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Optimising H_2O_2 digestion and quantifying microplastics in sediment and pacific oyster (*Crassostrea gigas*) samples

H. Brown [a], I.D. Williams [b,*]

[a] *School of Ocean and Earth Science, Faculty of Environmental and Life Sciences, University of Southampton, Waterfront Campus, European Way, Southampton, Hampshire SO14 3ZH, United Kingdom*

[b] *School of Engineering, Faculty of Engineering and Physical Sciences, University of Southampton, Highfield Campus, Southampton SO17 1BJ, United Kingdom*

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ABSTRACT

Microplastic pollution continues to threaten marine environments across the world, yet there is inadequate understanding regarding the sources, distribution and impacts of these particles. Marine microplastic pollution is commonly investigated with the use of biomonitors, such as bivalves. However, published methods on chemical tissue digestion lack clarification regarding reagent volumes for small tissue samples <5 g. Therefore, this study aimed to improve H_2O_2 digestion methods and quantify and categorise the microplastics found within sediment and *Crassostrea gigas* samples collected from Weston Shore, Southampton. Tissue samples of 1 g were digested in varying quantities of 30 % H_2O_2. 20 ml of 30 % H_2O_2 per gram of tissue was sufficient in digesting samples of 2 g or more; 1 g samples require further experimentation. Sediment samples were visually inspected under a light microscope, along with the oyster samples once the microplastics had been extracted using H_2O_2 digestion, followed by density separation using NaCl. For tissue samples ≤5 g, 20 ml H_2O_2 per g of tissue should be used for digestion. For tissue samples >6 g, 6× mass of the sample should be used for digestion. Sediment microplastic concentrations were found to decrease moving south east along the shore, with varying significance, whereas *C. gigas* microplastic loads did not show any significant spatial differentiation ($p = 0.3$). Both sediment and *C. gigas* samples were dominated by fibres (96 % and 97 %, respectively), which is consistent with similar studies worldwide. The new digestion method gives 50 % cost reduction and lessened environmental impacts.

1. Introduction

1.1. Microplastics in the marine environment

Plastic was first reported in the marine environment in the 1970s (Carpenter and Smith, 1972; Colton et al., 1974; Wong et al., 1974) and has since become ubiquitous across the oceans at all depth ranges (Chiba et al., 2018; Mountford and Maqueda, 2019; Parolini et al., 2023). The presence of plastic in the environment can pose serious health risks to marine organisms through ingestion and entanglement (Murphy et al., 2024) and has been found to impact all ecosystem services to some extent (Beaumont et al., 2019). Due to the cheap, durable and lightweight nature of plastics compared to other materials, such as glass, they have become incredibly popular. Consequently, 20 million tons of plastic enter the environment annually (IUCN, 2024).

Microplastics (MPs) are plastic particles that measure <5 mm in diameter and are either produced directly (primary MPs) or are created through a range of biotic and abiotic processes that cause plastics to fragment or deteriorate (secondary MPs) (Zhang et al., 2021). Both primary and secondary MPs threaten marine life at all trophic levels, either through direct consumption or indirectly through trophic transfer (Avio et al., 2015; Bhattacharya et al., 2010; Cole et al., 2015; Egbeocha et al., 2018; Sussarellu et al., 2016). Once ingested, MPs can bioaccumulate – the accumulation of chemicals or particles in an organism that takes place when the rate of ingestion exceeds the rate of excretion (Popek, 2018) – and biomagnify – the process by which contaminant concentrations increase in higher trophic levels through the consumption of contaminated prey (Popek, 2018). Damage caused by MPs can be physical (e.g. interactions between MPs and tissues), chemical (e.g. toxicity from contaminants and dyes associated with plastics) or biological (e.g. infections from bacteria colonies on the MPs) (Parolini et al., 2023) and can differ between taxonomic groups (Avio et al., 2015; Besseling et al., 2015; Bhattacharya et al., 2010; Green, 2016; Lu et al., 2016; Oliveira et al., 2013).

* Corresponding author.
E-mail address: idw@soton.ac.uk (I.D. Williams).

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These species-level implications can lead to negative impacts at the ecosystem scale, with reductions in secondary production (Troost et al., 2018), changes to bioturbation activity and nutrient cycling in sediments (Green et al., 2016) and altered population dynamics (Ferreira et al., 2016), all of which can change the structure of the community. Therefore, understanding, monitoring and managing MPs in the marine environment is essential for conserving the biodiversity and functioning of ecosystems. This helps to maintain the productivity of our oceans, which billions of people rely on for food security (FAO, 2022).

1.2. Bivalves as biomonitors for microplastics

A common way of monitoring environmental pollutants, such as MPs, is by using biomonitors (Multisanti et al., 2022). Biomonitors are living organisms that can be used to quantitatively assess levels of pollutants in the environment (Hatje, 2016). Bivalves are commonly used as biomonitors for MPs in the marine environment (Bendell et al., 2020; Ding et al., 2021; Ghazali et al., 2022) due to strong correlations between the MP concentration in their tissues and the environmental MP concentration (Qu et al., 2018). This is due to their feeding ecology; as filter feeders, bivalves process large volumes of water to obtain food particles using ciliated feeding organs. This is thought of as a passive feeding method, meaning bivalves cannot select for or against particles. There is some evidence to challenge this, however, with studies suggesting that bivalves can select and reject particles based on nutritional values (Espinosa et al., 2016) or particle size (Ding et al., 2021; Ward et al., 2019), which may hinder the reliability of their role as biomonitors. Furthermore, abiotic factors, such as temperature and salinity, can impact MP ingestion (Du et al., 2023; Stamataki et al., 2020), again resulting in MP concentrations that may not be representative of the environmental concentrations. Nevertheless, bivalve MP load can function as a good initial indicator of environmental MP pollution, and monitoring the concentration of MPs in bivalves allows spatial and temporal trends to be elucidated (Baechler et al., 2020; Walters et al., 2022).

Bivalves have been suggested as major vectors for the bioaccumulation of MPs in humans (Ding et al., 2021; Van Cauwenberghe and Janssen, 2014). Therefore, quantifying the MP load in bivalves can be used to enumerate human ingestion of MPs from the consumption of shellfish (Dao et al., 2023; Van Cauwenberghe and Janssen, 2014). Furthering this research can allow for greater accuracy in predicting human MP exposure through seafood. Globally, 3.3 billion people depend on seafood as their main source of protein, especially in developing countries (FAO, 2022). So, quantifying the extent of MP contamination in seafood is vital for global public health. Getting a comprehensive understanding on the state of MP contamination in bivalves worldwide is an effective initial step, as many bivalve species are commercially important, such as *Mytilus edulis*, *Crassostrea gigas* and *Chamelea gallina* (Song et al., 2024).

1.3. Southampton water

Southampton Water is a tidal estuary in the United Kingdom and exists north of the Solent, with riverine input from the rivers Test, Itchen and Hamble. It is a highly industrialised region subject to extensive anthropogenic activities – both commercial and recreational – that adversely affect water quality, including contributing to MP pollution (Soon et al., 2024; Xiong et al., 2023). Home to the UK's second largest container port and busiest cruise terminal, Southampton Water is inundated with heavy shipping traffic. Though often neglected as major MP sources, ships have been found to significantly contribute to environmental MP pollution through the disposal of grey water (wastewater produced from laundry, showers, sinks, etc.) (Peng et al., 2021) and degradation of paint and protective coatings (Song et al., 2014; Tamburri et al., 2022).

Similarly, various wastewater treatment works (WTWs) are distributed along Southampton Water's tributaries – Portswood WTW, Woolston WTW and Millbrook WTW. WTWs are known to release MPs into the environment through effluent discharge (Gies et al., 2018; Harley-Nyang et al., 2022; Murphy et al., 2016), which, in Southampton, includes industrial waste from Fawley refinery (Deng et al., 2023). Although research has argued the magnitude of this source (Carr et al., 2016; Napper et al., 2023), it should not be disregarded. At least three of the 15 storm overflows associated with the WTWs in Southampton are thought to be overflowing more frequently than DEFRA (Department for Environment, Food and Rural Affairs) requirements (Southern Water, 2022), releasing untreated water into the rivers and estuary. Other sources of MPs include road marking paints, tyre rubber and litter (Horton et al., 2017; Jaafarzadeh et al., 2024; Sommer et al., 2018).

1.4. Methods for microplastic extraction

Assessing MPs in the marine environment is important for conserving ecosystem health and concomitant ecosystem services. As such, having harmonised protocols of MP extraction from organic material with high efficiencies is vital for obtaining accurate, reliable and reproducible data (Enders et al., 2020), which can be used to inform policy makers and implement change. However, evaluation of methods should go beyond the extraction capacity of techniques. Potential hazards associated with the reagents, costs and how much time a researcher will has to spend extracting microplastics from samples are also essential factors (Thiele et al., 2019). There are a diverse number of methods for the extraction of MPs from organic material (Thiele et al., 2019), including acidic, such as nitric acid (De Witte et al., 2014), enzymatic, such as proteinase-K (Cole et al., 2014), basic, such as NaOH (Pfeiffer and Fischer, 2020) and oxidising, such as H_2O_2 (Enders et al., 2020; Li et al., 2015), each with their own advantages (Table 1). Methods can further vary depending on the exact protocol followed, with a range of enzymes, temperatures and filtration techniques that can be used.

1.5. Aims and objectives

Southampton Water has received ample attention regarding MP pollution (Gallagher et al., 2016; Rose et al., 2024; Stead et al., 2020; Stead, 2022; Zapata-Restrepo et al., 2025); however, research into organism MP contamination in the region remains minimal (Rose et al.,

Table 1
Advantages and disadvantages of common reagents used in methods of microplastic extraction from organic matter.

Reagent	Advantages	Disadvantages
NaOH	High digestion efficiency (Tuuri et al., 2024).	High levels of polymer damage (Tuuri et al., 2024).
H2O2	Disposal is environmentally friendly (Gao et al., 2020).	Exessive foaming can lead to sample losses (Thiele et al., 2019).
	High digestion efficiency (Tuuri et al., 2024).	May degrade or discolour synthetic ploymers (Karami et al., 2017). Production can be damaging to the environment (Gao et al., 2020).
Proteinase-K	High digestion efficiency (>96 %) Carrillo-Barragan et al., 2022). Minimal polymer damage (Carrillo-Barragan et al., 2022).	Expensive (Thiele et al., 2019).
HCl	High digestion efficiency at high concentrations (Karami et al., 2017).	Can cause polymer damage (Classens et al., 2013).
		May fuse synthetic polymer particles together, resulting in a reduced recovery rate (Karami et al., 2017).

2024; Zapata-Restrepo et al., 2025). Moreover, the extent of research into MPs in bivalves around the south coast of England is insufficient (Li et al., 2018; Scott et al., 2019; Zapata-Restrepo et al., 2025), leaving a clear gap for quantifying and assessing MPs in bivalves around the south of England.

To rectify this, the present studied aimed to investigate MP pollution in sediment and Pacific oyster (*Crassostrea gigas*) samples from Weston Shore. The abundance, accessibility and ecology (filter feeding, sessile organism) of *C. gigas* made it an ideal species to study. Given the extensive polluting influences in Southampton Water, it was assumed that MPs would be prevalent in the environment. In order to test this, refinements to H_2O_2 digestion methods for small tissue samples needed to be made. Full aims and objectives for this study can be found in Table 2.

2. Methods

2.1. Preliminary site survey

Weston Shore, Southampton, was selected as an ideal sampling site due to its accessibility and status as a Site of Special Scientific Interest (Mouchel, 2012). Prior to collecting samples from Weston Shore, a preliminary site survey was completed to assess the abundance and distribution of the species present, identify and map any sewage pipes and determine the best places for sampling. Twelve sewage outflow pipes were mapped along the length of Weston Shore (Fig. 1). Five sampling sites were chosen in order to assess the spatial variability of MPs along the shore (Fig. 2). All sampling sites had a high abundance of the bivalve *Crassostrea gigas* (Pacific oyster).

Table 2
Aims and objectives for the present study of microplastic analysis in the sediments and marine life of Southampton Water, with refinements of preexisting H_2O_2 digestion methods.

Aims	Objectives
i) Refine preexisting hydrogen peroxide digestion methods to provide clarification for small tissue (<5 g) samples	Observe the digestion efficiency of tissue samples in varying volumes of 30 % hydrogen peroxide
	Assess the extent of tissue digestion after 24, 48 and 72 h
ii) Investigate the extent of microplastic pollution in the sediments of Weston Shore, Southampton	Quantify the microplastic load in sediment samples
	Analyse spatial variations in sediment microplastic loads
	Analyse the differences between abundances of microplastic types in terms of shape and colour
iii) Investigate the extent of microplastic pollution in oysters in Southampton Water	Quantify the average microplastic load in oysters
	Analyse spatial variations in oyster microplastic loads
	Analyse the differences between abundances of microplastic types in terms of shape and colour

2.2. Collection and storage

Sampling took place between 0600 and 0800 GMT on 18/12/2024 during low tide. Specimens were collected by hand from five sampling sites (Fig. 2) and stored in plastic bags. The bags used were clean, new tie up plastics bags. This is not standard practice, due to the risk of contamination resulting in unreliable results. However, the glass jars taken to store the oysters were too small, so adaptations had to be made in the field.[1] Five specimens from each location were collected, giving a total of 25 individuals. All specimens were measured to ensure they met the IFCA guidelines of being larger than 70 mm. Sediment samples were collected from three of the five locations (Fig. 2) using a metal spoon and stored in glass jars to avoid plastic contamination. All samples were labelled with a number corresponding to their location on site.

The samples were taken to the National Oceanography Centre Southampton (NOCS), and were frozen at −20 °C to euthanise the organisms and prevent sample deterioration and decomposition. The samples were thawed at room temperature for 24 h prior to MP extraction.

2.3. Optimisation of tissue treatment using H_2O_2

There is a lack of detailed, harmonised methods for bivalve tissue digestion; published methods are often vague and incomplete. Enders et al. (2020) highlight the importance of sharing best-practice protocols in order to assist the harmonisation of MP quantification methods. Thiele et al.'s (2019) comprehensive method evaluation paper recommends the use of 10 % KOH as a suitable digestion technique for bivalve tissues. Nevertheless, treatment with H_2O_2 is very common (e.g. Li et al., 2015). The main objection to treatment with H_2O_2 is excessive foaming and subsequent sample loss (Thiele et al., 2019). However, this is not a significant problem for small tissue samples. Nevertheless, method statements for tissue treatment with H_2O_2 often lack sufficient detail and justification. To rectify this, experimental methods were used to determine a harmonised volume of H_2O_2 for the digestion of small samples of oyster tissue, in order to optimise pre-existing methods.

One individual of *C. gigas* was thawed and removed from its shell. Three beakers were set up and 1 g of tissue from the gills was added to each along with either 20 ml, 40 ml or 80 ml of 30 % H_2O_2. The beakers were loosely covered with a foil dish. After 24 h, the beakers were removed from the incubator to assess the progress of digestion. The beakers were incubated at 40 °C for another 24 h to assess whether further digestion may take place under the smaller quantities of H_2O_2. This process of incubate and assess was repeated for a total of 72 h, with the digestion assessment taking place every 24 h. The results from these experimental methods were used to refine the pre-existing methods implemented in section 2.6, ensuring H_2O_2 volume was tailored to small tissue samples.

2.4. Microplastic extractions

MP quantification in sediments followed methods outlined by Rose et al. (2024). Once fully thawed, 80 g of each sample was measured into separate foil dishes, which were pre-weighed at 2 g each, and placed in a drying oven at 50 °C for 24 h to remove the water content. After the samples were completely dry, they were reweighed to calculate their dry mass. Each of the three samples were split into five subsamples of 10 g for visual analysis under a light microscope using a magnification of × 63. Visual analysis was chosen, despite the caveats associated with human error and the lengthy process, to keep methods as eco-conscious and cost-effective as possible. Other methods with higher accuracies, such as density separation and wet peroxide oxidation (Rodrigues et al., 2018), often use many chemicals, which can be costly and environmentally damaging. As this study was already using large quantities of H_2O_2 and NaCl solution, visual inspection was the best way forward. The

[1] One author (Williams) is very familiar with this location and the challenges it provides for sampling. Hence, we took glass containers that are at the top end of what works practically and logistically. Larger glass containers that could have held the bivalves present at this location on the day of sampling are unsuitable for this type of sampling because of their size and weight, and because of the logistical and practical difficulties faced when subsequently storing and treating samples.

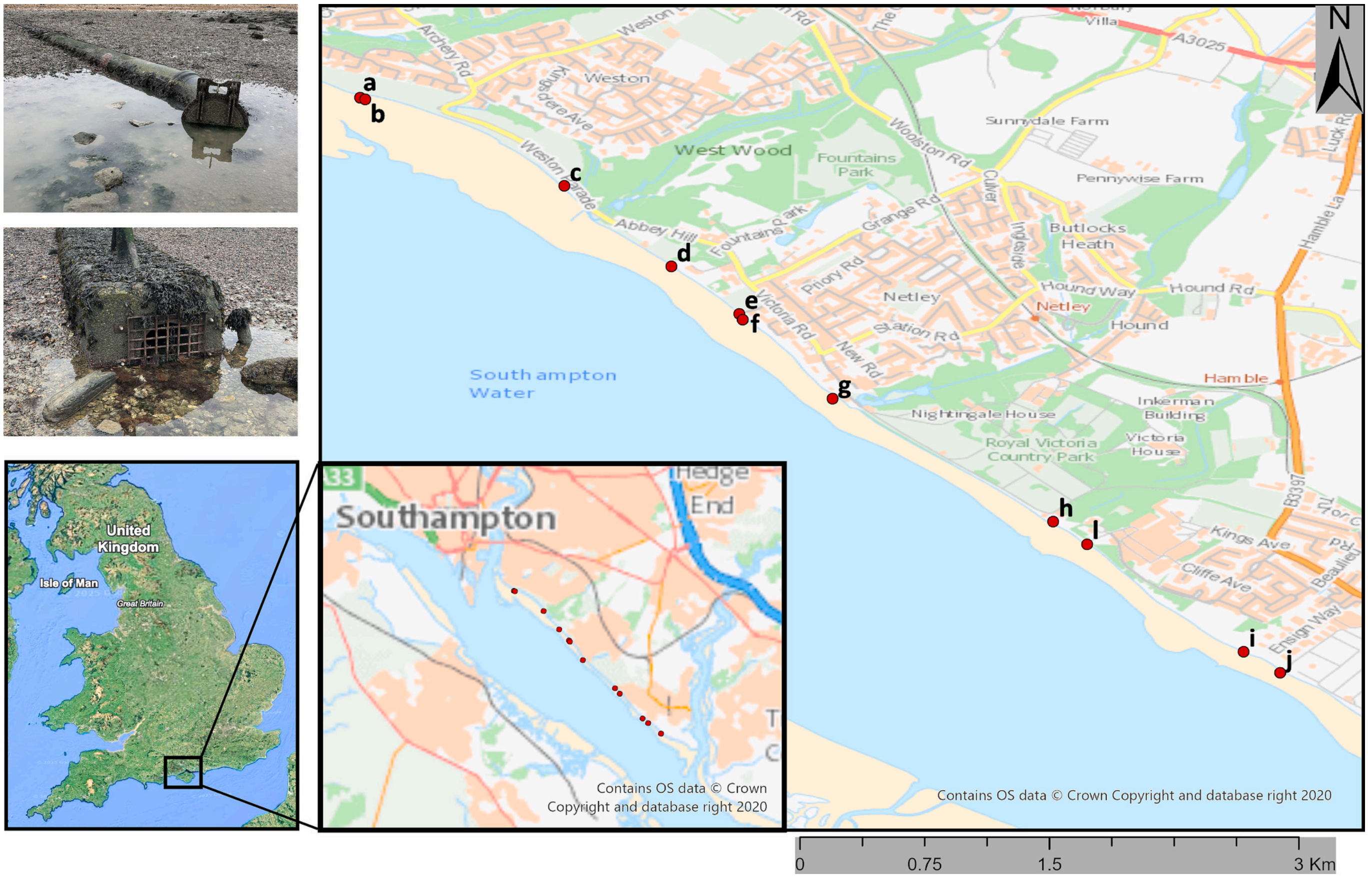

Fig. 1. Sewage outflow pipes mapped along Weston Shore, Southampton, during the preliminary site survey conducted on 04/11/2024 (a-c) and 06/11/2024 (d-j) prior to sediment and oyster sampling on the 18/12/2024. Examples of sewage pipes A (top) and C (bottom) are displayed in the corner.

subsamples were kept in labelled petri dishes until analysis to avoid airborne contamination of MPs.

Methods used for MP extraction from organic material were taken from Li et al. (2015) and Thiele et al. (2019) and fine-tuned using experimental methods as described above. Once thawed, shell length and total mass were recorded before the organism was extracted and the soft tissue was weighed. The adductor muscle and parts, or all, of the mantle tissue was removed from each specimen in order to reduce the mass of tissue to 4 or 5 g, allowing the volume of H_2O_2 to be reduced. A key part of this study was optimising digestion for small tissue samples. We thus removed the mantle as more plastics are likely to accumulate in the digestive tract and the gills. Furthermore, whilst MPs in the mantle are likely able to cause physical injuries which may lead to infections, knowing how many MPs are actually consumed is useful for determining the extent of biological related problems that may affect growth and reproduction. Each oyster was added to a separate beaker and 20 ml of 30 % H_2O_2 was added for each gram of tissue using a pipette. The H_2O_2 was not directly squirted on top of the tissue, but onto the side of the beaker instead, as this avoided vigorous fizzing. The beakers were loosely covered with a foil dish to avoid contamination from airborne MP particles and the samples were left to digest for 72 h at 40 °C to avoid polymer damage that can occur at higher temperatures (Thiele et al., 2019).

Once the organic material had been fully digested, the MPs were separated via floatation. Due to the cost-effective and environmentally friendly nature of NaCl compared to other salt solutions (Cutroneo et al., 2021), 200 ml of 3 M $NaCl_{(aq)}$ solution was added to each beaker, mixed and left for 24 h. The resultant solution was filtered over a 5 μm cellulose filter disk using a vacuum filter. We acknowledge that this frequently used standard method will filter out any beads of 5 um or less, increasing the risk of fibre domination in results. The disks were kept in labelled petri dishes to prevent airborne MP contamination until analysis under a light microscope using a magnification of ×63.

2.5. Data analysis

A Shapiro-Wilks test was used to test the data for a Gaussian distribution; this confirmed that the data did not follow a normal pattern, preventing a parametric statistical test from being used. Consequently, each site was subjected to the non-parametric Kruskal-Wallis test (χ^2) to investigate the statistical difference between MP quantities and morphologies between sites for both oyster and sediment samples. Further insight into the differences in morphologies were elucidated by a post-hoc analysis using the Dunn test (Z). This was run in accordance with the Bonferroni method to minimise the risk of false positives. Significant values were calculated at the 95 % confidence level ($p \leq 0.05$). All statistical testing was completed using R and RStudio (R Core Team, 2024; RStudio Team, 2024; Ogle et al., 2025).

3. Results

3.1. Quantification of H_2O_2 for tissue digestion

After 24 h in the incubator, the first assessment of tissue digestion was completed. The tissue that was exposed to 80 ml of H_2O_2 was completely digested, whereas the tissue samples that were exposed to 20 ml and 40 ml of H_2O_2 still had remnants of undigested tissue (Fig. 3a). After a further 24 h in the incubator (total 48 h), the tissue was almost fully digested in all conditions (Fig. 3b), however the 20 ml beaker appeared to have some tissue floating on the surface. After a total of 72

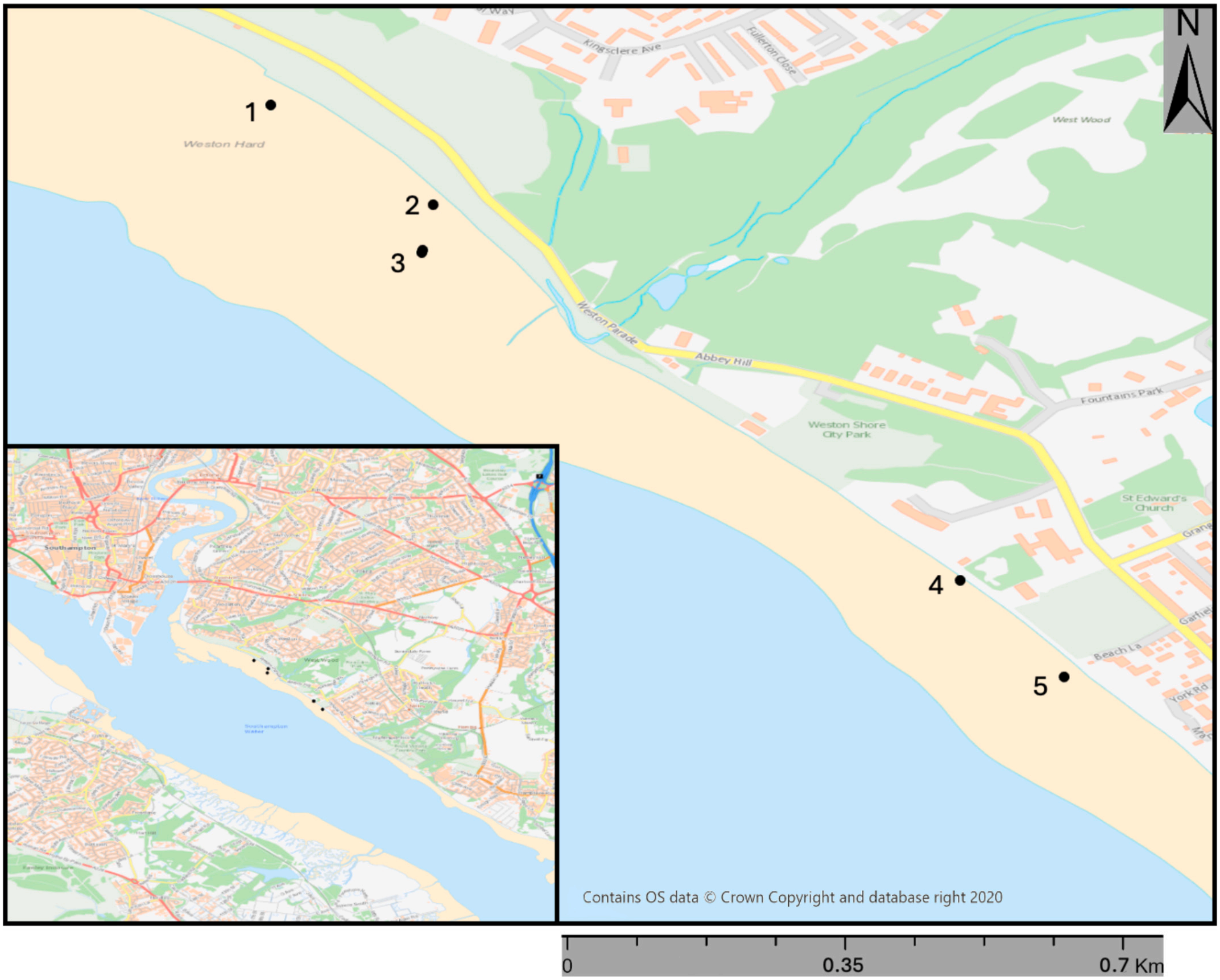

Fig. 2. Locations along Weston Shore, Southampton, where sediment (sites 1, 2 and 5) and oyster (sites 1–5) samples were collected.

h, the tissue samples in all conditions were fully digested, however the H_2O_2 in the 20 ml condition had fully evaporated (Fig. 3c).

3.2. Analysis of sediment microplastics

A visual inspection of the sediment samples from sites one, two and five using a light microscope revealed a total of 49 particles that could be classed as MPs with certainty across the three sampling sites (Appendix 2; Fig. 4). A potential maximum of 87 particles were recorded when including particles that instigated uncertainty (Fig. 4) and would require further testing, such as Raman spectroscopy or micro-Fourier transform infrared (μFT-IR) spectroscopy, for confirmation. When assessing the differences in plastic load and morphologies within and between sites, only the particles classed as 'certain' were considered and the particles that instigated uncertainty were disregarded. As a result, MP counts may be an underestimation.

Sampling site one had the greatest concentration of MPs, with an average (median) of 6 MPs/50 g of sediment, although there was large variation across subsamples (Fig. 5). Sites two and five were more consistent across subsamples and also had lower average MP loads of 1 and 0 MPs/50 g of sediment, respectively (Fig. 5). There is a general trend of decreasing sediment MP load moving from site one to site five, despite only site one and five significantly differing from one another ($Z = -3.00$, $p = 0.008$), with site one having a significantly greater quantity of MPs.

Fibres dominated the MP morphologies, with a total of 47 compared to only one bead and one fragment found across all sampling sites (Fig. 6), accounting for 96 % of the total MP load. As highlighted in Section 2.4, this is partially a consequence of the standard method used (i.e. filtration using 5 um pore size filters). Consequently, a range of colours were found among the fibres – black, white, and blue, supporting the findings of Zapata-Restrepo et al. (2025). The dominance of fibre colour is probably site and time dependent, and probably reflects prevailing local circumstances. White fibres were the most abundant, making up 91 % of the sample. Black and blue each made up 4.5 % of the total fibres. This contradicts the literature, with reports of black fibres dominating sediment samples on the opposite side of the estuary (75 %), followed by blue (22 %) and red (2 %) (Stead et al., 2020). White fibres remain largely unreported, with none (Stead et al., 2020) or very few (Gallagher et al., 2016) found. Despite the large variation between percentages, differences in fibre colour abundance was found to be insignificant at the 95 % confidence level ($\chi^2 = 5.08$, $p = 0.079$).

The difference in quantities of morphologies were also analysed between sites and only the number of fibres between sites one and five were found to be significantly different from one another ($Z = -2.93$, $p = 0.01$), with no statistical differences found between beads and

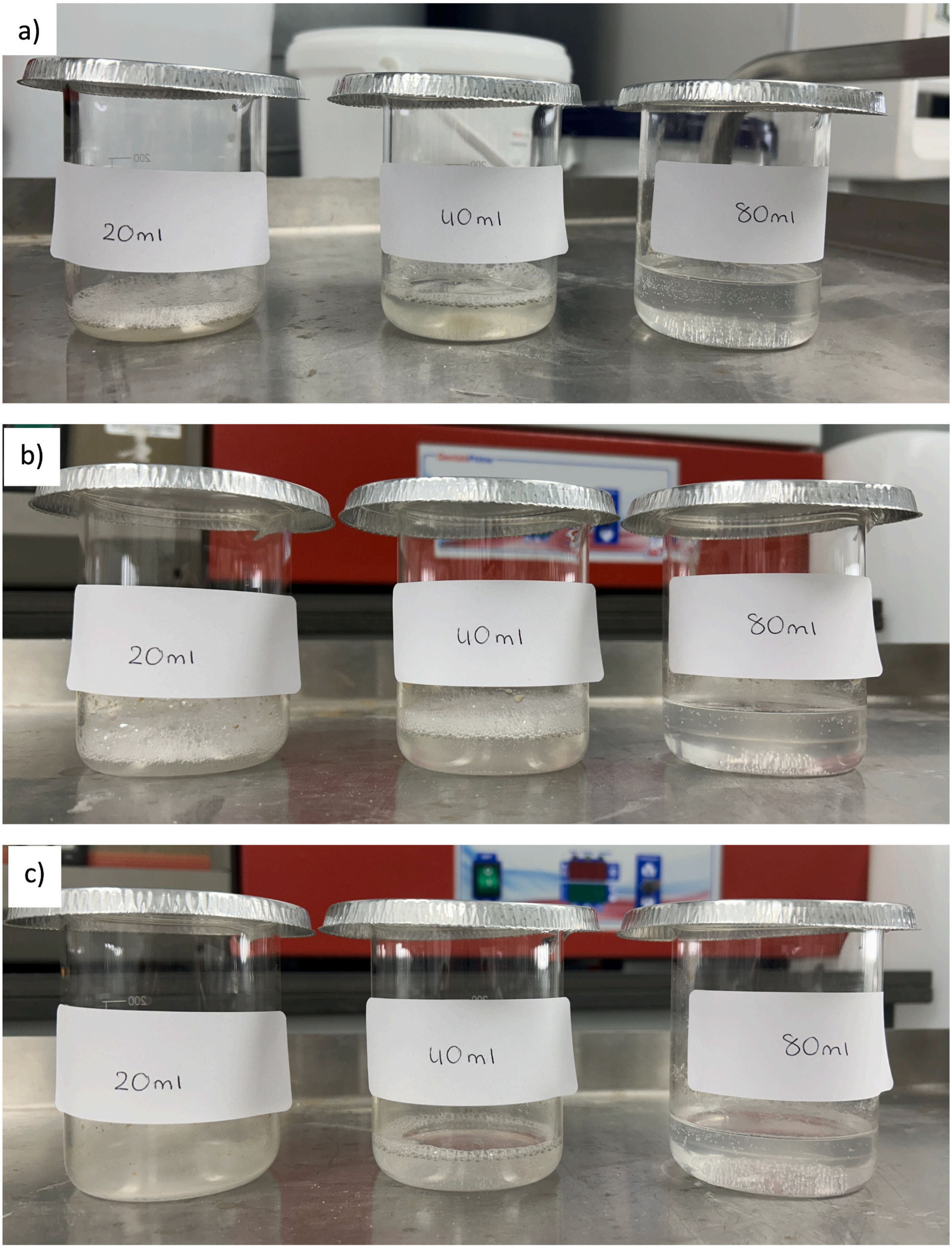

Fig. 3. a) 1 g of *C. gigas* tissue in 20 ml, 40 ml and 80 ml of 30 % H_2O_2 after 24 h incubated at 40 °C. b) Same as a) but after 48 h. c) Same as a) but after 72 h.

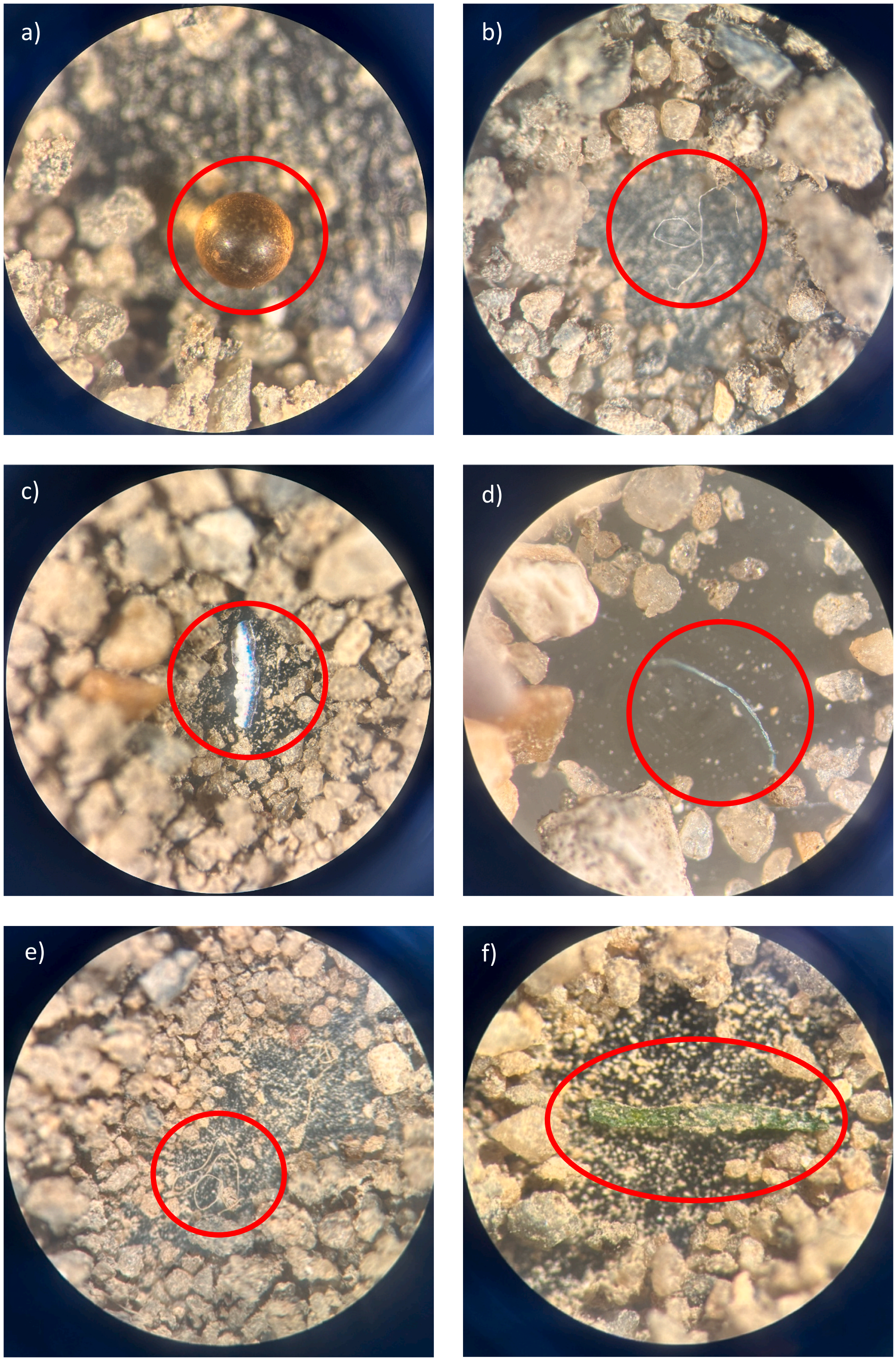

Fig. 4. Examples of microplastics found in sediment samples collected on 18/12/2024 from Weston Shore, Southampton. Microplastics were found by visual analysis using light microscopy with a magnification of $\times$ 63. a) Orange bead from site one. b) White fibre from site one. c) Blue and white fragment from site one. d) Blue fibre from site five. e) Uncertain fibre, potentially cellulose, from site one. f) Plant detritus fragment from site one. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fragments. There were no significant differences between sites two and one ($Z = 2.32$, $p = 0.062$) and two and five ($Z = -0.96$, $p = 1.00$).

Both site one and site two were found to have significantly greater quantities of fibres compared to beads ($Z = -2.88$, $p = 0.012$ and $Z = -2.27$, $p = 0.023$ for site one and two, respectively) and fragments ($Z = 2.88$, $p = 0.012$ and $Z = 2.27$, $p = 0.0229$ for site one and two, respectively). This is consistent with previous studies focussing on sediment in the area, finding fibres tend to dominate the plastic load within the sediment of Southampton Water (Stead et al., 2020). However, this contradicts the previously reported major morphology of MPs

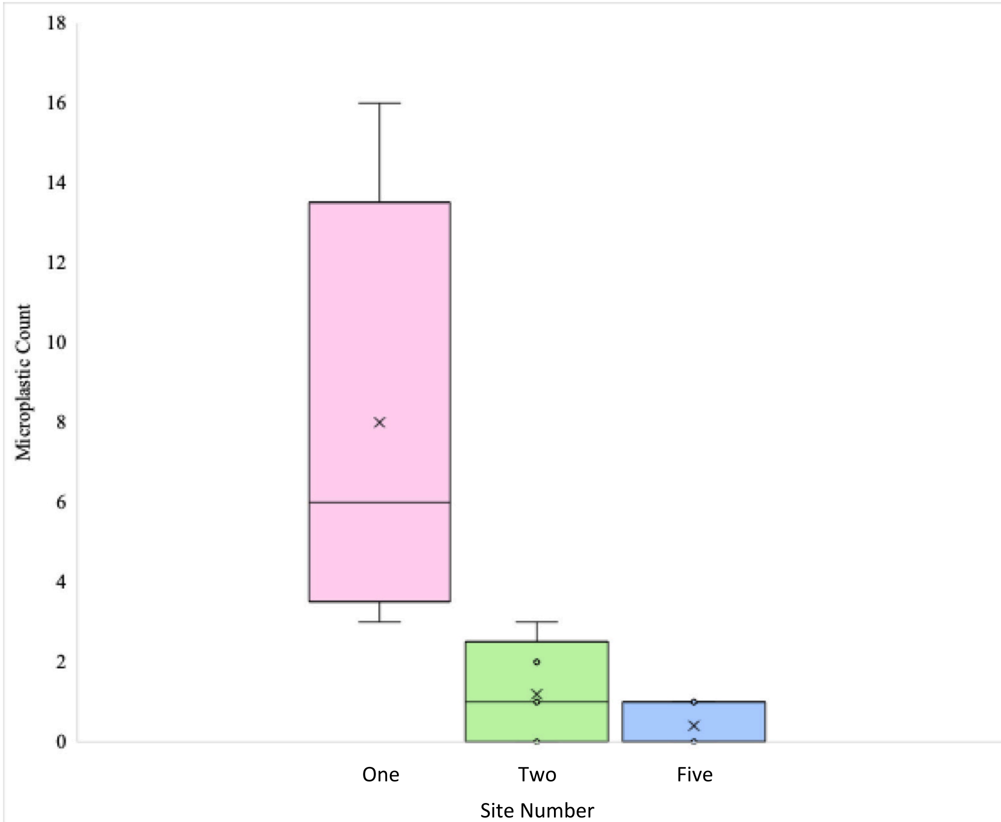

Fig. 5. Sediment microplastic loads in 50 g samples (10 g subsamples) collected from Weston Shore, Southampton on 18/12/2024. Samples were collected from three sites along Weston Shore – site one, site two and site five – between 0600 and 0800 GMT at low tide. Median values are depicted by the solid black line; mean values are depicted by the cross symbols.

in the water column of Southampton Water, where rounded particles were found to dominate (Gallagher et al., 2016). For both sites one and two, the quantity of beads and fragments did not significantly differ from one another within their respective sites, with only one bead and fragment found at site one, and no beads or fragments found at site two. This could be an underestimation however, as uncertain MPs were disregarded (Fig. 6). For site five, none of the morphologies significantly differed from one another ($\chi^2 = 4.3$, $p = 0.116$).

3.3. Analysis of oyster microplastics

Upon initial visual inspection of the 5 µm cellulose filter disks under a light microscope using a magnification of × 63, total plastic load (PL) was greatest at site one compared to a minimum total PL recorded at site two (Appendix 3; Fig. 7). Fibres dominated the morphology of MPs found (Fig. 8), with a total of 340 fibres across all sites, accounting for 97 % of the MP load. All fragments found were blue, whereas the fibres were present in four distinct colours – black, red, blue, and white – with varying abundances (Fig. 9). Inconsistent with the sediment samples, black fibres dominated the plastic load in the oysters (60 %), followed by white fibres (18.5 %), blue fibres (17 %) and red fibres (4.5 %). This is in alignment with previous reports of fibre colours (Stead et al., 2020; Gallagher et al., 2016). The average MP load for *C. gigas* on Weston Shore was calculated as (median ± IQR) 3.4±2.5 MPs/g wet weight (gww). This is much lower than previous reports of oyster MP loads in Southampton Water (Zapata-Restrepo et al., 2025).

None of the five sites significantly differed from one another at the 95 % confidence level in terms of *C. gigas* PL ($\chi^2 = 4.87$, $p = 0.3$) (Fig. 7). However, within each site, fibres were present in significantly greater quantities than fragments (site one: $\chi^2 = 3.86$, $p = 0.05$; site two: $\chi^2 = 4.09$, $p = 0.04$; site three: $\chi^2 = 3.97$, p = 0.05; site four: $\chi^2 = 4.35$, p = 0.04; site five: $\chi^2 = 3.86$, p = 0.05). This was in alignment with the sediment MP morphologies, as well as other bivalve MP morphologies from multiple different regions (Li et al., 2015; Ding et al., 2021; Wootton et al., 2022). There were no significant differences between occurrence of colours within sites, apart from a greater quantity of black fibres compared to red at site one (Z = 2.72, p = 0.04), site four (Z = 2.76, $p = 0.03$) and site five (Z = 2.79, p = 0.03). Overall, black fibres were found in greater quantities than all other colours (Fig. 9) and this was found to be significant at the 95 % confidence level (black vs blue: Z = 3.36, $p = 0.005$; black vs red: Z = 5.62, $p = 1.1 \times 10^{-7}$; black vs white: Z = 3.12, p = 0.02). There were no statistical differences between any of the other colours (Fig. 9).

4. Discussion

MPs were present in all sediment and *C. gigas* samples with consistent abundances across sites, apart from a significantly greater number in the sediment at site one compared to site five.

4.1. Refinement of H_2O_2 digestion method

Methods of MP extraction from organism samples are highly varied, with examples including H_2O_2 digestion, KOH digestion, proteinase-K digestion, and trypsin digestion, among others (Thiele et al., 2019; Enders et al., 2020). The varieties of MP extraction have gone under review many times in order to determine the most accurate and cost-effective approaches (Debraj and Lavanya, 2023; Enders et al., 2020; Thiele et al., 2019). This, however, can make it difficult to find a harmonised method for each approach, as each paper is reviewing a different version. Furthermore, there rarely seems to be an explanation for why the chosen quantity is being used, further adding to the

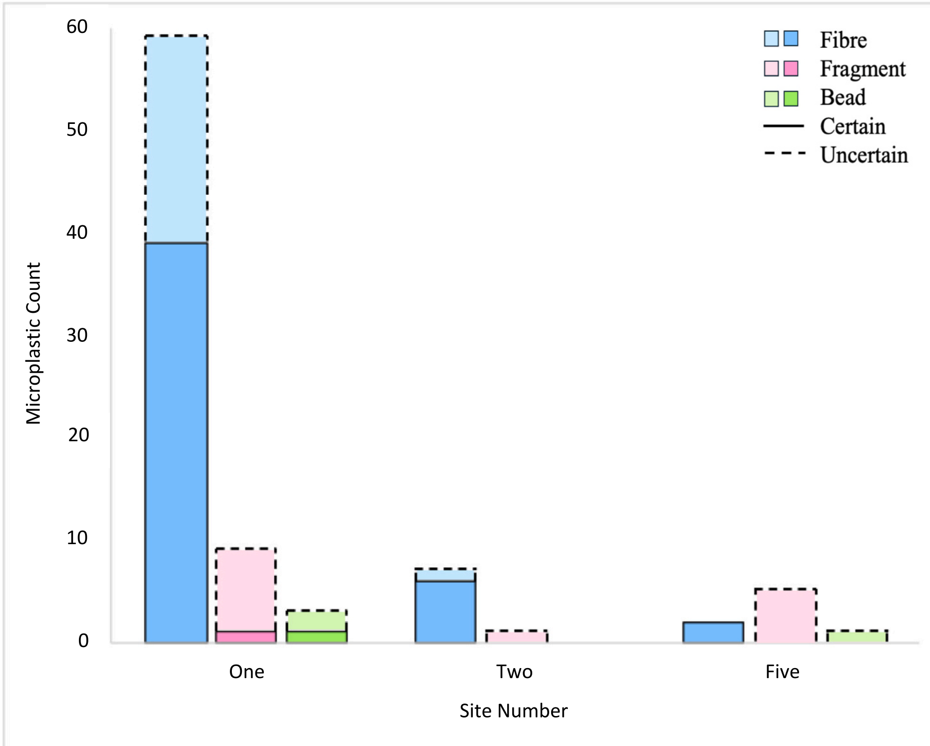

Fig. 6. Sediment microplastic loads and morphologies in 50 g samples (10 g subsamples) collected from Weston Shore, Southampton on 18/12/2024. Samples were collected from three sites along Weston Shore – site one, site two and site five. Certain microplastics are displayed by the solid lines and darker colours, uncertain microplastics are displayed by dashed lines and lighter colours. Uncertain counts are particles that would require further analysis to confirm whether they were plastic or not.

challenge of method selection.

Digestion using H_2O_2 was chosen due to the high digestion efficiency and minimal synthetic polymer damage associated with this reagent (Pfeiffer and Fischer, 2020; Tuuri et al., 2024). Whilst 20 ml of 30 % H_2O_2 was sufficient in digesting 1 g of tissue, the small volume of liquid was completely evaporated after 72 h, which was also the amount of time needed for the full digestion to occur. This suggests that a volume of 20 ml would be inappropriate to use for 1 g of tissue, but would be acceptable when scaled up with a larger amount of tissue. Therefore, for tissue samples $\leq$ 1 g, at least 40 ml of 30 % H_2O_2 would be needed, as this volume did not evaporate after 72 h of incubation; for samples of 2 g or more, 20 ml per gram would be sufficient for digestion. For the purpose of this study, 20 ml of 30 % H_2O_2 per 1 g of tissue was used as the benefits of reduced cost and eco-conscious practice outweighs the hindrance of an extra day of incubation compared to 80 ml of H_2O_2 per gram of tissue.

Thiele et al. (2019) suggested a volume of H_2O_2 6× that of the mass of tissue to be digested. Whilst this would indeed work to keep quantities of H_2O_2 minimal for large tissue samples, samples of 4 g or less would not follow this rule, as 20 ml would fully evaporate in the time needed for a complete digestion (Fig. 3). This could also be true of samples up to 8 g, however quantities of H_2O_2 between 20 ml and 40 ml were not tested, so cannot be reliably commented on. To reduce evaporation, samples could theoretically be incubated at lower temperatures – 30 °C for example – however, this is likely to increase incubation time and could consequently result in similar levels of evaporation anyway. Experimentation with different temperatures would be needed to determine whether it would be feasible.

Whilst using 20 ml of 30 % H_2O_2 per gram of tissue may work better than 6× the mass of the sample (Thiele et al., 2019) for small samples, it may not be an appropriate method for larger samples, as it would lead to unnecessarily large volumes of H_2O_2 being used. Therefore, it is suggested that for tissue samples of 5 g or less, 20 ml H_2O_2 per gram of tissue should be used; for samples >6 g, 6× the mass of the sample will suffice. The variation in reagent volumes per gram for different sample sizes displays non-linearity in reagent use. This can have implications for experiments scaling up, as less H_2O_2 is needed per gram as tissue samples get larger, making standardisation challenging.

Many studies are vague in reporting their methods, missing out detailed descriptions of quantities of reagents or sample masses. Li et al. (2015) were said to have used approximately 200 ml of H_2O_2 per sample, but did not report the sample mass. This lack of detail hinders the reproducibility and would lead to the overuse of H_2O_2. Had their methods been followed in this study, a total of 3 l of H_2O_2 would have been used, compared to the 1.4 l actually used. This cuts the purchase cost from ~£218.88 to ~£109.44 (from Vickers Laboratories, other manufacturers may vary); a 50 % cost reduction. Delivery and disposal costs are also calculated by volume, so would benefit from a reduced reagent quantity. Furthermore, by halving the volume of H_2O_2 used, other reagents used – NaCl solution in this case – can also be reduced. Not only does this emphasise the importance of clarifying what was done, but it also stresses the need to address the reasons behind why the chosen methods and quantities were used.

It is important that experimental methods use the smallest quantities of reagents possible in order to ensure the environmental impact of the methods are in line with the aims of the research – a study into environmental pollution, including MP pollution, should create as little impact on the environment as possible. This means considering the damage caused during production and disposal of harmful chemicals, such as H_2O_2. Whilst the disposal of diluted H_2O_2 may not have dire environmental consequences, as it decomposes into water and oxygen, the production of H_2O_2 is less eco-friendly. The manufacture of H_2O_2 is dominated by the anthraquinone (AQ) auto-oxidation (AO) method (Gao et al., 2020), which has numerous environmental implications. The

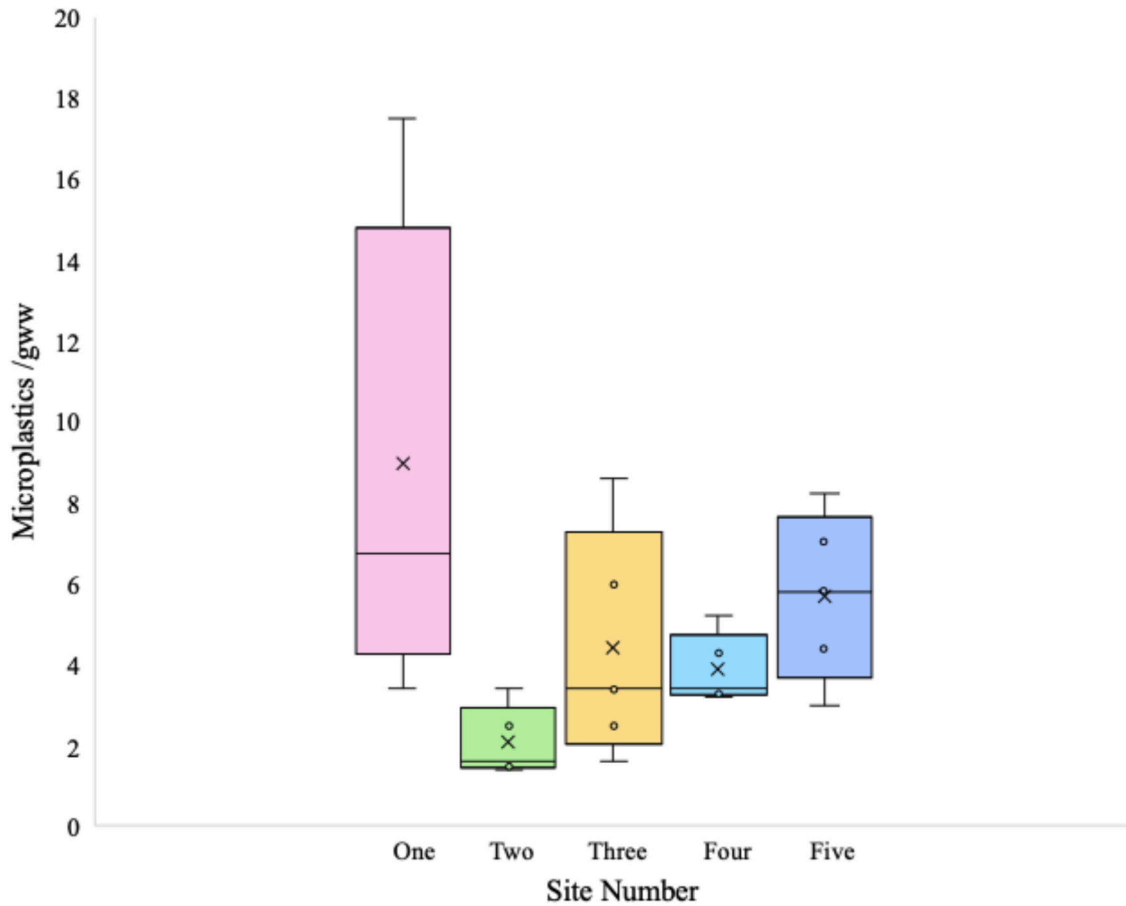

Fig. 7. Microplastic loads found in 15 *Crassostrea gigas* individuals across five sampling sites along Weston Shore, Southampton. Three oysters from each site were collected on 18/12/2024 between 0600 and 0800 GMT at low tide. Median values are depicted by the solid black line; mean values are depicted by the cross symbols.

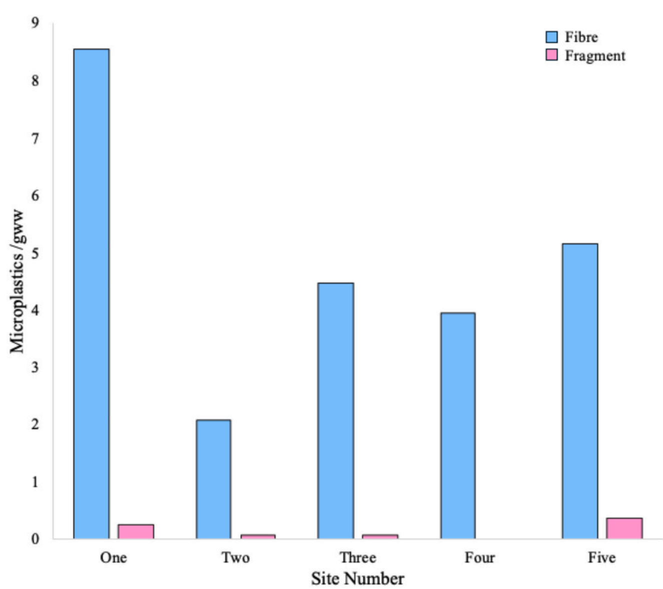

Fig. 8. Microplastic morphology in *Crassostrea gigas* samples taken from five sites along Weston Shore, Southampton. Three individuals from each site were collected on the 18/12/2024 between 0600 and 0800 at low tide.

AO method produces a significant amount of solid alumina (Al_2O_3) waste, which can accumulate in landfills, as well as exhaust waste containing aromatics, which are released into the atmosphere (Gao et al., 2020). Therefore, to conduct eco-conscious research, reagents must be used sparingly even if their disposal does not generate cause for concern. Furthermore, reducing the amount of reagent used to the smallest possible quantity creates more accessible methods that can be used by studies with a small budget.

We suggest refinements for already well-developed methods of tissue digestion using H_2O_2, with the aim of optimising reagent use for small tissue samples. The methods for tissue digestion and MP extraction should continue to be revised in order to optimise the approach; a necessary task as MP pollution continues to worsen and more research is needed to create effective management strategies.

4.2. Assessment of sediment microplastics

There does not appear to be a clear spatial trend in the concentration of MPs in the sediments of Weston Shore, with only site one and five significantly differing from one another ($p = 0.008$). The significantly greater abundance of MPs at site one compared to site five (Fig. 5) is likely to be attributable to the proximity of site one to sewage pipe C (Figs. 1 and 2), which was discharging wastewater effluent at the time of sampling. It is widely agreed that wastewater effluent is a source of MPs, although the extent to which effluent adds to environmental MP pollution is argued, with some studies estimating >90 % of MPs are removed within WTWs (Bayo et al., 2020; Carr et al., 2016; Mintenig et al., 2017; Talvitie et al., 2017), whereas others report much lower filtration efficiencies (Tadwusan and Babel, 2022).

Fibres were the dominant morphology of MPs found within the sediments of all sites (Fig. 6), although this was only significant at site one and two. This adds to the evidence of wastewater effluent as a

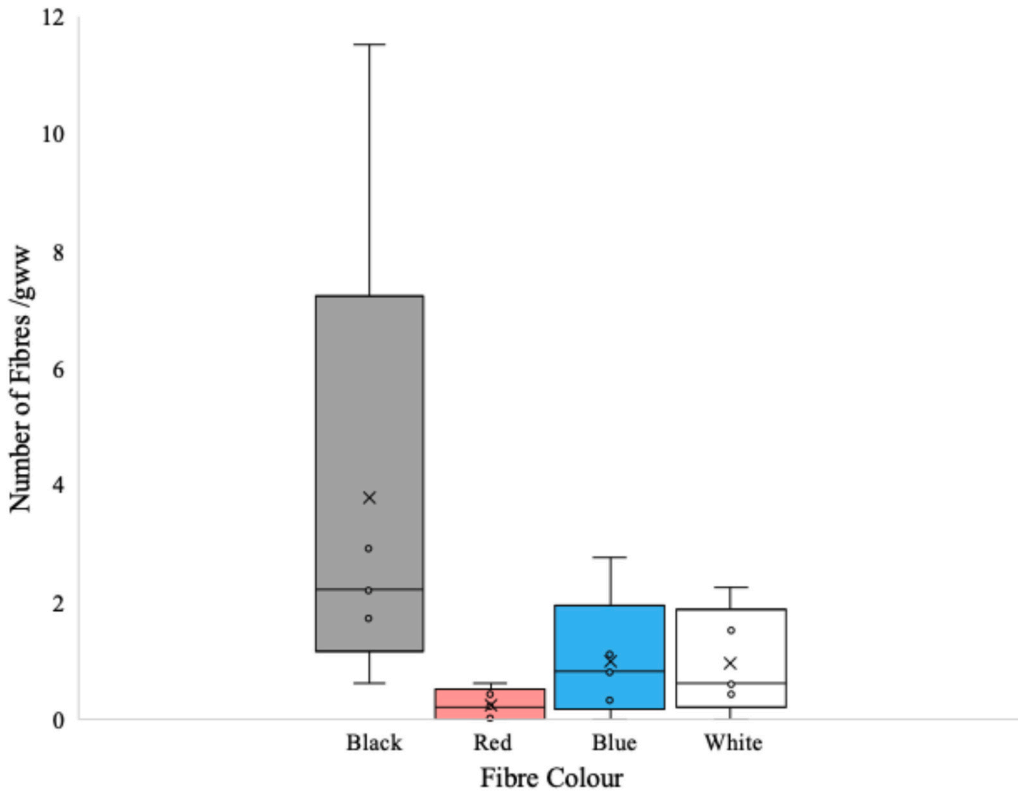

Fig. 9. Colours of fibres extracted from 15 *Crassostrea gigas* individuals collected from five sampling sites along Weston Shore, Southampton. Three oysters were collected from each site on the 18/12/2024 between 0600 and 0800 GMT during low tide. Median values are depicted by the solid black line; mean values are depicted by the cross symbols.

dominant MP source, due to the production of microfibres during laundry (Browne et al., 2011). It is likely that there are alternative sources of MPs, however, given the indifference in MP load between sites one and two (p = 0.062); this would be unlikely if wastewater effluent alone was the major contributor to MP pollution. Atmospheric deposition of MPs remains understudied but may actually be a significant source of microfibres into the marine environment (Napper et al., 2023). Wear and tear of synthetic fabrics that are not washed, such as flags, ropes, sails, etc. can be a major source of microfibres, especially in regions with considerable boating activities (Andrady, 2011), such as the Solent, Southampton Water and its tributaries. Moreover, the large population of Southampton, recorded as 264,957 in 2023 (Southampton City Council, 2025), adds to the likeliness of atmospheric deposition being a dominant source. Activities, such as driving (Brahney et al., 2021), and processes, such as wind abrasion at landfill sites (Hu et al., 2022), add to the atmospheric MP load and are intensified with larger populations. The similarity in MP loads between sites (Fig. 5) adds further evidence that atmospheric deposition may be a dominant source instead of point sources.

Dissimilar to the sediments, beads have been reported as the major MP morphology in the water column (Gallagher et al., 2016). The differences observed between the sediments and water may be due to changing sources. Since Gallagher et al's study in 2016, the UK government introduced a ban on microbeads in cosmetics products, which came into effect in January 2018 (GOV.UK, 2018). This will have limited the number of microbeads entering the marine environment through sewage effluent, accounting for the shift from bead dominated morphology to fibre dominated morphology. Assessment of the surface microlayer (SML) in late 2018 found fibres to be the dominant MP morphology (Stead et al., 2020), although the differences between these studies may be resultant from different sampling depths, instead of the microbead ban as polymer densities vary between materials (Duis and Coors, 2016), which controls their vertical distribution in the water column.

4.3. Assessment of oyster microplastics

MPs, especially fibres, were highly abundant within the oyster samples across all sites (Figs. 7 and 8). The consistency across sites indicates that MPs are likely to be abundant and well distributed within Southampton Water, or at least along the coast of Weston Shore. Much like the sediment plastic load, the greatest quantity of MPs were extracted from the oysters collected at site one. This, in combination with the sediment MP counts, provides further evidence that wastewater effluent is contributing to MP pollution. In both cases – sediment and oyster PLs – site one does not appear to be significantly different from other sites (apart from site five for sediment MPs). The spatial homogeneity suggests that there is a low MP concentration in the effluent in order to have such a small impact on the environment and organisms directly surrounding it. Alternatively, it may be that MPs are quickly dispersed throughout Southampton Water as a result of currents and tidal mixing. The ebb-dominant nature of the estuary combined with the intertidal mudflat structure of the benthos along Weston Shore may prevent suspended particles, such as MPs, from becoming trapped in this region (Quaresma et al., 2007), resulting in the spatial homogeneity observed within the sediment and oyster samples (Figs. 5 and 7). The rapid dispersal of MPs away from Weston Shore is further supported by the difference between average sediment MP loads (0.12, 0.02 and 0 MPs/g of sediment for sites one, two and five, respectively), compared to average *C. gigas* MP loads (3.4±2.5 MPs/gww). The much smaller sediment MP concentrations compared to *C. gigas* concentrations imply that MPs are not settling out of the water column.

Average MP load in *C. gigas* from Weston Shore was calculated as 3.4±2.5 MPs/gww, which is higher than *C. gigas* averages recorded in Europe, with a range of 0.11–0.47 MP/gww recorded (Bonello et al., 2018; Phuong et al., 2018), and worldwide, with a range of 0.02–1.48

MP/gww recorded (Abidli et al., 2019; Baechler et al., 2020; Cho et al., 2021; Covernton et al., 2019; Jang et al., 2020; Martinelli et al., 2020; Teng et al., 2019; Wootton et al., 2022). The greater-than-average MP load recorded in the present study is indicative of a highly polluted environment, which is common in urbanised regions (Jang et al., 2020). The European and worldwide studies were all conducted in less urbanised regions than Southampton Water, which can account for the lower average MP loads. Further differences between studies, such as extraction methods, may also account for the differences between averages. Efficacy of tissue digestion and MP recovery is variable between extraction methods (Carrillo-Barragan et al., 2022; Karami et al., 2017; Tuuri et al., 2024) and more variability can arise from filter sizes used and methods of analysis (visual vs spectroscopic).

When the average MP loads of other bivalve species are included, many studies around the world reported MP concentrations similar or greater than that of the present study (3.4±2.5 MPs/gww) (Bagheri et al., 2020; Birnstiel et al., 2019; Catarino et al., 2018; Digka et al., 2018; Khoironi et al., 2018; Renzi et al., 2018; Saha et al., 2021; Wang et al., 2019). However, looking at the south coast of England specifically, MP concentration in bivalve species tends to be lower than that of oysters in Southampton Water (Li et al., 2018; Scott et al., 2019), indicating spatial heterogeneity in MP pollution along the south coast of England.

A recent study into MPs in the European native oyster (*Ostrea edulis*) found an average of 54.04±16.96 MPs/gww for individuals collected from Weston Shore (Zapata-Restrepo et al., 2025). The major difference between Zapata-Restrepo et al's average and that of the current study (3.4±2.5 MPs/gww) may be attributed to species variability. *O. edulis* has been found to consume prey displaying a wider isotopic niche than *C. gigas* and, therefore, have a wider range of food sources (Ezgeta-Balić et al., 2020). Consequently, the presence of biofilms on MPs are more likely to create an isotopic signature that aligns with the isotopic niche of *O. edulis*, resulting in greater selection for MPs than *C. gigas*. Furthermore, the clearance rate of small particles (5–15 μm) is significantly lower in *O. edulis* than it is in *C. gigas* (Nielson et al., 2017). Thus, the greater MP load recorded in *O. edulis* (54.04±16.96 MPs/gww) (Zapata-Restrepo et al., 2025) compared to *C. gigas* (3.4±2.5 MPs/gww) may be explained by a combination of differences in particle selection and differing clearance rates.

The most abundant MP morphology found within the oysters was fibres (Fig. 8), which is in alignment with previous studies (Ding et al., 2021; Wootton et al., 2022; Zapata-Restrepo et al., 2025). The frequency of this observation suggests that oysters could be selecting for fibres over other morphologies of MPs due to preference of their shape, size or nutritional value (given the presence of a biofilm) (Cognie et al., 2001; Espinosa et al., 2016; Mladinich et al., 2022). Alternatively, studies have suggested that fibres are easily accumulated in bivalves due to the difficulty in removal compared to other MP morphologies (De Witte et al., 2014). Therefore, the abundance of microfibres in the environment in combination with their tendency to accumulate in bivalves (De Witte et al., 2014; Ding et al., 2021; Wootton et al., 2022; Zapata-Restrepo et al., 2025) may result in fibres posing a greater threat to marine ecosystems than other MP morphologies, due to their ability to contaminate marine life.

With multiple commercial fisheries occupying Southampton Water, the insights the present study provides for oyster MP consumption may have applications regarding commercial seafood production. Given the use of oysters as biomonitors for MP pollution, it can confidently be inferred that other bivalve and filter feeding species in Southampton Water are likely to contain MP particles; however, there are likely species variations in MP load, as seen between *O. edulis* (Zapata-Restrepo et al., 2025) and *C. gigas*. Many bivalve species, such as scallops, clams and cockles as well as finfish are harvested from Southampton Water and the Solent and are exported to continental Europe or sold in local markets. The Solent fishery trade boosts Southampton's local economy, valued at £13.85 million per year under current conditions, with the potential to increase to £14.31 million per year under improved water quality (Watson et al., 2020). This can be attributed to the negative health implications associated with the ingestion of MPs, including impaired filtration capacity, leading to less energy allocation for growth (Wegner et al., 2012), reduced fertility and reproduction (Sussarellu et al., 2016) and immune system implications (Détrée and Gallardo-Escárate, 2018). The economic benefit associated with the export of seafood from the Southampton and Solent area provides greater incentive to ensure that the water quality remains optimal.

The long-term human health implications associated with MP ingestion remain widely unknown. There is controversy surrounding the extent to which humans ingest MPs through the consumption of seafood; some studies suggest that the ingestion of household dust during a meal poses more of a threat than exposure through seafood (Catarino et al., 2018), whereas others suggest that seafood is a prominent exposure pathway (Van Cauwenberghe and Janssen, 2014). Whilst >90 % of ingested MPs are excreted (Wright and Kelly, 2017), persistent organic pollutants (POPs) and other toxins sorbed to MPs can leach from the particles and may remain in the body for longer (Hartmann et al., 2017). POPs are cause for concern due to their toxicity, which can result in hormone disruption, compromised reproductive health, and neurological and immunological disorders in humans and wildlife (Ashraf, 2017). Although there are many other pathways for MPs to enter the human body, including other food and drink sources (Kosuth et al., 2018; Kutralam-Muniasamy et al., 2020; Liebezeit and Liebezeit, 2015) and inhalation (Amato-Lourenço et al., 2021), reducing the MP load in water systems, and therefore seafood, would be beneficial for limiting exposure.

4.4. Comment on location

The sites at this location are close to Southampton city centre, 3 WTWs and a number of CSOs, and there is a double-high tide, all of which make the location rather unique. The WTWs handle industrial waste from Fawley Refinery and the area is popular for recreational boating and both commercial and industrial shipping vessels. In addition, it has recently become apparent that raw sewage wastewater – and therefore microplastics – have been entering into the river from apartment buildings situated just upstream of our sites for 35 years (Gilyeat and Ingham, 2025). Most studies on microplastics in *C. gigas* collected samples from relatively rural areas. For these reasons, and the general lack of testing in industrialised, city centre regions explains why Southampton Water is showing much higher MP loads compared to other studies.

5. Conclusions

This study aimed to optimise H_2O_2 digestion methods for the extraction of MPs from small tissue samples, as well as investigate MP pollution in sediment and *C. gigas* samples from along Southampton Water. For tissue samples ≤5 g, 20 ml H_2O_2 per g of tissue should be used for digestion. For tissue samples >6 g, 6× mass of the sample should be used for digestion. The need for greater volumes of H_2O_2 per gram of tissue for small samples compared to larger samples indicates non-linearity in the use of reagents for tissue digestion. This demonstrates the need for future research to identify sample size-specific reagent volumes in order to optimise harmonised protocols. This new digestion method gives 50 % cost reduction and lessened environmental impacts.

Sediment MP load declines moving south east along Weston Shore (Southampton Water), with averages of 6, 1 and 0 MPs per 50 g of sediment for sites one, two and five, respectively. However, there are varying degrees of significance associated with this trend, with only site one and five significantly differing from one another ($p = 0.008$). MP load in *C. gigas* samples did not reflect the declining trend, with no significant differences in MP load recorded between sites; consequently, MP load was calculated as 3.4±2.5 MPs/gww for *C. gigas* individuals on

Weston Shore. This value is much smaller than calculated values for similar species in the region, which can be attributed to species variability and differences in efficacy of extraction methods. The dominance of fibres in both the sediment and *C. gigas* samples indicates the prominence of wastewater effluent and atmospheric deposition as dominant sources of MPs in Southampton Water. MPs have negative implications for organism, ecosystem and human health, so understanding the main sources of MPs into an ecosystem is vital for creating management strategies to address the problem.

The sample sizes used in this study are small and all samples were collected on one day, giving no information on temporal variations. Nevertheless, our findings are important for analytical method development and can be used to inform policy makers. There are still knowledge gaps that must be addressed to guide and support the development of management strategies. Future studies should aim to map the distribution of MPs along the estuary, as this has not been done for a decade, in combination with identifying the main polymers. This will assist with identifying the main sources of MPs. Furthermore, regular testing of MPs in the sediments, water column and marine life should be conducted to observe the effects of any implemented changes, especially in urbanised and industrialised areas.

CRediT authorship contribution statement

H. Brown: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **I.D. Williams:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

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

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Data availability

Data will be made available on request.

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