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Assessing the spatial and temporal distribution of microplastics within the sediment of Plymouth Sound

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Abstract

The pollution of microplastics in the marine environment is a growing issue that impacts us globally. These invasive particles have been discovered in all regions of the world, including human body tissue. It is well understood that microplastics settle within seafloor sediment and hotspots are found close to human activity. However, the way microplastics are distributed both spatially and temporally in urban marine environments, and why this might be, is little known. Using the 'primary analytical process', a study was conducted in Plymouth Sound, United Kingdom, to assess how microplastics were distributed within the sediment here. General sediment samples and core sediment samples were extracted from 8 sites. Zinc Chloride was used to separate microplastics from sediment samples and the 'Guide to Microplastic Identification' was followed during analysis. Microplastics were present at every site and the distribution of them was closely related to human activity. Sites closer to land had a higher microplastic density than those further away. There was a significant difference between microplastic abundance across Plymouth Sound temporally, but not spatially. Sediment did not influence the number of microplastics present at each site. Synthetic textiles were found to be the main source of microplastics in Plymouth Sound, with microfibrils dominating sites. The results of this study provide evidence that microplastic pollution is growing and as the demand for plastic production grows, we can expect to see higher numbers in the future. The study highlights the need for further research as well as the need for managing plastic pollution in the marine environment.

Keywords: Microplastic, plastic, marine pollution, plastic pollution, sediment, Plymouth

Introduction

Plastic is one of the most widely produced materials worldwide due to it being inexpensive and versatile (PlasticsEurope, 2013). Each year, around 380 million metric tonnes of plastic is produced (Geyer *et al.*, 2017). Half of this plastic is single use, meaning that they are used once and then disposed of (UN Environment Programme, 2023.) As plastic is non-biodegradable and cannot decompose like organic materials do; it is either stored in landfill, recycled, or littered. Unfortunately, less than 10% of plastics are recycled and an estimated 25 trillion pieces are currently polluted in the Ocean (Eriksen *et al.*, 2014). The pollution of plastic in the marine environment is an increasing concern as by 2050, there will be more plastic than fish in the sea (Jennings *et al.*, 2021 and Wootton *et al.*, 2021).

Over time, plastics shed and break down into tiny pieces known as Microplastics (<5mm). Managing microplastic (MP) pollution is difficult due to their small size, vast abundance and the physical nature of the ocean (Serranti *et al.*, 2018). These tiny particles have been discovered in all regions of Earth; from on mountain tops to within the deepest of sea floor sediment (Zhang *et al.*, 2022, Cauwenberghe *et al.*, 2015). They have also been found in the atmosphere and in human body tissue, exposing us to potential harm (Ragusa *et al.*, 2021).

The main threat of microplastics (MPs) to marine life is through ingestion (Johnson-Arbor, 2022). Pollutants and heavy metals can stick to the surface area of MPs which can expose organisms to toxicity when ingested (Johnson-Arbor, 2022.) MPs provides no real nutrition, which can lead to starvation and malnourishment of marine life (Wang *et al.*, 2018). As well as this, MPs have been found to impact the growth of fish which reduces their chance of survival (Naidoo & Glassom 2019). This can consequently decrease fish stocks, impacting the availability of food to both humans and marine life. It is well established that MP production and distribution is influenced by urban activity and therefore, there is concern that people and animals living in these regions might suffer as plastic production grows (Qiu *et al.*, 2020).

Sources of microplastics in the marine environment

The main source of MPs originates from synthetic textiles such as polyester and acrylic, which are made from microfibers (Fig 1) (Boucher and Friot, 2017). When wearing and washing clothes, fibres shed into the air and waterways, which eventually meets the ocean (Boucher and Friot, 2017). MPs can also derive from car tyres, city dust and road markings (Boucher and Friot, 2017). Through run-off from land and sewage systems, MPs are easily washed into the marine environment. Urban areas are considered to be the largest source of MPs due to the intense anthropogenic activity occurring there (Qiu *et al.*, 2020). Qui *et al.* (2020) highlights the need for further research on the distribution and behaviour of MPs in urban environments.

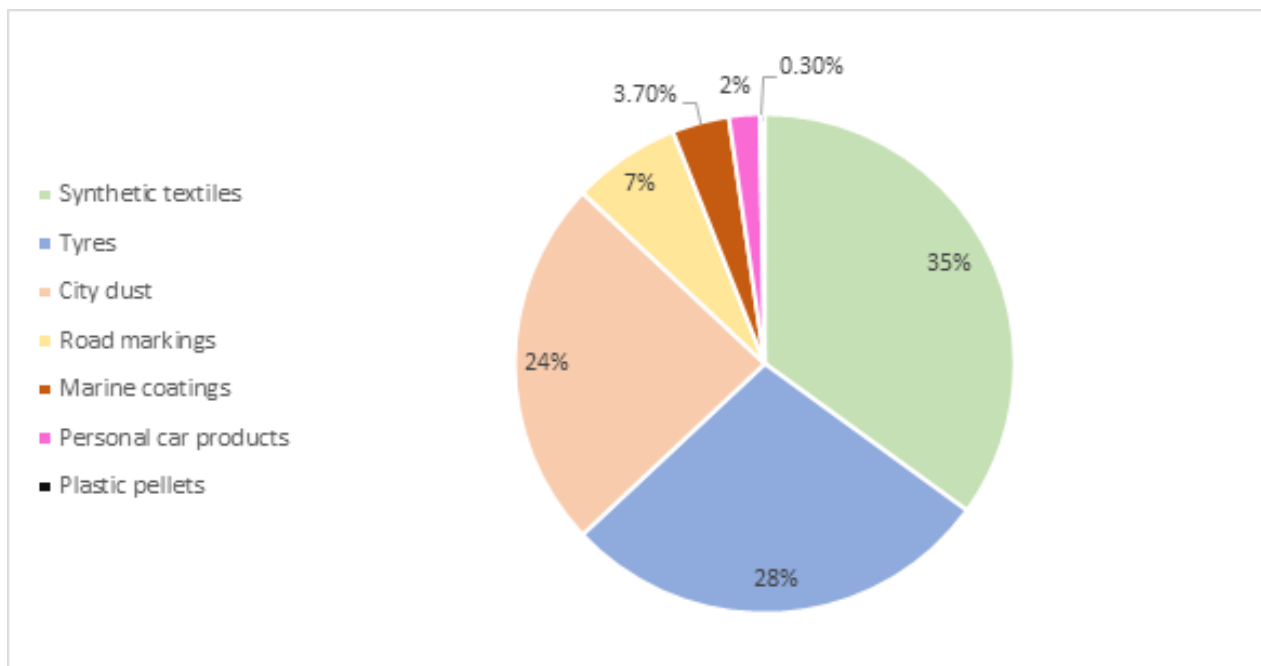


Figure 1: The main sources of microplastics into the marine environment. Data was reproduced from the International Union for Conservation of Nature's 2017 evaluation of the 'Primary Microplastics in the Ocean' (Boucher & Friot, 2017.)

Microplastic transportation in the marine environment

It is easy for macro-plastics (>5mm) and microplastics to transport through wind and into marine environments due to their light weight (Qiu *et al.*, 2020). 80% of all plastics in the ocean derive from rivers and coastal land where they are transported by wind and run-off (Richie, 2021). When in the sea, plastics break down further through wave action, currents and weathering (Costello and Ebert, 2020). MPs can be transported by sediment, ocean circulation and currents (Fig 2) (WHOI, 2022). Land and physical structures have been found to influence the distribution of sediment and the MPs within them (Collins, 2011). MPs settle to the seafloor when negatively buoyant, ingested by marine life or through 'Flocculation' (Fig 2) (Mountford & Morales Maqueda, 2019). Flocculation occurs when sediment sticks to each other when falling through the water column, forming a 'floc' (Fig 2) (Manning *et al.*, 2017). Since MPs are less dense than these grains, they can be easily trapped within a floc (Liu *et al.*, 2021). The settling velocity of these flocs depends on their size and density, with an increase in size decreasing the density, causing it to settle more slowly (HR Wallingford, 2013). When a floc settles slowly it can travel great distances and simultaneously transport MPs along with them (Guo *et al.*, 2021).

Sediment is one of the major components of suspended and settled matter in marine environments and the size of them can greatly impact the transportation of MPs (Shrestha & Blumberg, 2005). The size of sediments is classified as either cohesive (<2µm) or non-cohesive (<75µm) (Shrestha & Blumberg, 2005). Cohesive sediments consist mainly of clay and silt whereas non-cohesive sediments consist of sand and gravel (Shrestha & Blumberg, 2005). Cohesive sediment containing clays have electromagnetic properties that cause grains to bind together and form flocs (Shrestha

& Blumberg, 2005). Organic matter and life within sediment can also impact the binding properties of grains through bio-cohesiveness (Black *et al.*, 2002). Biological activity within sediment can change the properties of the grains and bind them together, which thus impacts the way MPs bind to sediment (Black *et al.*, 2002).

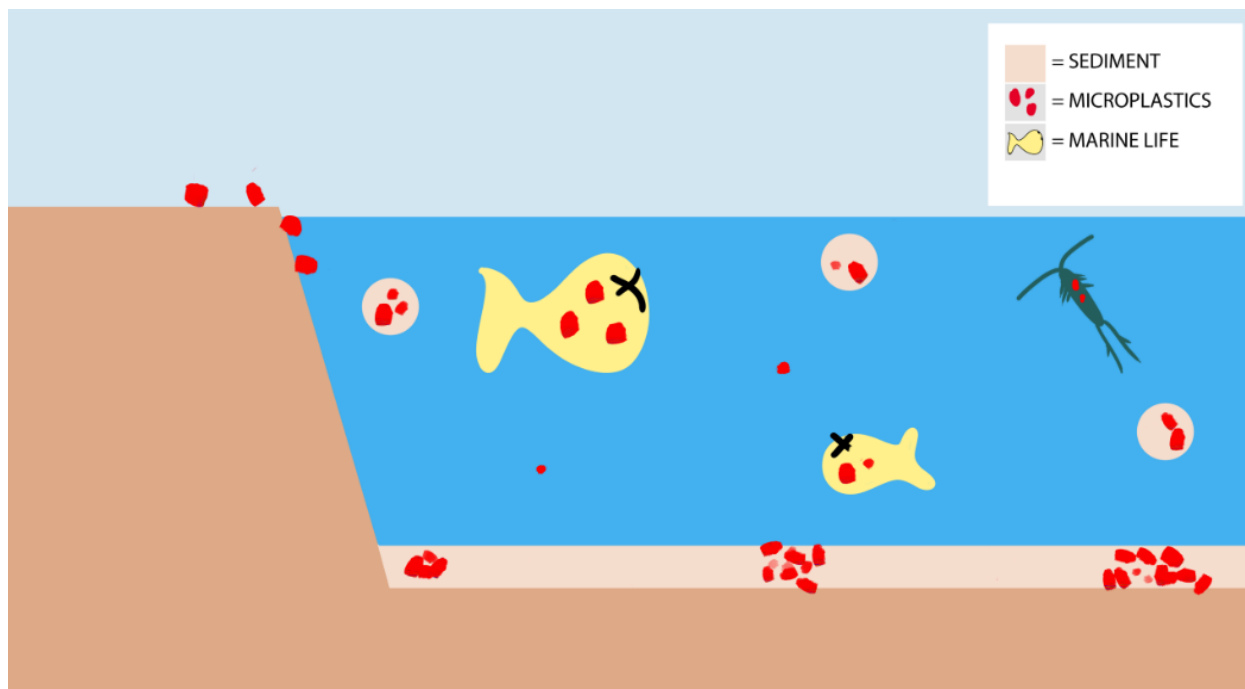


Figure 2: An infographic showing the different ways microplastics can be transported in the ocean.

Microplastics accumulate within seafloor sediment, forming ‘hotspots’ where large quantities of particles are found (Fig 2) (Cauwenberghe *et al.*, 2015). Due to sediment properties, MPs have been found to accumulate in certain marine environments including estuaries, fjords and coasts (Harris, 2020). Previous studies have found that the layers of seafloor can indicate time (Willis *et al.*, 2017). The top 10cm of sediment is matter estimated to have settled over the past 0-20 years and sediment between 10-30cm to have settled over the past 20-40 years (Willis *et al.*, 2017). Therefore, it is possible to estimate when MPs settled inside these layers. Furthermore, certain types of MPs have been found to accumulate in particular sediment types (Cauwenberghe *et al.*, 2015). For example, polyester is commonly found in sandy sediment and polyethylene is often found in muddy sediment (Al-Libhaibi *et al.*, 2019, Vianello *et al.*, 2013).

Study aims and objectives

In the urbanised coastal waters of Plymouth Sound, UK, a study was designed with the aim of assessing how microplastics are distributed in the environment. Sites were selected, sediment samples were collected and then analysed to answer the following questions: How are microplastics distributed in an urban marine environment? Has the abundance of microplastics changed over time in an urban marine environment? What

is the main type, and therefore possible source, of microplastics in an urban marine environment? 3 null hypotheses were stated to later accept or reject according to the results: Null Hypothesis 1 – There is no difference in the spatial distribution of microplastics across sites in Plymouth Sound. Null Hypothesis 2 – There is no difference in the temporal distribution (between sediment depths) of microplastics across site in Plymouth Sound. Null Hypothesis 3 – There is no difference in microplastic type across sites in Plymouth Sound.

Literature Review

Literature can assist in understanding what is already known on MPs, current research gaps and how best to study MPs in the marine environment. A study from the Tamar Estuary, UK, investigated the spatial distribution of MPs along this urbanised estuary (Browne *et al.*, 2010). It was found that MP density impacted the spatial distribution of them. In addition, most MPs studied were fragmented (Browne *et al.*, 2010). A similar study was conducted along the Southwest coast of the UK, which included Plymouth Sound, to assess how MPs were spatially distributed (Nel *et al.*, 2020). It was discovered that MPs accumulated in rural areas but derived from urban sources. Furthermore, MPs were found to be evenly distributed across sites of Plymouth Sound (Nel *et al.*, 2020). In the Derwent Estuary, Tasmania, research addressed how MP abundance changed with sediment depth in an urban estuarine environment (Willis *et al.*, 2017). More MPs were found in the top layers of sediment versus the old, indicating an increase in pollution over time. It was also found that microfibrils were the most common type of MP in sediment here (Willis *et al.*, 2017). Moreover, a study in Norway looked into the MP content surrounding wastewater discharge sites along an urbanised fjord (Haave *et al.*, 2019). It was discovered that particles under 1mm dominated the sites and hotspots were related to sediment type. Various methods were used to study MPs in these papers (Table 1). These methods provide insight as to how to investigate current research questions.

Literature highlights the need for further research on MP distribution covering a large spatial area including multiple estuaries (Browne *et al.*, 2010). It also points out that studying wider geographical areas will contribute to the understanding of MP hotspots around the world (Nel *et al.*, 2010). It is important to fully understand how MPs are distributed in the marine environment so that we can monitor and mitigate pollution. It is estimated that microplastic concentrations in sediment are increasing by 1.208 MPs per year, which escalates the need for solutions (Willis *et al.*, 2017).

Table 1: A comparison of the methodology used in previous, similar papers. Techniques used in these studies assisted with the development of methods for this current study and areas highlighted (yellow) were later selected to be used in the methods.

	Browne <i>et al.</i> , 2010	Nel <i>et al.</i> , 2020	Willis <i>et al.</i> , 2017	Have <i>et al.</i> , 2019
Data collection method	-Beach sediment collected into foil	-Sediment collected into jars -Citizen science data	-Core samplers	-Van Veen grab
MP separation method	-Sodium Chloride	-Zinc Chloride	-Hydrogen peroxide	-Zinc Chloride
MP analysis method	-Transmittance FT-IR -Spectral database	-Stereo microscope	-Stereo microscope	-Binocular microscope
Statistical tests used	-ANOVA	-Chi-squared	-T-tests	-ANOVA

Methodology

With the assistance of literature and previous studies, techniques were put into place to assess the distribution of MPs in Plymouth Sound. With consideration to the resources and time frame available, appropriate methodology was designed to provide understanding of how MPs were distributed over the area. The primary analytical process was followed which included sampling, extraction, quantitation, and quality assurance (Hanvey *et al.*, 2016).

Study area

Plymouth Sound (or The Sound), UK, was chosen as the study area to represent an urban marine environment (Fig 3). This area was selected due to its diverse environment consisting of estuaries, mudflats, harbours, and beaches. It is surrounded by a largely populated city with many sources of MPs including the River Plym, the River Tamar, run-off from land, sewage, tyre debris from the A38, a recycling centre, marine coatings from vessels and city dust (Nel *et al.*, 2020). It is already established that MPs in The Sound derive mainly from local sources (Nel *et al.*, 2020).

This location is highly productive with naval vessels, fishing boats and passenger ferries moving in and out each day. Due to a breakwater located on the perimeter of The Sound, sediment builds up in the centre, so it is dredged to maintain depth for large vessels to pass through (Fitri *et al.*, 2019). Therefore, there is constant overturning and deposition of sediment in certain areas of Plymouth Sound. The sediment type of Plymouth Sound varies from clay, mud, sand and rock (Fitzpatrick, 1999).

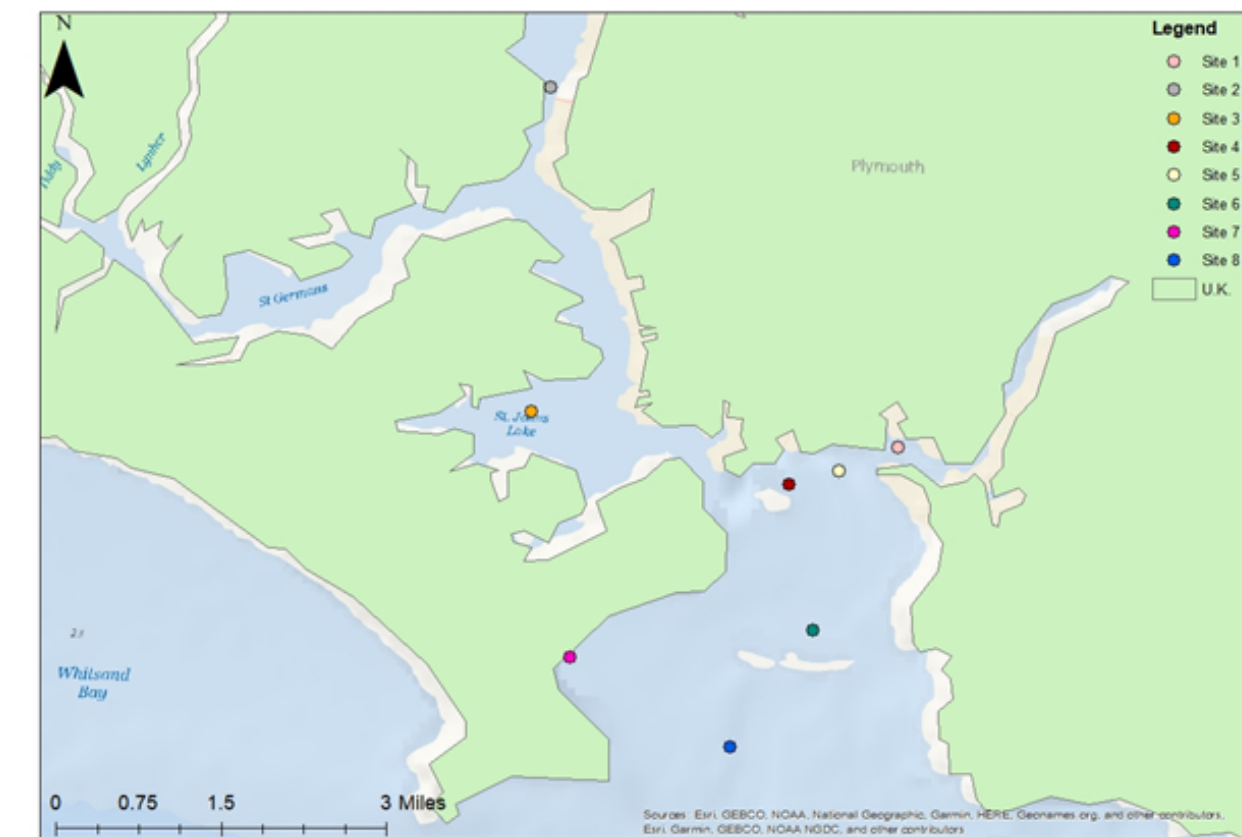


Figure 3: Arcmap showing the sites where samples were extracted. Site 1 is the River Plym, Site 2 is the Tamar Bridge, Site 3 is West Mud, Site 4 is Asia Pass, Site 5 is Smeaton's Pass, Site 6 is Echo Buoy, Site 7 is Cawsand Bay and Site 8 is the Control site. ArcGIS was used to develop this map on 16/03/2023 (GB National Outlines [SHAPE geospatial data], Scale 1:250000, Tiles: GB, Updated: 8 June 2005, Ordnance Survey (GB), Using: EDINA Digimap Ordnance Survey Service, <<https://digimap.edina.ac.uk>>, Downloaded: 2021-04-27 14:30:21.494).

Data collection

Sediment samples were collected on the 8th August 2022 from 9am to 12pm during low tide. 8 sites were chosen to cover a significant amount of The Sound and represent all features of the area (Fig 3). Site 8 was selected as a control site due to its offshore location, away from civilisation. A control site was used to compare other sites to and evaluate the pollution with more understanding. General sediment samples (GSS) were taken from each site using a Van Veen grab to collect seafloor sediment (Appendices: Fig 18). This instrument was used as it was readily available, cheap, and easy to operate (Haave *et al.*, 2019). Grab percentage varied from 5-95% due to the sediment type. A spade-full of GSS was wrapped in foil and stored in a freezer until later lab analysis (Browne *et al.*, 2010). All equipment was washed in-between sites using seawater to remove any sediment stuck to the surface. In addition, plastic instruments were avoided to prevent contamination of external MPs. At The River Plym (site 1), The Tamar Bridge (site 2) and Smeaton's Pass (site 5) (Fig 4), core sediment samples (CSS) were also collected to study the temporal distribution of MPs. Core sample sites were chosen due to the high activity of their locations. Before collecting the GSS at these sites, a new, hard plastic pipe was used to collect a CSS. Where plastic could not

be avoided, new hard plastic was used as it was unlikely to shed MPs and contaminate samples. Creating a vacuum to hold form, the core was removed and placed in foil. The size of each core was roughly 15cm long and all samples were kept in the freezer ready for analysis.

Microplastic extraction

Density separation is the most common way to extract MPs from sediment (Hanvey *et al.*, 2017). The microplastic separation method from Coppock *et al.*, (2017) was followed to extract MPs from GSS and CSS (Appendices: Figs 19 & 20). A teaspoon of GSS was wet-sieved using a 250-500um sieve to remove any large clumps of sediment (Fig 4). Sediment was transferred into a beaker using distilled water and then into a separating tube filled with Zinc Chloride ($ZnCl_2$) (Fig 4). $ZnCl_2$ was chosen due to it being 92-98% accurate and cheap with results in high MP recovery (Coppock *et al.*, 2017). The tube was topped up with $ZnCl_2$ so that the level was above the upper flexi tube to separate MPs (Fig 4). The contents were stirred with a stainless-steel rod before it was left for sediment to settle and MPs to float (Appendices: Fig 20). Sediment settling rate varied across samples; rocky sediment took up to an hour to settle whereas clay took up to 48 hours. The separation of all samples took 7 days in total. Once the sediment and MPs had separated, the upper flexi tube was released into a fresh beaker. A wash bottle containing $ZnCl_2$ was used to encourage plastics stuck on the tube into the beaker. Contents from the beaker were drained through a vacuum filter using 8cm wide grade 541 filters (Fig 4) (Appendices Fig 19).

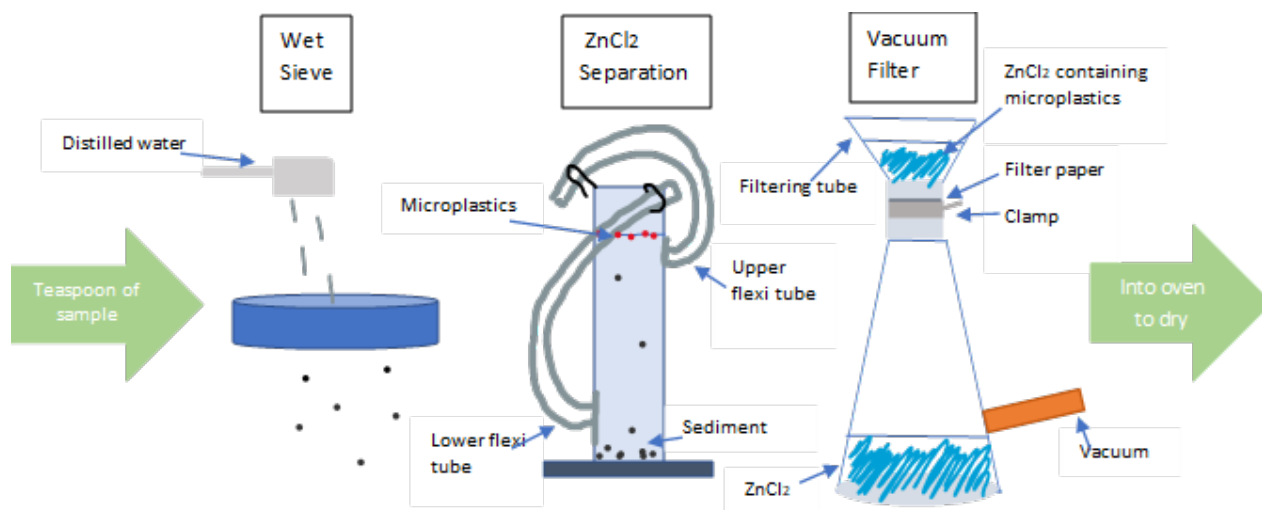


Figure 4: Figure showing a visual representation of the laboratory set up and process. $ZnCl_2$ = Zinc Chloride.

The filter with trapped MPs was dried in an oven set at 40°C for 30 minutes. Filtered samples were placed into individual glass petri dishes and stored in a secure tray, to await analysis. The same separation process was done for each CSS. However, instead of a teaspoon of sample, the top 1cm and bottom 1cm of CSS were taken. The top CSS and bottom CSS of one site was classed as two separate samples following separation. To ensure accuracy in the lab, metal instruments and glassware were used

to avoid contamination with external plastics. Filters were stored separately in containers to avoid material in the air from landing in the samples. These measures ensured that as little plastic as possible would contaminate the samples.

Microplastic analysis

Once the MPs were separated from the sediment, they were analysed under a stereo microscope using a camera attachment (Fig 5). Filters containing MPs were placed onto a piece of paper so as to move them around without disturbing the filter (Fig 5). Visual counting of particles from each site sample was done as this was the most common technique to use (Hanvey *et al.*, 2016). Although this technique is prone to human error, it was the most cost-efficient method available (Hanvey *et al.*, 2017). To analyse the MPs fairly, the same process was repeated. First, the top of the filter was inspected left to right, then the filter was moved down and inspected right to left. This was repeated until the filter was complete to ensure that no MPs were missed or counted twice (Marine & Environmental Research Institute, 2015) Images of each MP found were taken to refer back to. As well as counting MPs, the type and size of each one was identified using the 'Guide To Microplastic Identification' (Masura *et al.*, 2015.) The top and bottom sediments from each CSS were analysed following the same method. All data was combined into a spread sheet ready for statistical analysis and data processing.



Figure 5: Images of the microplastic analysis setup. On the left is the stereomicroscope with a camera attachment showing one of the paper filters. On the right is a closer image showing what could be seen through the microscope.

Flocculation of sediment samples

A selection of samples, representing all types of sediment in The Sound, were processed through a video-based LabSFLOC (Laboratory Spectral Flocculation Characteristics) instrument. A small sample from each sediment was placed into the LabSFLOC column where footage of settling velocity and floc size were observed through a computer (Fig 6) (Manning *et al.*, 2017). The floc porosity, fractal dimensions, floc dry mass and mass settling flux was calculated using image analysis algorithms (Manning *et al.*, 2017). Stokes law equation; $f = 6\pi r\eta u$ (where r is radius, η is viscosity and u is velocity) was used to calculate the settling velocity of each sample.

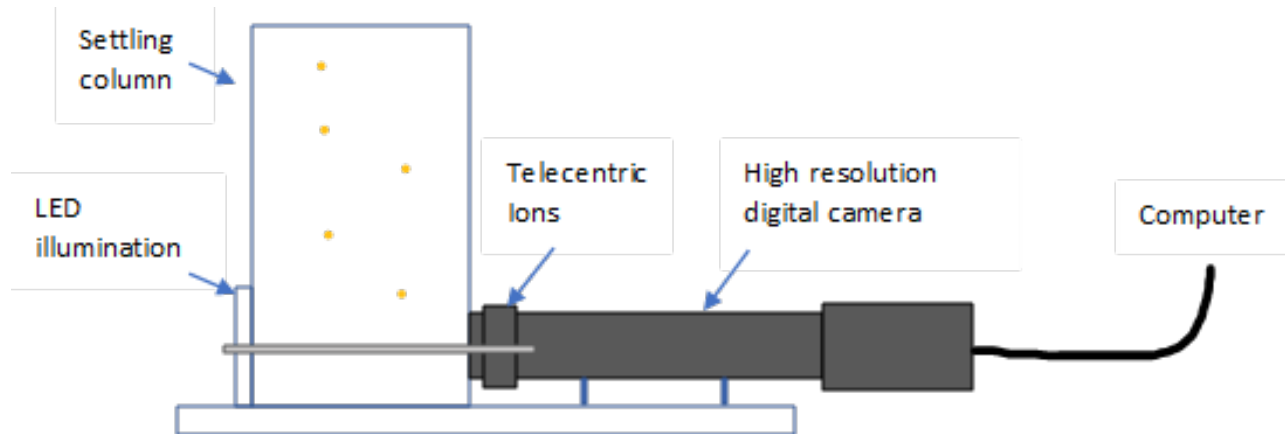


Figure 6: Set up of LabsFLOC instrument used to calculate settling properties of the sediment samples. Information replicated from HR Wallingford (2013.)

Statistical analysis and data processing

Statistical analysis of all data was conducted using Microsoft Excel (Office 16) and Matlab (R2021.a). General statistics were run to find mean, standard deviation, median, range and skew of the MP abundance of each sample. For sites where flocculation data was taken, average floc sizes were worked out. Coefficient Variation; $cv = \frac{\sigma}{\mu}$ (Eq. 1), was calculated for GSS and CSS to show how varied data was. In addition, Pearson Coefficient Correlation; [Equation] (Eq. 2), was calculated to see how strong the linear association was. Data was identified as quantitative and normally distributed. Statistical tests were used to find the significance of results. A Chi-Squared test; $\chi^2 = \sum (O_i - E_i)^2 / E_i$ (Eq. 3), was used to find the significance between MPs in GSS. The significance between CSS MP abundance was calculated using a Two-Sample T-test; [Equation] (Eq. 4). Two-sample t-tests (Eq. 4) were also performed to see if there were significant differences in MP types and relationships between abundance and length. A One Sample T-test; $t = \frac{\bar{x} - \mu}{s/\sqrt{n}}$ (Eq. 5), was used to see if there was any significance between sediment type and number of MPs. Lastly, the Coefficient of Determination; $R^2 = 1 - RSSTSS$ (Eq. 6) was carried out to test the significance of floc size on MP density. Data was processed into a variety of graphs to visually display findings using Microsoft Excel (Office 16), ArcGIS (10.8.1) and Matlab (R2021a).

Results

Overall, results showed equal spatial distribution of MPs across the sites and abundance changed over time. There was one dominating MP type and length found across Plymouth Sound (Fig 7 & 8).

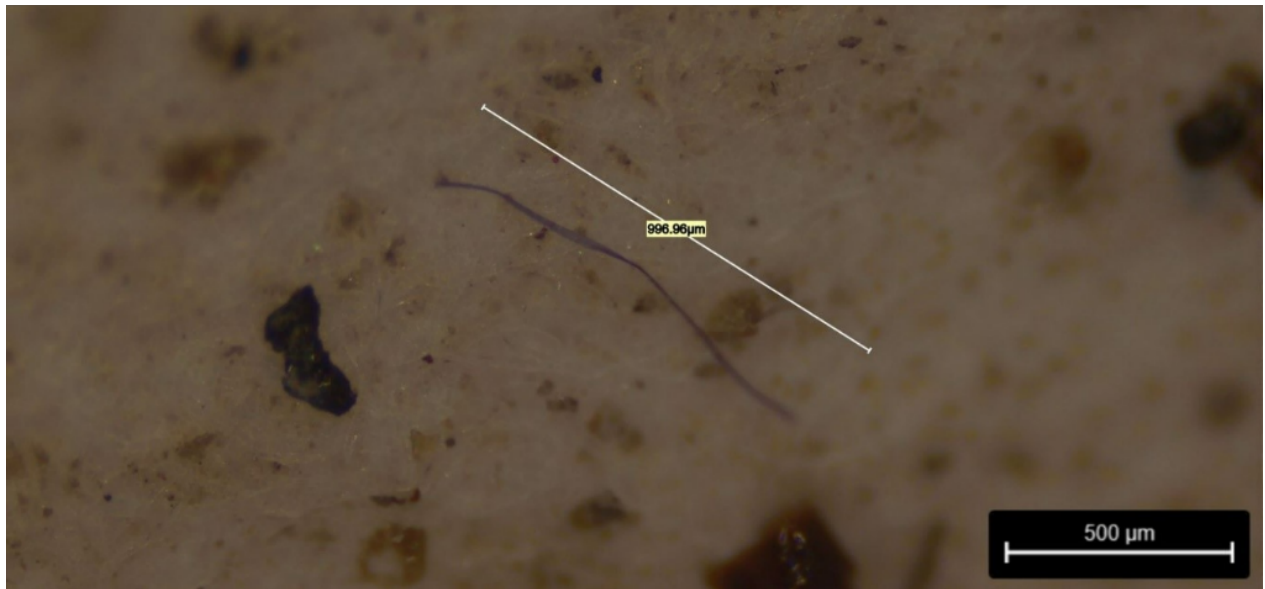


Figure 7: Image from a Leica stereo microscope showing a microplastic fibre from Site 1. The microplastic was extracted from the Plym Estuary on the 8th August, 2022 and was observed through a stereo microscope on the 10th of August, 2022.

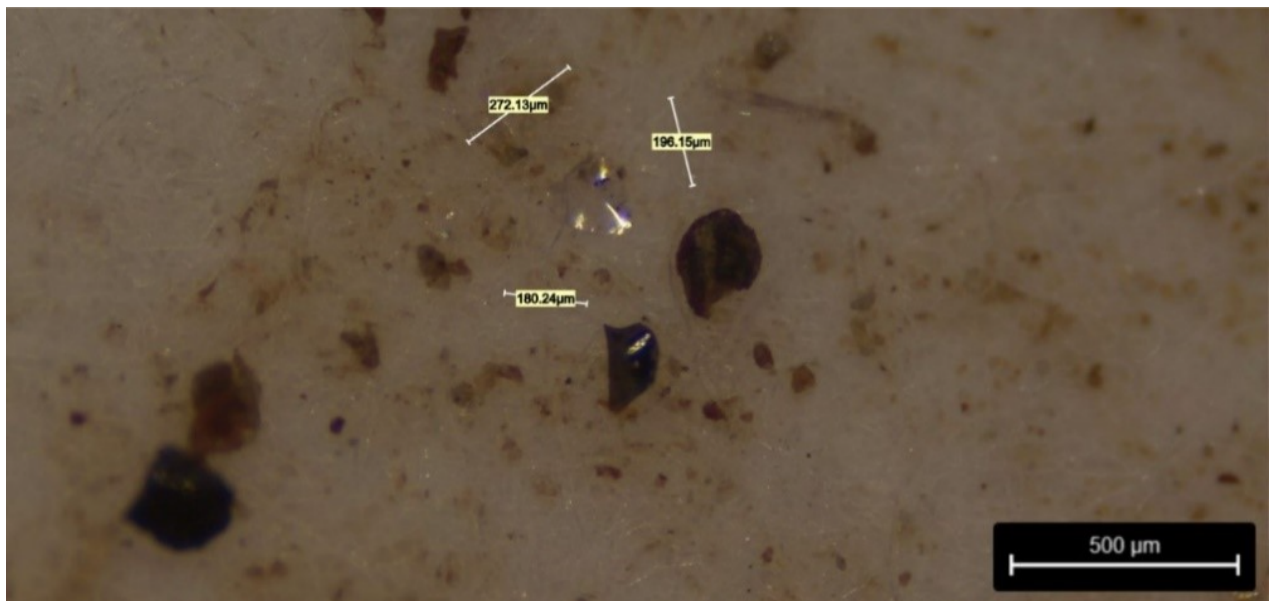


Figure 8: Image from a Leica stereo microscope showing a microplastic fragment from Site 5. The microplastic was extracted from the Meaton's Pass on the 8th August, 2022 and was observed through a stereo microscope on the 10th of August, 2022.

Sediment type and floc size did not seem to influence MPs found at sites. On average, 6.75 MPs were found at each site across Plymouth Sound (table 3). GSS data was approximately symmetric and evenly distributed with a large range between lowest and highest values (table 3). On the other hand, CSS data experienced highly skewed, unevenly distributed data (table 3). Coefficient of Variation (Eq. 1) is higher in the GSS data showing a greater level of dispersion around the mean (table 3). In addition,

Pearson Correlation (Eq. 2) value of the CSS data shows a negative correlation (table 3).

Table 3: The mean, standard deviation, median, range, skew and coefficient of variation of microplastic data across all samples. GSS is general sediment samples and CSS is core sediment samples. Pearson correlation has been done for comparison of top and bottom CSS.

	Mean abundance	Standard deviation	Median	Range	Skew	Coefficient of variation (CV)	Pearson correlation
GSS	6.75	3.2	7	8	0.23	47%	
Top CSS	4.67	1.15	4	2	1.75	25%	-1
Bottom CSS	1.67	0.58	2	1	-1.71	35%	

Distribution of microplastics across Plymouth Sound

The site with the highest density of MPs was site 1 whereas the lowest was site 8 (control) and site 6. In the sediment of site 1, almost four times more MPs were found compared to site 8 (Fig 9).

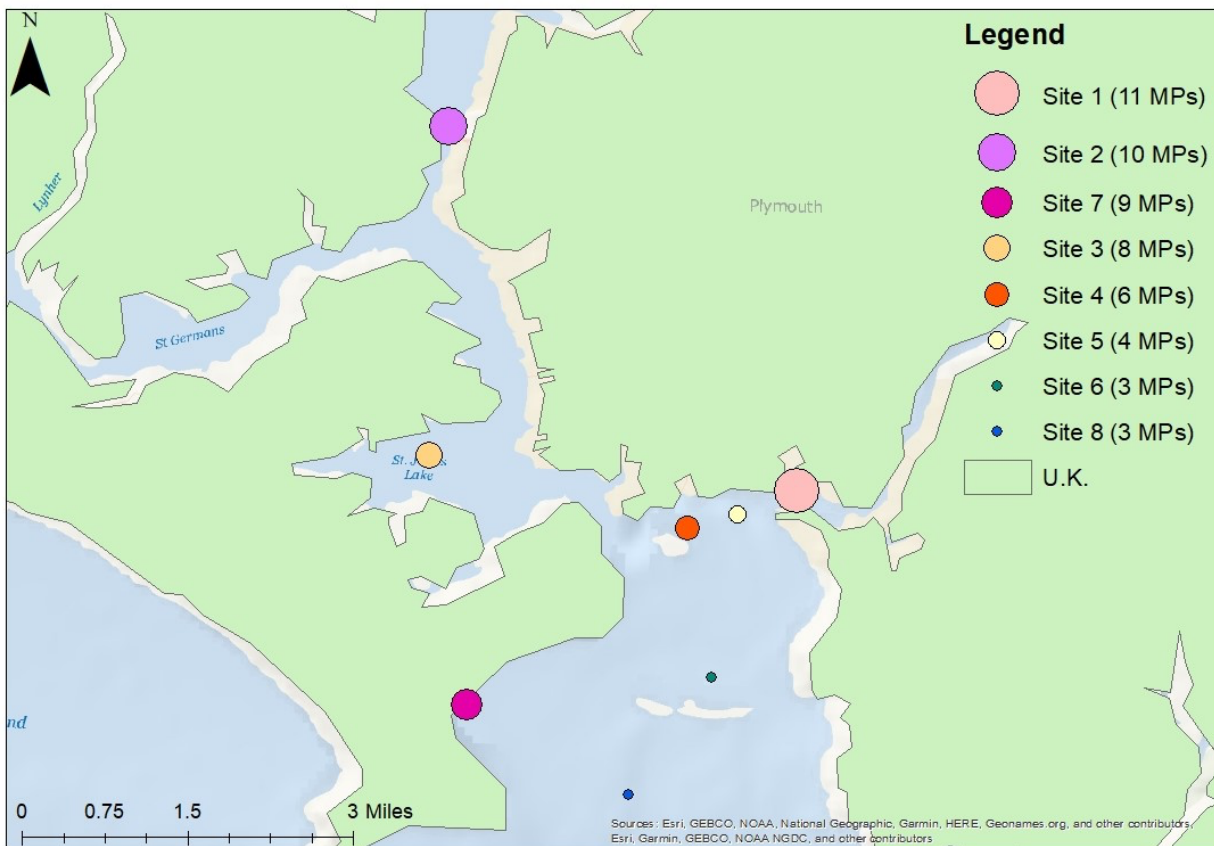


Figure 9: A distribution map showing microplastic proportional density across Plymouth Sound. The circle size visually represents the particle abundance. Site 1 is the River Plym, Site 2 is the Tamar Bridge, Site 3 is West Mud, Site 4 is Asia Pass, Site 5 is Smeaton’s Pass, Site 6 is Echo Buoy, Site 7 is Cawsand Bay and Site 8 is the Control site. Samples were collected between 9am and 11am on the 8th August 2022 during low tide. ArcGIS was used to develop this map on 16/03/2023 (GB National Outlines [SHAPE geospatial data], Scale 1:250000, Tiles: GB, Updated: 8 June 2005, Ordnance Survey (GB), Using: EDINA Digimap Ordnance Survey Service, <<https://digimap.edina.ac.uk>>, Downloaded: 2021-04-27 14:30:21.494).

At sites further away from land, the number of MPs was low compared to sites close to land (fig 9). The number of MPs in GSS at sites 1, 2, 3 and 7 were above average whereas sites 4, 5, 6 and 8 were below (Fig 10). However, there was no significant difference ($p > 0.05$) (Eq. 3) between MP abundance across GSS.

There were fewer particles found in CSS than in GSS (Fig 10). There was a significant difference ($p < 0.05$) (Eq. 4) between the number of MPs in the top versus bottom layers of sediment (Fig 10). Site 2 showed the highest MPs numbers in the top sediment and the lowest numbers in the bottom sediment (Fig 10).

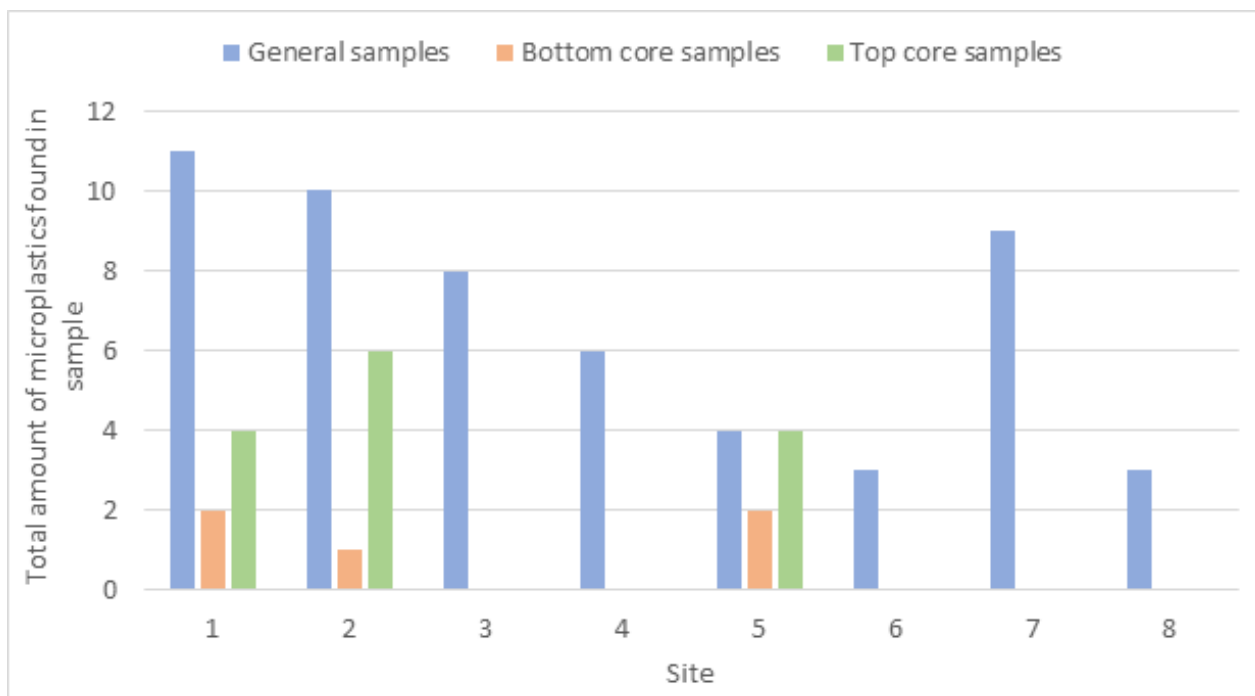


Figure 10: Microplastic abundances of all sites across Plymouth Sound including GSS and CSS. Samples were collected between 9am and 11am on the 8th August 2022 during low tide.

Dominating microplastic type and size across Plymouth Sound

Two of the seven MP types (Maura *et al.*, 2015) were found within the sediment of each site; fibres and fragments (Fig 11). Across GSS, there were 43 fibres and 11 fragments, showing a significant difference ($p < 0.05$) (Eq. 4) between the two types (Fig 11).

There were 4 fibres and 1 fragment found in bottom CSS (Fig 11). In the top CSS, there were 7 fibres and 7 fragments, showing an equal amount. There was no significant difference ($p > 0.05$) (Eq. 4) in MP type between the top and bottom sediment.

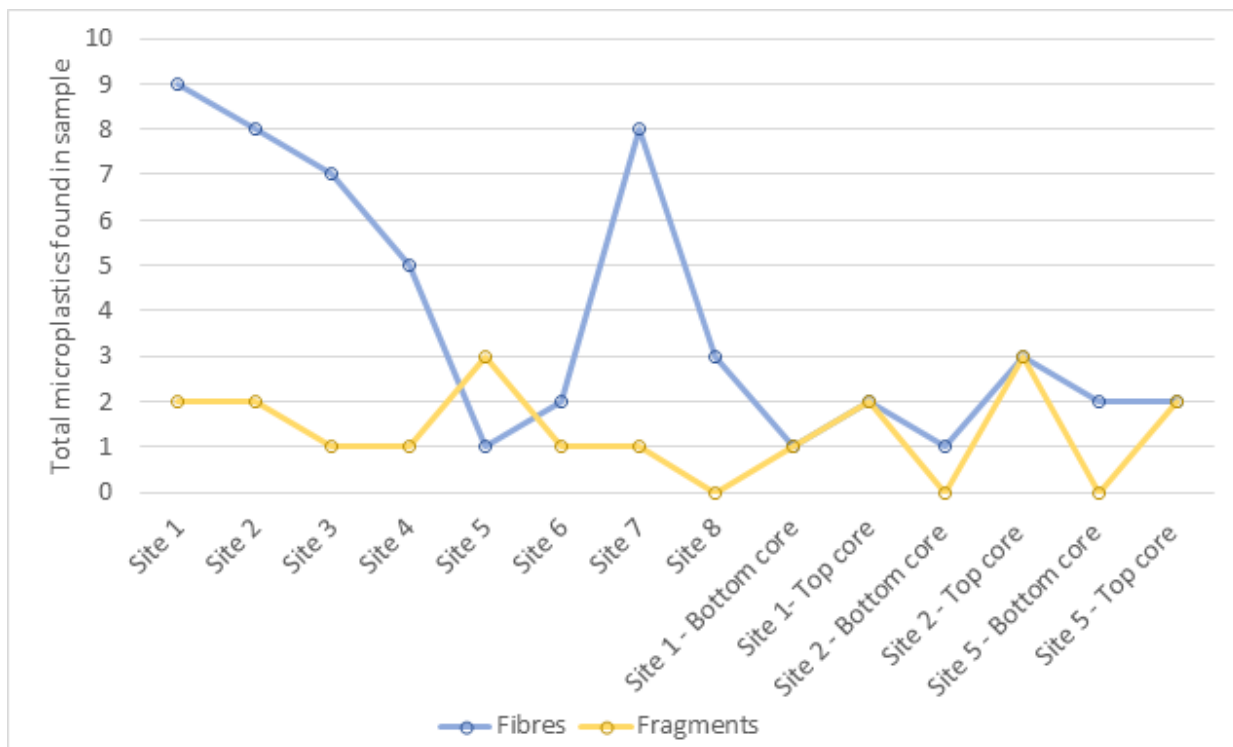


Figure 11: Line graph showing the types of microplastics found in each sample across all sites including bottom and top core samples. Samples were collected between 9am and 11am on the 8th August 2022 during low tide.

The size of MPs found in GSS of Plymouth Sound varied between <1mm to 5mm. There was no significant relationship between size of MPs and MP density ($p > 0.05$). Site 7 had the largest range in MP size with 4.03mm between the smallest and largest plastic. Site 5 had the smallest range in MP size with 0.8mm between the smallest and largest one. Overall, MPs <1mm dominated Plymouth Sound (Fig 12). Site 8, however, did not have a dominating size (Fig 12). In CSS, the dominant particle size in top sediment was <1mm whereas in bottom cores, the dominating size varied (Fig 12).

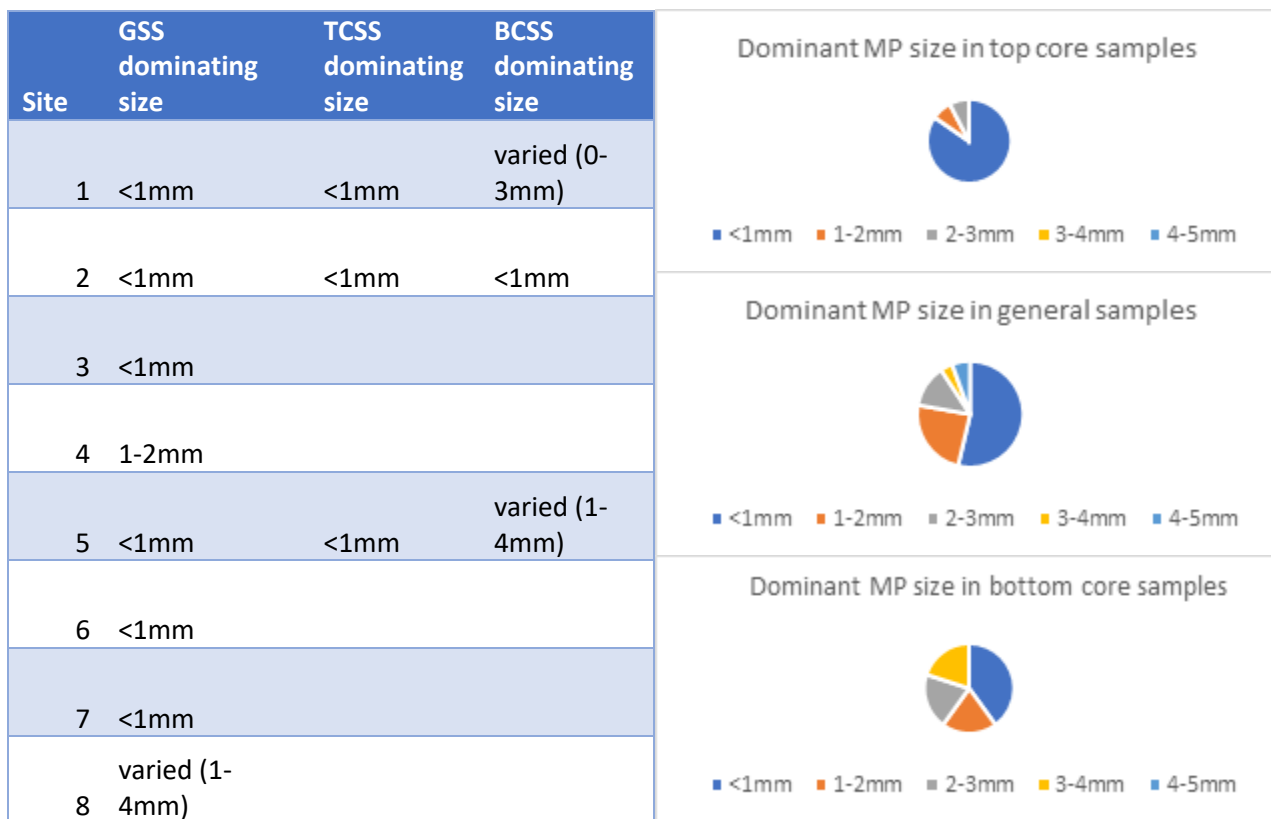


Figure 12: The most dominant microplastic sizes found in each site across the study area. Values in the table display dominant sizes of each site and pie charts show overall sizes of all sites. GSS indicates general sediment samples, TCSS is the top core sediment samples and BCSS is the bottom core sediment samples. Samples were collected between 9am and 11am on the 8th August 2022 during low tide.

Sediment type and floc impact on microplastic distribution

Sediment type varied at each site with clay being the most common across The Sound (Fig 13). Clay floc sizes were 109um and MP numbers were low compared to other sites (Fig 13). Muddy-sand sediment had the most MPs overall and an average floc size of 156um (Fig 13). The one sample consisting of rock (site 8) had the lowest number of MPs within it (Fig 13). There was no significant relationship ($p > 0.05$) (Eq. 5) between sediment type and MPs (Fig 13).

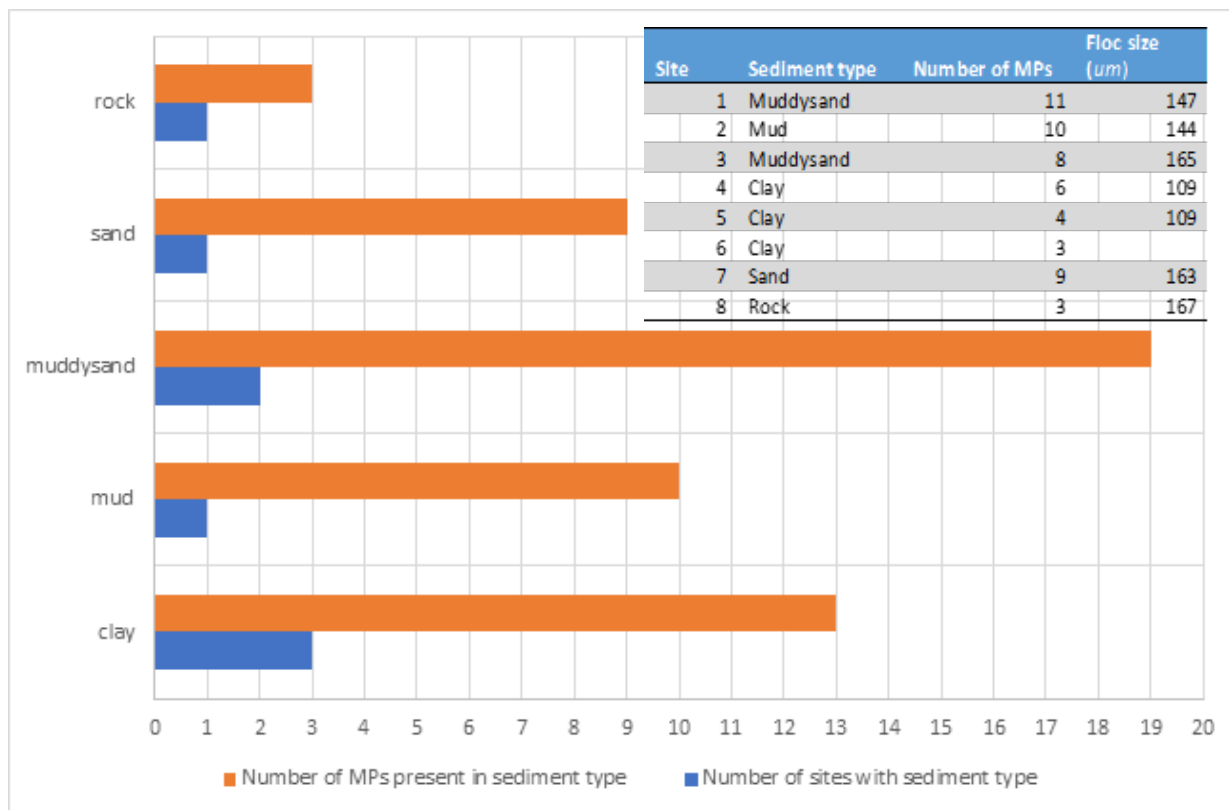


Figure 13: A visual overview of the number of microplastics (MPs) within certain sediments in relation to the sites consisting of that sediment. In the table, the number of microplastics present at each site according to sediment type and floc size is shown. Samples were collected between 9am and 11am on the 8th August 2022 during low tide.

On a plot, a curved trend line showed a relationship between number of MPs and floc size (Fig 14). Floccs between 140um and 150 um held the most amount of MPs in comparison to floccs <109um and >166um (Fig 14). The relationship between floc size and MPs, however, was not found to be significant ($p > 0.05$) (Eq. 6). In addition, an R^2 value of >0.05 shows the trend line does not approximate to the real data.

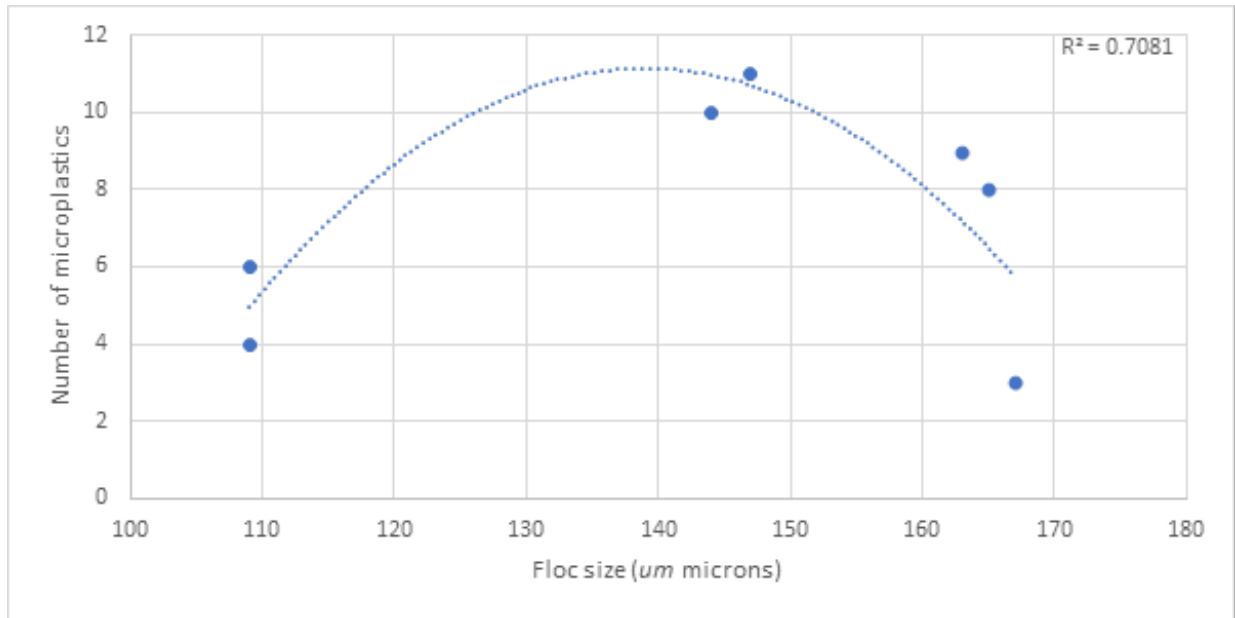


Figure 14: Scatter graph showing the number of microplastics from the sites plotted against the average floc size of the sediment. A curved trend line is displayed as a dotted blue line. Floc size is measured in microns (μm) and the R^2 value is shown to represent goodness of fit of the model.

Floc sizes of each sediment varied from $109\mu\text{m}$ to $167\mu\text{m}$ with an average floc size of $143\mu\text{m}$ (Fig 15). The majority of sites (1, 2, 4 and 5) had small floc sizes or ‘micro flocs’ of less than $160\mu\text{m}$. Sites 3, 7 and 8 had larger floc sizes known as ‘macro floc’ of above $160\mu\text{m}$.

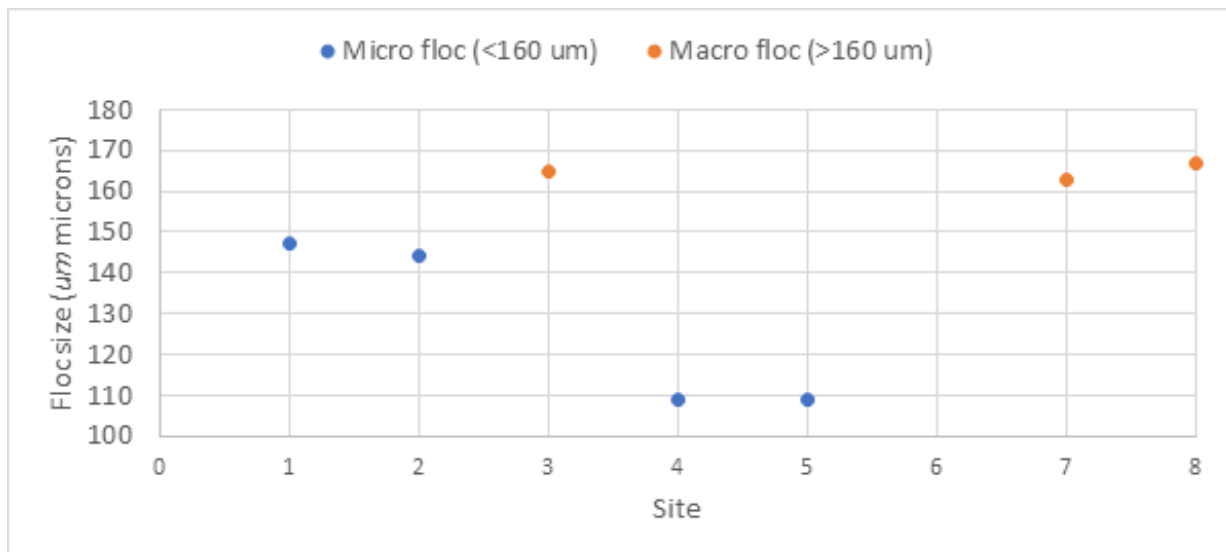
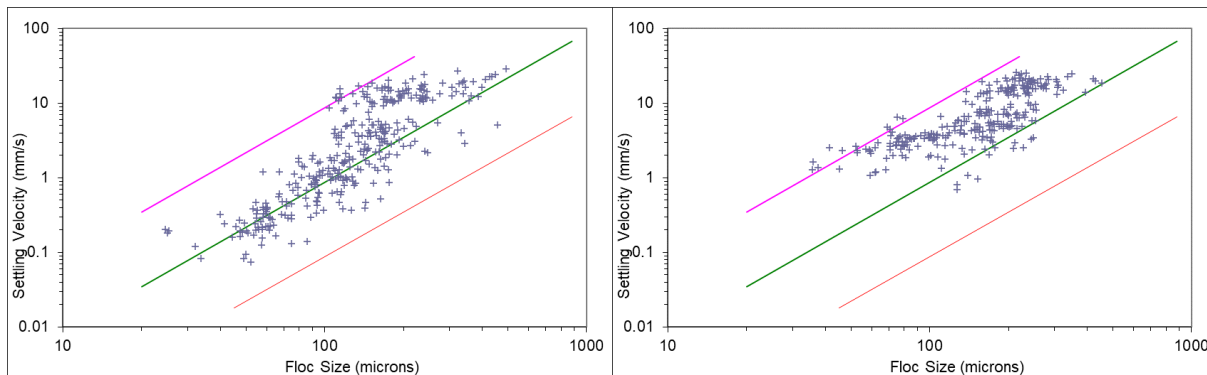


Figure 15: Scatter graph showing the ratio of microfloc ($<160\mu\text{m}$) to macrofloc ($>160\mu\text{m}$). Floc size is measured in microns (μm).

There was a positive correlation between floc size and settling velocity at all sites (Fig 16 & 17). The average settling velocity of flocs at all sites was 5.4mm/s and the average density was 444 kg/m³. Site 2 shows a slower settling velocity with a bigger range in floc size (Fig 16). Site 8 shows a faster settling velocity with a less varied floc size (Fig 17). Site 2 and site 8 represent two very different sediment types of The Sound.



Figures 16 and 17: Floc population scatter graphs showing settling velocity over floc size from site 2 (left) and the control site, 8 (right). Settling velocity is measured in mm/s which indicates millimeters per second.

Discussion

This study investigated the pollution of microplastics within the sediment of Plymouth Sound. Overall, particles were discovered in every sample, supporting the idea that MPs accumulate in sediment, forming hotspots (Cauwenberghe *et al.*, 2015). Questions previously asked in the introduction have been addressed; the spatial distribution of MPs here was equal, MP pollution has grown over time and the main type of MP found was fibre.

The spatial distribution of microplastics across Plymouth Sound

The results of this study indicate that the spatial distribution of MPs in sediment was evenly spread across Plymouth Sound. These findings support those found in Nel *et al.* (2020) that illustrate how there was no difference in MP density across Plymouth Sound. This could have been due to the sites being close together, experiencing similar conditions and therefore showing less variability (Nel *et al.*, 2020). On the other hand, it could have occurred because only one sample was collected per site, so it was hard to quantify a significant difference.

The control site (site 8) had fewer particles (3MPs) in comparison to other locations (>3MPs), which was anticipated (Fig 9 & 10). As the control site was further offshore than any other location, it experienced less human activity (Fig 9). Therefore, we can conclude that where areas are highly populated, plastic pollution is dense. These results are backed up by Wu *et al.* (2022) and Zhou *et al.* (2021) that found human activity had an impact on MP distribution. Although sediment type didn't have a significant impact on MP abundance, the rocks at site 8 might have influenced these findings (Fig 13). As rock is less cohesive than other sediment types, when settling, MPs do not accumulate in flocs (Shrestha & Blumberg, 2005). The small density of MPs at the control site could

have also been due to the Breakwater located between site 8 and Plymouth (Fig 9). This structure might have protected site 8 from urban MPs, consequently influencing the way MPs are distributed in the area. This idea is supported by Czuba *et al.* (2011) that illustrates how anthropogenic structures such as dams can influence sediment load and transport rate, which subsequently impacts MP distribution. These results provide insight into a possible measure to control MP distribution in a marine environment.

In parallel to this concept, other physical structures like land geomorphology could have influenced the way MPs were spatially distributed across the sites (Collins 2011). For example, at Cawsand Bay (site 7), MP density was high (9MPs) even though the sand sediment at this site did not impact MP density ($p > 0.05$) (Shrestha & Blumberg, 2005). Therefore, these results might have been due to the structure of the bay, creating a sink for MPs. In support to this theory, other sites surrounded by land; the River Plym (site 1) and West Mud (site 3) encountered MP levels above average (> 6.75 MPs) (Fig 9 & 10). This suggests that MPs accumulate at sites located in bays or in-between land. Further research should investigate how the structure of land impacts MP distribution. On the other hand, bio-cohesion might have been responsible for the high MP density at site 7 as many biological processes occur within sand, which can increase the stickiness of it (Shrestha & Blumberg, 2005).

Although sediment type was not found to have a significant impact on MP density, sites consisting of mud and muddy-sand experienced the highest levels of pollution (8-11MPs) (Fig 13). Mud is fine and cohesive so MPs 'stick' to it easily during flocculation (Manning *et al.*, 2017). Moreover, the flocculation of the sediment across Plymouth Sound did not significantly influence the distribution of MPs ($p > 0.05$) (Fig 14). Sites consisting of clay (sites 4, 5 and 6) experienced low MP densities which contradicts results found in He, *et al.*, 2020 and Cunningham, *et al.*, 2020 which concluded that clay sediment trapped high densities of MPs. The results of this current study might have been due to the small floc sizes (109 μ m) of sediment at these locations or due to human error (Fig 15).

A few sites in Plymouth Sound experienced dredging; Smeaton's Pass (site 5) and Echo Buoy (Site 6). In comparison to other sites, MP density was low at both areas (3-4MPs) indicating that dredging might have influenced the results (Fig 10). Dredging causes sediment to re-suspend into the water column, so any plastics within this sediment are constantly moved around (Ji *et al.*, 2021). These findings contradict those in Ji *et al.* (2021) that observed dredged sediment having high MP concentrations.

Sites close to land (sites 1, 2, 3 and 7) encountered high MP pollution (8-11MPs) (Fig 9). This further supports the idea that where human activity is high, plastic pollution is larger (Wu *et al.*, 2022 and Zhou *et al.*, 2021). The site with the highest density of MPs was the River Plym (site 1) (11MPs) (Fig 9 & 10). The River Plym was expected to have the largest density of MPs due to its location. Being close to a recycling centre, harbour and city, there are many possible sources of MPs at this site. Waste from the recycling centre and city storm drains contaminate the River Plym with MPs (Daly, 2022). In addition, marine coatings from boats in the harbour shed fragments that might also contribute to the River Plym's plastic pollution (Boucher & Friot, 2017). The Tamar Bridge (site 2) also experienced a large quantity of MPs (10MPs) (Fig 9 & 10). This site is close to land and within an estuary where it's known that MPs accumulate (Harris,

2020). Furthermore, above site 2 is a main road (A38) where tyre debris can directly enter the environment (Boucher & Friot, 2017).

Spatially dominating microplastics found across Plymouth Sound

Two types of MPs were found in the sediment of Plymouth Sound; fibres and fragments (*Fig 11*). However, there were significantly more fibres (43MPs) than fragments (11MPs) found in GSS ($p < 0.05$). These results support findings made by Willis *et al.* (2017) that found fibres to be the most abundant MP in an urban estuary. However, opposing results from Browne *et al.* (2010) stated that fragments dominated their sites across the same urban estuary as this study. This difference might be due to the main type of MP changing over time. With more fibres dominating the sites in this current study, it can be concluded that the main pollutant of Plymouth Sound is microfibre. It is understood that synthetic materials are the main polluter of fibres (Boucher & Friot, 2017). Therefore, the main source of MPs in Plymouth Sound is from sewage containing microfibres (Browne 2015). Smeaton's Pass (site 5), was the only site that had more fragments than fibres (*Fig 11*). As the main shipping channel for boats in Plymouth Sound, marine coating fragments from boats might have produced these results (Boucher & Friot, 2017).

MPs <1mm dominated this urban marine environment which reinforces results found in Haave *et al.* (2019) (*Fig 12*). Site 8 did not have a single dominating MP length, which might have been due to sample size limitations, sediment type, physical oceanographic conditions or the interference of the breakwater. The sizes of MPs found at Cawsand Bay (site 7) varied the most which might have been due to the dominating MP being polyester fibre (*Fig 12*). This reinforces the theory that polyester fibres accumulate in sandy sediment (Al-Libhaibi *et al.*, 2019). It also supports the idea that hotspots are related to sediment type (Haave *et al.*, 2019).

The temporal distribution of microplastics across Plymouth Sound

Results showed a significantly higher density of MPs in new sediment compared to old (*Fig 10*). Likewise, results from Willis *et al.* (2017) and Zhou *et al.* (2021) showed this same difference between sediment layers, indicating an increase in pollution over time. The bottom sediment was estimated to have settled 20-40 years ago whereas the top layer would have settled in the past 0-20 years (Willis *et al.*, 2017). The increase in MP correlates with the demand for plastic products (Kock *et al.*, 2020). Although recent efforts have been put into place to reduce plastic pollution, existing plastic continues to break down, so it is anticipated that MPs in the marine environment will continue to increase (Sul and Costa, 2014).

In all core samples, there were more fragments in the top layer of sediment compared to the bottom layer (*Fig 11*). This suggests that types of MPs have changed overtime, with more fragments being produced over the last 20 years. This hypothesis is backed up by Browne *et al.* (2010) who found more fragments in their samples where only the top-layer sediment was analysed (*Table 1*). It is known that after synthetic textiles, car tyres are the second largest producer of MPs (Boucher & Friot, 2017). As car travel has increased over the past 40 years (gov, 2021), tyres have shed more fragments, which explain these results. At the Tamar Bridge (site 2), where a main road runs across the sampling site, the number of fragments were the highest in the top layer of sediment

(Fig 11). This backs up the idea that more fragments have been produced by car tyres in recent years.

The size of particles varied in bottom sediments whereas plastics <1mm dominated top sediments (Fig 12). These results might be due to MPs breaking up into smaller pieces over the years. Furthermore, MPs in the top sediment are exposed to more erosion, weathering and tidal processes, meaning that they undergo more stress than those protected in layers of seafloor (Costello and Ebert, 2020). Wang *et al.* (2021) illustrates how few studies have explored MP size impact on density, so future studies should incorporate this concept.

Possible improvements to this current study

As the research on MPs is relatively new, there is no 'best standard' of measuring these particles in the marine environment (Farady, 2019). With one sample per site used in this current study, it was hard to quantify natural variability at each location and errors in the methodology. To improve this study, triplicate samples should be taken from each site to get a better understanding of MP abundance in specific areas. If this is too timely, research should focus on less sites, with more samples. With this, more significant and confident results can be gathered. Furthermore, deeper sediment core samples could be taken to show a larger time frame, with triplicate samples being taken. If researchers repeated this study, the selection of core sites should consider the environmental changes. In estuarine environments, the tidal range causes a lot of mixing which makes the results less accurate. Therefore, selecting a less turbid environment, such as a salt marsh, would provide a more accurate representation of time changing through layers. In addition, water property data could be collected to investigate the connections between these properties and the MPs present.

Conclusion

Overall, the aim of this study has been addressed as it is now understood how microplastics are distributed in the sediment of Plymouth Sound. There was a significant difference between microplastic abundance across Plymouth Sound temporally, but not spatially. MPs were evenly distributed in the sediment of The Sound showing a clear influence from human activity. Sediment did not influence the number of microplastics present at each site. In addition, the main type of MP was microfibre, likely originating from synthetic textiles. This suggested that the biggest source of MPs in the area derived from washing clothes into sewage outlets. The MP pollution of Plymouth Sound was found to have increased over time and as the demand for plastic production grows, we can expect to see higher numbers in the future. From these results, we can state that MP distribution is impacted by anthropogenic activity, physical structures and location in consideration to MP sources.

There are several implications of this research moving forward. Scientists may consider how physical structures influence MP distribution. In addition, understanding how MPs are distributed in deep seas and offshore environments, away from human influence, would be beneficial to compare these results with. Furthermore, there is a strong need to explore possible control mechanisms for MP distribution in the marine environment. In conclusion, a further understanding of how MPs behave in all types of marine environments is essential in order to manage MP pollution.

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