP m^{-3}). As fragments and fibres were the most observed MPs in SW, further analysis was conducted into these particle types in preparation for the experimental exposures with *A. aurita* polyps. The average diameter of fragments recorded at the sample sites ranged from 6.68 – 16.08 μm and fragments were predominantly blue in colour (65.78%) (table 5). The average length

of fibres at the sample sites ranged from 51.35 – 63.81 μm and the fibres were predominately blue (57.89%) (table 5).

The CTD bottle captured a total of 74 MPs across the four sample sites; 81.08% were fibres, 13.51% fragments, 2.7% beads and 2.7% film (table 4). The most MPs were recorded in the Upper Swinging Ground (70,000 MP m^{-3}), followed by Dock Head (32,000 MP m^{-3}), Marchwood (28,000 MP m^{-3}) and Mayflower (16,000 MP m^{-3}) (table 4). The average diameter of the 10 recorded fragments at each site ranged from 10 – 27.5 μm and were mostly blue in colour (80%) (table 5). The average length of the 60 recorded fibres ranged from 22.25 – 52.3 μm and were mostly blue in colour (48.33%) (table 5).

Table 5: Summary table of the average size, standard deviation (SD) and recorded colours of all fibres and fragments recorded from the offshore sample sites.

			Size	**Colour**				
Method	MP type	Site	Mean size (μm) and SD (±)	Blue	Red	Black	White	Other
Plankton net	Fibre	Upper SG	63.81 ± 41.36	37	2	27	0	0
Plankton net	Fibre	Dock Head	51.35 ± 31.01	18	2	10	1	0
Plankton net	Fibre	Mayflower	56.31 ± 39.60	35	4	28	0	0
Plankton net	Fibre	Marchwood	59.62 ± 37.54	29	8	8	0	0
Plankton net	Fragment	Upper SG	11.54 ± 9.03	48	18	22	1	0
Plankton net	Fragment	Dock Head	16.08 ± 15.92	70	1	24	14	1
Plankton net	Fragment	Mayflower	8.44 ± 7.01	77	4	22	1	1
Plankton net	Fragment	Marchwood	6.68 ± 5.37	53	2	16	2	0
Bottle capture	Fibre	Upper SG	49.59 ± 39.01	18	3	2	11	0
Bottle capture	Fibre	Dock Head	22.25 ± 13.80	6	2	4	0	0
Bottle capture	Fibre	Mayflower	47.83 ± 30.26	2	2	2	0	0
Bottle capture	Fibre	Marchwood	52.2 ± 30.65	3	2	2	1	0
Bottle capture	Fragment	Upper SG	6 ± 0	1	0	0	0	0
Bottle capture	Fragment	Dock Head	27.5 ± 10.61	1	0	0	1	0
Bottle capture	Fragment	Mayflower	10 ± 0	1	0	0	0	0
Bottle capture	Fragment	Marchwood	9.33 ± 4.41	5	0	1	0	0

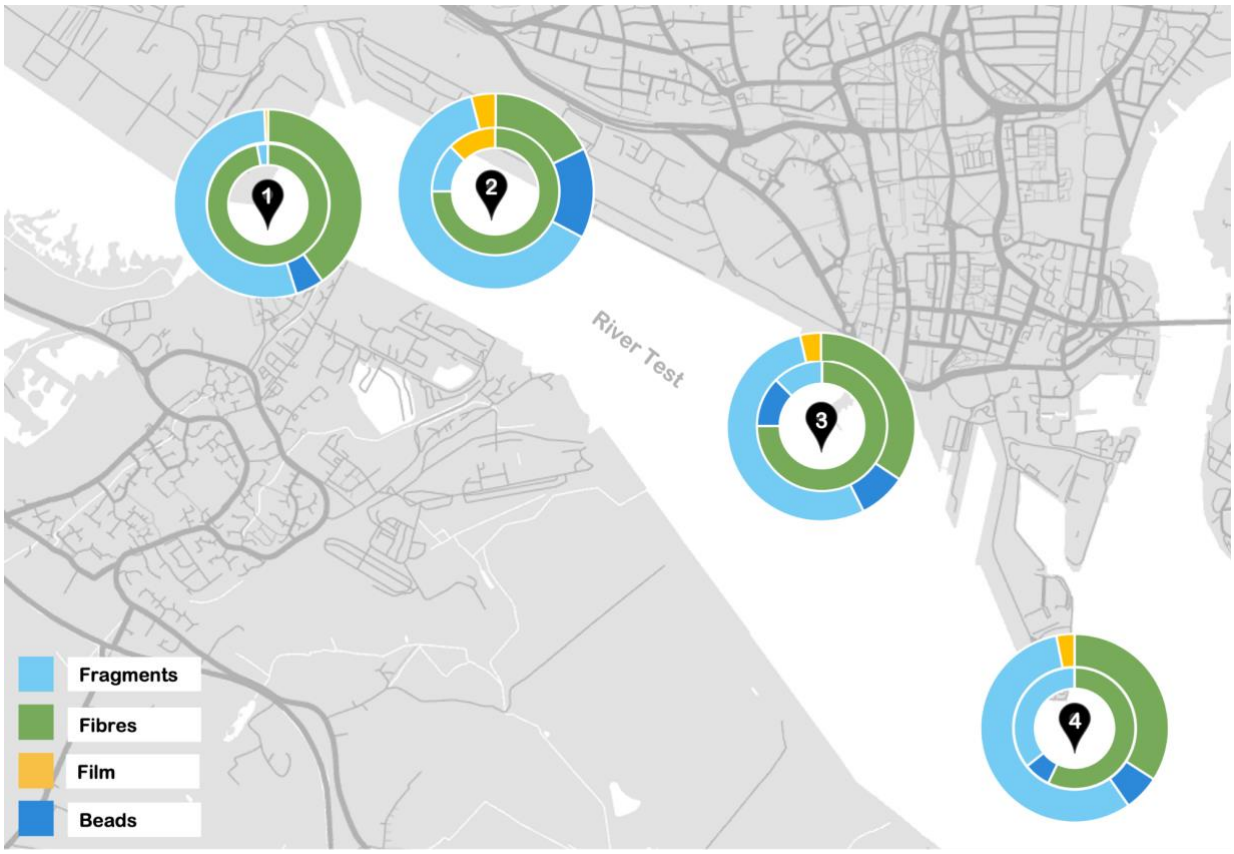


Figure 2. Map of offshore sample site location in the River Test. 1. Upper Swinging Ground. 2. Marchwood. 3. Mayflower. 4. Dock Head. The outer pie chart ring represents the MP abundance data from the plankton net trawling whilst the inner ring represents the MP abundance data from the CTD bottle captures. Colours are representative of the four different types of MPs located during sampling.

3.2 Inshore Microplastic Quantification

At Hamble Marina, 26 MPs were recorded inshore – 12 from a plankton net tow (2.44 MP m-3) and 14 from the glass plate method (GPM) (~46,700 MP m^{-3}) (table 6). Only one fragment was recorded at Hamble Marina using the GMP, measuring 10 μm and blue in colour (table 7). Fibres made up 84.62% of the total MPs, with an average length of 56.81 μm with the plankton net capture and 51.81 μm with the GPM (table 7). Of the 22 fibres recorded, 63.64% were blue (table 7).

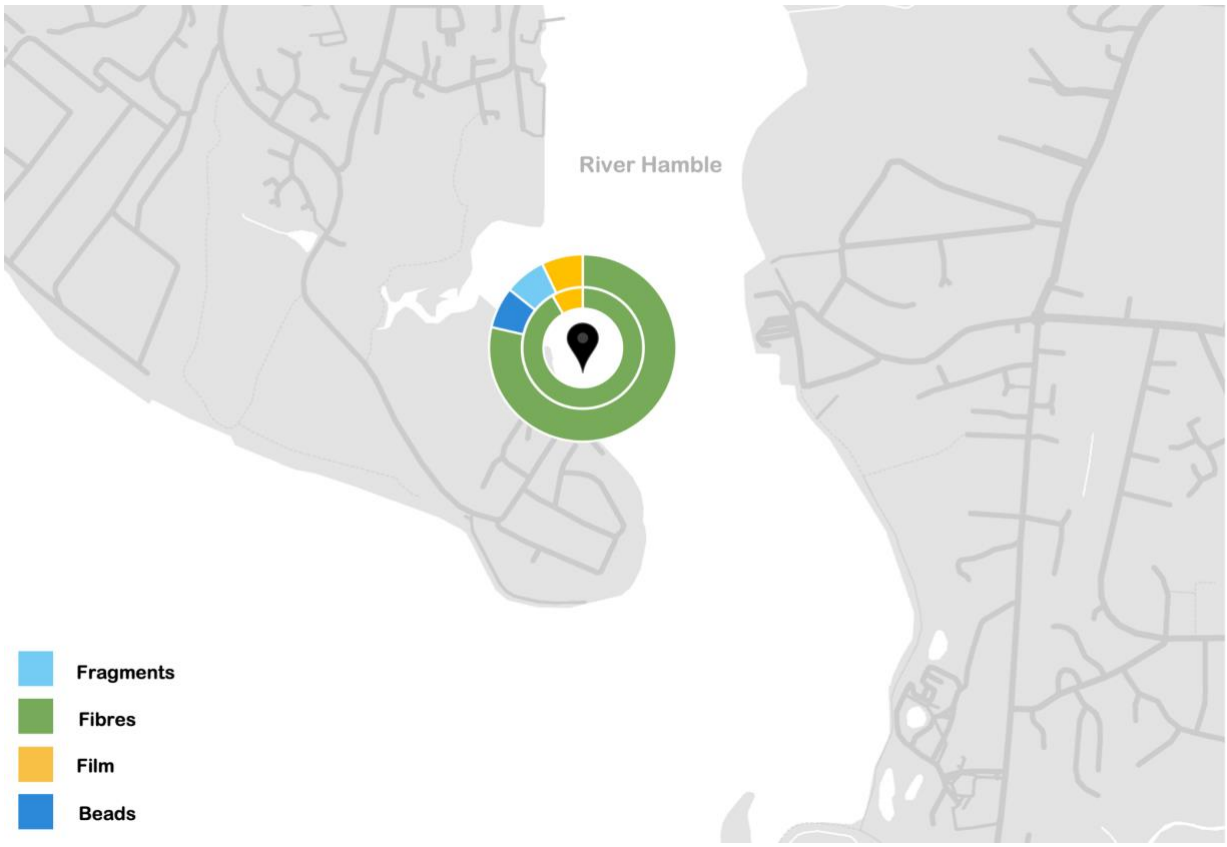


Figure 3. Map of the inshore sample location at Hamble Marina, River Hamble. The outer pie chart ring is representative of the data collected from the GPM sample; the inner pie chart ring is representative of the data collected from the hand-towed plankton net sample. The majority of MP collected were fibres, with some film but few beads or fragments.

In total, 36 MPs were recorded inshore in the Beaulieu River via two different collection methods: a hand-towed plankton net (2.24 MP m^{-3}) and the GPM (83,333 MP m^{-3}) (table 6). Fibres made up 75% of the total MPs, whilst fragments only made up 8.33%. The average diameter of fragments recorded at Bucklers' Hard using the plankton net was 11.5 μm and 10 μm using the GPM. The colours of fragments found were made up equally of blue, red, and black (33.33%) (table 7). The average length of fibres recorded at Buckers' Hard using the plankton net were 75 μm and 39.5 μm using the GPM. Blue was the predominant colour of the 27 fibres recorded (51.85%) (table 7).

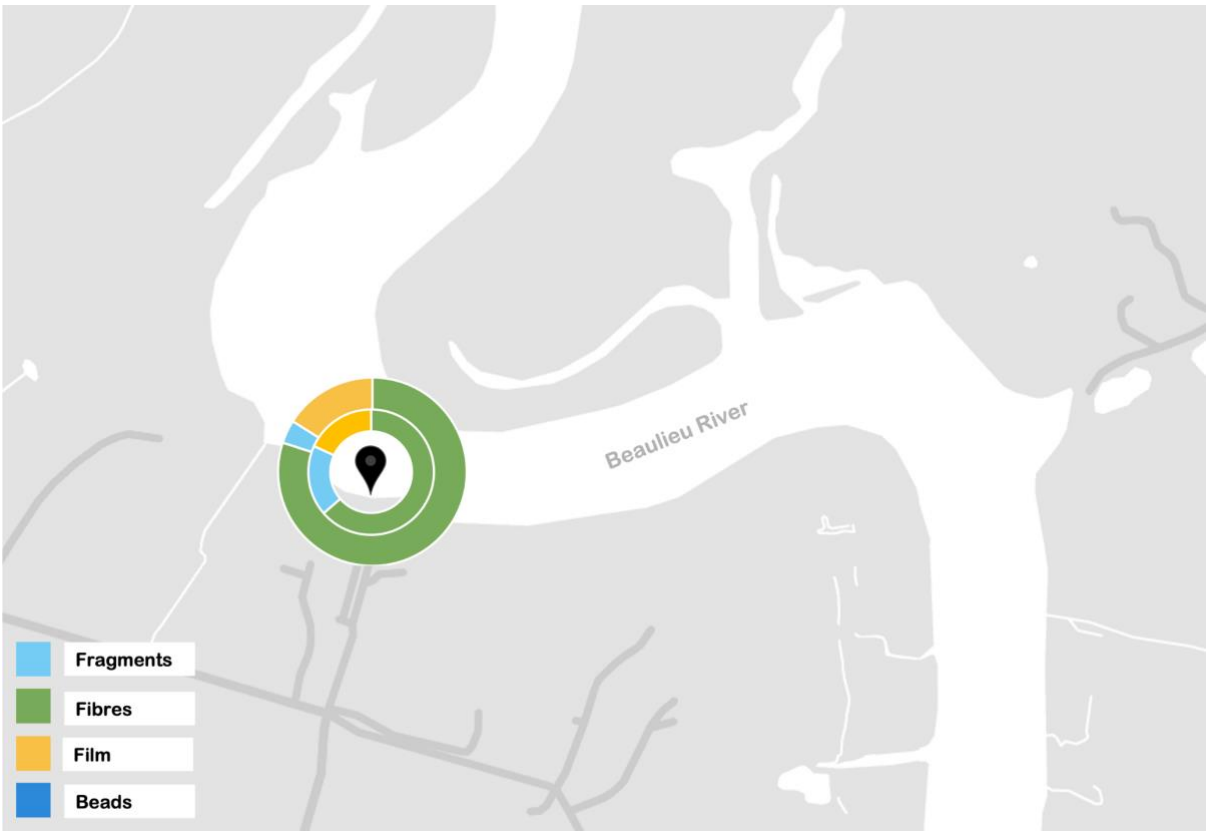


Figure 4. Map of the inshore sampling location Buckler's Hard, Beaulieu River. The outer pie chart ring is representative of the data collected from the GPM sample; the inner pie chart ring is representative of the data collected from the hand-towed plankton net sample. The majority of MP collected were fibres, with some film and fragmented MPs but no beads were recorded.

Table 6: Summary table of the type and abundance of MPs recorded, with respective collection method and volume of water samples from the inshore sample sites.

Site	Sample type	Vol of water (m^3)	Fibres	Beads	Fragments	Film	Total MPs
Hamble Marina	Plankton net	4.91	11	0	0	1	12
Bucklers' Hard	Plankton net	4.91	7	0	2	2	11
Hamble Marina	Glass plate	0.0003	11	1	1	1	14
Bucklers' Hard	Glass plate	0.0003	20	0	1	4	25

Table 7: Summary table of the average size, standard deviation (SD) and recorded colours of all fibres and fragments recorded from the inshore sample sites.

			Size	Colour				
Method	MP type	Site	Average length/ diameter (um)	Blue	Red	Black	White	Other
Plankton net	Fibre	Hamble Marina	56.81 ± 27.11	5	0	3	3	0
Plankton net	Fibre	Buckers' Hard	75 ± 68.68	4	2	0	1	0
Plankton net	Fragment	Hamble Marina	0 ± 0	0	0	0	0	0
Plankton net	Fragment	Buckers' Hard	11.5 ± 12.02	0	1	1	0	0
Glass plate	Fibre	Hamble Marina	51.81 ± 30.94	9	2	0	0	0
Glass plate	Fibre	Buckers' Hard	39.5 ± 27.81	10	3	4	3	0
Glass plate	Fragment	Hamble Marina	10 ± 0	1	0	0	0	0
Glass plate	Fragment	Buckers' Hard	10 ± 0	1	0	0	0	0

3.3 Polyp and *Artemia* Exposure Experiments

3.3.1 Statistical tests

The normality of the data collected from each experiment were tested with Shapiro-Wilk tests under the null hypothesis that the data would be normally distributed. Generally, the data were non-normally distributed, hence non-parametric Mann-Whitney U tests were performed to assess whether data varied significantly between experimental group pairs. Following the Mann-Whitney U tests, a Kruskal-Wallis test was used to further compare ingestion rates between more than two experimental groups.

3.3.2 Brief summary of key findings

Polyps exposed to MP fibres with no prey showed the largest variation in MP ingestion (1- 4 MPs, experiment 2), whilst polyps exposed to MP fragments with no prey showed a relatively low ingestion (0 – 2 MPs, experiment 3). Polyps exposed to MP fibres with prey showed the highest MP ingestion rate across all experiments (1 – 4 MPs, experiment 4), whereas polyps exposed to MP fragments with prey displayed low ingestion occurrence (0 – 2 MPs, experiment 5). The *Artemia* used in experiments 4 and 5 showed minimal MP particle ingestion, as did experiments 6 and 7 despite no predators being present (fig. 6).

3.3.3 Experiment 1 (Control)

Experiment 1 confirmed *A. aurita* polyps' prey on *Artemia* nauplii. On average, 8.08 $\pm$ 2 *Artemia* were ingested per 1 polyp / 24 hr (fig.5). The number of *Artemia* eaten per polyp was non-normally distributed (Shapiro-Wilk, W = 0.81703, p-value = 0.01473); a non-parametric one-sample Mann-Whitney U test was performed under the null hypothesis that the rate of *Artemia* ingested by *A. aurita* polyps in 24 hr is significantly different from the average 8.08 $\pm$ 2 (V = 67, p-value = 0.03002). The rejection of the null suggests ingestion rates of *Artemia* by *A. aurita* polyps differs significantly at the individual level.

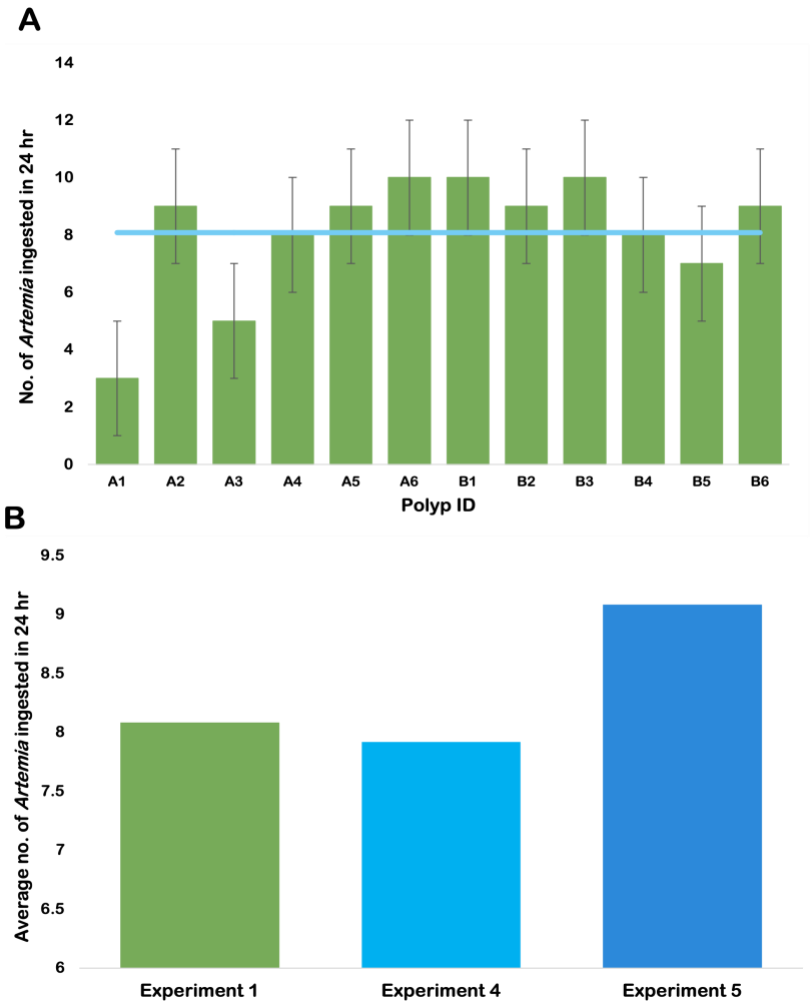


Figure 5. A) Bar chart displaying the number of *Artemia* eaten by each polyp in Experiment 1. Blue line represents the average number of *Artemia* eaten per polyp in the experiment (8.08 ± 2). Error bar represents the ± 2 disparities of the number of *Artemia* put into each well plate with a polyp at the start of the experiment. B) Bar chart comparing the average number of *Artemia* eaten by polyps in experiment 1, 4 and 5 – experiment 5 ingested the most (9.08 ± 2) and experiment 4 (7.92 ± 2) ingested the least but differences were insignificant when compared to the control average ingestion rate (section 4.3.5).

3.3.4 Experiment 2 and 3

Experiment 2 showed *A. aurita* polyps ingest MP fibres without the presence of natural prey, at an occurrence rate (number of contaminated polyps / total number of polyps in experiment) of 100% (n = 12). Quantities ingested ranged from 1 – 4 MPs / polyp, with an average ingestion rate (±1 standard deviation, SD) of 2.3 (± 1.15 SD) MPs /

polyp. A Shapiro-Wilk test highlighted the data was non-normal (W = 0.85907, p-value = 0.0476).

Experiment 3 showed that *A. aurita* polyps ingest MP fragments without the presence of natural prey, at an occurrence rate of 92% (n = 12). Quantities ingested ranged from 1 – 2 MPs / polyp (only considering the contaminated polyps), with an average ingestion rate of 1.3 (± 0.78 SD) MPs / polyp. A Shapiro-Wilk test highlighted the data was non-normal (W = 0.77716, p-value = 0.005213).

Due to the non-normal data distribution of this small data set, a Mann-Whitney U test was performed on the data from experiments 2 and 3 under the null hypothesis that polyps ingest fibres at the same rate as fragments without natural prey in a 24 hr. The experiments were significantly different (W = 106, p-value = 0.04395), therefore the null hypothesis can be rejected.

3.3.5 Experiment 4 and 5

Experiment 4 demonstrated polyps ingest fibres with natural prey at an occurrence rate of 100% (n = 12). Quantities ingested ranged from 1 – 4 MPs / polyp, with an average ingestion rate of 2.6 (± 1.16 SD) MPs / polyp. Data from this experiment was normally distributed, (Shapiro-Wilk W = 0.86638, p-value = 0.05881), but the p value suggested very low normality. The polyps ingested an average of 7.92 ± 2 *Artemia* / 24 hr, but this was not significantly different from the control average (Mann-Whitney, W = 77, p-value = 0.7883). The *Artemia* showed low levels of MP fibre ingestion (average 0.3 MPs / well).

Experiment 5 demonstrated polyps ingest fragments with natural prey at an occurrence rate of 82% (n = 12). Quantities ingested ranged from 1 -2 MPs / polyp (only considering contaminated polyps), at an average ingestion rate of 1.2 (± 0.72 SD) MPs / polyp. Data from this experiment were non normally distributed (Shapiro-Wilk W = 0.81833, p-value = 0.01526). The polyps ingested an average of 9.08 ± 2 *Artemia* / 24 hr, but this was not significantly different from the control average (W = 55.5, p-value = 0.3261). The *Artemia* showed low MP fragment ingestion (average of 0.4 MPs / well).

As the Shapiro-Wilk test for experiment 4 determined a non-significant but low p-value, a non-parametric Mann Whitney U test was performed on the data from experiments 4 and 5 under the null hypothesis that polyps ingest fibres and fragments at the same rate with natural prey present in 24 hr. Experiments 4 and 5 were significantly different (W = 112, p-value = 0.01669), therefore the null hypothesis can be rejected; polyps ingest more fibres than fragments in 24 hr with natural prey present.

3.3.6 Experiment 2 – 5 Kruskal-Wallis Test

The Kruskal-Wallis Test determined the occurrence rate of MPs in polyps was not significantly different between exposure experiments 2, 3, 4 and 5 (Kruskal-Wallis chi-squared = 3.7202, df = 3, p-value = 0.2933).

3.3.7 Experiment 6 and 7 – Artemia with microplastics and no predator

Experiment 6 indicates *Artemia* ingest little to no MP fibres upon exposure, without a predator. On average, 0.8 fibres were ingested by 10 ± 2 *Artemia* / 24 hr; most fibres were recorded in the well plate water (average 4.2 MPs / well). The data in this

experiment were non-normally distributed (Shapiro-Wilk, W = 0.80247, p-value = 0.009997).

Experiment 7 indicates *Artemia* ingest virtually no MP fragments upon exposure, without a predator. On average, 0.4 fragments were ingested by 10 ± 2 *Artemia* / 24 hr. Most fragments were recorded in the well plate water (average 4.6 MPs / well). The data in this experiment were non-normally distributed (Shapiro-Wilk, W = 0.67433, p-value = 0.0004823).

Due to the abnormal data distribution in this small data set, a non-parametric Mann-Whitney U test was performed on the data collected from Experiments 6 and 7 under the null hypothesis that *Artemia* ingest an equal number of fibres and fragments without natural predators present over 24 hr. Experiments 6 and 7 were not significantly different (W = 92.5, p-value = 0.2003), therefore the null hypothesis can be accepted – *Artemia* ingest MP fibres and fragments at an equal rate.

3.3.8 Experiment 4 – 7 Kruskal-Wallis Test

The occurrence of MPs in *Artemia* was significantly different between the exposure experiments (Kruskal-Wallis chi-squared = 10.373, df = 3, p-value = 0.01565). A pairwise Wilcoxon rank sum test with a Benjamini-Hochburg correction found experiments 5 and 6 were significantly different (p-value = 0.0067), suggesting *Artemia* exposure to fragments and a predator ingested less MPs than *Artemia* exposed to no predator and fibres.

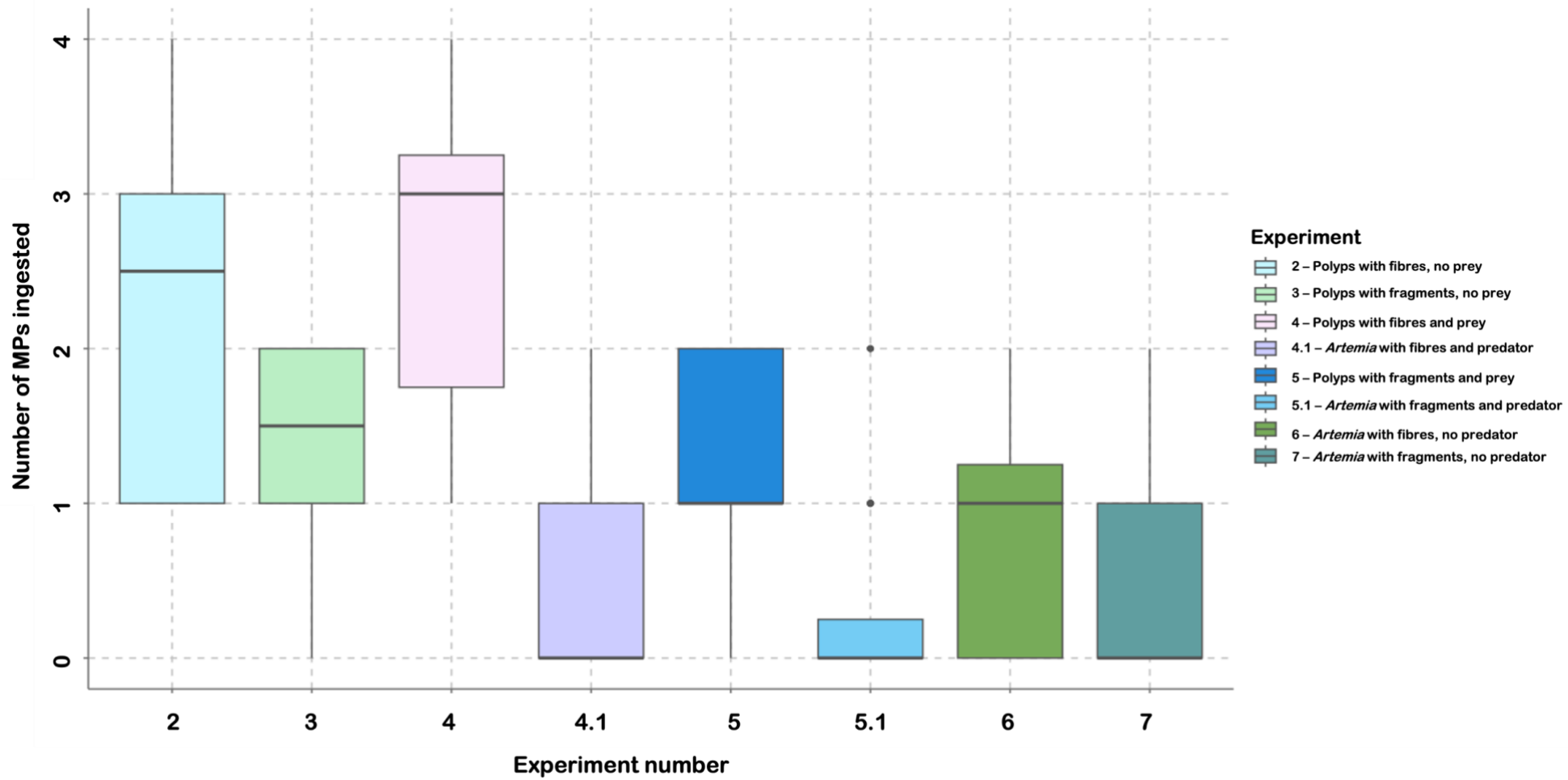


Figure 6. 2) Polyps exposed to MP fibres with no prey in the well showed the largest variation in MP ingestion rates for all individual organisms across the experiments, with a median of 2.5 fibres ingested and number of fibres ingested per polyp ranging from 1 – 4 fibres. 3) Polyps exposed to MP fragments with no prey showed relatively low ingestion of MP particles, with a maximum of 2 fragments ingested per individual polyp. 4) Polyps exposed to MP fibres with prey in the well showed the highest ingestion rates of any species across all experiments, with a median of 3 fibres ingested and a minimum of 1 fibre and maximum of 4 fibres ingested. 4.1) Artemia exposed to MP fibres with a predator in the well showed very low ingestion rates, with a maximum of 2 fibres ingested in a population of 10 $\pm$2 Artemia and median of 0 fibres ingested. 5) Polyps exposed to MP fragments with prey displayed the same range of particles consumed as Experiment 3 but with a lower median, which was 1 fragment. 5.1) Artemia exposed to MP fragments with a predator in the well showed the lowest quantity of MPs ingested by any organism across all experiments. With a median of 0 fragments ingested, the Artemia populations that consumed 1 or 2 fragments are considered outliers. 6) Artemia exposed to MP fibres with no predators in the well displayed a higher median consumption rate than the Artemia in Experiment 4.1, although the maximum number of fibres consumed was the same in both experimental populations. 7) Artemia exposed to MP fragments with no predators in the well displayed a similar consumption rate to the Artemia population in Experiment 6, but consumption of MPs were vastly higher than in Experiment 5.1 in the presence of a predator.

4 Discussion

4.1 Microplastic Quantification

The first aim of this study was to quantify MP characteristics in SW estuary. Of the total number of MPs recorded from the offshore plankton net trawl (663 MPs), fragments made up 56.85% (table 4). At the remaining offshore and inshore sites, fibres made up the majority of MPs (>75% per site) (table 4, 6). The high abundance of MP fragments in the River Test from the plankton net trawl was unexpected, as nearly all MP quantification studies find predominantly fibres (Anderson et al., 2018; Gallagher et al., 2016; Aytan et al., 2016; Steer et al., 2017; Schmidt et al., 2018; Desforges et al., 2014). Large quantities of MP fragments have been recorded in the Northern Hemisphere (Andrady, 2011), which somewhat validates this study's findings. Gallagher et al. (2016) recorded 35 'irregular' and 115 'rounded' MPs in the River Test; assuming the 'irregular' MPs are fragments, Gallagher et al. (2016) provide further evidence of this MP type being identified in the River Test. Furthermore, this study found blue MPs were present in > 48% of all sample sites, in keeping with other studies (Nelms et al., 2018; Clark et al., 2016). The average size of fibre and fragments ranged between 6 – 75 μm at all sample sites, which is relatively small considering most studies report MPs to range between 500μm – 5mm (Hidalgo-Ruz et al., 2012); this could be a result of abrasion from strong tidal currents associated with SW estuary (Quaresma et al., 2007).

Ultimately, this study emphasises how few MP beads 'naturally' occur in SW estuary (7.76% of total MPs), as predicted in the study aims. SW estuary contains 6.57 times as many fragments and 4.95 times as may fibres than beads. Studying the influence of MP beads in zooplankton and other marine organisms results in an inaccurate

understanding of how MPs affect these species *in situ* as they are unlikely to encounter them. Thus, it was necessary to gather evidence to suggest that a zooplankton species ingest 'naturally' occurring MPs to provide a basis for further research regarding MP pollution.

4.2 Microplastic sampling

The second aim of this study was to assess the usability and efficiency of MP collection methods. Offshore plankton net trawling sampled a higher volume of water (m^3) than any other method, collecting the highest quantity of MPs but lowest concentration of MPs m^{-3}. For example, 1.8 MP m^{-3} were recorded offshore at Mayflower during a plankton new trawl, but 16,000 MP m^{-3} were recorded offshore at the same location with a CTD bottle capture. Firstly, this suggests the 200 μm plankton net does not efficiently capture MPs passing through the mesh. Secondly, the data highlight the possibility of uneven distribution of MPs in the water column (Lebreton et al., 2012). Although the wake of the research vessel may have disturbed MPs floating in the water column throughout trawling, the inshore plankton net tow captured a similar concentration of MPs (2.44 M^{-3} Hamble, 2.24 M^{-3}, Beaulieu). Aytan et al. (2016) also recorded similar MP concentrations to this study in the Black Sea after conducting a series of plankton net trawls (0.31 – 3.32 MP m^{-3}). Trawling appears to be a limiting MP capture method in comparison to the CTD bottle capture at offshore sites. Moreover, Gallagher et al. (2016) recorded 348 MPs in the River Test using a 300 μm mesh plankton net. This value is nearly half of the 663 MP recorded in the same river as this study, albeit comparisons of MPs m^{-3} in SW are not possible as Gallagher et al. (2016) did not provide a volume of water sampled. The larger mesh size used by Gallagher et al., (2016) may have limited the quantity of MPs collected, but MP

abundance may have increased in the River Test in the last six years. Quantifying MPs in offshore sites via plankton net trawling is commonly practised in marine science (Steer et al., 2017; Schmidt et al., 2018). Yet, the CTD bottle capture in this study collected more MPs m^{-3}, less debris and was less time-consuming. Hence, this study suggests that CTD bottle capture should be practiced more frequently during offshore MP collection in the upper most layer of the water column.

The MPs collected inshore further display the fallible nature of plankton net trawling. At Hamble Marina and Buckler's Hard, the plankton net caught 2.44 MP m^{-3} and 2.24 MP m^{-3}, respectively, compared to the GPM which captured 46,000 MP m^{-3} and 83,333 MP m^{-3}, respectively. When devising the GPM, Anderson et al. (2018) found between 42,100 – 93,000 MP m^{-3} at sample sites in Hamble and Beaulieu River - concentrations comparable to this study. From the results of this study, the newly devised GPM by Anderson et al. (2018) seems a rapid, efficient inshore MP collection method. Yet, this method is limited as beads and fragments do not appear to adhere well to the glass surface, despite the plankton net tow showing evidence that these MP types are found inshore at Hamble and Beaulieu.

The high concentrations of MPs found in SW water estuary could be a result of tidal activity, geographical constraints, and anthropogenic activity (Lebreton et al., 2012). The sample sites in this study were selected due to their proximity to key ports and marinas in SW, but future research should be directed at assessing whether there are significant differences between MP abundances in these areas and pinpointing the primary cause of pollution.

4.3 Polyp and *Artemia Exposure Experiments*

4.3.1 Microplastic adherence

Whilst adherence to animal tissue has been identified as a pathway for the uptake of MPs in some organisms, Rocha et al. (2020) found the average number of MPs adhering to coral polyp epidermis to be 1.2 MPs / 96 hr with a MP quantity of 1 mg / mL, sized 63 – 125 μm. This study recognises that MPs may be adhering to the polyps but is likely to occur infrequently and thus any MPs found on the polyp filter papers were considered to be ingested. In future research, examination between adherence and ingestion rates of MPs in polyps should be evaluated.

4.3.2 Experiment 1 (Control)

Experiment 1 confirmed *A. aurita* polyps ingest *Artemia* as a natural prey (Sullivan et al., 1994). The polyps showed slight variation in the quantity of *Artemia* ingested in experiment 1 (fig. 5). Environmental variables like light, food, temperature, and salinity can influence polyp growth and reproduction (Hubot et al., 2017), but as these variables were kept the same throughout the polyp incubation, other factors may have influenced feeding rate. Polyp size and health could have influenced feeding rates in experiment 1. In future studies, recording the size of each polyp before exposure experiments would be useful. But, as the average feeding rate was not significantly different between the polyps, the differences in feeding rate are not a limitation in this study.

4.3.3 Experiment 2 and 3

One aim of this study was to assess whether *A. aurita* polyps ingest MPs, without the presence of natural prey. Experiments 2 and 3 suggest polyps ingest MP fibres and

fragments without prey, rejecting H01. Polyps ingested significantly more fibres than fragments in the exposure period according to a Mann Whitney U test but not a Kruskal-Wallis test, questioning the validity of the significance. Evidence suggests certain zooplankton copepod species selectively ingest specific MP shapes, with preference over other shapes (Botterell et al., 2020). It is thought that different feeding strategies or species-specific capacity to ingest an MP shape increase ingestion probability (Botterell et al., 2020). Being long, thin, and pliant, MP fibres could become entangled in polyp tentacles more easily than irregularly shaped, hard MP fragments. Fibres are also similar in shape to the thin, tapered *Artemia* nauplii that *A. aurita* polyps' prey upon. Hence, the shape and material properties of MP fibres may have contributed to higher ingestion rates in *A. aurita* polyps. The uptake of MPs is not influenced by shape in all zooplankton species (Klein et al., 2021), therefore size should also be considered as a variable. Regardless of the identical size ranges used for both MP types in this study (1 – 60 μm), fragments have a larger surface area than fibres, which may have made them harder to ingest for the polyps. Yet, *A. aurita* polyps are capable of ingesting *Artemia* nauplii which can grow to 517 μm in size (Léger et al., 1987). This insinuates that the polyps had a particular infinity for ingesting MP fibres. Fibrous MP material was identified in abundance in SW estuary (fig. 2, 3, 4). However, the ratio of MPs to water volume (1 MP / mL) is an inaccurate reflection of the number of MPs found *in situ* (table 4). Whilst experiment 2 shows *A. aurita* polyps ingest MP fibres at a relatively high rate, this polyp species may not be at such a high risk of ingesting these MPs in SW estuary. Ideally, future research should focus on collecting polyp samples from the estuary to find evidence of *in situ* MP ingestion.

4.3.4 Experiment 4 and 5

Another aim of this study was to assess whether *A. aurita* polyps ingest MPs that commonly occur in SW with the presence of natural prey. Prey is thought to stimulate feeding behaviour in some zooplankton species during exposure experiments, resulting in the uptake of more MPs (Hall et al., 2015). Contrary to other studies (Romero-Kutzner et al., 2022; Hall et al., 2015), the addition of *Artemia* in experiments 4 and 5 did not significantly increase the ingestion of MPs by *A. aurita* in this study. In other words, MP ingestion rate by polyps was not influenced by the presence of prey. As the polyps maintained MP ingestion rates, the H02 can be rejected. The polyps also maintained an affinity for ingesting more fibres than fragments. Cnidarian polyps are considered to have an indiscriminate appetite (Schiariti et al., 2008), but experiments 2 – 5 show MP ingestion selectivity for fibres in this polyp species. Furthermore, the ingestion of *Artemia* by polyps in experiments 4 and 5 did not significantly change in the presence of MPs compared to experiment 1, suggesting that the ingestion of MPs did not negatively impact natural feeding rates in the polyps.

4.3.5 Experiment 6 and 7

The final aim of this study was to assess whether *Artemia* nauplii ingest MPs with or without a predator. MP size range small enough to account for the size of particles ingested by *Artemia* nauplii (≤ 20 μm) (Wang et al., 2019) were used in the experimental exposures. However, extremely low quantities of MPs (average 0.8 fibres, 0.4 fragments) were ingested between 10 ± 2 *Artemia* / 24 hr, even without a predator. *Artemia* did ingest significantly more fibres without a predator than fragments with a predator (p-value = 0.0067), but this could have been due to differences in polyp feeding rates. Overall, the H03 can be rejected, although some research implies MP

ingestion in zooplankton occurs from trophic transfer of contaminated prey (Sucharitakul et al., 2020; Setälä et al., 2014; Costa et al., 2020). Future studies should feed *Aretmia* nauplii a higher concentration of MP ≤ 20 μm – size was a limiting factor in this study as MPs were taken from filtered water samples in SW, ordinarily MP beads are ordered in a specific size and quantity for experiments.

Regardless, this emphasises that MP ingestion in *A. aurita* polyps is not governed by trophic interactions with prey. Whilst Setälä et al. (2014) found certain macrozooplankton ingest MPs from contaminated mesozooplankton, this study did not contain any cnidarian species and only used MP beads to demonstrate the trophic transfer of MPs, thus does not challenge the findings in this study. Another study has shown evidence of *Artemia* nauplii ingesting MP beads (Sucharitakul et al., 2020), but no evidence was collected to investigate whether other MPs shapes were ingested in significant quantities. The same study (Sucharitakul et al., 2020) found that *Aurelia coerulea* ephyrae were 35 times more likely to be contaminated with MPs via trophic transfer than direct ingestion. As few to no MP beads are found during MP quantification studies, (Aytan et al., 2016; Steer et al., 2017), the findings of Sucharitakul et al. (2020) study are not a strong representation of potential MP ingestion in marine organisms *in situ*. If research focus is maintained on MP bead contamination in zooplankton, our understanding of how MPs influence cnidarian zooplankton species will be impaired. As *Artemia* in experiments 6 and 7 were exposed to a higher ratio of MPs to water volume than were found in SW estuary, but low ingestion rates were still observed, it is unlikely trophic transfer of MP fibres or fragments is occurring between *A. aurita* polyps and *Artemia* in the estuary.

Conclusion

Firstly, this study has demonstrated that MPs are abundant in SW estuary. Fibres and fragments were the predominantly recorded MP shapes, but beads and film were also recorded. There was great diversity in size of MPs, ranging from 2 – 220 μm in length and the colour of MPs was predominately blue. MPs were plentiful in both inshore and offshore sample sites within the estuary. Of the three methods used to collect MPs, using CTD bottles for an instant water capture sample was the fastest and most efficient method. Therefore, this study recommends using CTD bottle capture and at least one other method during MP future quantification studies to accurately gauge MP abundance.

Secondly, this study has found evidence to suggest the cnidarian polyp *A. aurita*, a local species in SW estuary, ingests MP fibres and fragments in laboratory exposure experiments. Trophic transfer from natural prey did not appear to be a leading cause of ingestion in this polyp species. The polyps showed an affinity for ingesting fibres; when considering how many fibres were collected from SW estuary, this species is likely to be at high risk of ingesting MP fibres in SW. This study encourages further research into how fibre ingestion may impact the health of *A. aurita* polyps and the collection of polyps from SW estuary to investigate whether they ingest fibres *in situ*.

References

Alabi, O.A., Ologbonjaye, K.I., Awosolu, O. and Alalade, O.E., 2019. Public and environmental health effects of plastic wastes disposal: a review. *J Toxicol Risk Assess*, *5*(021), pp.1-13.

Anderson, Z.T., Cundy, A.B., Croudace, I.W., Warwick, P.E., Celis-Hernandez, O. and Stead, J.L., 2018. A rapid method for assessing the accumulation of microplastics in the sea surface microlayer (SML) of estuarine systems. *Scientific Reports*, *8*(1), p.9428.

Andrady, A.L., 2011. Microplastics in the marine environment. *Marine pollution bulletin*, *62*(8), pp.1596-1605.

Aytan, U., Valente, A., Senturk, Y., Usta, R., Sahin, F.B.E., Mazlum, R.E. and Agirbas, E., 2016. First evaluation of neustonic microplastics in Black Sea waters. *Marine environmental research*, *119*, pp.22-30.

Barros, H.P. and Valenti, W.C., 2003. Ingestion rates of Artemia nauplii for different larval stages of Macrobrachium rosenbergii. *Aquaculture*, *217*(1-4), pp.223-233.

Botterell, Z.L., Beaumont, N., Dorrington, T., Steinke, M., Thompson, R.C. and Lindeque, P.K., 2019. Bioavailability and effects of microplastics on marine zooplankton: A review. *Environmental Pollution*, *245*, pp.98-110.

Botterell, Z.L., Beaumont, N., Cole, M., Hopkins, F.E., Steinke, M., Thompson, R.C. and Lindeque, P.K., 2020. Bioavailability of microplastics to marine zooplankton: effect of shape and infochemicals. *Environmental Science & Technology*, *54*(19), pp.12024-12033.

Boucher, J. and Friot D. (2017). Primary Microplastics in the Oceans: A Global Evaluation of Sources. Gland, Switzerland: IUCN. 43pp.

Browne, M.A., Galloway, T.S. and Thompson, R.C., 2010. Spatial patterns of plastic debris along estuarine shorelines. *Environmental science & technology*, *44*(9), pp.3404-3409.

Celis-Hernandez, O., Cundy, A.B., Croudace, I.W. and Ward, R.D., 2022. Environmental risk of trace metals and metalloids in estuarine sediments: An example from Southampton Water, UK. *Marine Pollution Bulletin*, *178*, p.113580.

Claessens, M., De Meester, S., Van Landuyt, L., De Clerck, K. and Janssen, C.R., 2011. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. *Marine pollution bulletin*, *62*(10), pp.2199-2204.

Clark, J.R., Cole, M., Lindeque, P.K., Fileman, E., Blackford, J., Lewis, C., Lenton, T.M. and Galloway, T.S., 2016. Marine microplastic debris: a targeted plan for understanding and quantifying interactions with marine life. *Frontiers in Ecology and the Environment*, *14*(6), pp.317-324.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J. and Galloway, T.S., 2013. Microplastic ingestion by zooplankton. *Environmental science & technology*, *47*(12), pp.6646-6655.

Cole, M., Lindeque, P., Halsband, C. and Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. *Marine pollution bulletin*, *62*(12), pp.2588-2597.

Costa, E., Piazza, V., Lavorano, S., Faimali, M., Garaventa, F. and Gambardella, C., 2020. Trophic transfer of microplastics from copepods to jellyfish in the marine environment. *Frontiers in Environmental Science*, *8*, p.571732.

Croudace, I.W. and Cundy, A.B., 1995. Heavy metal and hydrocarbon pollution in recent sediments from Southampton Water, Southern England: a geochemical and isotopic study. *Environmental science & technology*, *29*(5), pp.1288-1296.

De Sá, L.C., Oliveira, M., Ribeiro, F., Rocha, T.L. and Futter, M.N., 2018. Studies of the effects of microplastics on aquatic organisms: what do we know and where should we focus our efforts in the future? *Science of the total environment*, *645*, pp.1029-1039.

Desforges, J.P.W., Galbraith, M., Dangerfield, N. and Ross, P.S., 2014. Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean. *Marine pollution bulletin*, *79*(1-2), pp.94-99.

Desforges, J.P.W., Galbraith, M. and Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the Northeast Pacific Ocean. *Archives of environmental contamination and toxicology*, *69*, pp.320-330.

Dyer, K.R., 1982. Localized mixing of low salinity patches in a partially mixed estuary (Southampton Water, England). In *Estuarine comparisons* (pp. 21-36). Academic Press.

Eerkes-Medrano, D., Thompson, R.C. and Aldridge, D.C., 2015. Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. *Water research*, *75*, pp.63-82.

Gallagher, A., Rees, A., Rowe, R., Stevens, J. and Wright, P., 2016. Microplastics in the Solent estuarine complex, UK: an initial assessment. *Marine Pollution Bulletin*, *102*(2), pp.243-249.

Gewert, B., Plassmann, M.M. and MacLeod, M., 2015. Pathways for degradation of plastic polymers floating in the marine environment. *Environmental science: processes & impacts*, *17*(9), pp.1513-1521.

Hall, N.M., Berry, K.L.E., Rintoul, L. and Hoogenboom, M.O., 2015. Microplastic ingestion by scleractinian corals. *Marine Biology*, *162*, pp.725-732.

Hays, G.C., Doyle, T.K. and Houghton, J.D., 2018. A paradigm shift in the trophic importance of jellyfish?. *Trends in ecology & evolution*, *33*(11), pp.874-884.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C. and Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environmental science & technology*, *46*(6), pp.3060-3075.

Hierl, F., Wu, H.C. and Westphal, H., 2021. Scleractinian corals incorporate microplastic particles: identification from a laboratory study. *Environmental Science and Pollution Research*, *28*(28), pp.37882-37893.

Hubot, N., Lucas, C.H. and Piraino, S., 2017. Environmental control of asexual reproduction and somatic growth of Aurelia spp.(Cnidaria, Scyphozoa) polyps from the Adriatic Sea. *PLoS One*, *12*(6), p.e0178482.

Jacobsen, J.K., Massey, L. and Gulland, F., 2010. Fatal ingestion of floating net debris by two sperm whales (Physeter macrocephalus). *Marine Pollution Bulletin*, *60*(5), pp.765-767.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R. and Law, K.L., 2015. Plastic waste inputs from land into the ocean. *Science*, *347*(6223), pp.768-771.

Kaposi, K.L., Mos, B., Kelaher, B.P. and Dworjanyn, S.A., 2014. Ingestion of microplastic has limited impact on a marine larva. *Environmental science & technology*, *48*(3), pp.1638-1645.

Kooi, M., Nes, E.H.V., Scheffer, M. and Koelmans, A.A., 2017. Ups and downs in the ocean: effects of biofouling on vertical transport of microplastics. *Environmental science & technology*, *51*(14), pp.7963-7971.

Kosore, C., Ojwang, L., Maghanga, J., Kamau, J., Kimeli, A., Omukoto, J., Ngisiag'e, N., Mwaluma, J., Ong'ada, H., Magori, C. and Ndirui, E., 2018. Occurrence and ingestion of microplastics by zooplankton in Kenya's marine environment: first documented evidence. *African Journal of Marine Science*, *40*(3), pp.225-234.

Kowalski, N., Reichardt, A.M. and Waniek, J.J., 2016. Sinking rates of microplastics and potential implications of their alteration by physical, biological, and chemical factors. *Marine pollution bulletin*, *109*(1), pp.310-319.

Lebreton, L.M., Greer, S.D. and Borrero, J.C., 2012. Numerical modelling of floating debris in the world's oceans. *Marine pollution bulletin*, *64*(3), pp.653-661.

Léger, P., Bengtson, D.A., Sorgeloos, P., Simpson, K.L. and Beck, A.D., 1987. The nutritional value of Artemia: a review. *Artemia research and its applications*, *3*, pp.357-372.

Li, W.C., Tse, H.F. and Fok, L., 2016. Plastic waste in the marine environment: A review of sources, occurrence and effects. *Science of the total environment*, *566*, pp.333-349.

Lucas, C.H., 2001. Reproduction and life history strategies of the common jellyfish, Aurelia aurita, in relation to its ambient environment. In *Jellyfish Blooms: Ecological and Societal Importance: Proceedings of the International Conference on Jellyfish Blooms, held in Gulf Shores, Alabama, 12–14 January 2000* (pp. 229-246). Springer Netherlands.

Lucas, C.H., Graham, W.M. and Widmer, C., 2012. Jellyfish life histories: role of polyps in forming and maintaining scyphomedusa populations. *Advances in marine biology*, *63*, pp.133-196.

Lucas, C.H. and Williams, J.A., 1994. Population dynamics of the scyphomedusa Aurelia aurita in Southampton Water. *Journal of Plankton Research*, *16*(7), pp.879-895.

Lucas, C.H., Williams, D.W., Wiliams, J.A. and Sheader, M., 1995. Seasonal dynamics and production of the hydromedusan Clytia hemisphaerica (Hydromedusa: Leptomedusa) in Southampton water. *Estuaries*, *18*, pp.362-372.

Michels, J., Stippkugel, A., Lenz, M., Wirtz, K. and Engel, A., 2018. Rapid aggregation of biofilm-covered microplastics with marine biogenic particles. *Proceedings of the Royal Society B*, *285*(1885), p.20181203.

Murphy, F., Ewins, C., Carbonnier, F. and Quinn, B., 2016. Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. *Environmental science & technology*, *50*(11), pp.5800-5808.

Nelms, S.E., Duncan, E.M., Broderick, A.C., Galloway, T.S., Godfrey, M.H., Hamann, M., Lindeque, P.K. and Godley, B.J., 2016. Plastic and marine turtles: a review and call for research. *ICES Journal of Marine Science*, *73*(2), pp.165-181.

Nelms, S.E., Galloway, T.S., Godley, B.J., Jarvis, D.S. and Lindeque, P.K., 2018. Investigating microplastic trophic transfer in marine top predators. *Environmental pollution*, *238*, pp.999-1007.

Nobre, C.R., Santana, M.F.M., Maluf, A., Cortez, F.S., Cesar, A., Pereira, C.D.S. and Turra, A., 2015. Assessment of microplastic toxicity to embryonic development of the sea urchin Lytechinus variegatus (Echinodermata: Echinoidea). *Marine pollution bulletin*, *92*(1-2), pp.99-104

Persson, L., Carney Almroth, B.M., Collins, C.D., Cornell, S., de Wit, C.A., Diamond, M.L., Fantke, P., Hassellöv, M., MacLeod, M., Ryberg, M.W. and Søgaard Jørgensen, P., 2022. Outside the safe operating space of the planetary boundary for novel entities. *Environmental science & technology*, *56*(3), pp.1510-1521.

PlasticsEurope, E., 2012. Plastics–The Facts 2012: An analysis of European plastics production, demand and waste data for 2011.

Quaresma, V.D.S., Bastos, A.C. and Amos, C.L., 2007. Sedimentary processes over an intertidal flat: A field investigation at Hythe flats, Southampton Water (UK). *Marine geology*, *241*(1-4), pp.117-136.

Rekstad, M.E., Majaneva, S., Borgersen, Å.L. and Aberle, N., 2021. Occurrence and habitat characteristics of Aurelia sp. polyps in a high-latitude fjord. *Frontiers in Marine Science*, *8*, p.684634.

Richardson, A.J., 2008. In hot water: zooplankton and climate change. *ICES Journal of Marine Science*, *65*(3), pp.279-295.

Rocha, R.J.M., Rodrigues, A.C.M., Campos, D., Cícero, L.H., Costa, A.P.L., Silva, D.A.M., Oliveira, M., Soares, A.M.V.M. and Silva, A.P., 2020. Do microplastics affect the zoanthid Zoanthus sociatus?. *Science of The Total Environment*, *713*, p.136659.

Romero-Kutzner, V., Tarí, J., Herrera, A., Martínez, I., Bondyale-Juez, D. and Gómez, M., 2022. Ingestion of polyethylene microspheres occur only in presence of prey in the jellyfish Aurelia aurita. *Marine Pollution Bulletin*, *175*, p.113269.

Rotjan, R.D., Sharp, K.H., Gauthier, A.E., Yelton, R., Lopez, E.M.B., Carilli, J., Kagan, J.C. and Urban-Rich, J., 2019. Patterns, dynamics and consequences of microplastic ingestion by the temperate coral, Astrangia poculata. *Proceedings of the Royal Society B*, *286*(1905), p.20190726.

Schiariti, A., Kawahara, M., Uye, S. and Mianzan, H.W., 2008. Life cycle of the jellyfish Lychnorhiza lucerna (Scyphozoa: Rhizostomeae). *Marine Biology*, *156*, pp.1-12.

Schmidt, N., Thibault, D., Galgani, F., Paluselli, A. and Sempéré, R., 2018. Occurrence of microplastics in surface waters of the Gulf of Lion (NW Mediterranean Sea). *Progress in Oceanography*, *163*, pp.214-220.

Small, C. and Nicholls, R.J., 2003. A global analysis of human settlement in coastal zones. *Journal of coastal research*, pp.584-599.

Setälä, O., Fleming-Lehtinen, V. and Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the planktonic food web. *Environmental pollution*, *185*, pp.77-83.

Soulsby, P.G., Lowthion, D. and Houston, M.C.M., 1985. The identification and evaluation of environmental quality in Southampton Water, UK, using limited manpower resources. *Estuarine management and quality assessment*, pp.187-201.

Steer, M., Cole, M., Thompson, R.C. and Lindeque, P.K., 2017. Microplastic ingestion in fish larvae in the western English Channel. *Environmental pollution*, *226*, pp.250-259.

Sucharitakul, P., Pitt, K.A. and Welsh, D.T., 2020. Limited ingestion, rapid egestion and no detectable impacts of microbeads on the moon jellyfish, Aurelia aurita. *Marine Pollution Bulletin*, *156*, p.111208.

Sullivan, B.K., Garcia, J.R. and Klein-MacPhee, G., 1994. Prey selection by the scyphomedusan predator Aurelia aurita. *Marine Biology*, *121*, pp.335-341.

Sun, X., Li, Q., Zhu, M., Liang, J., Zheng, S. and Zhao, Y., 2017. Ingestion of microplastics by natural zooplankton groups in the northern South China Sea. *Marine pollution bulletin*, *115*(1-2), pp.217-224.

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W., McGonigle, D. and Russell, A.E., 2004. Lost at sea: where is all the plastic? *Science*, *304*(5672), pp.838-838.

Townend, I., 2008. A conceptual model of Southampton Water. *Environment Agency, DEFRA, ABP MER*.

Turner, J.T., 2015. Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. *Progress in Oceanography*, *130*, pp.205-248.

Van Cauwenberghe, L., Vanreusel, A., Mees, J. and Janssen, CR., 2013. Microplastic pollution in deep-sea sediments. *Environmental pollution, 182,* pp.495-499.

Van Sebille, E., Wilcox, C., Lebreton, L., Maximenko, N., Hardesty, B.D., Van Franeker, J.A., Eriksen, M., Siegel, D., Galgani, F. and Law, K.L., 2015. A global inventory of small floating plastic debris. *Environmental Research Letters*, *10*(12), pp.124006.

Vroom, R.J., Koelmans, A.A., Besseling, E. and Halsband, C., 2017. Aging of microplastics promotes their ingestion by marine zooplankton. *Environmental pollution*, *231*, pp.987-996.

Wang, Y., Zhang, D., Zhang, M., Mu, J., Ding, G., Mao, Z., Cao, Y., Jin, F., Cong, Y., Wang, L. and Zhang, W., 2019. Effects of ingested polystyrene microplastics on brine shrimp, Artemia parthenogenetica. *Environmental Pollution*, *244*, pp.715-722.

Wayman, C. and Niemann, H., 2021. The fate of plastic in the ocean environment–a minireview. *Environmental Science: Processes & Impacts*, *23*(2), pp.198-212.

Worm, B., Lotze, H.K., Jubinville, I., Wilcox, C. and Jambeck, J., 2017. Plastic as a persistent marine pollutant. *Annual Review of Environment and Resources*, *42*, pp.1-26.

Wright, S.L., Thompson, R.C. and Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. *Environmental pollution*, *178*, pp.483-492.

Yonkos, L.T., Friedel, E.A., Perez-Reyes, A.C., Ghosal, S. and Arthur, C.D., 2014. Microplastics in four estuarine rivers in the Chesapeake Bay, USA. *Environmental science & technology*, *48*(24), pp.14195-14202.

Zheng J., and Suh S., 2019. Strategies to reduce the global carbon footprint of plastics. *Nature Climate Change*, *29(5),* pp.374-8.

Appendix

Table 8: The abundance, shape and colour of all MPs recorded in this study, relative to the sample site and collection method used.

Site	Sample type	Filter No.	No. of MPs	Blue fibre	Blue fragme	Blue bead	Red fibre	Red fragmer	Red bead	Black fragment	Black fibre	Black bead	White Fragment	White Fibre	Beads (other)	Fragments (other)	Film
Upper S.G (test)	Boat net	1	49	27	4	1	1	2	5	2	6	0	0	0	0	0	1
Upper S.G (test)	Boat net	1.1	15	2	1	1	1	1	0	5	3	0	0	0	1	0	0
Upper S.G (test)	Boat net	1.2	38	7	18	0	0	2	0	4	6	0	1	0	0	0	0
Upper S.G (test)	Boat net	1.3	27	7	8	0	0	2	0	6	4	0	0	0	0	0	0
Upper S.G (test)	Boat net	1.4	35	1	17	0	0	11	0	5	1	0	0	0	0	0	0
Dock Head	Boat net	2	12	0	2	2	1	0	1	2	1	1	1	0	0	0	1
Dock Head	Boat net	2.1	29	3	7	0	0	0	4	5	3	2	3	0	0	0	2
Dock Head	Boat net	2.2	34	5	12	1	1	0	1	5	4	3	1	0	0	0	1
Dock Head	Boat net	2.3	22	1	8	2	0	0	0	5	0	1	1	0	2	0	2
Dock Head	Boat net	2.4	16	2	10	0	0	1	0	1	0	0	0	0	1	0	1
Dock Head	Boat net	2.5	11	1	5	0	0	0	0	1	1	0	2	0	1	0	0
Dock Head	Boat net	2.6	20	4	10	0	0	0	0	3	0	0	1	0	2	0	0
Dock Head	Boat net	2.7	30	2	16	1	0	0	0	2	1	1	5	1	0	1	0
Mayflower	Boat net	3	17	5	5	0	0	0	2	4	0	1	0	0	0	0	0
Mayflower	Boat net	3.1	19	4	4	0	1	2	0	2	3	0	0	0	1	1	1
Mayflower	Boat net	3.2	37	6	15	2	2	1	0	4	4	0	0	0	3	0	0
Mayflower	Boat net	3.3	27	2	14	0	1	1	0	2	2	0	0	0	2	0	3
Mayflower	Boat net	3.4	22	4	9	0	0	0	1	4	4	0	0	0	0	0	0
Mayflower	Boat net	3.5	26	7	9	0	0	0	0	2	6	0	0	0	0	0	2
Mayflower	Boat net	3.6	11	3	7	0	0	0	0	0	1	0	0	0	0	0	0
Mayflower	Boat net	3.7	17	2	8	0	0	0	0	2	4	0	0	0	1	0	0
Mayflower	Boat net	3.8	19	1	6	1	0	0	0	2	5	0	1	0	2	0	1
Marchwood	Boat net	4	17	4	7	0	3	0	0	1	1	0	1	0	0	0	0
Marchwood	Boat net	4.1	11	3	5	0	0	0	0	2	1	0	0	0	0	0	0
Marchwood	Boat net	4.2	18	6	9	1	1	0	0	1	0	0	0	0	0	0	0
Marchwood	Boat net	4.3	22	4	11	1	1	0	0	1	3	1	0	0	0	0	0
Marchwood	Boat net	4.4	32	7	13	1	0	1	1	5	3	0	0	0	0	0	1
Marchwood	Boat net	4.5	13	2	3	0	1	1	0	3	1	1	0	0	0	0	1
Marchwood	Boat net	4.6	17	3	5	0	0	0	1	3	1	1	1	0	0	0	2
Upper S.G (test)	Boat bottle	1	27	12	1	0	1	0	0	0	2	0	0	11	0	0	0
Upper S.G (test)	Boat bottle	1.1	8	6	0	0	2			0	0	0	0	0	0	0	0
Dockhead	Boat bottle	2	16	6	1	0	2	0	0	0	4	0	1	0	0	0	2
Mayflower	Boat bottle	3	8	2	1	0	2	0	1	0	2	0	0	0	0	0	0
Marchwood	Boat bottle	4	15	3	5	1	2	0	0	1	2	0	0	1	0	0	0
Hamble Marina	Net	1	10	3	0	0	0	0	0	0	3	0	0	3	0	0	1
Hamble Marina	Net	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Bucklers' Hard	Net	1	6	3	0	0	1	0	0	0	1	0	0	0	0	0	1
Bucklers' Hard	Net	2	5	1	0	0	0	1	0	1	0	0	0	1	0	0	1
Hamble Marina	Glass plate	1	8	5	0	0	1	0	1	0	0	0	0	0	0	0	1
Hamble Marina	Glass plate	1	6	4	1	0	1	0	0	0	0	0	0	0	0	0	0
Bucklers' Hard	Glass plate	1	15	8	0	0	1	0	0	0	1	0	0	2	0	0	3
Bucklers' Hard	Glass plate	2	10	2	1	0	2	0	0	0	3	0	0	1	0	0	1

Table 9: The categorised size and average size of all MP fibres recorded this study, relative to the sample site and collection metho used

Site	Sample type	Filter No.	>5	5 to 10 um	11 to 20 um	21 to 30um	31 to 40 um	41 to 50 um	51 to 60 um	61 to 70 um	71 to 80 um	81 to 90 um	91 - 100 um	<100 um	Total	Average
Upper S.G (test)	Boat net	1	0	3	4	5	9	3	2	1	4	0	2	1	1447	42.56
Upper S.G (test)	Boat net	1.1	0	1	0	0	1	1	2	0	0	0	0	1	361	60.17
Upper S.G (test)	Boat net	1.2	0	0	0	0	0	2	1	1	1	0	1	7	1440	110.78
Upper S.G (test)	Boat net	1.3	0	0	2	0	1	0	1	3	1	0	1	2	730	66.37
Upper S.G (test)	Boat net	1.4	0	0	0	0	0	0	0	0	1	1	0	0	170	85
Dockhead	Boat net	2	0	1	0	0	0	0	0	0	1	0	0	0	88	44
Dockhead	Boat net	2.1	0	0	0	0	1	1	1	0	1	0	0	2	470	78.3
Dockhead	Boat net	2.2	0	0	3	2	1	3	0	1	0	0	0	0	352	35.2
Dockhead	Boat net	2.3	0	0	0	0	0	0	0	0	0	1	0	0	90	90
Dockhead	Boat net	2.4	0	0	1	1	0	0	0	0	0	0	0	0	45	22.5
Dockhead	Boat net	2.5	0	0	0	0	1	0	0	0	0	0	1	0	132	68
Dockhead	Boat net	2.6	0	0	0	0	1	0	1	0	2	0	0	0	260	65
Dockhead	Boat net	2.7	0	0	1	1	1	0	0	0	1	0	0	0	155	38.75
Mayflower	Boat net	3	0	1	1	0	0	0	0	0	2	0	0	1	332	66.4
Mayflower	Boat net	3.1	0	1	1	0	0	1	0	2	2	0	0	1	480	60
Mayflower	Boat net	3.2	0	1	3	2	1	1	0	1	0	1	0	2	649	54.08
Mayflower	Boat net	3.3	0	0	0	0	1	0	1	0	2	0	0	1	366	73.2
Mayflower	Boat net	3.4	0	0	0	0	1	0	2	1	0	0	1	3	682	85.25
Mayflower	Boat net	3.5	0	2	6	3	0	0	0	1	1	0	0	0	324	24.92
Mayflower	Boat net	3.6	0	0	0	1	1	1	0	0	0	1	0	0	200	50
Mayflower	Boat net	3.7	0	0	0	1	1	1	0	2	0	0	0	1	363	60.5
Mayflower	Boat net	3.8	0	0	1	0	0	1	1	0	0	1	1	1	377	62.83
Marchwood	Boat net	4	0	0	0	0	2	1	0	0	0	0	1	4	705	88.13
Marchwood	Boat net	4.1	0	0	0	2	0	0	1	1	0	0	0	0	185	46.25
Marchwood	Boat net	4.2	0	0	1	0	1	1	1	1	0	0	0	2	488	69.71
Marchwood	Boat net	4.3	0	0	1	0	1	1	1	1	2	0	1	0	478	59.75
Marchwood	Boat net	4.4	0	3	2	1	0	1	2	1	0	0	0	0	324	32.4
Marchwood	Boat net	4.5	0	0	0	1	0	0	0	1	1	0	0	1	280	70
Marchwood	Boat net	4.6	1	0	0	1	1	0	0	0	0	0	0	1	178	44.5
Upper S.G (test)	Boat bottle	1	27	12	1	0	1	0	0	0	2	0	0	11	0	0	0
Upper S.G (test)	Boat bottle	1.1	8	6	0	0	2			0	0	0	0	0	0	0	0
Dockhead	Boat bottle	2	16	6	1	0	2	0	0	0	4	0	1	0	0	0	2
Mayflower	Boat bottle	3	8	2	1	0	2	0	1	0	2	0	0	0	0	0	0
Marchwood	Boat bottle	4	15	3	5	1	2	0	0	1	2	0	0	1	0	0	0
Hamble Marina	Net	1	10	3	0	0	0	0	0	0	3	0	0	3	0	0	1
Hamble Marina	Net	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Bucklers' Hard	Net	1	6	3	0	0	1	0	0	0	1	0	0	0	0	0	1
Bucklers' Hard	Net	2	5	1	0	0	0	1	0	1	0	0	0	1	0	0	1
Hamble Marina	Glass plate	1	8	5	0	0	1	0	1	0	0	0	0	0	0	0	1
Hamble Marina	Glass plate	1	6	4	1	0	1	0	0	0	0	0	0	0	0	0	0
Bucklers' Hard	Glass plate	1	15	8	0	0	1	0	0	0	1	0	0	2	0	0	3
Bucklers' Hard	Glass plate	2	10	2	1	0	2	0	0	0	3	0	0	1	0	0	1

Table 9: The categorised size and average size of all MP fragments recorded this study, relative to the sample site and collection metho used

Site	Sample type	Filter No.	>5	5 to 10 um	11 to 20 um	21 to 30um	31 to 40 um	41 to 50 um	51 to 60 um	61 to 70 um	71 to 80 um	81 to 90 um	91 - 100 um	<100 um	Total	Average
Upper S.G (te	Boat net	1	1	5	1	0	0	1	0	0	0	0	0	0	98	12.25
Upper S.G (te	Boat net	1.1	0	5	2	0	0	0	0	0	0	0	0	0	60	8.57
Upper S.G (te	Boat net	1.2	5	9	9	2	0	0	0	0	0	0	0	0	268	10.72
Upper S.G (te	Boat net	1.3	0	12	3	1	0	0	0	0	0	0	0	0	170	10.63
Upper S.G (te	Boat net	1.4	7	12	10	2	1	0	1	0	0	0	0	0	431	13.06
Dockhead	Boat net	2	2	2	0	0	0	0	0	0	1	0	0	0	99	19.80
Dockhead	Boat net	2.1	3	5	3	1	3	0	0	0	0	0	0	0	227	15.13
Dockhead	Boat net	2.2	1	8	5	4	0	0	0	0	0	0	0	0	254	14.11
Dockhead	Boat net	2.3	6	4	2	1	0	1	0	0	0	0	0	0	114	10.29
Dockhead	Boat net	2.4	5	4	1	1	1	0	0	0	0	0	0	0	123	10.25
Dockhead	Boat net	2.5	3	2	0	1	0	1	0	1	0	0	0	0	163	20.38
Dockhead	Boat net	2.6	2	5	3	3	0	1	0	0	0	0	0	0	208	14.86
Dockhead	Boat net	2.7	1	5	5	8	2	2	0	0	0	1	0	0	581	24.21
Mayflower	Boat net	3	4	4	1	0	0	0	0	0	0	0	0	0	56	6.22
Mayflower	Boat net	3.1	1	4	2	2	0	0	0	0	0	0	0	0	122	13.56
Mayflower	Boat net	3.2	10	7	3	0	0	0	0	0	0	0	0	0	148	7.40
Mayflower	Boat net	3.3	6	5	5	1	0	0	0	0	0	0	0	0	146	8.59
Mayflower	Boat net	3.4	5	7	1	0	0	0	0	0	0	0	0	0	77	5.92
Mayflower	Boat net	3.5	5	3	3	0	0	0	0	0	0	0	0	0	82	7.46
Mayflower	Boat net	3.6	1	5	1	0	0	0	0	0	0	0	0	0	56	8.00
Mayflower	Boat net	3.7	3	4	2	1	0	0	0	0	0	0	0	0	92	9.20
Mayflower	Boat net	3.8	1	6	1	0	0	1	0	0	0	0	0	0	107	11.89
Marchwood	Boat net	4	4	4	1	0	0	0	0	0	0	0	0	0	53	5.89
Marchwood	Boat net	4.1	2	4	0	1	0	0	0	0	0	0	0	0	61	8.71
Marchwood	Boat net	4.2	0	10	0	0	0	0	0	0	0	0	0	0	74	7.40
Marchwood	Boat net	4.3	6	5	1	0	0	0	0	0	0	0	0	0	67	5.58
Marchwood	Boat net	4.4	7	11	1	0	0	0	0	0	0	0	0	0	102	5.37
Marchwood	Boat net	4.5	3	2	2	0	0	0	0	0	0	0	0	0	51	7.29
Marchwood	Boat net	4.6	2	6	0	0	1	0	0	0	0	0	0	0	80	8.89
Upper S.G (te	Boat bottle	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1.00
Upper S.G (te	Boat bottle	1.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
Dockhead	Boat bottle	2	0	0	1	0	1	0	0	0	0	0	0	0	55	27.50
Dockhead	Boat bottle	3	0	1	0	0	0	0	0	0	0	0	0	0	10	10.00
Dockhead	Boat bottle	4	0	4	2	0	0	0	0	0	0	0	0	0	56	9.33
Hamble Mari	Net	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
Hamble Mari	Net	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
Bucklers' Har	Net	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
Bucklers' Har	Net	2	1	0	1	0	0	0	0	0	0	0	0	0	23	11.50
Hamble Mari	Glass plate	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
Hamble Mari	Glass plate	1	0	1	0	0	0	0	0	0	0	0	0	0	10	10.00
Bucklers' Har	Glass plate	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
Bucklers' Har	Glass plate	2	0	1	0	0	0	0	0	0	0	0	0	0	10	10.00

Table 10: The number of MPs found after 24 hr in the polyp, *Artemia* and well water of each experiment.

Polyp No	No. of fibres in polyp	No. of frags in polyp	No. of fibres in *Artemia*	No. of frags in *Artemia*	No. of fibres in well water	No. of frags in well water	No. of Artemia eaten
A1							3
A2							9
A3							5
A4							8
A5							9
A6							10
B1							10
B2							9
B3							10
B4							8
B5							7
B6							9
C1	3				2		
C2	2				3		
C3	4				1		
C4	2				3		
C5	1				4		
C6	1				4		
D1	4				1		
D2	3				2		
D3	1				4		
D4	3				2		
D5	1				4		
D6	3				2		
E1		1				4	
E2		1				4	
E3		2				3	
E4		2				3	
E5		2				3	
E6		2				3	
F1		1				4	
F2		2				3	
F3		2				3	
F4		0				5	
F5		1				4	
F6		0				5	

G1	4		0		1		7
G2	2		0		3		9
G3	4		0		1		6
G4	3		1		1		9
G5	1		0		4		4
G6	2		1		2		5
H1	3		0		2		9
H2	1		2		2		9
H3	4		0		1		10
H4	1		0		4		9
H5	3		0		2		10
H6	3		0		2		8
I1		1		0		4	9
I2		2		0		3	9
I3		1		0		4	9
I4		2		0		3	9
I5		1		0		4	10
I6		2		0		3	9
J1		2		1		2	8
J2		1		2		2	9
J3		0		1		4	9
J4		1		1		3	8
J5		0		0		5	10
J6		1		0		5	10
K1			2		3		
K2			1		4		
K3			2		3		
K4			0		5		
K5			2		3		
K6			0		5		
L1			1		4		
L2			0		5		
L3			1		4		
L4			0		5		
L5			1		4		
L6			0		5		

M1				0		5	
M2				0		5	
M3				0		5	
M4				0		5	
M5				1		4	
M6				1		4	
N1				0		5	
N2				0		5	
N3				0		5	
N4				0		5	
N5				2		3	
N6				1		4	