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Novel Methods of Microplastic Identification and Mitigation in Water

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Novel Methods of Microplastic Identification and Mitigation in Water

MRes Biological Sciences

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1. Acknowledgments

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2. Abstract

Microplastics present an encroaching danger to the health of global and regional ecosystems, including inland freshwater habitats, the organisms that dwell within them, and potentially human health. As such, the need for identification, monitoring, and mitigation of microplastic pollution in aquatic environments has become ever more pressing.

With an increasing need to test water samples for microplastic pollution, previously utilised methods prove slow and labour-intensive. This thesis aims to develop a quicker, efficient methodology of filtering water samples for microplastics, utilising the natural fluorescent properties of plastics to allow for a more accurate analysis. Using a mechanical pump glass filtration system, water samples from a selection of sites across the length of Great Britain and In North Wales (and even Corkscrew Swamp in Florida, USA) were filtered through glass fibre filter papers, dried, and examined via dissection microscopy, using the microscope's own lighting system, and a separate fluorescence lamp (excitation 440 to 460 nm). A significantly (p <0.05) greater quantity of microplastics L^{-1} were found utilising fluorescence than using the microscope's light at 7/ 14 sites: Afon Cegin, Chester Reed Bed, Corkscrew Swamp, Lake Windemere, Llyn Cefni, River Black Water, River Thames and Ullswater. A lack of significant difference (p >0.05) was most likely the result of generally low populations of microplastic pollution (e.g., Loch Lomond) or due to issues with tests for significance (e.g., River Irwell, River Tame). The success of this methodology allows for its inclusion alongside other standard water-monitoring processes and infers microplastic levels could be higher than previously anticipated, as previous methods of environmental microplastic identification rarely use fluorescence. However, it is noted that visual inspection should not be utilised as a replacement for chemospectroscopy methods of identification.

There is little to no constant monitoring of microplastic levels across the UK coasts, and no previous studies upon the presence of microplastics around the isle of Anglesey, North Wales. Five sites around the Anglesey coast were selected for a pilot study, at three corners of the island (Porth Dafarch, Penmon Point, Cemaes Bay), and two from opposite sides of the Menai Strait (Bangor and Menai Bridge). A significant difference (p <0.05) was found between each of the surveyed sites and control samples of distilled water, verifying the presence of microplastic pollution along the Anglesey coast. A significantly greater average number of microplastics $^{L-1}$ were found on the Bangor side (7.958 ± 0.52) of the Menai Strait than the Menai Bridge side (2 ± 0.87). The more-populated tourist beach of Cemaes Bay had a higher average number of microplastics L^{-1} (1.7 ± 0.29) than the less-populated Porth Dafarch (1.1 ± 0.13), but less than the less-populated Penmon Point (1.95 ± 0.27). Causation was

speculated to result from human population, distance from shoreline, distance from human habitation, weather, and tidal current speed. The presence of nanoplastics within the Bangor samples was confirmed with high-end fluorescence microscopy. Significantly more fragment-type microplastics than other types were found at every site barring Cemaes Bay. These results demonstrate the need for further sampling for microplastics across not only Anglesey, but across the UK and worldwide.

Efforts must be taken to investigate methods to reduce the proliferance of microplastics in the natural environment as potential mitigation strategies to hamper the consequences of MP pollution in a world where single use plastics are pandemic. One such method could be the utilisation of CTWs (Constructed Treatment Wetlands); a cheap, effective, and durable system already successfully being utilised to treat wastewater from industry, and commercial and residential areas. Previous studies have already shown promise, with high microplastic retention rates of CTWs being observed. This study aims to use small-scale CTWs to further bolster these results, prove that treatment works on a smaller scale, and distinguish the effectiveness between wetland habitats and their substrata at retaining microplastics. Four small wetland microcosms had two to four litres of custom microplastic-polluted water added to them daily (with ~500 microplastic particles per litre, initially starting with four litres/ ~2000 microplastics), and two litres of sample water were taken daily, which were then filtered and examined to determine the loss of microplastics. Control microcosms consisting of four empty microcosms and two microcosms consisting of the wetland pebble substrate underwent the same procedure. Significantly more microplastics (p <0.05) were found to be retained by the wetland treatments over the 15-day sampling period than the control and pebbles treatments, with the pebbles treatment having a higher retention rate than the plain water treatment. Though there was a significant difference (p <0.05) found between the wetland and water treatments on each day, no significant difference was found between the water and pebbles treatments on any day, and on many days (1-4, 13-15) there was no significant difference between the wetland and pebble treatments. These findings give credence to CTWs being effective at retaining microplastics, with the wetland plants/ habitat as a whole being the driving force behind microplastic retention.

3. Literature Review for Luke Fears' MRes Thesis Submission

3a. The Extent of Plastics

Plastics remain one of the world's most consumed manufactured materials, with production increasing from 359 million tonnes in 2018 to 368 million tonnes in 2019 (Plastics Europe, 2020) at least 8 million tonnes of which enter the Earth's oceans, making up 80% of marine debris (IUCN, 2020). The production of single use plastics, plastic objects intended for a single usage before disposal, has doubled since the year 2000, with >40% of these plastics being disposed of in landfills rather than being recycled or renewed (Chen et al., 2021). The huge extent of plastic pollution has given rise to the term "plasticine era" being used by some to describe our current high-plastic use timeframe (Reed, 2015).

3b. Definitions

Microplastics are defined as plastic particles of microscopic size, and there is a lack of a clear consensus as to their actual size categorization (Hidalgo-Ruz et al., 2012) with examples including 5 mm or smaller (Thevenon, Carroll & Sousa, 2014), > 1.6 µm (Ng and Obbard, 2006), and < 1mm (Claessens et al., 2013), with macroplastics referring to plastic particles with sizes beyond these thresholds, e.g., > 5mm by Thevenon, Carroll & Sousa's (2014) definition. The definition of nanoplastics is currently under debate, with the two most-debated boundary sizes being 1000 nm and 100 nm (Gigault et al., 2018). MPs come under two broad categories, as defined by Boucher and Friot (2017); primary microplastics are released directly into the natural environment by being manufactured specifically to that size (e.g., microbeads in cleaning products and cosmetics) or by abrasion of macroplastics, such as car tyre wear and fibre release from fabrics during washing. Secondary microplastics are formed by the breakdown of macroplastics once exposed to the natural/ marine environment through weathering processes like photodegradation. There are a variety of ways in which to subcategorize microplastics, such as chemical composition, size (Zhang et al., 2017), colour (Dahms, van Rensburg & Greenfield, 2020), and most popularly, microplastic type, categorizing the microplastics by their shape and structure. Microplastic shape definitions can vary wildly between studies, for example Magnusson and Norén (2014) classifying MPs as fibre, fragment and flake ("very thin partivles"), and Zhu et al. (2019) utilising categorization of fragments, fibres, films, pellets and foams.

3c. The Issues of Microplastics

The extent of microplastic prevalence within the earth's ecosystems, particularly the oceans, is not accurately known, but some estimations have been made, with Eriksen et al. (2014) and Sebille et al. (2015) estimating that 93- 68 kilotons of MPs are currently polluting the Earth's oceans (Boucher and Friot, 2017). Due to its prevalence, microplastic pollution is proving to have, or have the potential to have significant ecological impacts. Microplastics enter the food web at a low trophic level (e.g., in phytoplankton (Cole et al., 2013)), and remain in the food web at higher trophic levels, having even been found to have been consumed by humans (Batel et al., 2014; Eriksen et al. 2014). Fibre microplastics from synthetic fabrics have great potential to enter the planet's ecosystems, as evident from De Falco et al. (2019) who found an average length of 360-660 µm and an average diameter of 12–16 µm of fibres from washed fabrics, which could easily pass through filtration systems at wastewater treatment facilities. Microplastic particles have also been identified embedded in the tissues and organs of organisms as a result of respiratory activity after feeding and consuming microplastics (Claessens et al., 2013). Microplastics can also act as vectors for harmful chemicals and pathogens, which may leach into the tissues of organisms (Cole et al., 2013; Ziccardi et al., 2016), potentially causing brain damage and behavioural disorders, as seen in fish with high-microplastic diets (Mattson et al., 2017). Furthermore, microplastics have been shown to cause issues with buoyancy when ingested by organisms, as well as interfering with photosynthesis (Kooi et al., 2016), and transmit persistent organic pollutants, which accumulate and leech into an organisms' tissues (Ziccardi et al, 2016), and have even been observed to have been consumed by humans (Eriksen et al., 2014). Despite these observations, there is still limited data on the complete cycle and effects of microplastics as environmental pollutants, so the extent to which they effect the Earth's ecosystems has yet to be determined in full (Van Sebille, 2015). As such, more consistent monitoring of all the Earth's water sources is required in order to better understand the proliferance of microplastic contamination within the Earth's ecosystems.

With the increasing prevalence of microplastics and the severity of the effects of said pollution becoming increasingly researched and evident, the need for proficient identification methods is ever more necessary, in order to help better establish current trends in microplastic pollution and consistently monitor said levels. Furthermore, mitigation and management strategies are required in order to manage the negative effects of microplastic pollution.

3d. Microplastic Sampling

There are three main aspects to the process of microplastic identification in water samples: sample collection, processing and analysis. In terms of sample capture, there are three main avenues of sampling methods. Selective sampling involves capturing microplastic particles directly from the environment (e.g., suspended in water), but this method is time-exhaustive and difficult to provide large quantities of samples due to MP distribution, and so is primarily used for obtaining particles from land (Hidalgo-Ruz et al., 2012). Bulk sampling involves the taking of samples directly from the environment to be processed after their capture, with one of the most common methods of bulk sampling is the storing of water samples in containers for later examination, for example Felismo, Helm and Rochman (2021) and Watkins et al. (2019) utilising 1L glass amber bottles. Finally, volume reduced sampling consists of the reduction of the sample size as the sample is being taken to increase the concentration of particles in said samples, for example be sieving sediment samples to a particular size category, and after separating microplastics from water (often by running a net through), adding these microplastics to water to create a new, volume-reduced sample (Hidalgo-Ruz et al., 2012).

3e. Sample Processing

As attempting to analyse water samples for MPs suspended in themselves is problematic and futile, filtration is the main method of processing water samples ready for analysis. All studies this researcher found during the course of my investigation utilised some variance of filtration. Often, water samples are pre-sieved through mesh (often stainless steel) of varying pore densities to separate out macro-particulates prior to further processing (e.g., Ta and Babel, 2020). Organic digestion involves the destruction of organic components from a sample by the addition of chemicals, such as hydrogen peroxide in wet peroxide oxidation (WPO) (Masura et al., 2015), potassium hydroxide (Luo et al., 2019), alkaline NAOH (Su et al., 2020), hydrochloric acid (Ma et al., 2020), and sulphuric acid (Dikerva and Simon, 2019). Density separation works by increasing the density of the water sample by the addition of compounds such as sodium chloride (Masura et al., 2015), NaI, zinc chloride, potassium fornate (Zhang et al., 2018), sodium tungstate dihydrate, sodium polytungstate, potassium formate, and oil (Stock et al., 2019), after which the solution is left and the microplastics float to the top of the solution and are then extracted. Vacuum pumping is a popular method of sample filtration, requiring a vacuum pump to suck the water sample through a filter, said filters varying in material and pore density, e.g., Whatman Grade GF/C glass fibre filters (Dunn et al., 2020) and nylon filters (Cai et al., 2018). One of the most influential pieces of literature upon the processing (i.e., filtering) of water

samples for microplastics (and helping standardize the microplastic size definition as being > 5mm) is the NOAA's protocol (Masura et al. 2015), providing a standardized methodology of bulk sample capture, various levels of filtration, WPO, density separation, and final filtration and gravimetric analysis (weighing) to prepare microplastics on filters ready for visual or chemoscopic analysis.

3f. Sample Analysis – Visual

There is a huge range of methodologies for analysing samples for microplastic particles. Visual analysis is one of the most common and allows the visual sorting of microplastics into specific groups of colours, shapes, and sizes., based on visual recognition. This method however can be victim to a large degree of human error, with microplastics not visible to the naked eye remaining uncounted, alongside those camouflaged against the backdrop of the filters, with a much higher error rate present for microscope counts (Browne et al., 2011; Schymanski et al., 2018; Weis, 2020). Microplastics can also be overestimated, with non-manufactured particles being confused for microplastics due to having similar shapes and sizes (though this becomes less likely with organic digestion and density separation methods performed beforehand). Ergo, additional techniques can be employed as a visual aid, including utilising filters with gridlines (Estahbanati and Fahrenfeld, 2016, and the hot needle test, wherein a hot needle is pressed against the particle in question to see if reacts to the heat as a plastic would (melting, curling, etc.); but this only works with particles large enough (Egessa et al., 2020; Irfan et al., 2020; Payton, Beckingham & Dustan (2020). Fluorescence microscopy is being utilised as an identification method increasingly more frequently, wherein a fluorescence adapter is added to a stereomicroscope or a specialised fluorescence microscope is utilised. Stains such as the lipophilic Nile Red (Plenderleith, Swift and Rimmer, 2014) can be used to better illuminate the microplastics by bonding to them and fluorescing under excitation (though unfortunately it does not bond to some compounds like black particles from car tyres, and also bonds to organic matter, which can lead to confusion in identification) (Araujo et al., 2018; Chrichton et al., 2017; Stanton et al., 2019). Another stain is the Rose-Bengal, which dyes organic matter (cytoplasm), helping to distinguish organic and inorganic matter from each other under fluorescence (Gbogbo et al., 2020). There have been previous attempts at utilising fluorescence microscopy sans-stain, such as by Payton (2017), utilising the natural fluorescent properties of the microplastics themselves. Electron microscopy is a method that has been used sparingly due to its expense and difficulty but has been proven successful in imaging smaller microplastics and nanoplastics, for example by Zarfi (2019.)

3g. Sample Analysis – Chemospectroscopy

A more accurate field of MP identification methodology is chemospectroscopy, utilising methods such as Raman spectroscopy and Fourier-Transform Infra-Red spectroscopy (FTIR). FTIR consists of three modes: reflectance (in which IR is reflected off of samples (Vianello et al., 2013)), transmission (IR is transmitted through translucent particles) and attenuated total reflectance (ATR), in which the sample makes direct contact with the ATR crystal, which allows for a quicker and more accurate analysis with fewer reflectance errors). Unfortunately, even ATR-FTIR takes a long time and has to rely on focussing on each individual particle (Ivleva, Wiesheu & Niessner, 2017), which can be circumnavigated with the use of focal plane array (FPA), featuring several detectors in a grid pattern, allowing for the detection of a wide array of spectra over a large area quickly, and forming a map image of spectra from the sample area (Levin and Bhargava, 2005). Unfortunately, environmental chemicals such as water can interfere with the IR, and so filters must be dried before analysis. Conversely, the other popular method of chemospectroscopy, Raman spectroscopy, is not affected by water, as it uses visible light instead of IR. Unfortunately, this method can only target and analyse a single particle at a time, though recent innovations in automatic analysis are rapidly increasing analysis speed. Thermography identification is utilised much less and involves methods such as Pyrolysis Gas Chromatography/ Mass Spectrometry (Py-GC/MS), which involves heating the sample into a gas (destroying it in the process) and conducting gas chromatography/ mass spectrometry upon it to determine its chemical makeup (Ivleva, Wiesheu & Niessner, 2017). Chen et al. (2020) compared FTIR to Raman, finding FTIR provides "identification of polar functional groups and could not detect MPs smaller than 10 μm. In contrast, Raman spectroscopy has a better resolution with down to a size of 1 μm and can identify substances with aromatic bonds which IR has weak intensity", encouraging the combine usage of both techniques.

3h. Reducing Microplastic Pollution Upstream

The main strategy in reducing microplastic pollution is the prevention of their creation in the first place (Eriksen et al., 2018; Prata et al., 2019). This can occur by means of reducing primary microplastics, for example by banning the inclusion of microbeads in cosmetic products, as done so by countries such as the USA in 2015 (FDA, 2020) and the UK in 2018 (Gove, 2018), and by reducing secondary microplastics by reducing the overall number of plastic items being consumed by the world's population (e.g., Nestlé switching all their Smarties packaging to paper (Ridler, 2021) and Chester, UK, banning plastic straws (Chester West and Chester, 2020). Prata et al. (2019) identified 10 factors for stakeholders to reduce plastic pollution: regulation of production and consumption; eco-design;

increasing the demand for recycled plastics; reducing the use of plastics; use of renewable energy for recycling; extended producer responsibility over waste; improvements in waste collection systems; prioritization of recycling; use of bio-based and biodegradable plastics; and improvement in recyclability of e-waste (e-waste meaning electronics and appliances). These factors reflect the findings of Eriksen et al. (2018), who determines that intervention at various points along the pathway of micro/macroplastics from creation to entry into the natural environment is the best solution to reducing plastic pollution, and responsibility falls to waste handlers, plastic producers, government, product/packaging manufacturers, and consumers (Ogunola, Onada & Falaye, 2018). A novel strategy may be the pyrolysis of sewage sludge (an important MP source), which removes significant quantities of microplastics (Ni et al., 2020). Filtration systems consisting of membranes at wastewater treatment plants (WWTPs) (which remove low quantities of microplastics by themselves, with 7.2 billion MPs day^{-1} entering rivers from WWTPs (Liu et al., 2021)) already in use have proven effective in removing up to 99% of MPs from influent. Other tested methods of removing microplastics from wastewater before they reach the environment include electrocoagulation and photo catalytic degradation (Patil et al. 2021). The components and formation of WWTPs and sewage farms can also be altered to increase efficiency at trapping microplastics within (Padervand et al., 2020).

3i. Reducing Microplastic Pollution Downstream

Microplastic pollution mitigation strategies to remove MPs already present in the natural environment are regularly attempted, such as beach cleans; community-based procedures in which plastics (and general litter) is removed from coastlines, quite often by volunteers, and has been proven effective at preventing plastic waste left on beaches from entering the water column. Organisation of these beach cleans, and similar litter-cleanse projects can be conducted on a local, national, and even international scale. Unfortunately, this method requires a great deal of frequent and intensive work and is not effective at preventing plastics reaching the ocean via air dispersal or in less-populated areas not cleaned and does not account for MPs already present in the water column and requires the interest of participants to succeed. Ogunola, Onada & Falaye, (2018) determined that for a significant effect to be had, beach cleans should occur at 2-year intervals, where the average residence time of the waste is greater than time taken to produce the same amount. Ogunola, Onada & Falaye (2018) also commented on the great potential of biotechnology in reducing plastic waste, either by the implementation of ‘bio-plastics’; materials made from organic matter which will decompose naturally past their use-by date, or by the usage of microorganisms themselves to degrade, or by the usage of their decompositional and degrading compounds such as enzymes, lipases, esterases, cutinases,

peroxidases, hydrolases, hydroxylases, and oxido-reductases, but further research is required to implement this technology beyond a laboratory setting and to develop methodologies for these processes to be implemented on a mass scale cost-effectively (Ogunola, Onada & Falaye, 2018). Organic compounds such as organosilanes have been shown to accumulate microplastics exposed to them (Sturm, Horn & Schuhen, 2021). Other methods of removing microplastics from the water course include their direct removal from the littoral zone via contraptions designed to specifically remove microplastics. Macroorganisms also have the potential to help remove microplastic pollution, for example mushroom coral (*Danafungia scruposa*) which has been proven to remove MPs from the water by passive (adhesion toto body surface) and active (ingestion) mechanisms (Corona et al., 2020), as has green algae by passive mechanisms (Padervand et al., 2020). More mechanical methods include the creation of an artificial 'coastline' at the water's surface in which MPs can be trapped (The Ocean Cleanup, 2021). Despite these advances and technologies, the upstream capture of microplastics prior to exposure to the world's oceans is much more preferable, and even more preferable to that is the cessation/ reduction of the creation of primary microplastics and macroplastics which degrade into secondary microplastics (Eriksen et al., 2018; Ogunola, Onada & Falaye, 2018; Verschoor et al., 2016).

3j. Knowledge Gaps, Aims and Hypotheses

Despite the advantages chemospectroscopy and other chemical analysis methods present over visual analysis, the lengthy time it takes to utilise certain methods (e.g., Raman spectroscopy) and the prohibitive expensiveness of the equipment (Hidalgo-Ruz et al., 2012) deems chemospectroscopy unsuitable for mass-scale usage in identifying microplastics. As such, beyond development of chemospectroscopy methods and technologies to ensure greater efficiency and affordability, the other sector of MP identification to be developed (beyond novel methods) would be the visual analysis school of MP ID. Although far less accurate than chemospectroscopy (Browne et al., 2011; Schymanski et al., 2018; Weis, 2020), the utilisation of fluorescence excitation has been proven to be able to help identify microplastics previously undistinguishable through the usage of stains, which have their own drawbacks of requiring extra steps and preparation prior to visual inspection compared to non-fluorescence microscopy, and potential issues from stains staining non-MPs leading to false-positives (Araujo et al., 2018; Chrichton et al., 2017; Stanton et al., 2019), and although attempts at using fluorescence microscopy sans-stain have been attempted (e.g., Payton (2017)), there has been little comparison between fluorescent and non-fluorescent visual ID methods sans-stain. As such, this researcher aims to distinguish between the effectiveness of fluorescence vs non-fluorescence microscopy in identifying MPs in water samples and hypothesises that more will be found via the

former ID method. If this is proven to be accurate, the relative inexpense of fluorescence adapters for stereomicroscopes would allow fluorescence microscopy to be adopted as a more standardised methodology in MP ID, allowing for a more accurate level of analysis compared to non-fluorescence microscopy at a greater number of sample sites (though obviously not so much as chemospectroscopy methods).

Beyond policy control and the actions of stakeholders to discourage plastic usage, provide plastic alternatives, increase recycling and reuse effectiveness, and reduce plastic product creation (Prata et al., 2019), the microplastics already present in natural environments or found in wastewater on its way to said natural environments needs to be stemmed. One of the most promising aspects include the usage of WWTPs, which naturally trap some levels of microplastics within themselves (Lieu et al., 2021); WWTPs can consist of environments which replicate natural habitats, such as Constructed Treatment Wetlands (CTWs) (Kadlec and Wallace, 2008). There have been some previous studies which have shown CTWs as retaining microplastics from (waste)water influxes (e.g., Coalition Clean Baltic (2017), Townsend et al. (2019), Ziajahromi et al. (2020)) and so the usage of CTWs could provide effective for treating wastewater at plants, or in general alongside water courses potentially infected with MP pollution. However, said studies have been utilised in large scales, and are not easily replicable in a smaller, more intimate and budget-restricted research setting. Furthermore, whether it is the wetland units as a whole, or their sediment alone which is responsible for trapping microplastics is not well known, and so a distinction of the effectiveness of these two factors is required to determine if more effort should be put into utilising CTWs as a whole, or if just using their substrata is necessary. This researcher aims to recreate a generalised CTW microplastic-retention study but on a much smaller scale for implementation in more intimate lab-settings, utilising wetland, substrata and control treatments to determine if it is the wetland unit as a whole responsible for MP retention and hypothesises that significantly more MPs will be emitted by the effluent of control and substrata-based treatments than replication CTWs.

3k. Thesis Plan

In this thesis, this researcher aims to conduct an investigation comparing the usage of visual identification of microplastics, stereomicroscopy, by using its normal light exposure settings, and under fluorescence excitation, for a number of various sites, and hypothesises that significantly more microplastics will be found under fluorescence than the standard visual identification method. After this study is completed, provided the null hypothesis is rejected, this researcher will conduct another study utilising the fluorescence microscopy methodology to determine the microplastic pollution

levels of a nearby extended area to determine if there are significant quantities of microplastics in said area (and further prove the efficacy of this method), hypothesising that a significant quantity of microplastics will be found in samples. Finally, this researcher wishes to tackle the issue of removing microplastics from natural water sources and wastewater by devising a miniature set of CTWs and analysing microplastic output with a constant input, with three treatments consisting of wetland microcosms, microcosms formed of the wetland substrate minus the biota, and a control treatment. It is hypothesised that there will be a significant difference in microplastic output between the treatments, with the control and substrate treatments emitting significantly more microplastics than the wetland treatments. Should the null hypotheses be rejected, it would confirm that these smaller microcosms are indeed a viable option for lab-based experimentation, potentially opening up an avenue for various variances in the construction of said microcosms, and will also prove that it is wetlands as a whole, and not just the substrata which is responsible for microplastic retention, and thus potential future CTWs constructed for the purpose of eliminating microplastics will need to go all in on their wetland components.

3l. References

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4. Utilising Fluorescence Microscopy to Identify Microplastics in Water

4a. Abstract

Microplastics present an encroaching danger to the health of global and regional ecosystems, including inland freshwater habitats, the organisms that dwell within them, and potentially human health. As such, the need for identification, monitoring, and mitigation of microplastic pollution in aquatic environments has become ever more pressing. With an increasing need to test water samples for microplastic pollution, previously utilised methods prove slow and labour-intensive. This thesis aims to develop a quicker, efficient methodology of filtering water samples for microplastics, utilising the natural fluorescent properties of plastics to allow for a more accurate analysis. Using a mechanical pump glass filtration system, water samples from a selection of sites across the length of Great Britain and In North Wales (and even Corkscrew Swamp in Florida, USA) were filtered through glass fibre filter papers, dried, and examined via dissection microscopy, using the microscope's own lighting system, and a separate fluorescence lamp (excitation 440 to 460 nm). A significantly (p <0.05) greater quantity of microplastics L^{-1} were found utilising fluorescence than using the microscope's light at 7/ 14 sites: Afon Cegin, Chester Reed Bed, Corkscrew Swamp, Lake Windemere, Llyn Cefni, River Black Water, River Thames and Ullswater. A lack of significant difference (p >0.05) was most likely the result of generally low populations of microplastic pollution (e.g., Loch Lomond) or due to issues with tests for significance (e.g., River Irwell, River Tame). The success of this methodology allows for its inclusion alongside other standard water-monitoring processes and infers microplastic levels could be higher than previously anticipated, as previous methods of environmental microplastic identification rarely use fluorescence. However, it is noted that visual inspection should not be utilised as a replacement for chemospectroscopy methods of identification.

4b. Introduction

The most common method of identifying microplastics in water samples utilises filtering of the sample, followed by visual inspection of the filter with a microscope (as shown by the NOAA protocol by Masura et al. (2015), a standardised method for the analysis of water, beach, and bed samples, created by the US Department of Commerce's National Oceanic and Atmospheric Administration), has been utilised in over 200 research papers (e.g. Masonet al., 2016; Estahbanati and Fahrenfeld, 2016).).

The water samples are filtered by being poured through metal mesh of increasing pore density, dried, undergo Wet Peroxide Oxidation to remove organic components and density separation to separate the microplastics from the rest of the sample, which are then sequestered onto a custom mesh filter, before being examined by dissection microscopy. This method eliminates the sediment which would otherwise obstruct views of microplastics. However, it is a multi-step process which takes several days to process a sample with a single piece of kit, and multiple microplastic fragments may be overlooked during inspection as a result of blending in with the filter and the rest of the sample. As plastic particles are known to fluoresce when exposed to various wavelengths of light (Langhals, Zgela & Schluker, 2014), the usage of a fluorescence lamp to microscope inspection should allow for easier and more accurate detection of microplastics. Furthermore, a standard electrical pump-operated glassware filtration system (pictured in Figure 2) would take mere minutes to filter an entire sample (dependnat on levels of sediment within), and microplastics amongst any sediment caught by filters should likely be visible under fluorescence due to the fluorescing of the microplastics allowing them to stand out.

This study aims to determine if using fluorescence in tandem with dissection microscopy will help identify microplastics more clearly, with greater efficiency and ease. It is hypothesised that there will be a larger quantity of microplastic particles per litre identified using fluorescence microscopy than microscopy on its own, with a higher number of fragment-type microplastic particles observed than other types.

This study was conducted by me and fellow researcher Jedd Owens, who together captured certain samples, and filtered and analysed all of them. The data gathered from this study was utilised by Dunn et al. (2020) (Christian Dunn being our project supervisor), to release a publication outlining the method and general aspects and implications therein. As such, this paper will be similar to Dunn et al. (20120) but provide a more in-depth explanation and analysis of the methods, with this researcher's own take on the collected data. The results and conclusions in this study will be similar, if not possibly identical to those in Jedd Owen's paper, as he will be using the same data and methods of statistical analysis, and likely drawing similar conclusions as a result.

4c. Methodology

Sample Collection:

Grab samples were collected from water sources utilising 1L amber bottles, with at least three litres collected from each site. Samples were collected from the maximum depth possible to reach at the site, at a minimum of ~60 cm. Amber bottles were rinsed with water from sample sites before samples

were taken to help eliminate any microplastics already present in the bottles. To take samples, bottles were held underwater vertically, allowing all the air to escape, before the bottle cap screwed on underwater to prevent loss of sample and also rinse the cap in the process to prevent air contamination. Sample sites were: Dickie's Boatyard (Afon Cegin), Llyn Cefni, River Tame, River Blackwater, River Irwell, Loch Lamond, Falls of Dochart, Cors Goch, River Thames, Chester Reed Bed, Llyn Padrig, Ullswater, Lake Windemere, and Corkscrew Swamp (Florida, USA). Samples were collected from June 2018 to March 2019. Geographical locations of sample sites can be found on Table 1, and a map of locations can be found as Figure 1. Dickie's Boatyard was chosen due to its close proximity to the university and ease of access for initial trials of this method. Cors Goch was chosen due to the researchers regularly volunteering at the reserve. The number of samples gathered was dependent upon the logistical feasibility of obtaining and transporting said samples, though we tried to take as many as we could for greater validity of data. The rest of the samples were provided by fellow researchers and stakeholders interested in this field of research and wishing to know the levels of microplastics present in the samples they provided. As the provision of samples was on a voluntary basis and dependant on the factors affecting said sample provider's abilities to provide said samples, the number of samples provided to us varied between sites. Individuals providing myself, Dunn and Owens with samples were Nunnerley, L., Kirby, J., Armstrong, OL., Thomas, PJ., Aberg, D., Gilder, W., Green, D., Antwis, RE., and Freeman, C. The number of samples from each site can be seen in Table 16.

Figure 1. Map of UK locations from where samples were taken.

Sample Filtration and Identification of Number of Microplastic Types:

Masura et al.'s (2015) methodology was used as the basis for the filtration. Water samples were filtered through Whatman GF/C-grade (1.2 µm pore density) glass-fibre filters via a glass vacuum electrical pump filtering system, utilising a MilliporeSigma™ Glass Vacuum Filter Holder and Büchner flask. Sample bottles and the sample collection glass were rinsed with distilled waterto remove any lingering potential MPs and sediment. Filters were placed into glass petri dishes and dried in an oven at 60°C overnight. Four control samples per treatment site utilising distilled water were also filtered.

Dried filter samples were analysed for microplastics by visual confirmation using a dissection microscope (magnification 10-40), both without fluorescence (utilising the microscope's own lamp),

and with fluorescence only, utilising a NIGHTSEA Stereo Microscope Fluorescence Adapter, excitation 440-460 nm (Royal Blue), emission 500 nm. The number of microplastics of each type was counted four times. Free et al. (2014) was used as a basis for categorizing microplastics into five types (fragment, fibre, film pellet, and foam), with additional guidance from Dunn (2018) on distinguishing microplastics from non-synthetic polymers. Confirmation bias was avoided by double-blind testing; one person counted the number of microplastic particles in each sample without being let know which sample they were analysing, until at least 2 counts by each individual for each sample were conducted. To confirm if a disputed particle was a microplastic, a hot needle test (De Witte et al., 2014)) was conducted to determine if the particle melted/ curled when heat was administered. Control samples to eliminate the presence of contaminating microplastics were created and analysed utilising the aforementioned method, replacing the sample water with pure distilled water.

Each litre sample of water varied considerably with how long it took to filter. Higher levels of sediment or solute particles infused with the water led to much longer filtering times, with cleaner samples (e.g., Loch Lomond) taking less than a minute each, while dirtier samples (such as River Tame or River Thames) taking up to six hours to filter one litre. This was thought to be due to the sediment settling onto the filter, preventing the continued suction of water by the pump. As such, dirtier samples were often split between multiple filters, and a method of pouring was utilised where the sample water would be poured slowly and carefully into the filtration equipment so as to leave as much of the sediment in the bottom of the bottle as possible, before it was all washed into the final filtration. Separate filters from the same litre samples had their counts combined. To count the number of each type of microplastic, each filter paper took anywhere from two minutes, to five minutes on filters with more sediment or microplastics, with three repetitions needed, for a total of at least eight to twenty minutes spent on each filter paper.

Contamination Prevention:

Latex gloves were always worn when handling samples, and clothing (including cotton lab coats) was cleaned with lint roller. All possible plastic materials (containers, petri dishes, etc.) were replaced with glass and/ or rubber counterparts to reduce fragment pollution by said containers. Samples always remained covered by petri dish lids, apart from when being analysed. Amber bottles were rinsed thoroughly with distilled water before and after usage. Petri dishes were sealed with parafilm between usages. Open glassware was covered with tin foil to help prevent contamination during filtration. The use of control samples also helped to mitigate the effects of environmental contamination; by counting the average number of microplastics found in 4 litre-samples of distilled water (the same

water used to wash down the sample water during filtration), and subtracting these averages from the average sample results, a normalised, more accurate analysis of microplastic presence in these samples was formed. Control populations can be seen in Table 16.

Statistical Analysis:

Averages for each type of microplastic per individual sample, and per sample site were calculated, as were the averages of the control samples for each site. Averages for each type of microplastic from controls was subtracted from the averages from the sample sites to help eliminate the effect of contamination.

Normality of data sets was determined using Kolmogorov-Smirnov and Shapiro-Wilkes tests, and homogeneity using Levene's equality of variance tests. All data was found to be non-parametrically distributed, so independent samples Mann-Whitney U tests were conducted to determine significance of data. To determine significant differences between quantities L^{-1} of different types of microplastics found, individual Independent Samples Kruskal-Wallis Pairwise Comparison tests were conducted. Software used was IBM SPSS Statistics 25, Microsoft Word, Microsoft Excel, and Google Maps.

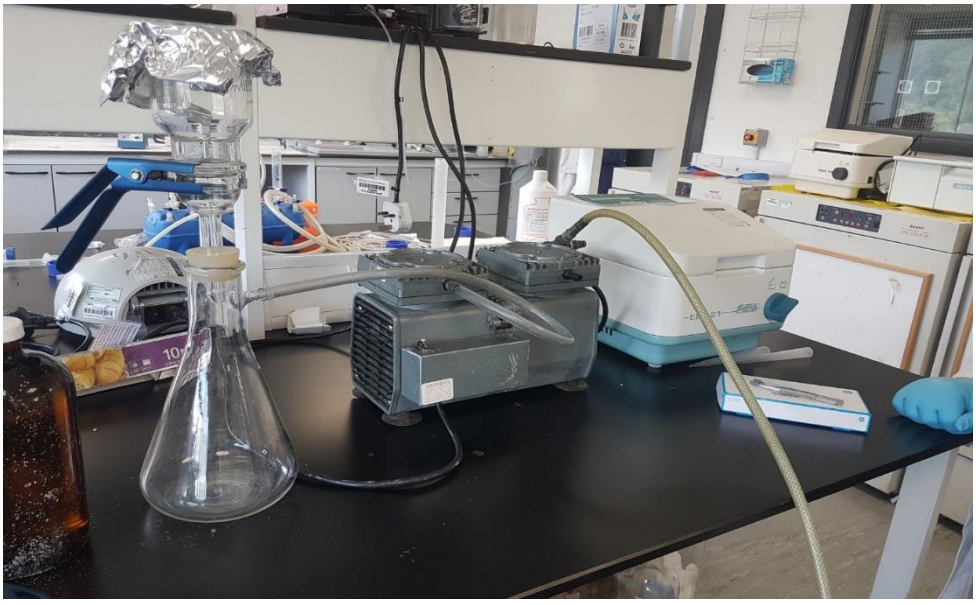


Figure 2. Filtration equipment setup, including electrical pump, MilliporeSigma™ Glass Vacuum Filter Holder and Büchner flask, rubber tubes, vice, tin foil cover, and 1L amber sample bottle.

4d. Results

A significant difference in average number of microplastics L^{-1} was found between fluorescent and non-fluorescent microscope analysis for the following sites: Afon Cegin (Fluorescence *Mdn* = 5.667, Non-Fluorescence *Mdn* = 1, *U* = 305, *z* = -2.192, *r* = -0.283, *p* = 0.028), Chester Reed Bed (Fluorescence *Mdn* = 1.063, Non-Fluorescence *Mdn* = 0, *U* = 88, *z* = -3.277, *r* = -0.518, *p* = 0.002), Corkscrew Swamp (Fluorescence *Mdn* = 0.15, Non-Fluorescence *Mdn* = 0, *U* = 184, *z* = -3.017, *r* = -0.427, *p* = 0.003), Lake Windermere (Fluorescence *Mdn* = 3.500, Non-Fluorescence *Mdn* = 0.091, *U* = 43.5, *z* = -6.085, *r* = -0.786, *p* <0.001), Llyn Cefni (Fluorescence *Mdn* = 7.625, Non-Fluorescence *Mdn* = 0, *U* = 10, *z* = -5.281, *r* =-0.835, *p* <0.001), River Blackwater (Fluorescence *Mdn* = 1.438, Non-Fluorescence *Mdn* = 0, *U* = 140, *z* = -1.755, *r* = -0.277, *p* <0.001), River Thames (Fluorescence *Mdn* = 1.688, Non-Fluorescence *Mdn* = 0.188, *U* = 116.5, *z* = -2.324, *r* = -0.367, *p* = 0.023), Ullswater (Fluorescence *Mdn* = 4.833, Non-Fluorescence *Mdn* = 0, *U* = 6.5, *z* = -6.687, *r* = -0.863, *p* <0.001).

No significant difference was for the sites: River Irwell (Fluorescence/ Non-Fluorescence *Mdn* = 0, *U* = 3043.00, *z* = -0.676, *r* = -0.053, *p* = 0.499), Cors Goch (Fluorescence *Mdn* = 0.414, Non-Fluorescence *Mdn* = 0.267, *U* = 169, *z* = -0.852, *r* = -0.135, *p* = 0.414), Falls of Dochart (Fluorescence/ Non-Fluorescence *Mdn* = 0, *U* = 157.5, *z* = -1.418, *r* = -0.224, *p* = 0.253), Llyn Padrig (Fluorescence *Mdn* = 0.167, Non-Fluorescence *Mdn* = 0, *U* = 97, *z* = -1.809, *r* = -0.33, *p* = 0.539), Loch Lomond (Fluorescence/ Non-Fluorescence *Mdn* = 0, *U* = 177, *z* = -0.768, *r* = -0.121, *p* = 0.547), River Irwell (Fluorescence/ Non-Fluorescence *Mdn* = 0, *U* = 216, *z* = 0.520, *r* = 0.082, *p* = 0.678), River Tame – (Fluorescence/ Non-Fluorescence *Mdn* = 0 *U* = 200, *z* = 0, *r* = 0, *p* = 1.000).

Despite the massive disparity between the number of microplastics (namely fragments) observed in the River Tame samples between Fluorescence and Non-Fluorescence (Table 1, Figure 4), no significant difference was found. This could likely be due to size of the disparity between analytical methods leading to medians of 0, and the lack of 'groups' for the Mann-Whitney U test to analyse creating a *p* of 1.000 and 0.678. However, it is clear to see that there is a great disparity between the numbers of Fragment microplastics found from each investigation method, and as such it is assumed that p <0.05 and there is a significant difference. The same applies to River Irwell where despite a large observable disparity between fragment-type microplastics under fluorescence (average of 84.88 ± 17.01 microplastics L^{-1}) and non-fluorescence (average of 18.75 ± 3.18 microplastics L^{-1}), no significant difference was found (Table 2). As such, this researcher argues that either the samples were faulty, or the Mann Whitney-U tests were not powerful enough or unable to be correctly utilised to show a significant difference between fluorescence and non-fluorescence analysis, despite a much greater number of microplastic particles being observed via the former analytical method (Table 1, Figure 4).

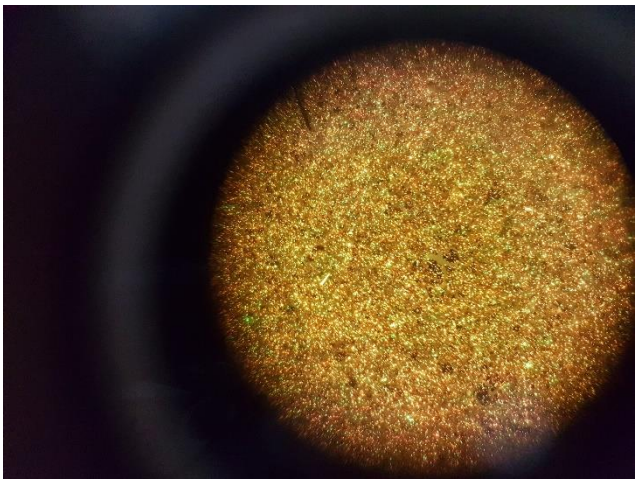


Figure 3. View of a River Tame sample showing an estimated >1000 microplastics fluorescing under fluorescent excitation.

Table 1. Average number of microplastics L^{-1} minus averages of control samples for each type of microplastic (Fibre, Fragment, Film Pellet, Foam) and totals for each sample site (with geographic location) with standard error.

Site	Location	Microplastic Type											
		Fibre		Fragment		Film		Pellet		Foam		Total	
		Fluorescence	Non-Fluorescence	Fluorescence	Non-Fluorescence	Fluorescence	Non-Fluorescence	Fluorescence	Non-Fluorescence	Fluorescence	Non-Fluorescence	Fluorescence	Non-Fluorescence
Afon Cegin	53°13'53.3"N 4°06'39.4"W	14.83 ± 5.19	11 ± 2.96	49.67 ± 8.7	22.33 ± 3.12	5.67 ± 3.04	1 ± 0.62	2.67 ± 1.02	0 ± 0	4 ± 3.12	0 ± 0	76.83 ± 16.33	34.33 ± 6.15
River Irwell	53°29'19.2"N 2°16'07.9"W	0 ± 0.32	0.44 ± 0.15	84.88 ± 17.01	18.75 ± 3.18	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	84.88 ± 17.26	19.19 ± 3.18
River Tame	53°27'44.6"N 2°06'03.9"W	0 ± 0	0.38 ± 0.26	>1000	20.38 + 4.79	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	>1000	20.75 ± 4.75
River Blackwater	51°43’34.9”N 0°45’23.7”E	3 ± 0.61	0.63 ± 1.32	10.69 ± 1.89	4.81 ± 1.13	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.44 ± 0.5588	0 ± 0	15.13 ± 2.14	5.44 ± 1.3
Loch Lomond	56°06'43.9"N 4°37'25.8"W	0.94 ± 0.3	0.06 ± 0.06	1.5 ± 0.31	1.44 ± 0.27	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	2.44 ± -0.51	1.5 ± 0.26
Falls of Dochart	56°27'45.2"N 4°19'13.2"W	1.06 ± 0.06	0.06 ± 0.29	2.19 ± 0.4	1 ± 0.33	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3.25 ± 0.63	1.06 ± 0.34
River Thames	51°30'30.7"N 0°06'37.0"W	6.4 ± 0.99	0.31 ± 0.2	74.38 ± 5.16	8.56 ± 1.39	1.06 ± 0.24	0 ± 0	0.06 ± 0.06	0 ± 0	1.69 ± 0.44	0.19 ± 0.1	84.13 ± 6.02	9.06 ± 1.35
Llyn Cefni	53°16'12.4"N 4°20'22.4"W	7.44 ± 1.1	0 ± 0	16.81 ± 2.34	2.88 ± 0.62	7.63 ± 1.54	0.06 ± 0.06	8.44 ± 1.46	0 ± 0	2.878 ±0.9	0 ± 0	43.19 ± 5.07	2.94 ± 0.65
Chester Reedbed	53°12'28.6"N 2°54'12.0"W	1.81 ± 0.33	0.31 ± 0.19	4.25 ± 0.99	0.75 ± 0.29	0.13 ± 0.08	0 ± 0	0.38 ± 0.3	0 ± 0	1.06 ± 0.43	0 ± 0	7.63 ± 1.51	1.06 ± 0.43
Ullswater	54°34'30.4"N 2°54'29.4"W	4.92 ± 0.65	0.21 ± 0.1	14.04 ± 1.31	1.25 + 0.31	3.25 ± 0.55	0 ± 0	4.83 ± 0.87	0 ± 0	2.46 ± 0.37	0 ± 0	29.5 ± 2.89	1.46 ± 0.35
Lake Windemere	54°22'37.5"N 2°56'14.5"W	3.58 ± 0.71	0.33 ± 0.24	14.67 ± 1.46	1.15 ± 0.31	2.21 ± 0.45	0.09 ± 0.08	0.7 ± 0.21	0 ± 0	3.5 ± 0.48	0 ± 0	24.65 ± 2.85	1.57 ± 0.47
Cors Goch	53°18'31.9"N 4°15'22.2"W	1.1 ± 0.32	0.44 ± 0.14	3.03 ± 0.56	0.47 ± 0.43	0.41 ± 0.16	0.14 ± 0.1	0 ± 0	0.13 ± 0	0.14 ± 0.09	0.27 ±0.13	4.68 ± 0.96	1.45 ± 0.67
Corkscrew Swamp	26°22'31.5"N 81°36'33.3"W	0 ± 0.18	0 + 0.05	2.75 ± 0.42	0.25 ± 0.1	0.1 ± 0.07	0 ± 0	0.15 ± 0.08	0 ± 0	0.3 ± 0.1	0 ± 0	3.3 ± 0.55	0.25 ± 0.10
Llyn Padrig	53°13'35.5"N 4°27'10.8"W	0.33 ± 0.18	0 ± 0	1.83 ± 0.42	0.08 ± 0.08	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.17 ± 0.1076	0 ± 0	2.33 ± 0.57	0.08 ± 0.08

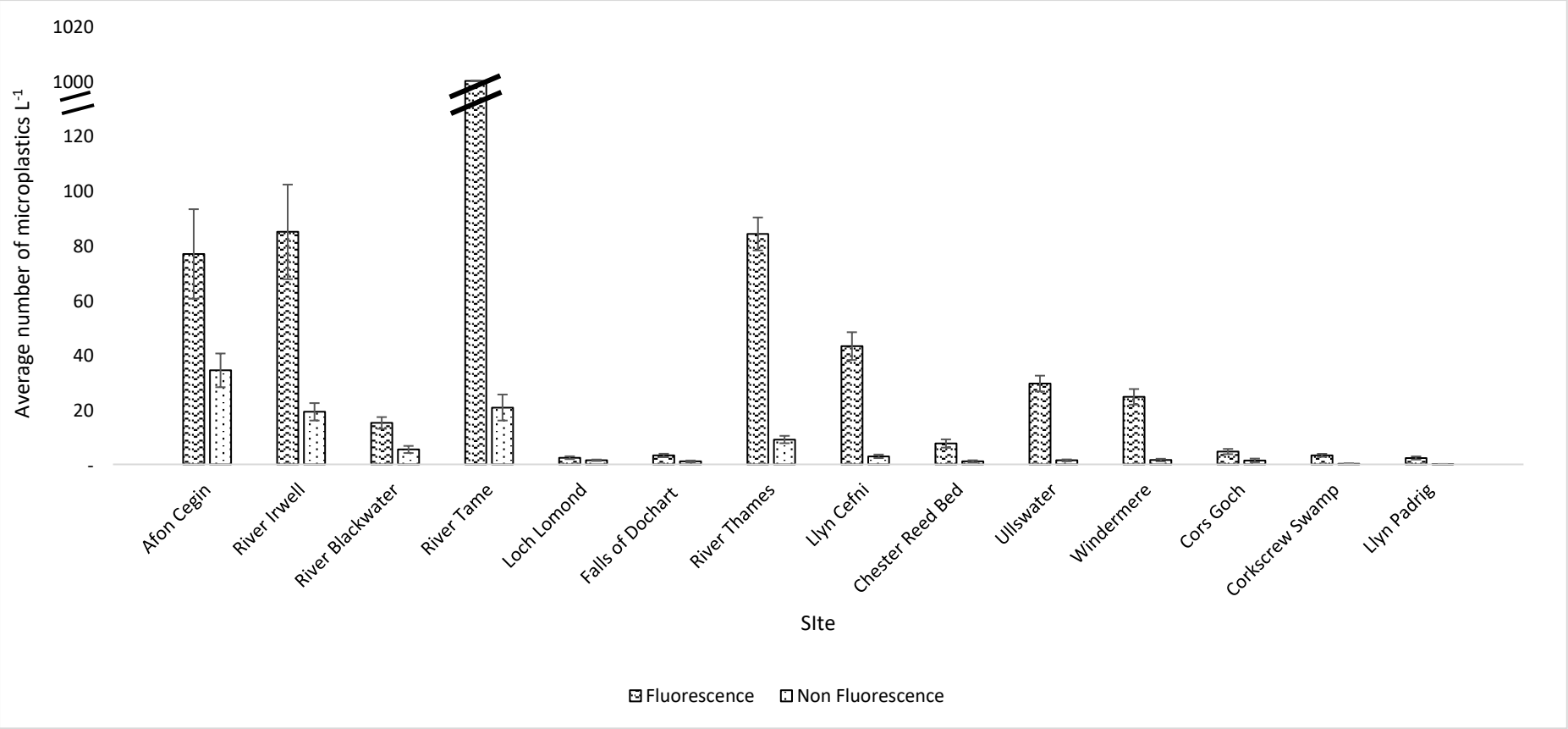
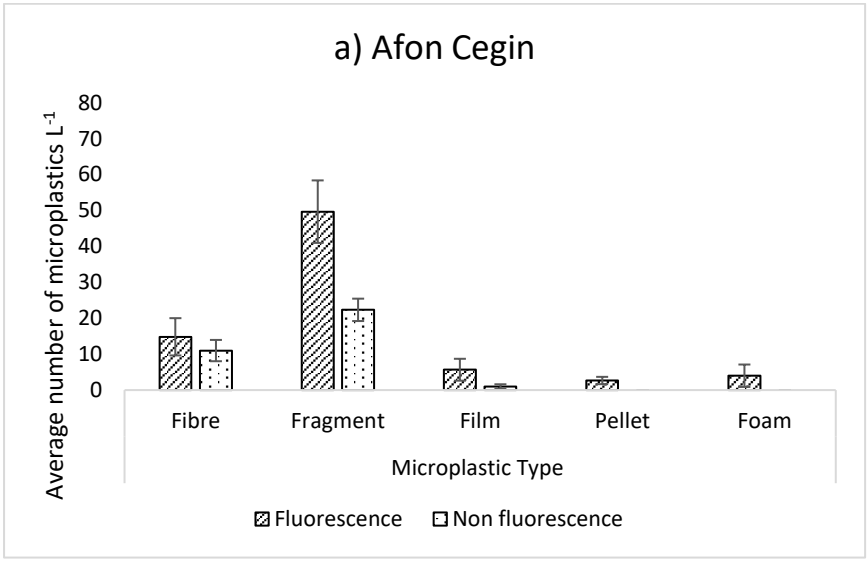
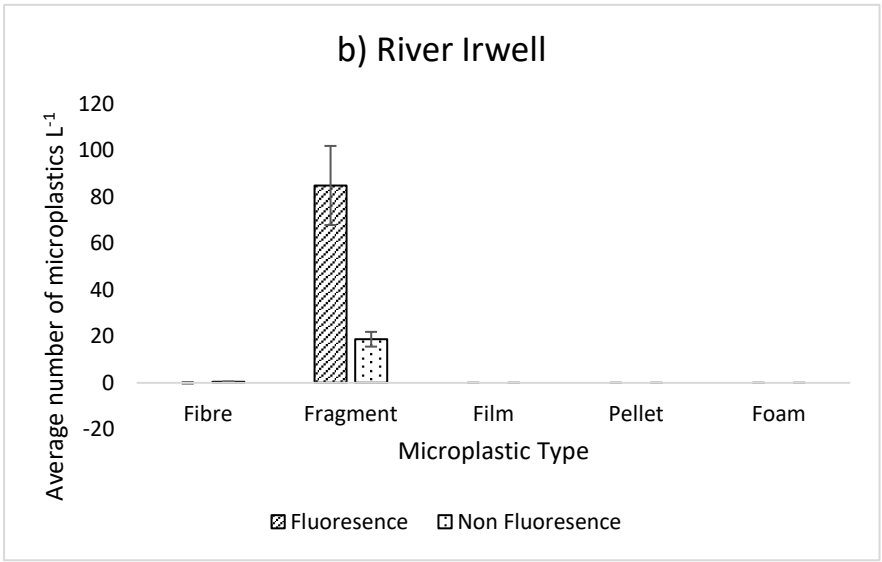
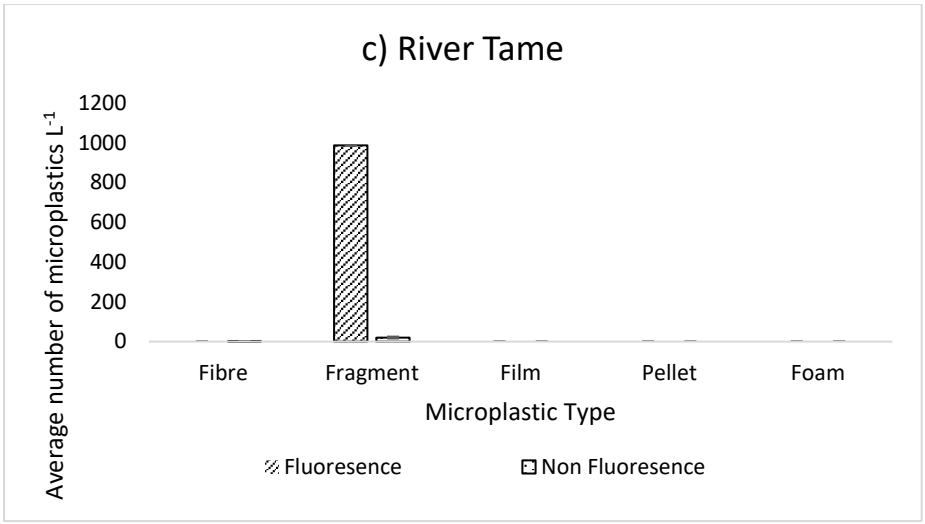


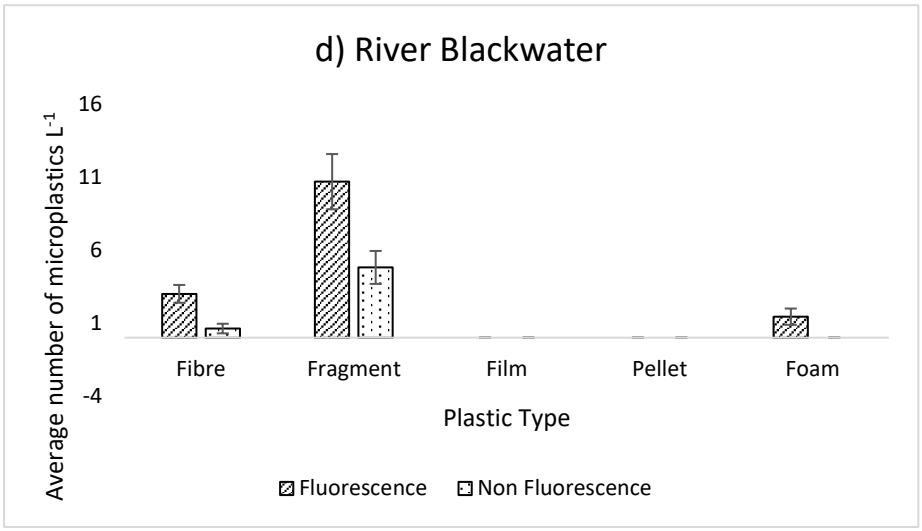
Figure 4. Average number of microplastics L^{-1} minus average control samples, per site, and by fluorescence method (dashed), and non-fluorescence method (dotted) with standard error bars.

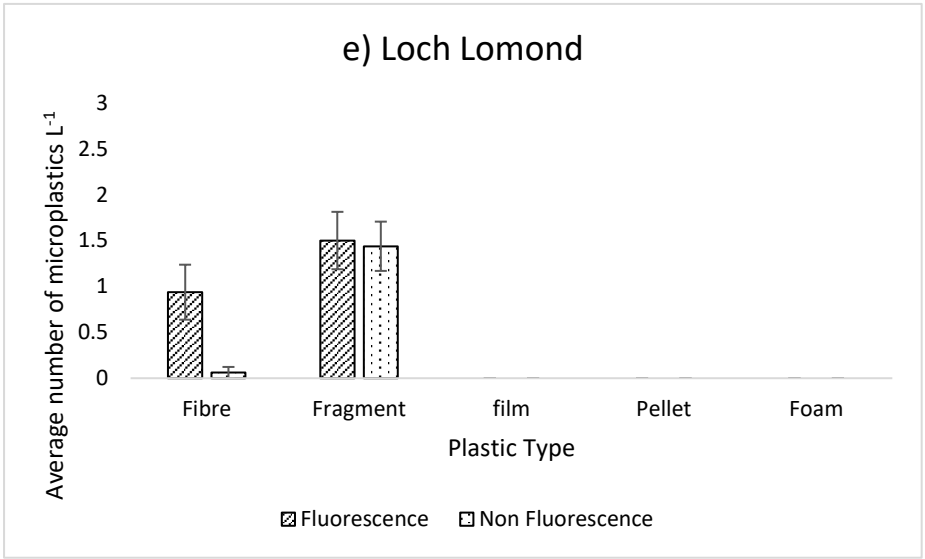
As can be seen from Figure 4, a far greater average quantity of microplastics L^{-1} were observed using the fluorescence method than non-fluorescence, with quantities of up to over 1000 microplastics L^{-1} (River Tame) being observed under fluorescence, compared to 20.75 ± 4.75 microplastics L^{-1} without fluorescence. This trend was even observed for sites with low microplastic occurrence, for example in River Blackwater, wherein 15.13 ± 2.14 microplastics L^{-1} were found under fluorescence, and 5.44 ± 1.3 microplastics L^{-1} without fluorescence. A significant difference was found between fragments and all the other types of microplastic for every site sampled, with a greater average quantity of fragments found within each sample. Please refer to Tables 2 to 14 within the appendix section for results of independent Sample Kruskal-Wallis Pairwise Comparisons tests for fluorescence analysis of individual sites, testing for significant differences between microplastic type populations.

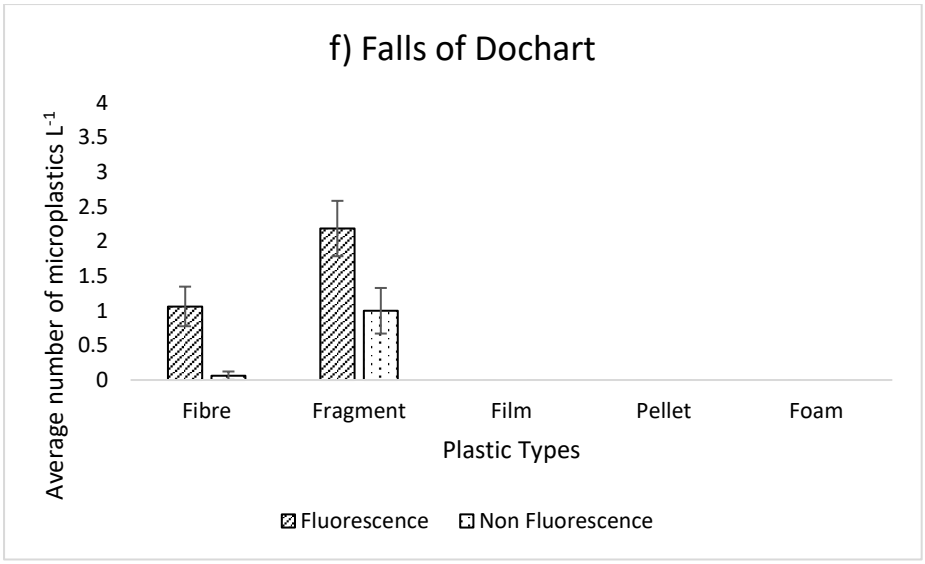


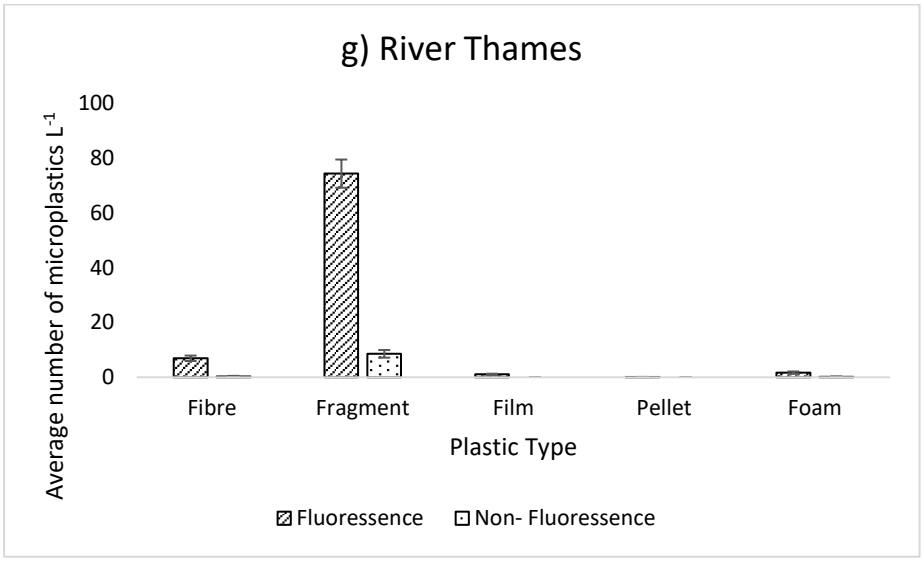


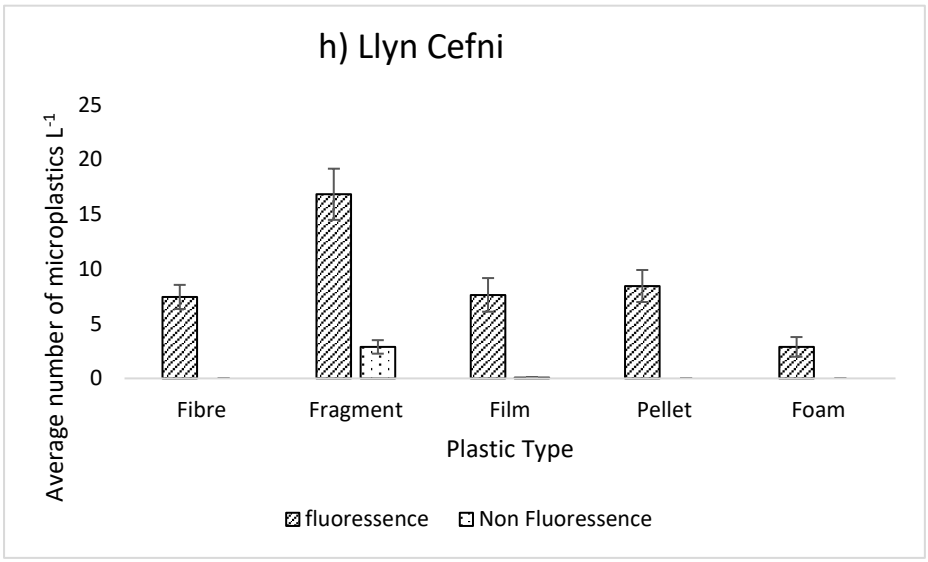

c) River Tame
Average number of microplastics L-1
1200
1000
800
600
400
200
0
Fibre
Fragment
Film
Pellet
Foam
Microplastic Type
Fluoresence
Non Fluoresence

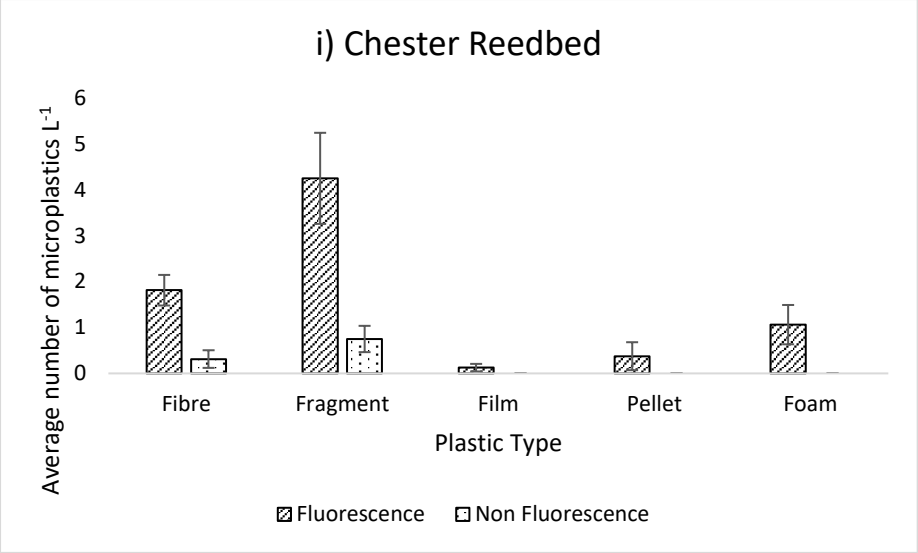

d) River Blackwater
Average number of microplastics L-1
16
11
6
1
-4
Fibre
Fragment
Film
Pellet
Foam
Plastic Type
Fluorescence
Non Fluorescence

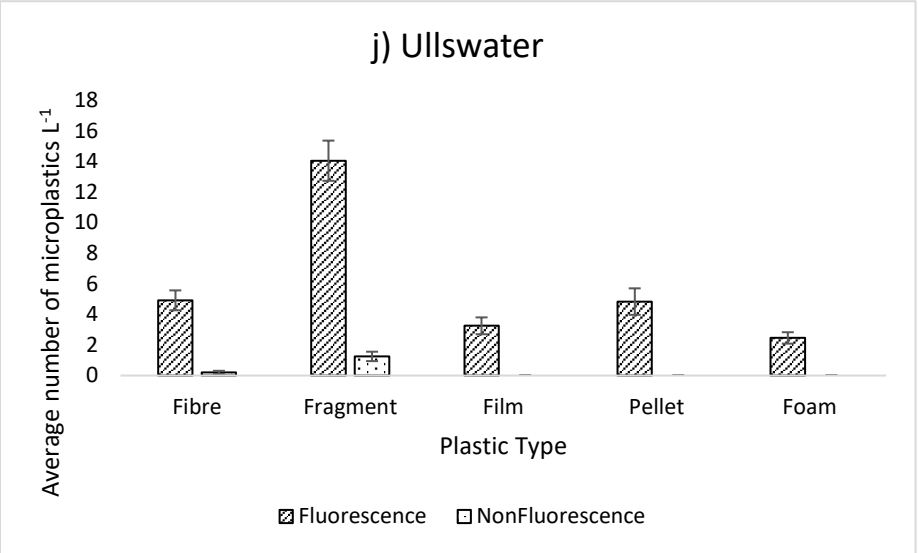

e) Loch Lomond
Average number of microplastics L-1
3
2.5
2
1.5
1
0.5
0
Fibre
Fragment
film
Pellet
Foam
Plastic Type
Fluorescence
Non Fluorescence

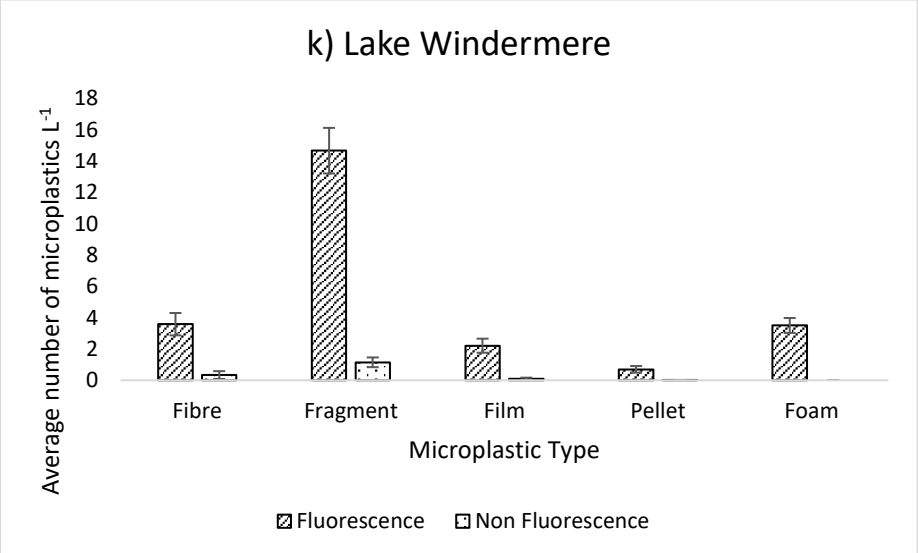

f) Falls of Dochart
Average number of microplastics L-1
4
3.5
3
2.5
2
1.5
1
0.5
0
Fibre
Fragment
Film
Pellet
Foam
Plastic Types
Fluorescence
Non Fluorescence

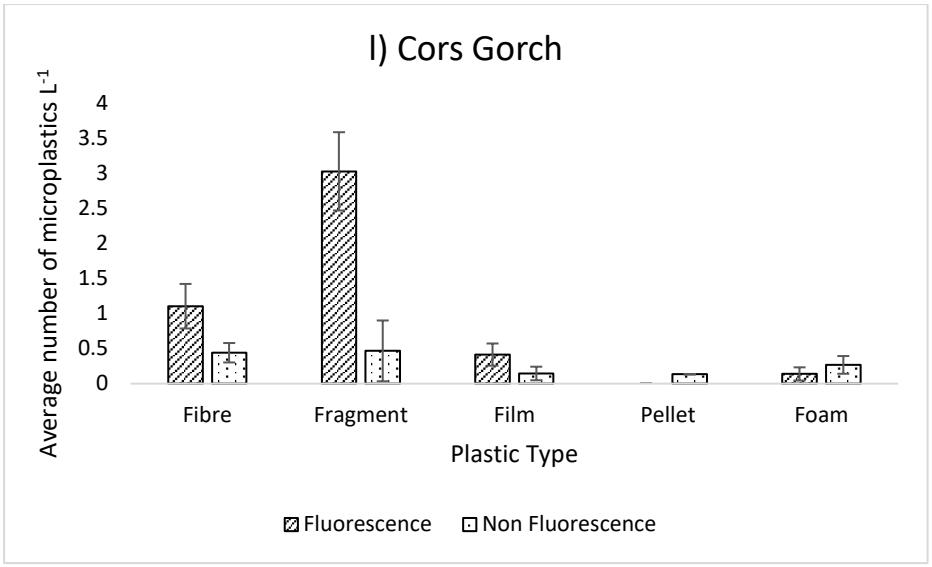

g) River Thames
Average number of microplastics L-1
100
80
60
40
20
0
Fibre
Fragment
Film
Pellet
Foam
Plastic Type
Fluoressence
Non- Fluoressence

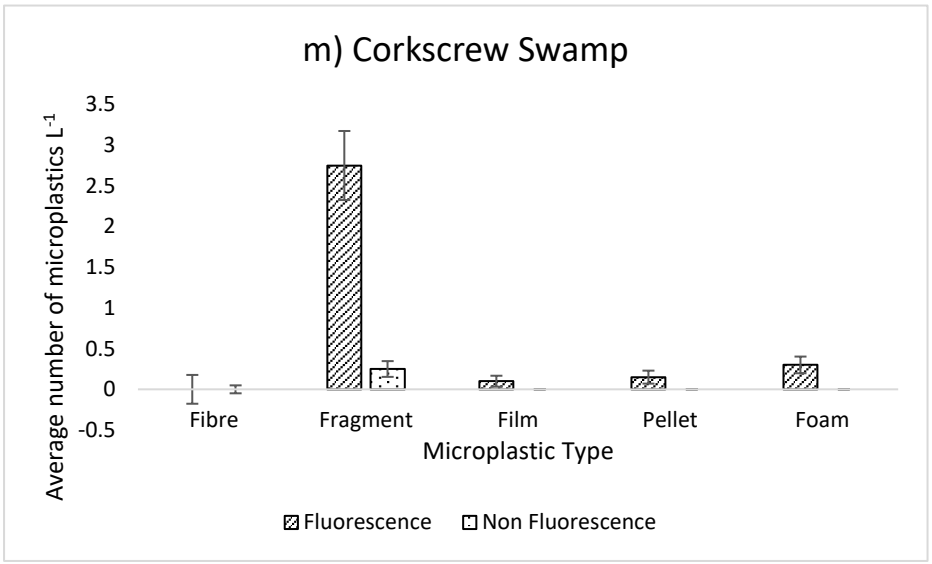

h) Llyn Cefni
Average number of microplastics L-1
25
20
15
10
5
0
Fibre
Fragment
Film
Pellet
Foam
Plastic Type
fluoressence
Non Fluoressence


i) Chester Reedbed
Average number of microplastics L-1
0
1
2
3
4
5
6
Fibre
Fragment
Film
Pellet
Foam
Plastic Type
Fluorescence
Non Fluorescence


j) Ullswater
Average number of microplastics L-1
0
2
4
6
8
10
12
14
16
18
Fibre
Fragment
Film
Pellet
Foam
Plastic Type
Fluorescence
NonFluorescence


k) Lake Windermere
Average number of microplastics L-1
0
2
4
6
8
10
12
14
16
18
Fibre
Fragment
Film
Pellet
Foam
Microplastic Type
Fluorescence
Non Fluorescence


l) Cors Gorch
Average number of microplastics L-1
4
3.5
3
2.5
2
1.5
1
0.5
0
Fibre
Fragment
Film
Pellet
Foam
Plastic Type
Fluorescence
Non Fluorescence


m) Corkscrew Swamp
Average number of microplastics L-1
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
Fibre
Fragment
Film
Pellet
Foam
Microplastic Type
Fluorescence
Non Fluorescence

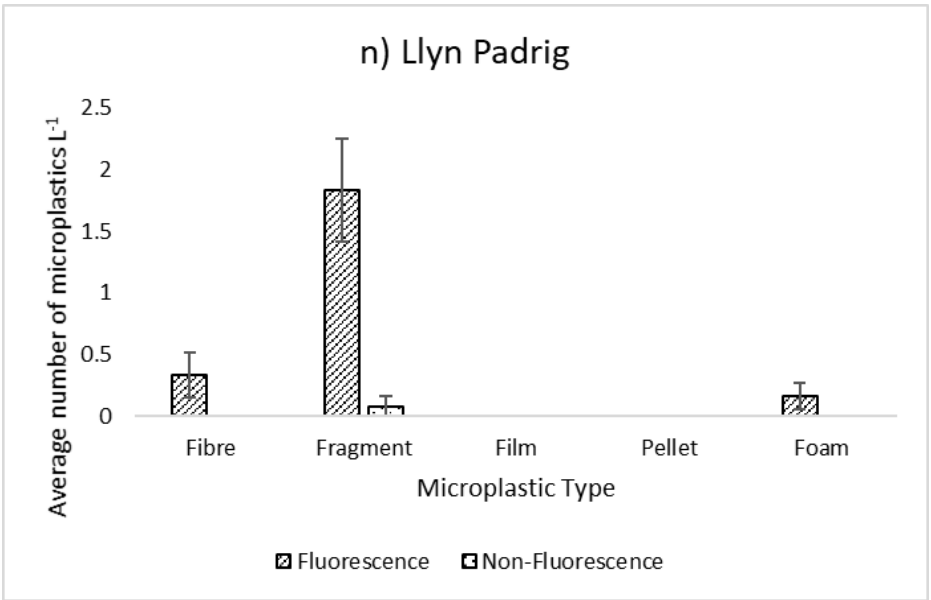

n) Llyn Padrig
Average number of microplastics L-1
2.5
2
1.5
1
0.5
0
Fibre
Fragment
Film
Pellet
Foam
Microplastic Type
Fluorescence
Non-Fluorescence

Figure 5. Average number of microplastics L^{-1} for sites **a)** Afon Cegin, **b)** River Irwell, **c)** River Tame, **d)** River Blackwater, **e)** Loch Lamond, **f)** Falls of Dochart, **g)** River Thames, **h)** Llyn Cefni, **i)** Chester Reed Bed, **j)** Ullswater, **k)** Lake Windermere, **l)** Cors Goch, **m)** Corkscrew Swamp, **n)** Llyn Padrig, by microplastic type and investigation using fluorescence microscopy (dashed) and non-fluorescence microscopy (dotted), adjusted for controls, with standard error bars.

4e. Discussion

As can be seen from the significant difference (p <0.05) between the use of dissection microscopy with fluorescence and without fluorescence for seven of the fourteen sites sampled, with a significantly higher number of average microplastics L^{-1} found via the former method than the latter (Table 1, Figures 4 and 5), our method of fluorescence microscopy has proven that it can be effective at analysing microplastic particles within water. For cases where no significant difference was found between analytical methods, this was quite often due to low levels of average microplastics L^{-1} found both between fluorescence and non-fluorescence analyses (e.g., Falls of Dochart fluorescence: 3.25 ± 0.63 L^{-1}, non-fluorescence: 1.06 ± 0.34 L^{-1}) (Table 1), or due to probable errors with statistical test algorithms. As such, this brings into question previously-held beliefs about microplastic quantities in fresh (and sea) water sources. Multiples of previous studies, such as Bouwman et al. (2018), Felismino, Helm & Rochman (2021), and Payton (2016) utilise visual inspection of microplastics via microscopy sans-fluorescence; thus the actual microplastic quantities could potentially be dramatically higher than reported, affecting pre-conceived notions about current levels of microplastic pollution found in water bodies from studies only utilising standard dissection microscopy as a visualisation method. The sampling methods utilised by these studies also used bulk sampling as I did; for example Bouwman et al. (2018) and Payton (2016) utilised bulk sampling through scooping water via a metal bucket and pouring it directly through a sieve, and Felismino, Helm & Rochman (2021) used amber bottles, not affecting the potential for the number of microplastics L^{-1}, as would a method utilising volume reduced sampling such as nets would, and avoiding the trap of bulk sampling producing higher MP yields than volume reduced sampling (Green et al., 2018).

The ease and effectiveness of this method of fluorescence microscopy makes it an ideal candidate to be utilised alongside other water quality measurement practices. Current water quality measurement practices, utilised by organisations such as Dŵr Cymru Welsh Water and Severn Trent Water (Drinking Water Inspectorate, 2018) do not include microplastic measurement as a pre-requisite. With microplastic pollution becoming more of an issue (Nizzetto, Langaas & Futter, 2016), an efficient method of analysing water samples for microplastic pollution is required as a further step to determining the health of water bodies. Furthermore, this fluorescence microscopy method allows

the analysis of different types of microplastics, and as such could be used to help identify the most prevalent types and thus infer the sources of plastic pollution, helping in aiding the cessation of pollution from plastic waste. The ease of microscope analysis in this method allows for quick results more reliable than standard stereomicroscopy, and as such could be utilised as the measurement method for widespread studies of microplastic pollution.

There was a markedly significant difference in average microplastics L^{-1} between types of microplastics found, with fibres, and fragments especially, being far more prevalent than the foam, film and pellet types, as is evident from the Kruskal-Wallis pairwise comparisons in Tables 2 to 15 (no pairwise comparison available for Corkscrew Swamp due to no significant difference being found by Independent Samples Kruskal Wallis test). This reflects the observations of Cole et al. (2013) and Rillig (2012) upon the high abundance of fragments resulting from the deconstruction and breakdown of macroplastics into microplastics, and those of De Falco et al. (2019) who found that significant numbers of fibres were released from clothing in every wash (640,000 to 1.5 million per kilogram of fabric washed), and an average length of 360-660 μm and an average diameter of 12–16 μm of fibres, which could easily pass through filtration systems at wastewater treatment facilities and enter the Earth's marine and freshwater ecosystems. This pattern of a significant majority of fragments is present in most sample sites, with specific significant differences between fragments and the other plastic types varying between sites (Tables 2-14). This could potentially be a result of the more rural nature of Loch Lomond and Falls of Dochart, away from wastewater effluent of modern living, with much of the microplastics present as a result of tourism and wind dispersal (Rezaei et al., 219). Further study would be required to find the exact origin of much of the microplastic pollution present in inland waters.

Photobleaching of microplastics exposed to fluorescence excitation was considered on whether to be a factor affecting the colouration and potentially fluorescing properties of the microplastics, but it was determined that this would likely not be the case as photobleaching of plastics has been observed to be an issue at lower wavelengths/ higher frequency (Sullivan & Gugliada, 2018). Nevertheless, this did inform our decision to utilise a Royal Blue (440-460 nm) fluorescence adapter, as the Ultraviolet version had a lower wavelength (360 nm to 380 nm), and thus has greater potential for photobleaching, and as budgetary restraints only allowed us the purchase of a single adapter, the research team settled on Royal Blue.

During this study, it was noted that fluorescence was shown by organic elements such as plant matter and dead invertebrates. This was to be expected, as the natural world is abundant with examples of organisms bioluminescening or fluorescing under excitation by various light wavelengths.

Fluorescence is notable in various species of Longhorn beetle such as *Sternotomis virescens and Anoplophora elegans* and the weevil *Pachyrrhynchus gemmatus purpureus* (Welch et al., 2012). Resilin is a protein found in the cuticles of arthropods, which emits a blue-green fluorescence when excited by long-wave UV light (310 nm- 340 nm most excitation, limited to 420 nm) (Anderson, 1963), and is commonly found in a variety of structures found in riparian insects, such as the venom injectors of a honeybee and legs of cockroaches (Weisenborn, 2011). As the insects were not identified, and the excitation used was 440-460 nm ((Royal) Blue light), it is unlikely that resilin was involved, but could likely be some other fluorescing chemical, as is evident with species such as the tick *Dermacentor variabilis* (Shade, 2018). Chlorophyll fluorescence too has been a long-documented phenomenon. Chlorophyll and various plant proteins, when exposed to (visible or invisible) spectra, will emit fluorescence of specific wavelengths when exposed to specific wavelengths of radiation, for example blue at 440 nm excitation, and green at 520 nm excitation (Buschmann, Langsdorf & Lichtenthaler, 2000). This excitation is an integral component of photosynthesis and is present in all photosynthesizing plants. (Bilger, Johnsen & Schreiber, 2001). Furthermore, inorganic particles (thought to be sand) appeared to reflect the light of the fluorescent lamp, appearing bright, which could potentially be confused as fluorescence. To avoid miscounts because of these, large degrees of scrutiny and conservative counts were utilised, alongside hot needle tests, to rule out any miscounts. Several particles were observed which were too small to be thoroughly identified but were counted in the fragment category rather than as its own category as this microplastic type was observed to be the most common found, and therefore it was assumed that nanoparticles would most likely be the fragment type of nanoplastics. This researcher is aware of the invalidity of this method, which luckily would not likely have an effect on statistical outcomes of data sets as very few of these particles were identified, and since the completion of this study has adapted the identification methodology of this study to count these particles as their own category. The usage of chemospectroscopy would put to bed any such issues in determining the chemical composition of these nanoparticles.

It is worth noting that the method developed in this study is intended to be utilised for quick visual analyses of micro and nanoplastic contamination within water samples, and as such is dependent upon the discretion and analytical prowess of the examiner in visually discriminating between microplastic particles and particles of a similar size and appearance, and thus is not intended as a replacement for the more accurate method of compound identification, chemospectroscopy. Previous analyses of microplastic contamination in water utilising dissection microscopy and chemospectroscopy have found that misidentification of microplastics during visual inspection is fairly common, varying from researcher and samples; for example, Löder and Gerdts (2015) found that only 1.4% of particles visually resembling microplastics from North Sea sediment samples were of synthetic

polymer origin when analysed with FPA-µFTIR (Focal Plane Array Micro Fourier-Transform InfraRed Spectroscopy). Owens, Dunn and I used extremely conservative counts when analysing samples for microplastics and practiced analysing various samples before undertaking this study to build up our analytical ability to ensure our analyses were as accurate as we could make them. We would have liked to have utilised FTIR or Raman spectroscopy on at least some of the samples to verify our findings, but unfortunately, we lacked the time and resources to do so. As such, this researcher recommends that this method also be utilised in tandem with chemospectroscopy if a more accurate analysis is required.

4f. Appendices

Table 2. Kruskal-Wallis Pairwise Comparisons of average microplastics L^{-1} between microplastic types, displaying Test Statistic, Standard Error, and Significance, for site Afon Cegin, analysis with fluorescence.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Foam-Pellet	.667	5.062	.895
Foam-Film	1.583	5.062	.754
Foam-Fibre	8.250	5.062	.103
Foam-Fragment	17.000	5.062	.001
Pellet-Film	.917	5.062	.856
Pellet-Fibre	7.583	5.062	.134
Pellet-Fragment	16.333	5.062	.001
Film-Fibre	6.667	5.062	.188
Film-Fragment	15.417	5.062	.002
Fibre-Fragment	-8.750	5.062	.084

Table 3. Kruskal-Wallis Pairwise Comparisons of average microplastics L^{-1} between microplastic types, displaying Test Statistic, Standard Error, and Significance, for site River Irwell, analysis with fluorescence.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Film-Fibre	2.000	3.183	.530
Pellet-Fibre	2.000	3.183	.530
Foam-Fibre	2.000	3.183	.530
Film-Fragment	10.500	3.183	.001
Pellet-Fragment	10.500	3.183	.001
Foam-Fragment	10.500	3.183	.001
Film-Pellet	.000	3.183	1.000
Film-Foam	.000	3.183	1.000
Pellet-Foam	.000	3.183	1.000
Fibre-Fragment	-8.500	3.183	.008

Table 4. Kruskal-Wallis Pairwise Comparisons of average microplastics L^{-1} between microplastic types, displaying Test Statistic, Standard Error, and Significance, for site River Tame, analysis with fluorescence.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Fibre-Film	.000	2.902	1.000
Fibre-Pellet	.000	2.902	1.000
Fibre-Foam	.000	2.902	1.000
Fibre-Fragment	-10.000	2.902	.001
Film-Fragment	10.000	2.902	.001
Pellet-Fragment	10.000	2.902	.001
Foam-Fragment	10.000	2.902	.001
Film-Pellet	.000	2.902	1.000
Film-Foam	.000	2.902	1.000
Pellet-Foam	.000	2.902	1.000

Table 5. Kruskal-Wallis Pairwise Comparisons of average microplastics L^{-1} between microplastic types, displaying Test Statistic, Standard Error, and Significance, for site River Blackwater, analysis with fluorescence.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Film-Fibre	9.750	4.044	.016
Pellet-Fibre	9.750	4.044	.016
Film-Fragment	14.000	4.044	.001
Pellet-Fragment	14.000	4.044	.001
Film-Pellet	.000	4.044	1.000
Film-Foam	-6.250	4.044	.122
Pellet-Foam	-6.250	4.044	.122
Foam-Fibre	3.500	4.044	.387
Foam-Fragment	7.750	4.044	.055
Fibre-Fragment	-4.250	4.044	.293

Table 6. Kruskal-Wallis Pairwise Comparisons of average microplastics L^{-1} between microplastic types, displaying Test Statistic, Standard Error, and Significance, for site River Loch Lomond, analysis with fluorescence.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Film-Fibre	9.000	3.565	.012
Pellet-Fibre	9.000	3.565	.012
Foam-Fibre	9.000	3.565	.012
Film-Fragment	8.500	3.565	.017
Pellet-Fragment	8.500	3.565	.017
Foam-Fragment	8.500	3.565	.017
Film-Pellet	.000	3.565	1.000
Film-Foam	.000	3.565	1.000
Pellet-Foam	.000	3.565	1.000
Fragment-Fibre	.500	3.565	.888

Table 7. Kruskal-Wallis Pairwise Comparisons of average microplastics L^{-1} between microplastic types, displaying Test Statistic, Standard Error, and Significance, for site Falls of Dochart, analysis with fluorescence.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Film-Fibre	8.750	3.706	.018
Pellet-Fibre	8.750	3.706	.018
Foam-Fibre	8.750	3.706	.018
Film-Fragment	11.250	3.706	.002
Pellet-Fragment	11.250	3.706	.002
Foam-Fragment	11.250	3.706	.002
Film-Pellet	.000	3.706	1.000
Film-Foam	.000	3.706	1.000
Pellet-Foam	.000	3.706	1.000
Fibre-Fragment	-2.500	3.706	.500

Table 8. Kruskal-Wallis Pairwise Comparisons of average microplastics L^{-1} between microplastic types, displaying Test Statistic, Standard Error, and Significance, for site River Thames, analysis with fluorescence.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Pellet-Film	3.750	4.147	.366
Pellet-Foam	-4.625	4.147	.265
Pellet-Fibre	10.625	4.147	.010
Pellet-Fragment	14.750	4.147	.000
Film-Foam	-.875	4.147	.833
Film-Fibre	6.875	4.147	.097
Film-Fragment	11.000	4.147	.008
Foam-Fibre	6.000	4.147	.148
Foam-Fragment	10.125	4.147	.015
Fibre-Fragment	-4.125	4.147	.320

Table 9. Kruskal-Wallis Pairwise Comparisons of average microplastics L^{-1} between microplastic types, displaying Test Statistic, Standard Error, and Significance, for site Llyn Cefni, analysis with fluorescence.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Foam-Film	6.125	4.180	.143
Foam-Fibre	6.250	4.180	.135
Foam-Pellet	8.250	4.180	.048
Foam-Fragment	14.375	4.180	.001
Film-Fibre	.125	4.180	.976
Film-Pellet	-2.125	4.180	.611
Film-Fragment	8.250	4.180	.048
Fibre-Pellet	-2.000	4.180	.632
Fibre-Fragment	-8.125	4.180	.052
Pellet-Fragment	6.125	4.180	.143

Table 10. Kruskal-Wallis Pairwise Comparisons of average microplastics L^{-1} between microplastic types, displaying Test Statistic, Standard Error, and Significance, for site Chester Reed Bed, analysis with fluorescence.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Film-Pellet	-1.375	4.118	.738
Film-Foam	-5.500	4.118	.182
Film-Fibre	9.125	4.118	.027
Film-Fragment	14.000	4.118	.001
Pellet-Foam	-4.125	4.118	.317
Pellet-Fibre	7.750	4.118	.060
Pellet-Fragment	12.625	4.118	.002
Foam-Fibre	3.625	4.118	.379
Foam-Fragment	8.500	4.118	.039
Fibre-Fragment	-4.875	4.118	.237

Table 11. Kruskal-Wallis Pairwise Comparisons of average microplastics L^{-1} between microplastic types, displaying Test Statistic, Standard Error, and Significance, for site Ullswater, analysis with fluorescence.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Foam-Film	4.083	5.058	.419
Foam-Pellet	5.250	5.058	.299
Foam-Fibre	13.000	5.058	.010
Foam-Fragment	20.583	5.058	.000
Film-Pellet	-1.167	5.058	.818
Film-Fibre	8.917	5.058	.078
Film-Fragment	16.500	5.058	.001
Pellet-Fibre	7.750	5.058	.125
Pellet-Fragment	15.333	5.058	.002
Fibre-Fragment	-7.583	5.058	.134

Table 12. Kruskal-Wallis Pairwise Comparisons of average microplastics L^{-1} between microplastic types, displaying Test Statistic, Standard Error, and Significance, for site Lake Windemere, analysis with fluorescence.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Pellet-Film	6.333	5.074	.212
Pellet-Foam	-14.500	5.074	.004
Pellet-Fibre	15.167	5.074	.003
Pellet-Fragment	24.000	5.074	.000
Film-Foam	-8.167	5.074	.108
Film-Fibre	8.833	5.074	.082
Film-Fragment	17.667	5.074	.000
Foam-Fibre	.667	5.074	.895
Foam-Fragment	9.500	5.074	.061
Fibre-Fragment	-8.833	5.074	.082

Table 13. Kruskal-Wallis Pairwise Comparisons of average microplastics L^{-1} between microplastic types, displaying Test Statistic, Standard Error, and Significance, for site Cors Goch, analysis with fluorescence.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Pellet-Foam	-2.500	4.123	.544
Pellet-Film	7.000	4.123	.090
Pellet-Fibre	10.750	4.123	.009
Pellet-Fragment	14.750	4.123	.000
Foam-Film	4.500	4.123	.275
Foam-Fibre	8.250	4.123	.045
Foam-Fragment	12.250	4.123	.003
Film-Fibre	3.750	4.123	.363
Film-Fragment	7.750	4.123	.060
Fibre-Fragment	-4.000	4.123	.332

Table 14. Kruskal-Wallis Pairwise Comparisons of average microplastics L^{-1} between microplastic types, displaying Test Statistic, Standard Error, and Significance, for site Corkscrew Swamp, analysis with fluorescence.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Fibre-Pellet	-.653	4.141	.875
Fibre-Film	-1.778	4.141	.668
Fibre-Foam	-7.153	4.141	.084
Fibre-Fragment	-14.028	4.141	.001
Pellet-Film	1.125	4.872	.817
Pellet-Foam	-6.500	4.872	.182
Pellet-Fragment	13.375	4.872	.006
Film-Foam	-5.375	4.872	.270
Film-Fragment	12.250	4.872	.012
Foam-Fragment	6.875	4.872	.158

Note: Kruskal-Wallis Pairwise Comparison not available due to lack of values in sample groups for site Corkscrew swamp

Table 15. Number of 1 litre samples filtered and analysed in this study.

Site	Number of samples
Afon Cegin	6
River Irwell	4
River Tame	4
River Blackwater	4
Loch Lomond	4
Falls of Dochart	4
River Thames	4
Llyn Cefni	4
Chester Reed Bed	4
Ullswater	6
Lake Windemere	6
Cors Goch	4
Corkscrew Swamp	5
Llyn Padrig	3

Table 16. Average number of microplastics L^{-1} in control samples for each site.

Site	ID Method	Microplastic Type					
		Fibre	Fragment	Film	Pellet	Foam	Total
Afon Cegin	Fluorescence	16	24	1	1	5	47
	Non-Fluorescence	0	0	0	0	0	0
River Irwell	Fluorescence	1.5625	13.0625	0.125	0.0625	0	14.8125
	Non-Fluorescence	0	0	0	0	0	0
River Tame	Fluorescence	1.5625	13.0625	0.125	0.0625	0	14.8125
	Non-Fluorescence	0	0	0	0	0	0
River Blackwater	Fluorescence	0	1.5	0	0	0	1.5
	Non-Fluorescence	0	0	0	0	0	0
Loch Lamond	Fluorescence	0	0.75	0	0	0	0.75
	Non-Fluorescence	0	0.375	0	0	0	0.375
Falls of Dochart	Fluorescence	0	0.75	0	0	0	0.75
	Non-Fluorescence	0	0.375	0	0	0	0.375
River Thames	Fluorescence	0.25	1.75	0	0	0	2
	Non-Fluorescence	0.5	0	0	0	0	0.5
Llyn Cefni	Fluorescence	0.5	1	0	0	0	1.5
	Non-Fluorescence	0	0	0	0	0	0
Chester Reed Bed	Fluorescence	0	0.25	0	0	0	0.25
	Non-Fluorescence	0	0	0	0	0	0
Ullswater	Fluorescence	0.75	1	0	0	0	1.75
	Non-Fluorescence	0	0	0	0	0	0
Lake Windemere	Fluorescence	0.75	1.5	0	0	0	2.25
	Non-Fluorescence	0	0.5	0	0	0	0.5
Cors Goch	Fluorescence	0	0.25	0	0	0	0.25
	Non-Fluorescence	0	0	0	0	0	0
Corkscrew Swamp	Fluorescence	1	1.25	0	0	0	2.25
	Non-Fluorescence	0.25	0	0	0	0	0.25
Llyn Padrig	Fluorescence	0	0	0	0	0	0
	Non-Fluorescence	0	0	0	0	0	0

4g. References

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5. Pilot Study Investigation of the Presence of Microplastics within the Coastal Waters of Anglesey, North Wales

5a. Abstract

There is little to no constant monitoring of microplastic levels across the UK coasts, and no previous studies upon the presence of microplastics around the isle of Anglesey, North Wales. Five sites around the Anglesey coast were selected for a pilot study, at three corners of the island (Porth Dafarch, Penmon Point, Cemaes Bay), and two from opposite sides of the Menai Strait (Bangor and Menai Bridge). A significant difference (p <0.05) was found between each of the surveyed sites and control samples of distilled water, verifying the presence of microplastic pollution along the Anglesey coast. A significantly greater average number of microplastics $^{L-1}$ were found on the Bangor side (7.958 ± 0.52) of the Menai Strait than the Menai Bridge side (2 ± 0.87). The more-populated tourist beach of Cemaes Bay had a higher average number of microplastics L^{-1} (1.7 ± 0.29) than the less-populated Porth Dafarch (1.1 ± 0.13), but less than the less-populated Penmon Point (1.95 ± 0.27). Causation was speculated to result from human population, distance from shoreline, distance from human habitation, weather, and tidal current speed. The presence of nanoplastics within the Bangor samples was confirmed with high-end fluorescence microscopy. Significantly more fragment-type microplastics than other types were found at every site barring Cemaes Bay. These results demonstrate the need for further sampling for microplastics across not only Anglesey, but across the UK and worldwide.

5b. Introduction

There is little to no data on the prevalence of microplastic contamination in the coastal waters of North Wales. While there have been analyses of other pollution indicators besides microplastics in the North Welsh coast (for example concentrations of zinc by Ireland et al. (1973) and Walker et al. (1975)), constant monitoring of microbial colonies in coastal waters (at Cemaes Bay, a 2019 summer peak of 164 colonies of Intestinal enterococci per 100 ml, and 540 colonies of *E. coli* per 100 ml; levels

regarded as water quality (Natural Resources Wales, 2020)), and at least one study into microplastic levels in Irish coastal waters of the Irish Sea (Green et al., 2018), there is a distinct lack of any publication or report on microplastic levels in the Menai Strait and the Irish Sea surrounding North Wales. As such, analysis of North Welsh coastal waters is a requirement to garner a better understanding of the spread and severity of microplastic pollution not only in the area, but to form part of the global investigation into microplastic pollution.

This study aims to perform a preliminary analysis of microplastic concentrations in areas covering the coastal waters of the Isle of Anglesey, North Wales, and produce a rudimentary picture of its state of microplastic pollution to be built upon by further analyses. This study hypothesises that there will be significantly more microplastics in the North Welsh coastal waters and control samples, especially in areas closer to human habitations; the greater-populated tourist-orientated Cemaes Bay shows only a 'Sufficient' level of bathing water quality in 2018, compared to 'Excellent' quality of Porth Dafarch (Natural Resources Wales, 2018), and as such microplastic levels could reflect these trends.

The collection, processing and analysis of samples was conducted by me and Jedd Owens, who will not be conducting his own version of this study in his own MRes submission.

5c. Methodology

Sample Collection:

Grab samples were collected from water sources utilising 1L amber bottles, with at least three litres collected from each site. Samples were collected from the maximum depth possible to reach at the site, at a minimum of ~60 cm. Amber bottles were rinsed with water from sample sites before samples were taken to help eliminate any microplastics already present in the bottles. To take samples, bottles were held underwater vertically, allowing all the air to escape, before the bottle cap screwed on underwater (rinsing the cap in the process) to prevent loss of sample and also to prevent air-contamination. Sample sites were Penmon Point, Cemaes Bay, Porth Dafarch, the Menai Strait on the Anglesey side by the School of Ocean Sciences in Menai Bridge, and from the Menai Strait on the Bangor side by a beach near Bangor Pier. Samples were collected in close proximity to the shoreline for all sites excluding Menai Bridge, where the sample was collected from further into the Menai Strait

via a pontoon (as the sea wall formation prevented a closer-to-shore access point). Samples were collected on 16/03/2019. Geographical locations of sample sites can be found on Table 1. Map of sample sites can be found as Figure 1. Porth Dafarch, Cemaes Bay and Penmon Point were chosen as sample sites due to their positioning at the extreme corners of the island (that were accessible), thus allowing a greater area to be sampled overall and providing a well-rounded study on the coast of the isle as a whole. The Menai Strait sites were chosen to include data from beneath the isle and in the strait, and from the Bangor side and Menai Bridge side to compare prevalence by the city of Bangor with the smaller with the less-populated Menai Bridge on the opposite side of the Strait. 6 samples were collected from Menai Strait – Bangor, whilst Anglesey sites provided us with 5 samples each, due to availability of amber bottles.

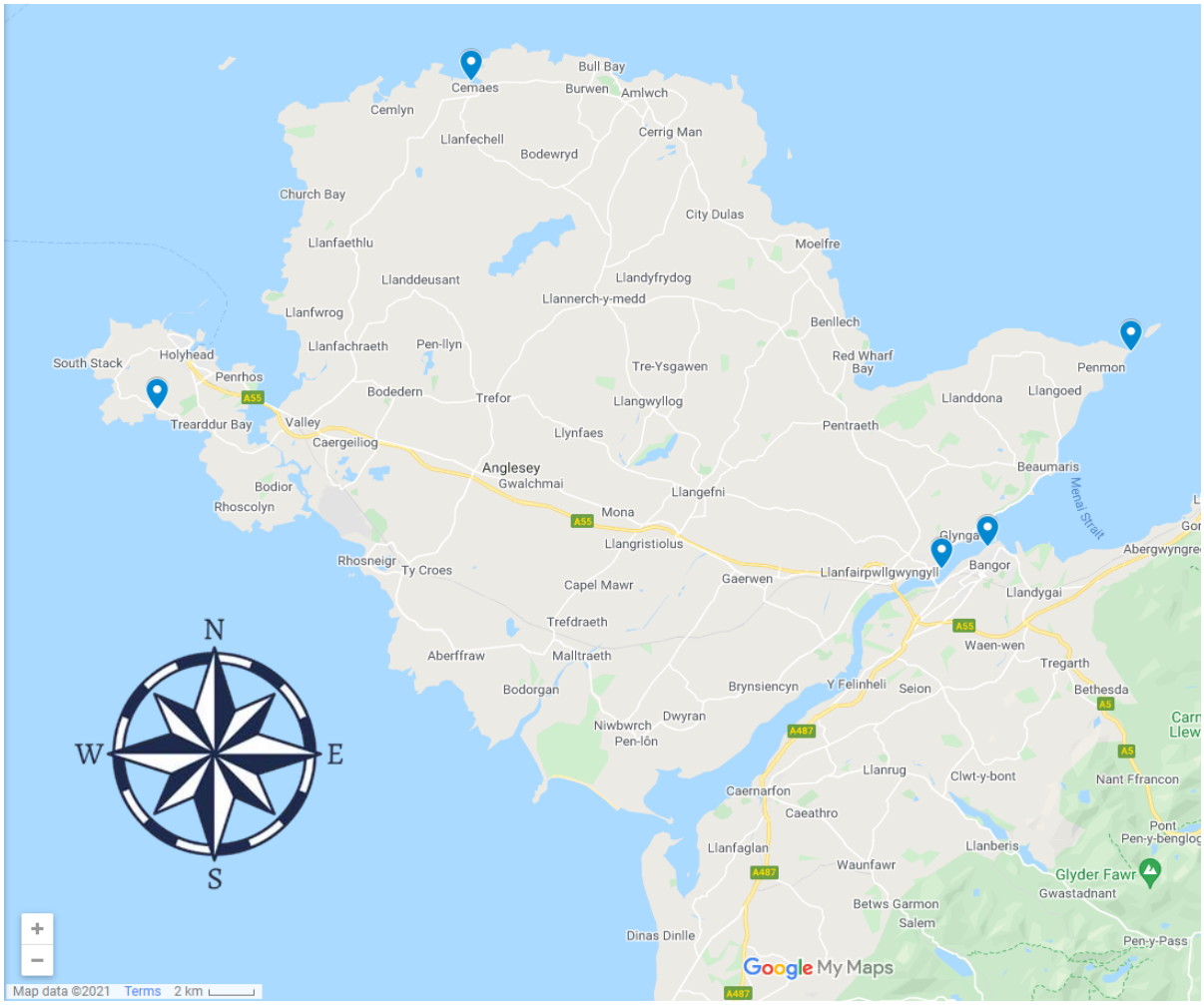

Figure 1. Map of sample collection sites.

Sample Filtration and Identification of Number of Microplastic Types:

The methods of filtration and analysis utilising fluorescence exposure and dissection microscopy are detailed by Dunn et al. (2020) and Fears (2021). Samples were filtered through Whatman Grade GF/C glass fibre filters, using an electric air pump and MilliporeSigma™ Glass Vacuum Filter Holder with Büchner flask, being washed down with pure distilled water to catch remnants of the samples stuck to the side of the filter intake, before being dried overnight at 60°C, and analysed under fluorescence using a dissection microscope to visually determine the number of each type of microplastic. Blind testing and three repetitions allowed for a reduction in confirmation bias and increased validity of results. Control samples to eliminate the presence of contaminating microplastics were created and analysed utilising the aforementioned method, replacing the sample water with pure distilled water. Whilst cleaner samples like the controls and those taken from Menai Bridge filtered easily and quickly in around one minute, samples with a greater concentration of sediment took much longer, around 30 minutes per sample. Identification of microplastics took on average three minutes per sample repetition (more when microplastic densities were higher), for a minimum of 12 minutes per sample. One sample (sample 6) from Menai Bridge was analysed at the Bangor University School of Ocean Sciences using a GXM L2800 Biological Upright Research Grade Microscope with fluorescence illuminator and camera adaptor, magnification 0.0151 nm per pixel, in order to determine the presence of nanoplastic particles invisible to the naked eye or fluorescence dissection microscopy (more would have been conducted but time restraints and the Covid-19 pandemic shutting down research facilities prevented us from doing so). As with Dunn et al. (2019) and Fears et al. (2020), identification of microplastics as being such was dependent upon the discretion of the researcher, and any attempts to utilise chemospectroscopy for a more robust analysis were thwarted by the Covid-19 pandemic forcing research facilities to close.

Contamination Prevention:

Latex gloves were always worn when handling samples, and clothing (including cotton lab coats) was cleaned with lint roller. Amber bottles and all equipment were thoroughly rinsed with distilled water before and after usage. All possible plastic materials (containers, petri dishes, etc.) were replaced with glass and/ or rubber ones to reduce fragment pollution by said containers. Samples always remained covered by petri dish lids, apart from when being analysed. Petri dishes were sealed with parafilm

between usages. Open glassware was covered with tin foil to prevent contamination during filtration. The use of control samples also helped to mitigate the effects of environmental contamination; by counting the average number of microplastics found in 4 litre-samples of distilled water (the same water used to wash down the sample water during filtration), and subtracting these averages from the average sample results, a more accurate analysis of microplastic presence in these samples was formed. In actuality, no microplastics were identified of any variety in any of the samples, and so subtraction of any values from those generated by analysis of samples was unnecessary.

Statistical Analysis:

Averages for each type of microplastic per individual sample, and per sample site were calculated, as were the averages of the control samples. Averages for each type of microplastic from controls was subtracted from the averages from the sample sites to help eliminate the effect of contamination.

Normality of data sets was determined using Kolmogorov-Smirnov and Shapiro-Wilkes tests, and homogeneity using a Levene's equality of variance test. All data was found to be non-parametrically distributed, so independent samples Mann-Whitney U and Kruskal-Wallis tests were conducted to determine significance of data. Differences between populations of different microplastics within data sets was determined by Kruskal-Wallis Pairwise Comparisons. Software used was IBM SPSS Statistics 25, Microsoft Word, Microsoft Excel and Google Maps.

5d. Results

A significant difference was found between the control samples (*Mdn* = 0) and all sites; Menai Strait – Bangor (*Mdn* = 7.75) ($U = 60$, $z = -6.388$, $r = -0.825$, $p < 0.001$), Menai Strait – Menai Bridge (*Mdn* = 1) ($U = 225.5$, $z = -2.815$, $r = -0.398$, $p = 0.005$), Cemaes Bay (*Mdn* = 1.75) ($U = 162.5$, $z = -3.886$, $r = -0.55$, $p < 0.001$), Penmon Point (*Mdn* = 2.25) ($U = 200$, $z = -3.261$, $r = -0.461$, $p = 0.001$), Porth Dafarch (*Mdn* = 1.25) ($U = 225$, $z = -2.815$, $r = -0.398$, $p = 0.005$).

There was a good range between average microplastic concentrations from Anglesey sample sites, troughing at a total of 1.1 ± 0.13 microplastics L^{-1} at Porth Dafarch, to 2 ± 0.87 microplastics L^{-1} in the Menai Strait (Menai Bridge). Significantly more fragments (p <0.001) were found at each site except Cemaes Bay than all other types of microplastics, with fibres having the second greatest quantities. (Table 1, Tables 3- 7, Figure 2).

A significant difference was found between the average concentrations of microplastics found in samples collected from the Menai Strait - Menai Bridge (*Mdn* = 1), and from Menai Strait - Bangor (*Mdn* = 7.75) (U = 599, z = 3.924, r = 0.529, p <0.001), with the Menai Bridge side demonstrating an average microplastic concentration of 2 ± 0.87 microplastics L^{-1}, and the Bangor side 7.96 ± 0.52 microplastics L^{-1}. Unexpectedly, despite showing a greater number of microplastics, the Bangor sampling site was 15 metres further away from the nearest commercial/ residential area than the Menai Bridge site. This is converse to the other three sites, with average number of microplastics L^{-1} increasing as the distance decreased (Table 2). There has been little data on levels of littering around Anglesey, with most numbers being garnered through litter cleans and published in local newspapers. For example, over 500 kg of litter was picked up by an RSPCA litter clean around three Anglesey beaches in 2018 (Forgrave, 2019), and the organisation Surfers Against Sewage held four beach clean-ups collecting over 100 bags of waste by mid-2017 (Wyn-Williams, 2017). A survey by Williams, Randerson & Alharbi (2014) compared levels of litter on beaches across Wales between 2002 and 2012, finding that levels remained at Grade A (best levels) for the North Wales beaches at Penmaenmawr and Llandudno, and remained at Grade B for the Anglesey beaches of Porth Dafarch and Llanfynach. Samples from Bangor and Penmon Point were taken one hour before high tide, when the current was flowing past Penmon Point and into the Northwest delta of the Menai Strait at a speed of 0.7-1.2 knots, westerly at Cemaes Bay at speeds of 0.4-0.8 knots and south-westerly at 0.7-1.2 knots at Porth Dafarch at high tide, and into the Northwest delta of the Menai Strait at 0.3-0.5 knots when the Menai Bridge samples were taken one hour after high tide (CMBHA, 2020; CS&PF, 2020). On the day that samples were taken, there were few (~20) vessels upon the water around the Anglesey coast, most being passenger vessels (Figure 4) (ShipAIS, 2020).

Table 1. Average number of microplastics L^{-1} minus average control samples for each type of microplastic (Fibre, Fragment, Film Pellet, Foam) and totals for each sample site (with geographic location) with standard error.

Sample Site	Location	Average number of microplastics L^{-1}					
		Fibre	Frag	Film	Pellet	Foam	TOTAL
Menai Strait - Menai Bridge	53°13'31.3"N 4°09'33.6"W	0.125 ± 0.0625	1.9375 ± 0.8119	0 ± 0	0 ± 0	0 ± 0	2 ± 0.866
Menai Strait - Bangor	53°14'03.0"N 4°07'48.6"W	1.875 ± 0.3048	4.25 ± 0.243	1.2083 ± 0.3196	0.0833 ± 0.0481	0.5417 ± 0.038	7.9583 ± 0.5185
Porth Dafarch	53°17'13.9"N 4°39'05.9"W	0.15 ± 0.0894	0.95 ± 0.1304	0 ± 0	0 ± 0	0 ± 0	1.1 ± 0.1342
Cemaes Bay	53°24'52.8"N 4°27'15.0"W	0.6 ± 0.1517	1.05 ± 0.3421	0.05 ± 0.0447	0 ± 0	0 ± 0	1.7 ± 0.2864
Penmon Point	53°18'35.8"N 4°02'25.0"W	0.5 ± 0.1414	1.45 ± 0.1643	0 ± 0	0 ± 0	0 ± 0	1.95 ± 0.2683

5e. Discussion

The prevalence of microplastics in the waters around Anglesey presents great concern, especially when considering that North Welsh coastal waters are some of the cleanest in regard to other indicators of pollution (Natural Resources Wales, 2020). However, this is not to say that this is not just novel for North Welsh waters, as due to a lack of microplastic surveys of waters globally (WHO, 2019), these numbers could be overall miniscule or much greater than the concentrations found in this study. As such this highlights the necessity of regular, accurate surveying of microplastic pollution across all ecosystems to fully grasp their prevalence.

As can be seen in Figure 2/ Table 1, a significantly higher concentration of microplastics was found in the Bangor side of the Menai Strait than the Anglesey side (5.96 microplastics L^{-1}, or 297.92% more), causing a significant difference (p <0.001) between the two sites . This could be a result of the sampling location for the Bangor side being close to a pier featuring tourist destinations and regular visitors, where pollution and littering would be higher. As such, it could be expected that the Bangor site has a closer proximity to commercial or residential areas, as is the case with the other three sites, which show a trend of the average number of microplastics L^{-1} found increasing as distance to nearest commercial/ residential area decreases, from 1.1 ± 0.13 microplastics L^{-1} at 110 metres at Porth Dafarch to 1.95 ± 0.27 microplastics L^{-1} at 35 metres at Penmon Point. Comparing Menai Bridge to Bangor however, the result is the opposite, with Bangor showing a higher average number of microplastics L^{-1} (7.956 ± 0.52) than Menai Bridge (2), despite being 15 metres further away from human habitation (Table 2). A factor which could explain this trend is the proximity to the shoreline from which the samples were taken; the Bangor samples were taken from water less than a metre deep (from manually walking out to the sampling spots) very close to the shoreline, while the Anglesey samples were extracted from water several metres deep via a jetty erected into the Strait. As such plastic litter pollution could be lesser for the Anglesey side due to being further away from where litter is more likely dropped. However, the samples from the other Anglesey sites were taken at a similar depth and distance from the shoreline as the Bangor Menai Bridge samples, and showed a similar, if not lower degree of microplastic pollution than the Menai Bridge samples, indicating a higher level of pollution along the Bangor coastline. Visual analysis of the Bangor sample site found the presence of litter, compared to no litter at Menai Bridge. There were only 20 vessels (primarily composed of passenger vessels) out on the Anglesey coastline on the sampling day (ShipAIS, 2020), so barring

unreported and unlikely specific incidents of large-scale littering into the sea by persons aboard these vessels, it is unlikely that these vessels would have contributed significantly enough to affect results.

Another factor affecting MP populations could be the speed of the current flowing into the Menai Strait, with a higher speed (0.7-1.2 knots) being present when the Bangor samples were taken, compared to a slower speed (0.3-0.5 knots) when the Menai Bridge samples were taken (CMBHA, 2020; CS&PF, 2020). The higher speed could potentially deliver a greater number of microplastics per unit time (provided microplastic density was uniform or in groups of high concentrations, which could be determined via volume reduced sampling using a net and flowmeter (Stock et al., 2019)), and thus a higher number of microplastics overall, or even the force of the water could potentially dislodge more microplastics into its course (this researcher theorises). Further proof of this is Cemaes Bay providing a lower average number of microplastics (1.7 ± 0.29 L^{-1}) at a lower current speed (0.4-0.8 knots) than Penmon Point (average of 1.95 ± 0.27 microplastics L^{-1}, 0.7-1.2 knots). However, this is converse to Porth Dafarch, which showed a greater current speed (0.7-1.2 knots) than Cemaes Bay yet provided less microplastics on average (1.1 ± 0.13 L^{-1}) (CMBHA, 2020; CS&PF, 2020), though this could be explained due to other factors mentioned. A major reason is most likely the populations of Bangor and Menai Bridge, with Bangor's 2018 census showing a population of 18,709 (population density 4666/km²), compared to Anglesey's population of 4861 (population density 2,368/km²) (City Population, 2019), with a greater population creating a greater amount of waste (though to be sure, studies investigating microplastic/ plastic output into the seas/ water courses from certain areas would need to be conducted).

The samples analysed with the Fears (2021) method failed to account for levels of nanoplastics contaminating water. Nanoplastics are plastic particles only nanometres in size and thus invisible to the naked eye and extraordinarily difficult to view with a dissection microscope. One sample (Sample 6) from the Menai Strait - Bangor set was analysed at Bangor University School of Ocean Science, utilising a GXM L2800 Biological Upright Research Grade Microscope with fluorescence illuminator and camera adaptor, magnification 0.0151 nm per pixel. The analysis revealed a total of 448 nano/ microplastics L^{-1}, 56.28 x higher than the average of 7.96 microplastics L^{-1} found throughout the samples from the same set using a Dissection microscope with fluorescence, with a significant difference being present between the two analyses (Dissection Microscope $Mdn = 1.208$, GXM L28000 Biological Upright Research Grade Microscope $Mdn = 10$, $U = 25$, $z = 2.619$, $r = 0.828$, $p = 0.008$) (Figure 3). While not as much is known about nanoplastics as microplastics and macroplastics, there is

increasing interest of research into the causes, proliferance and effects of nanoplastic pollution, with the conclusion that they pose a significant threat to human health and the environment, particularly through the adsorption and ingestion of nanoplastics in (marine) organisms leading to bioaccumulation, bioamplification (de Costa et al., 2016), and interference with bodily tissues and processes (Claessens et al., 2013; Cole et al., 2013; Kooi et al., 2016; Ziccardi et al., 2016).

For future expansion of this study, a greater number of samples would be collected and analysed for each site to provide an even higher validity of data. A greater number of sites would also be surveyed (unfortunately, only 5 sites were able to be sampled at one time due to logistical issues, and the Covid-19 pandemic preventing further sample capture), and more sample sites at evenly spread and distant locations would provide a broader and more accurate record. Furthermore, samples could be taken from coastal waters around mainland North Wales (i.e., Gwynedd/ Caernarfonshire, Denbighshire, County Conwy, Flintshire, Wrexham) to provide an improved view of microplastics across the entirety of North Wales. Samples were taken from across the UK for studies on identifying microplastics with fluorescence dissection microscopy by Dunn et al. (2020) and Fears (2021), utilising the same method as this paper, and were found to have a microplastic presence at every site surveyed (the lowest being 2.33 ± 0.57 L^{-1} at Llyn Padrig), providing an impression of Anglesey being less polluted by microplastics than the rest of the country. Results gathered from Anglesey studies could be compared to nation-wide studies such as these to provide a clearer picture of the UK, or even to studies across various countries across the world to provide a global perspective. All the sites visited were public beaches bar Menai Strait – Menai Bridge, and as such are likely to have a microplastic presence as a result of secondary microplastics from littering, and thus breakdown of macroplastics and presence of fragments, plus primary microplastics via fibres from visitors' clothing (Boucher and Friot, 2017). Sampling at remote sites away from regular human contact would provide a clearer picture of the spread of microplastics to the natural environment. Usage of the GXM L2800 Biological Upright Research Grade Microscope with fluorescence illuminator and camera adaptor on all samples would also be useful in identifying the presence of nanoplastic particles and thus ascertain their spread across the Anglesey coast (this was not used for all the samples in this study due to the Covid-19 pandemic closing university facilities), and the implementation of chemospectrographic methods would ascertain to a much higher degree of accuracy in MP detection. The tracking of water vessels in the surrounding areas, current flows and tide count could also be measured at the time of sample creation for more accurate results. Finally, samples could be taken at multiple times through the year, and in varying weather conditions, to analyse the effects of seasonality and weather (samples in this

study were taken on a day with wind speeds reaching 39.149 mph (Time and Date, 2020), which could have affected the flow of the water sources and the microplastics within them). It must be noted that visual microscopy, even fluorescence microscopy will likely have a much lower accuracy than chemospectroscopy methods in identifying microplastics from samples, and so this researcher advises the usage of chemospectroscopy whenever possible (Fears, 2021).

5f. Appendix

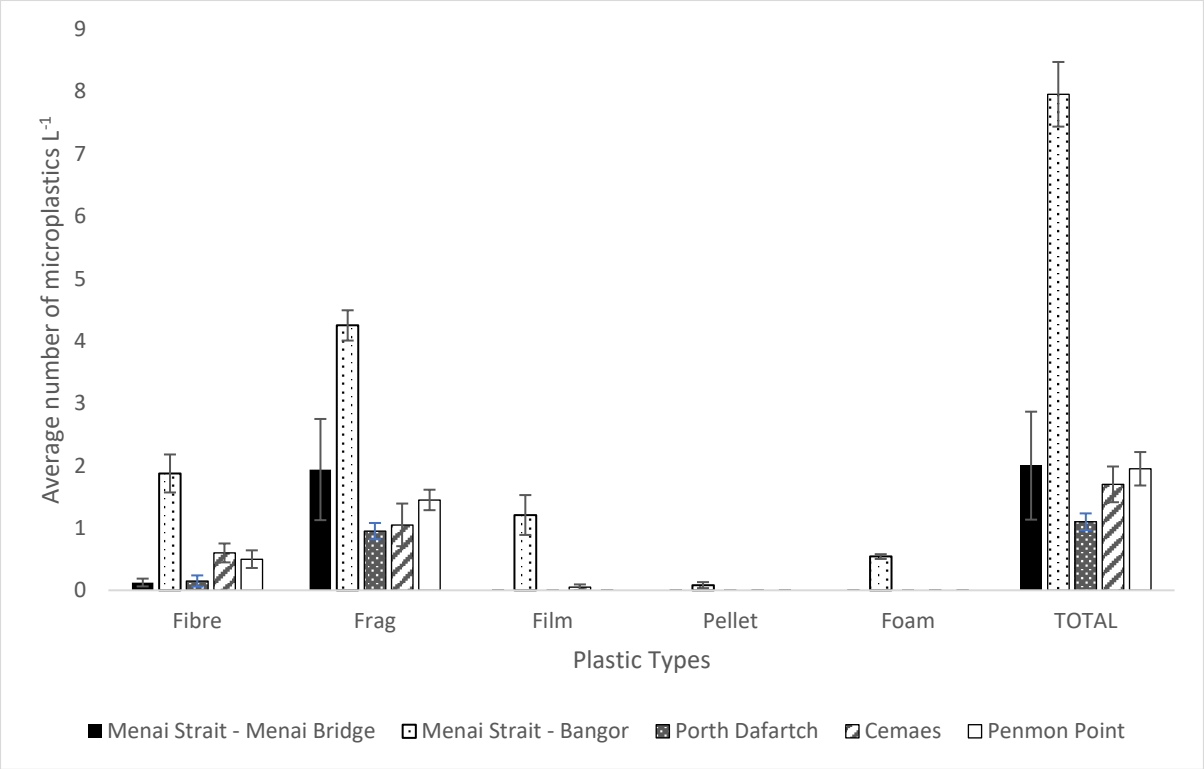

Figure 2. Average number of microplastics L^{-1} for each type of microplastic and total microplastics, minus controls for each site with standard error (black – Menai Strait (Menai Bridge), dotted – Menai Strait (Bangor), black speckled – Porth Dafarch, zig-zagged – Cemaes Bay, white – Penmon Point).

Table 2. Average Total Number of Microplastics L^{-1} adjusted for controls found from each sample site, with distance from said site from the closest residential/ commercial area.

Sample Site	Average Total Number of Microplastics	Distance from Closest Residential/ Commercial Area (metres)
Menai Strait - Menai Bridge	2	95
Menai Strait - Bangor	7.9583	110
Porth Dafarch	1.1	130
Cemaes Bay	1.7	58
Penmon Point	1.95	35

Table 3. Kruskal-Wallis Pairwise Comparisons of average microplastics L^{-1} between microplastic types, displaying Test Statistic, Standard Error, and Significance, for site Menai Strait – Menai Bridge.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Film-Fibre	4.000	3.680	.277
Pellet-Fibre	4.000	3.680	.277
Foam-Fibre	4.000	3.680	.277
Film-Fragment	13.500	3.680	.000
Pellet-Fragment	13.500	3.680	.000
Foam-Fragment	13.500	3.680	.000
Film-Pellet	.000	3.680	1.000
Film-Foam	.000	3.680	1.000
Pellet-Foam	.000	3.680	1.000
Fibre-Fragment	-9.500	3.680	.010

Table 4. Kruskal-Wallis Pairwise Comparisons of average microplastics L^{-1} between microplastic types, displaying Test Statistic, Standard Error, and Significance, for site Menai Strait – Bangor. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

Total N	30
Test Statistic	.452
Degree Of Freedom	5
Asymptotic Sig.(2-sided test)	.994

Table 5. Kruskal-Wallis Pairwise Comparisons of average microplastics L^{-1} between microplastic types, displaying Test Statistic, Standard Error, and Significance, for site Porth Dafarch.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Film-Fibre	4.000	3.682	.277
Pellet-Fibre	4.000	3.682	.277
Foam-Fibre	4.000	3.682	.277
Film-Fragment	13.500	3.682	.000
Pellet-Fragment	13.500	3.682	.000
Foam-Fragment	13.500	3.682	.000
Film-Pellet	.000	3.682	1.000
Film-Foam	.000	3.682	1.000
Pellet-Foam	.000	3.682	1.000
Fibre-Fragment	-9.500	3.682	.010

Table 6. Kruskal-Wallis Pairwise Comparisons of average microplastics L^{-1} between microplastic types, displaying Test Statistic, Standard Error, and Significance, for site Cemaes Bay.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Foam-Pellet	1.400	4.314	.746
Foam-Film	4.600	4.314	.286
Foam-Fibre	9.700	4.314	.025
Foam-Fragment	14.300	4.314	.001
Pellet-Film	3.200	4.314	.458
Pellet-Fibre	8.300	4.314	.054
Pellet-Fragment	12.900	4.314	.003
Film-Fibre	5.100	4.314	.237
Film-Fragment	9.700	4.314	.025
Fibre-Fragment	-4.600	4.314	.286

Table 7. Kruskal-Wallis Pairwise Comparisons of average microplastics L^{-1} between microplastic types, displaying Test Statistic, Standard Error, and Significance, for site Penmon Point.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Film-Fibre	8.300	3.987	.037
Pellet-Fibre	8.300	3.987	.037
Foam-Fibre	8.300	3.987	.037
Film-Fragment	14.200	3.987	.000
Pellet-Fragment	14.200	3.987	.000
Foam-Fragment	14.200	3.987	.000
Film-Pellet	.000	3.987	1.000
Film-Foam	.000	3.987	1.000
Pellet-Foam	.000	3.987	1.000
Fibre-Fragment	-5.900	3.987	.139

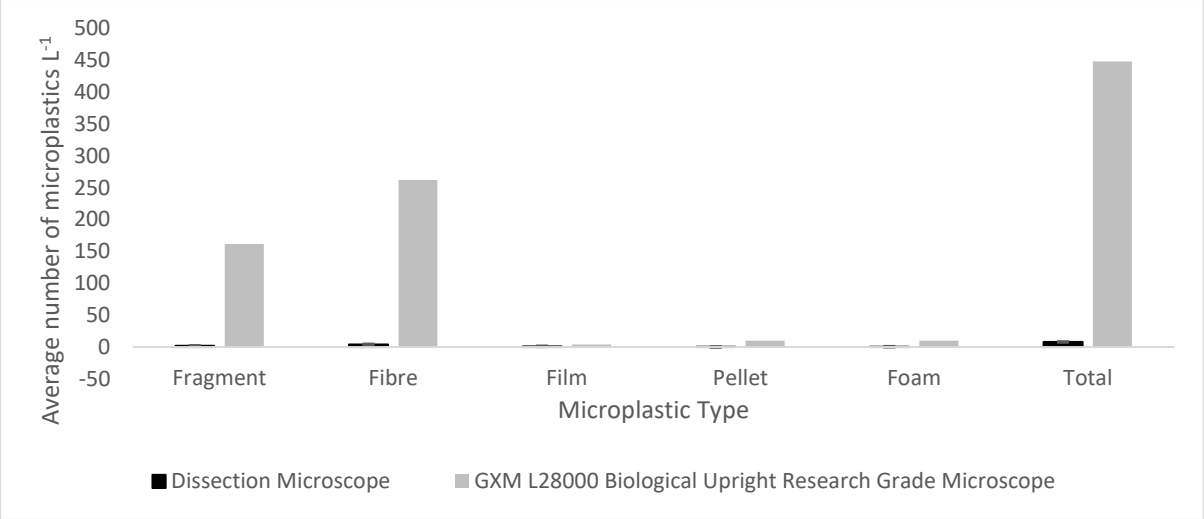

Figure 3. Average number of microplastics L^{-1} for each microplastic type and in total for the Menai Strait – Bangor site sample 6 for analysis by dissection microscope (black) and GXM L2800 Biological Upright Research Grade Microscope (grey).

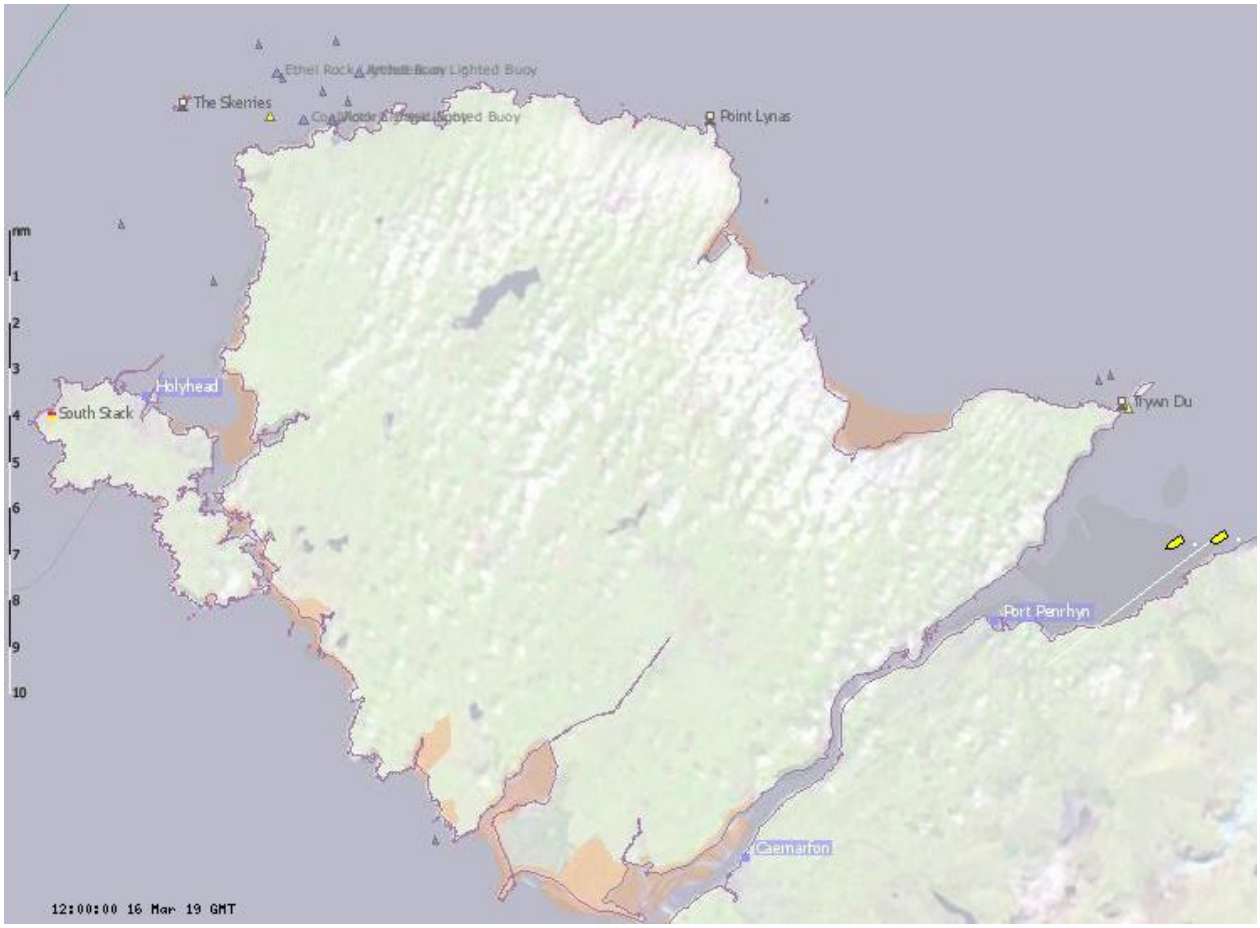

Figure 4. Presence of ships and vessels around Anglesey on day of sampling. Colour code: Green – Cargo, Red – Tanker, Blue – Passenger, White – **Hi Speed Craft**, Yellow – Unspecified/ Other, Black – Tug (ShipAIS, 2020).

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6. CTWs (Constructed Treatment Wetlands) as a Method of Removing Microplastics from Water

6a. Abstract

Efforts must be taken to investigate methods to reduce the proliferance of microplastics in the natural environment as potential mitigation strategies to hamper the consequences of MP pollution in a world where single use plastics are pandemic. One such method could be the utilisation of CTWs (Constructed Treatment Wetlands); a cheap, effective, and durable system already successfully being utilised to treat wastewater from industry, and commercial and residential areas. Previous studies have already shown promise, with high microplastic retention rates of CTWs being observed. This study aims to use small-scale CTWs to further bolster these results, prove that treatment works on a smaller scale, and distinguish the effectiveness between wetland habitats and their substrata at retaining microplastics. Four small wetland microcosms had two to four litres of custom microplastic-polluted water added to them daily (with ~500 microplastic particles per litre, initially starting with four litres/ ~2000 microplastics), and two litres of sample water were taken daily, which were then filtered and examined to determine the loss of microplastics. Control microcosms consisting of four empty microcosms and two microcosms consisting of the wetland pebble substrate underwent the same procedure. Significantly more microplastics (p <0.05) were found to be retained by the wetland treatments over the 15-day sampling period than the control and pebbles treatments, with the pebbles treatment having a higher retention rate than the plain water treatment. Though there was a significant difference (p <0.05) found between the wetland and water treatments on each day, no significant difference was found between the water and pebbles treatments on any day, and on many days (1-4, 13-15) there was no significant difference between the wetland and pebble treatments. These findings give credence to CTWs being effective at retaining microplastics, with the wetland plants/ habitat as a whole being the driving force behind microplastic retention.

5b. Introduction

Constructed treatment wetlands (CTWs) are wetland environments designed for wastewater treatment and engineered to be environmentally friendly by optimising natural process found in natural environments for increased sustainability. Wetland plants and the biofilms which grow on and around them neutralize harmful pathogens and pollutants (such as nitrogenous compounds, phosphorus, heavy metals) by their natural processes, making them a cheap, effective, and ecologically-conscious method of treating wastewater, particularly from industry and sewage (Kadlec & Wallace, 2008). One possible method of treating water for microplastic pollution is the usage of CTW environments to trap microplastic particles. Townsend et al. (2019) observed that surface-floating microplastics had a tendency to become trapped in the dense vegetation of wetlands in the Greater Melbourne Region in Victoria, Australia, and "a dominant share of the plastic supplied to the marine environment is retained nearshore in estuarine, beach, and wetland sediments, although the physical mechanisms of this process have not been investigated" (Townsend et al., 2019). Ziajahromi et al. (2020) found up to 203% more microplastics held within the sediment of floating constructed wetlands at the inlet than the outlet. Coalition Clean Baltic (2017) is one of the most comprehensive studies, finding that a variety of Wastewater Treatment Plant (WWTP) wetlands held microplastic retention rates of up to 100% for particles of varying shapes and sizes. Thus, the utilisation of wetlands holds potential for some level of wastewater or stormwater treatment. Despite these promising results, the study of CTW environments as potential mitigators of microplastic pollution has been very limited, and more research is required to further validate the effectiveness of CTWs in retaining microplastics, with a controlled influx of microplastics ruling out the effects of contamination. Furthermore, control samples featuring just the sediment of CTWs, and environments with no obstacles (i.e., free-standing water) can rule out the effect of the factor of buoyancy of microplastics and their microplastics settling into their environment in their retention. Moreover, Prati et al. (2019) found that MPs became sedimented in WWTPs, perhaps inferring that it is the sediment/ substrate of a WWTP/ CTW which is responsible for the retention of MPs. Testing CTW sediment without wetland biota can determine the efficiency of the CTW as a whole compared to CTWs devoid of plants and consisting of only substrate, in capturing microplastics. Also, this study will be on a much smaller scale and budget, using far more rudimentary methods than that of said previous studies, and thus aims to prove that these studies can be easily undertaken with this level of simplicity, and thus easy to replicate for mass-experimentation.

As such, this study aims to assess the ability of CTW environments to retain microplastic particles and determine if it is the CTW as a whole, its sediment only, or the natural buoyancy of microplastics which allow microplastics to be retained if at all. Moreover, this study will assess the feasibility of using

miniature CTW microcosms in microplastic retention studies of this nature for future usage. This study hypothesises that there will be a significant difference between the number of microplastics released from CTW, sediment, and water control samples over time. Furthermore, it is hypothesised that a loading potential will be reached, were a significant increase in the number of microplastics released occurs for all treatments.

Jedd Owens and I conducted the entirety of the setup and gathering and analysis of data equally, and he is writing his own study based on the data gathered. The results and conclusions in this study will be similar, if not possibly identical to those in Jedd Owen's paper, as he will be using the same data and methods of statistical analysis, and likely drawing similar conclusions as a result.

6c. Methodology

Treatment Water Creation:

Microplastic particles approximately 1-3 mm in length, 0.2 mm width, resembling the fibre and fragment types were created by finely-cutting up nets used to hold oranges. Approximately 500 microplastic particles were administered to each litre of water, ascertained by adding the average weight for 500 particles (0.100g) from three counts. Microplastics were added to 1 L amber bottles via a funnel, being washed into bottles via tap water; the remainder of the bottles were filled with tap water to create litre samples of microplastic-polluted water.

Wetland Unit Creation and Sampling:

Three treatment sets were utilised, each consisting of four (two in the case of the pebbles treatment) plastic 15 L garden troughs with a hole at the base, sealed with a rubber bung, to form a microcosm. The water treatment consisted of empty troughs. The wetland treatment consisted of *Typha latifolia* (common bulrush, common cattail) grown in a substrate consisting of pebbles and gravel, on top of which a biofilm formed, and represented a scaled-down version of CTWs popularly used, these CTWs themselves being generic and non-specific wetland types. The pebbles treatment consisted only of the substrate (pebbles and gravel, most likely quartzite, ranging from ~4 mm to 20 mm) utilised in the wetland microcosms. Unfortunately, only two pebbles microcosms were able to be utilised, due to un-sealable cracks forming from the bung holes of two of the microcosms. Microcosms were placed upon

thick polystyrene foam sheets to allow enough elevation from the ground to allow the sample collection bottles to be placed into position for sample capture. The sheets were replaced by wide, tall plastic rings on Day 8 (see Discussion as to why). Plain water microcosms were chosen to be studied to act as a control, to help determine if the settling and flotation of microplastics had any effect on the microcosms' ability to retain them. Ideally the placement of the microcosms would have been randomised, but the availability of space in the study area and the necessity to expose the wetland units to the sun whilst giving them sufficient cover from winds on the elevated study area (atop the roof of a 5 story building), plus the lack of materials with which to elevate the microcosms necessitated the bunching of said microcosms.

Prior to the first day of sampling, four litres (with ~2000 microplastics) of treatment water were added to each microcosm. Commencing with Day 1, two litres of sample water were extracted into 1 L amber bottles via the microcosm bungholes, which was replaced with two litres (with ~1000 microplastics) of treatment water. Unfortunately, due to several factors (please refer to Discussion), the total water level of each microcosm declined over time due to water loss, making it impossible to retrieve two litres of sample water from the wetland and pebble treatments by Day 4. To remedy this, three litres (with ~1500 microplastics) of treatment water were added daily after each sampling to the microcosms from Day 4. Two litres of sample water were still taken from the pebbles and wetland microcosms, while three litres were taken from each of the water microcosms daily from Day 7, as a result of a greater retention of water and microplastics (due to floating and lack of water loss) compared to the other treatments and risk of over-filling of microcosms. Samples were taken roughly around every 24 hours and pre-made treatment water was administered immediately after samples were taken and being analysed to allow it to settle. Sampling took place from Tuesday 24th September 2019 to Tuesday October 8th 2019. The number of sample litre bottles extracted from each treatment can be seen in Tables 2-4.

Figure 1. Wetland treatment microcosms (front) and pebble treatment microcosms (rear) atop a polystyrene base.

Sample Filtration and Analysis:

The method of filtration is detailed by Dunn et al. (2020) and Fears (2021b). Samples were filtered using a mechanical air pump and MilliporeSigma™ Glass Vacuum Filter Holder with Büchner flask through Whatman Grade GF/C glass filters. Samples were dried in an oven at 60°C overnight, before being examined under a dissection microscope and by eye sans-microscope (possible due to the bright coloration and obvious size/ presence of the MP particles) for microplastic particles. As only the microplastics specifically created for the study were counted, no distinction between types needed to be made, and the bright colours of the plastic against the white of the filters allowed for easy identification. Filtering was quick, with each litre sample taking on average 3 minutes to be filtered. Each filter took at least 2 minutes to look through, with more-densely packed filters taking up to an average of 10 minutes. Four blind counts were undertook on each filter, for an average of 8 to 40 minutes per filter in total. When counting the water treatment samples, frequently there would be too many microplastics to accurately visually analyse the complete number without spending an exorbitant amount of time on analysis, and so a cut-off of 500 particles was decided upon.

Contamination Control and Sample Protection:

As only bright orange and red microplastics (i.e., the ones created for the experiment) were to be counted, the need for the strict controls of experiments by Dunn et al. (2019) and Fears (2021b) was eliminated. However, a tin foil covering of the filtration cup was still utilised, as was parafilm to seal glass petri dish samples, to prevent loss of microplastics, and cotton lab coats and nitrile gloves were worn during sample filtration and analysis. Microcosms were kept outside, under roof-cover, with the wetland treatment being exposed to the sun.

Statistical Analysis:

Kolmogorov-Smirnov and Shapiro-Wilkes tests for normality were conducted. Non-normal distribution was found on virtually all days (normal distribution found for days 10 and 12 of the wetland samples). Levene's test of homogeneity tests found no equality of variance any sample (p <0.05). Independent samples Kruskal-Wallace tests were utilised to determine significant differences in microplastics released between treatments overall, and Kruskal-Wallis Pairwise Comparison tests were used to determine significant differences in microplastics released between days within and between treatments. Programmes used were Microsoft Word, Microsoft Excel and IBM SPSS Statistics 25.

6d. Results

A significant difference (p <0.05) in number of microplastics sampled was found overall between each treatment over the entire 15-day period (Wetland *Mdn* = 4.5, Pebbles *Mdn* = 71.125, Water *Mdn* 225.464, H (2) = 189.022) (Figure 3), with a mean difference of -225.25 microplastics L^{-1} between the wetland and water treatments, -87.45 L^{-1} microplastics between the wetland and pebble treatments, and -137.8 microplastics L^{-1} between the pebbles and water treatments (Table 5). There was a significant difference (p <0.05) between each of the treatments overall for each day, though there often was no significant difference (p >0.05) between the wetland and pebble treatments (days 1-4, 13-15), and to a greater extent the water and pebble treatments on every single day (Tables 9 to 24). As can be seen from Figure 2 and Table 1, the average number of microplastics released from the wetland treatments remained consistently low over the 15 days, peaking at 26.97 L^{-1} on Day 10, with a trough of 0.0 L^{-1} on Day 2, and finishing the experimental run releasing 2.53 L^{-1} on Day 15. The pebbles treatment showed an overall average increase from 27.06 L^{-1} on Day 1, to 139.67 L^{-1} on Day 5, before declining to 61.06 L^{-1} on Day 7, then increasing to 231.75 L^{-1} on Day 11, declining again to 62.94 L^{-1} on Day 14, before rising again to 81.31 L^{-1} on Day 15. Average number of microplastics released from the water treatment remained higher than the wetland and pebble treatments, save for Day 5 and Day 6 where numbers declined to lower than the pebbles treatment (troughing at 12.5 L^{-1} on Day 6), before rising to 413.79 L^{-1} on Day 15. Each of the treatments saw a significant difference (p <0.05) in number of microplastics over the 15 day period (Water - H (14) = 54.613, P <0.001, Pebbles - H (14) = 44.395, p <0.001, Wetland - H (14) = 54.613, p <0.001) (Figures 3 to 5), with the water treatment featuring significant differences most obviously between days 5 to 8 and 12 to 15, the pebbles treatment days 1 to 3 and days 8 to 12, and the wetland treatment on days 2 to 6 and 9 to 11. When comparing the first day to the final day, there was no significant difference for the water (p = 0.303) and wetland (p = 0.247) treatments, but there was for the pebbles treatment (p = 0.029) (Tables 6 to 8), indicating that the general outputs of the water and wetland treatments remained the same (and thus inferring the wetland treatment succeeding in retaining its input of microplastics significantly), whilst the pebbles treatment had a significantly greater output by the end, and thus failed in its mission to retain MPs.

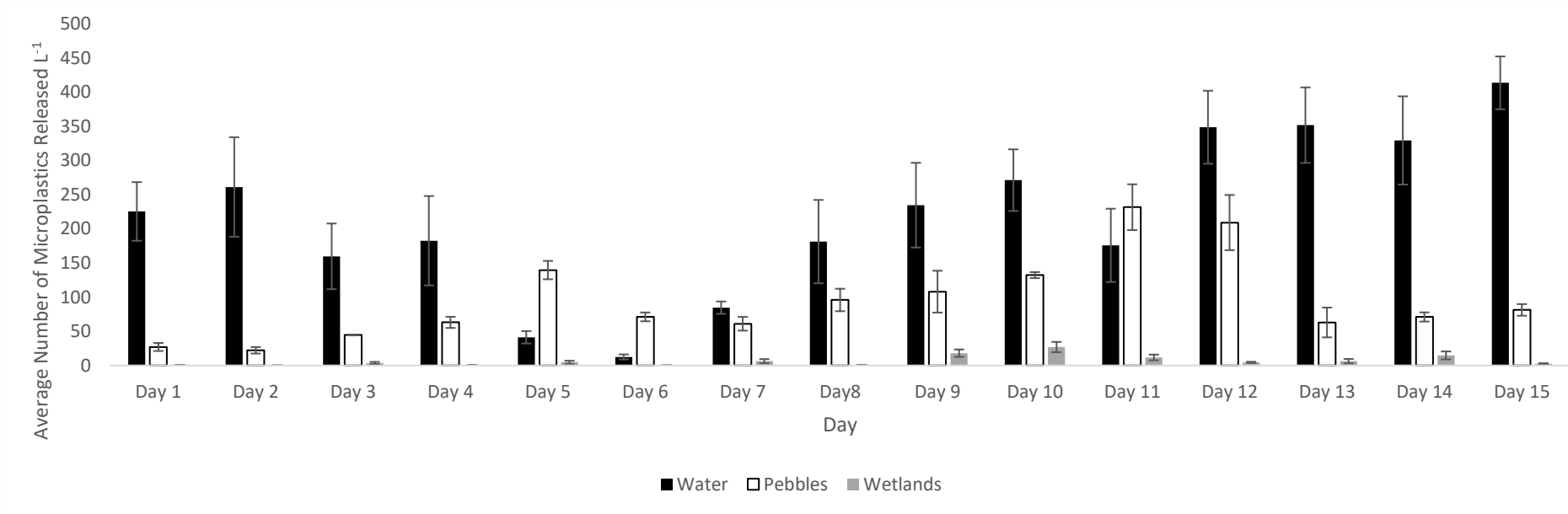

Figure 2. Average number of microplastics released L^{-1} from water (black), pebble (white) and wetland (grey) treatments per day, with standard error bars.

Table 1. Average number of microplastics L^{-1} released from each treatment per day with standard errors.

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
Water	225.46 ± 42.94	261.28 ± 72.87	159.75 ± 48.09	182.66 ± 65.45	41.25 ± 9.09	12.5 ± 3.67	84.6 ± 9.08	181.35 ± 61	234.71 ± 62.03	271.29 ± 45.12	175.85 ± 53.69	348.79 ± 53.43	351.85 ± 55.32	329.46 ± 64.58	413.79 ± 38.71
Pebbles	27.06 ± 5.89	22.19 ± 4.72	44.63 ± 0.63	63.08 ± 8.19	139.67 ± 13.46	71.13 ± 6.46	61.06 ± 10.02	95.88± 16.43	108.06 ± 30.63	132.25 ± 4.4194	231.75 ± 33.52	209.19 ± 40.41	62.94 ± 21.77	71 ± 6.76	81.31 ± 8.52
Wetland	0.44 ± 0.13	0 ± 0	3.63 ± 1.59	0.35 ± 0.19	4.66 ± 2.23	0.09 ± 0.06	6.25 ± 3.09	0.57 ± 0.28	18 ± 5.4	26.97 ± 7.53	11.719 ± 4.23	4.5 ± 0.87	6.28 ± 3.29	14.69 ± 5.82	2.53 ± 0.74

6e. Discussion

Due to the significant difference (p <0.05 (Figure 2, Table 5)) between the wetland, pebbles and water treatments, with the wetland treatment showing consistent lower yields of microplastics than the others (Figure 1, Table 1 to 23), it can be determined that the CTW environments successfully captured microplastic pollution from the treatment water and could potentially be an effective method of treating water microplastic pollution. Furthermore, this also proves that a wetland unit as a whole, or in the very least *Typha latifolia* is far more effective at filtering microplastics than the pebble substrate alone, though the pebble substrate was still somewhat effective in retaining microplastics when compared to the control water treatment, as is observable from the significant difference (p <0.05) between the treatments, and from lower levels of microplastic expulsion over the majority of the sample days (Figure 1, Table 1 to 23). The success of this study also proves that this scaled-down methodology is effective at producing valid, replicable results.

Unfortunately, only two out of the four pebbles microcosms created could be used as a result of cracks around the bungholes which allowed water to escape, despite efforts to fix the issue, and as replacements could not be found on time, the study went ahead using only two working pebbles microcosms. One of these microcosms too had a crack in the base, but it was successfully sealed via the application of Flex Tape. Utilising only two pebbles microcosms compared to the four of the other treatments likely marginally reduced the validity of the pebbles data, affecting the standard error and thus the robustness of statistical analyses, so for future studies, fluid loss tests will need to be conducted to ensure there are no leakages in a timely manner to allow the successful repair or replacement of leaking microcosms.

Some water from each microcosm was lost when taking samples in between removing the microcosm plug and adjusting the sample bottle nape to the flow of the water, and also as a result of wind blowing water away when trying to obtain a sample of windy days. This loss was accounted for by the addition of extra water from Day 4 (also because of loss of water due to environmental factors); an extra litre per microcosm for a total of three litres. A potential future method to prevent sample loss would be to utilise a faucet in place of a bung, which could easily be turned on and off once the sample collection bottle is in place. However, this runs the risk of microplastics becoming lodged within the faucet and around its exterior extending into the microcosm from its bunghole, leading to underestimation of microplastic emission.

Environmental conditions caused by the weather were also observed to have an effect upon the level of treatment water administered to the microcosms. The wetland treatment microcosms were left exposed to the sun to allow sunlight to maintain the health of the bulrush, and the pebbles treatment

microcosms were slightly more sheltered under a section of roof behind the wetland microcosms due to spacing issues, though still exposed to the elements. Exposure to the sun, and wind speeds of up to 30 mph (Time and Date, 2020) caused a noticeable decline in water levels between the administering of treatment water and the taking of samples (though uptake of water by the bulrush could also be a factor (Baeza et al., 2009)), leading to an increasing difficulty in obtaining two litres from each microcosm. Conversely, the water treatment microcosms were sheltered under a part of the roof and saw a much smaller drop in overall water levels, even after experiencing windy days. To remedy this, from Day 4, three litres were added to each microcosm of each treatment after samples were taken, including to the water microcosms to keep the influx of microplastics constant between all treatments. Adding the addition water ensured that the visible water line remained the same level as prior to Day 1 of sample collection and allowed for the collection of at least two litres of sample water from all microcosms. For future replications of this study, the locations of the microcosms would be randomised or calculated to mitigate the effect environmental conditions would have upon the microcosms, or to monitor how said environmental conditions would affect the microcosms specifically.

Unfortunately, with the addition of an extra litre to the water treatment microcosms per day, little of which were lost due to environmental factors, and the removal of only two litres of water per day, the water level within the water microcosms began to rise, and risked spilling over. Furthermore, all the microplastics in the water microcosms were observed to float, keeping them from being collected during sampling, and causing counts to decline (Days 5 and 6 on Figure 2, Tables 1 and 4). To remedy this, from Day 7, three litres of sample water were taken from each water treatment microcosm, allowing for the capture of microplastics and preventing over-spill (Days 7 to 15 in Figure 2, Tables 1 and 4).

On the morning of Day 8, it was discovered that the wetland and pebbles microcosms had been disturbed and some wetland and pebbles microcosms tipped, as the polystyrene base on which the microcosms were elevated upon was stolen. As such, tall and wide plastic rings were found and used to uplift the microcosms for the rest of the experiment. This seemed to have had little effect on the pebbles microcosm, which saw an increase of 34.81 microplastics counted from the day before but could have resulted in the significant decline of -5.68 microplastics in Day 8 from Day 7, (p = 0.043 (Table 8)).

The microplastics in question utilised in the study were created by hand by cutting up polyethylene and/ or polypropylene plastic netting, and as a result the microplastics varied greatly in size, length and shape, with some probable instances of plastic particles being cut longer than 5mm, making them

macroplastics by definition. In future studies, it could prove prudent for microplastics to instead be purchased from manufacturers, which would hold a greater likelihood of uniformity of type and size, which could enable studies into the efficiency of CTWs upon retaining each different type of microplastic, or even different chemical compositions of plastic, e.g., polystyrene, PVC, etc.

Typha latifolia was utilised in the wetland microcosms due to its proliferation in various forms of wetland habitats across the globe, making it perfect for a simulation of a general CTW. Pebbles were also used as a substrate as they would allow the *Typha* to grow and survive and are commonly utilised within CTWs (Kadlec & Wallace, 2008). However, *T. latifolia* is not the only plant found in wetlands, which vary in type dependent upon the plants, sediment, and water type. For future studies, a variety of simulation wetlands could be constructed within microcosms to test their effectiveness in removing microplastics, e.g., mangrove plants and saltwater for mangrove forests, *Sphagnum* moss and peat for peat bogs, grasses, sedges and mineral-rich-lower/ medium pH soil for fens (Finlayson, Milton & Prentice, 2018).

There are numerous different water delivery and flow systems for CTWs. For example, vertical flow, horizontal flow, and French vertical flow (in which water is administered to the wetland via inlet pipes within the sediment). For this study, water was delivered to the microcosms via being poured from amber bottles directly onto the surface of the substrate, and thus reflects the surface flow wetland type, in which water flows into the wetland via the wetland surface rather than into the substrate, but as the water samples were extracted from the base of the microcosm, it also resembled a vertical flow wetland (Kadlec & Wallace, 2008). For future studies, various other methods of water flow/ insertion could be utilised to determine if they had an effect on microplastic retention.

Each of the microcosms were held within a fifteen-litre gardening trough, with pebble substrates reaching ~40 cm in height. As such, it is not known if the size and shape of the microcosms, as well as the quantity of substrate and/or wetland plants had any effect upon microplastic retention. Future studies could vary in the shape and size of microcosm units, as well as the amount of substrate utilised to grow varying number so plants, to determine if this has any effect upon microplastic retention by the wetlands. The microcosms used for this study were only small representations of CTWs, and advancing on from using larger experimental microcosms, an even more effective method of determining CTW effectiveness would be to create a new CTW from scratch to test its microplastic retention ability, or alternatively retrofit and repair an existing natural wetland in poor condition into a CTW, or even use the multitude of CTWs already in use around the world for industry, farming and wastewater management.

A factor which would account for the trends in the number of microplastics released from each treatment could be the treatment's loading potential or saturation point; the point at which the microcosm becomes inundated with microplastics so that an equal quantity of, if not more microplastics are emitted from the microcosm than are currently being inputted, or in simpler terms the maximum load the microcosm can hold before large constant levels of microplastic excretion (Li et al., 2021). This is reflected by the trends in microplastic release for the water treatment. Due to the floating nature of microplastics and the input of water exceeding the output from Day 4 to Day 6, numbers of microplastics declined from 182.66 L^{-1} on Day 4 to 12.5 L^{-1} on Day 6, but after the removal of three litres of sample water each day (but still an excess of microplastics entering the system than exiting), the numbers of microplastics released increased, peaking at 413.79 L^{-1} on Day 15, as the microcosms got closer to loading potential. As the peak was on the final day, it is possible that the loading potential had not been reached yet, though a longer study period would shed light on this. Conversely, the pebbles treatment showed the saturation point to potentially be reached multiple times, with two and a half cycles of microplastic expulsion reaching a peak then troughing. Microplastic release rose to 139.67 L^{-1} on Day 5, before declining to 61.06 L^{-1} on Day 7, then peaking at 231.75 L^{-1} on Day 11, then declining to 62.94 L^{-1} on Day 13 and beginning to rise again before the study ended on Day 15 with 81.31 microplastics L^{-1} released. The two peaks could likely be where the loading potential for the system was hit, releasing its max yield for four litres at that point in time, before declining to a trough level of microplastic expulsion. As the second peak on Day 11 was significantly (p <0.001) higher by 92.08 microplastics L^{-1} than the first peak on Day 5, it can be inferred that the loading potential of the microcosm increased as microplastics continued to be added. However, this could also be an effect of the increase in treatment water to the microcosms from Day 4, causing an increase in overall levels of microplastics and water, thus allowing more to be expulsed. However, this likely would not account for the rise, trough, and secondary rise of microplastic expulsion from Day 8 (although the day 8 increase in microplastic effluent could be accounted for by the upsetting of the treatments, unsettling the microcosms and allowing for more MPs to be expunged). It must also be noted that there was no immediate drop off from peak microplastic release to trough microplastic release the next day, with a number of microplastics lower than the peak but higher than the trough the day after peaking; this could be a natural phenomenon of the microcosm releasing excess microplastics from when the saturation point was reached. It is likely that the loading potential of the wetland treatment was not reached at any point over the study, due to the consistent low levels of microplastic expulsion (peaking at 26.97 microplastics L^{-1} on Day 10, when ~5600 microplastics were introduced overall into each microcosm). As such it can be fully determined that

CTWs with plants rather than just substrate have a higher retention rate and loading potential than those without plants.

An issue leftover from the experiment was the presence of microplastics still remaining within the wetland microcosms. It is not known whether the presence of microplastics would have any long-lasting or short-term effects upon the health of the CTWs, both on the plants themselves and other biota associated with the CTWs. As such, further study onto the effect of microplastics upon wetland plants, biota, and natural processes need to be undertaken to determine if the positives of using CTWs to remove microplastics outweigh the negatives. There are however numerous past, current, and future investigations into microplastic disposal and elimination, such as the usage of the bacterium *Ideonella sakaiensis* to breakdown PET (Yoshida et al.,2016), and the usage of Ferrofluid to attract microplastics (Bendix, 2019), which could potentially be used hand-in-hand with CTWs as an effective method for completely eradicate trapped microplastics. Microplastic pollution in coastal and freshwater environments is becoming an increasing concern, with microplastics being found in freshwater bodies across the length of Great Britain, even in remote areas (Dunn et al., 2020; Fears, 2021b), and even in coastal areas which have low levels of pollution in other factors, such as around Anglesey, North Wales (Fears, 2021a). As such, there is the potential to utilise CTWs to help alleviate the presence of these microplastics from these large bodies of water. One solution could be to strategically place CTWs at water flow inputs to these areas, i.e., around the banks of rivers, or even in their centres provided CTWs were strong enough to resist flow rates, preventing microplastics from polluting further downstream. CTWs could also potentially be placed around the banks of these affected large water bodies to remove microplastics travelling via the flow of the water. Floating CTWs have already been proven as a sustainable method of treating waste and stormwater for contaminants by their natural biological processes (Shahid et al., 2018), and so could be placed more centrally in large water bodies to not only treat water for microplastics, but other contaminants. CTWs have already been proven to be proficient at removing pollutants from brackish water (Shi et al., 2011) and so could be utilised to treat brackish water systems by coastal areas. With wetland areas such as mangrove swamps found around brackish and saline environments such as the Florida peninsula and Keys (Office of Resilience and Coastal Protection, 2019), there is also the potential for saltwater-based CTWs to function as a method of trapping microplastics, although further testing is required to determine the effectiveness of these specific wetland types.

As this study has shown that CTWs with plants have a higher loading potential than those without plants, there is a great deal of potential for investigating the efficiencies of different plants and mixtures of plants in retaining microplastics; while this study used *Typha latifolia*, common in British wetlands and CTWs, other common forms of plants such as *Sphagnum* (peat moss), sedges, marsh

grasses (e.g., *Spartina anglica*, or common cord grass) and various other species of reeds like *Typha* could be utilised to determine their CTW saturation points.

6f. Appendix

Table 2. Number of microplastics released L^{-1} from wetland treatment, per day, including sample averages. Red N/A panels indicate when samples were unable to be taken.

Sample No.	Number of microplastics released per sample per day														
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
1	1	0	3	0	1	0	3	1	54	11	2	5	26	45	0
1	1	0	3	0	1	1	3	1	49	11	2	5	25	45	0
1	0	0	1	0	2	0	3	1	52	11	2	5	24	45	0
1	0	0	3	0	1	0	3	1	62	11	2	5	25	45	0
AVERAGE	0.5	0	2.5	0	1.25	0.25	3	1	54.25	11	2	5	25	45	0
2	0	0	0	0	0	0	6	2	25	20	0	6	23	17	3
2	0	0	0	0	0	0	6	2	23	20	0	6	20	17	3
2	0	0	0	0	0	0	4	2	20	18	0	6	18	18	2
2	0	0	0	0	0	0	4	2	23	18	0	6	16	19	2
AVERAGE	0	0	0	0	0	0	5	2	22.75	19	0	6	19.25	17.75	2.5
3	0	0	1	0	12	0	1	0	9	34	26	7	1	2	0
3	0	0	1	0	14	0	1	0	9	34	26	7	1	2	0
3	2	0	3	0	11	0	1	0	9	28	26	7	1	2	0
3	1	0	1		4	0	1	0	9	31	26	7	1	2	0
AVERAGE	0.75	0	1.5	0	10.25	0	1	0	9	31.75	26	7	1	2	0
4	0	0	0	1	21	0	6	0	19	2	11	2	3	3	5
4	0	0	0	1	19	0	6	0	21	2	11	2	3	3	5
4	0	0	0	1	20	0	4	0	21	2	11	2	3	3	5
4	0	0	0	1	17	0	6	0	17	2	11	2	3	3	5
AVERAGE	0	0	0	1	19.25	0	5.5	0	19.5	2	11	2	3	3	5
5	0	0	6	1	0	0	5	0	18	3	5	4	0	2	0
5	0	0	7	0	0	0	5	0	18	3	5	4	0	2	0
5	4	0	20	1	0	0	6	0	18	3	5	4	0	2	0
5	0	0	8	1	0	0	6	0	18	3	5	4	0	2	0
AVERAGE	1	0	10.25	0.75	0	0	5.5	0	18	3	5	4	0	2	0
6	0	0	6	N/A	2	0	0	0	11	36	7	8	0	0	5
6	0	0	12	N/A	4	0	0	0	11	36	7	8	0	0	5
6	3	0	6	N/A	4	2	0	0	13	36	7	8	0	0	5
6	0	0	6	N/A	1	0	0	0	11	36	7	8	0	0	5
AVERAGE	0.75	0	7.5	N/A	2.75	0.5	0	0	11.5	36	7	8	0	0	5
7	0	N/A	N/A	N/A	2	0	0	1	0	45	31	4	1	38	4
7	0	N/A	N/A	N/A	1	0	5	1	0	45	31	4	1	38	4
7	0	N/A	N/A	N/A	1	0	0	1	0	45	31	4	1	38	4
7	0	N/A	N/A	N/A	0	0	0	1	0	45	31	4	1		4
AVERAGE	0	N/A	N/A	N/A	1	0	1.25	1	0	45	31	4	1	38	4
8	0	N/A	N/A	N/A	2	0	28	N/A	9	68	N/A	0	1	10	3
8	0	N/A	N/A	N/A	3	0	32	N/A	9	68	N/A	0	1	9	4
8	0	N/A	N/A	N/A	3	0	26	N/A	9	68	N/A	0	1	9	4
8	2	N/A	N/A	N/A	3	0	29	N/A	9	68	N/A	0	1	11	4
AVERAGE	0.5	N/A	N/A	N/A	2.75	0	28.75	N/A	9	68	N/A	0	1	9.75	3.75

Table 3. Number of microplastics released L^{-1} from pebbles treatment, per day, including sample averages. Red N/A panels indicate when samples were unable to be taken.

Sample No.	Number of microplastics released per sample per day														
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
1	38	35	49	49	116	31	25	79	126	133	296	316	38	87	51
1	42	32	44	49	155	95	39	83	128	121	304	334	34	94	51
1	42	31	46	42	96	70	48	85	115	109	265	349	40	92	51
1	41	34	42	37	63	61	54	86	120	146	313	349	39	80	56
AVERAGE	40.75	33	45.25	44.25	107.5	64.25	41.5	83.25	122.25	127.25	294.5	337	37.75	88.25	52.25
2	40	14	41	84	191	38	44	152	65	123	333	132	44	55	90
2	39	14	44	84	155	66	56	117	65	139	272	132	44	55	96
2	34	14	52	67	143	64	69	176	68	130	291	137	44	55	102
2	34	16	33	79	159	56	33	155	67	127	314	144	44	55	92
AVERAGE	36.75	14.5	42.5	78.5	162	56	50.5	150	66.25	129.75	302.5	136.25	44	55	95
3	18	25	44	79	128	114	55	82	36	143	164	149	32	59	89
3	17	30	47	75	136	81	65	78	40	149	160	139	32	64	84
3	14	31	42	61	171	81	56	94	54	159	148	141	32	62	87
3	14	34	50	51	163	87	56	101	39	138	156	144	32	59	87
AVERAGE	15.75	30	45.75	66.5	149.5	90.75	58	88.75	42.25	147.25	157	143.25	32	61	86.75
4	15	14	52	N/A	N/A	57	71	69	198	128	174	221	134	86	92
4	15	11	40	N/A	N/A	62	85	54	205	127	172	245	145	82	95
4	15	9	44	N/A	N/A	97	103	67	214	119	184	201	141	77	82
4	15	11	44	N/A	N/A	78	118	56	189	125	162	214	132	74	96
AVERAGE	15	11.25	45	N/A	N/A	73.5	94.25	61.5	201.5	124.75	173	220.25	138	79.75	91.25

Table 4. Number of microplastics L^{-1} released from water treatment, per day, including sample averages. Blue panels stating 500. Red N/A panels indicate when samples were unable to be taken. Blue 500 panels represent when counts were cut off at 500 particles.

Sample No.	Number of microplastics released per sample per day														
	DAY 1	DAY 2	DAY 3	DaY 4	DAY 5	DAY 6	DAY 7	Day 8	Day 9	day 10	Day 11	Day 12	Day 13	Day 14	Day 15
1	155	500	92	500	47	4	111	500	53	500	500	61	500	57	500
1	149	500	87	500	47	4	121	500	53	500	500	61	500	58	500
1	154	500	73	500	36	4	118	500	53	500	500	61	500	58	500
1	161	500	77	500	48	4	142	500	53	500	500	61	500	58	500
AVERAGE	154.75	500	82.25	500	44.5	4	123	500	53	500	500	61	500	57.75	500
2	157	36	116	500	86	6	44	1	500	256	42	500	68	500	500
2	183	34	118	500	88	6	44	1	500	231	42	500	68	500	500
2	185	37	108	500	90	6	44	1	500	266	42	500	68	500	500
2	189	39	98	500	95	6	44	1	500	249	42	500	68	500	500
AVERAGE	178.5	36.5	110	500	89.75	6	44	1	500	250.5	42	500	68	500	500
3	159	234	186	32	40	9	35	2	500	73	63	500	500	18	500
3	149	274	207	34	46	9	35	2	500	89	62	500	500	18	500
3	164	280	216	24	42	9	35	2	500	76	63	500	500	18	500
3	158	168	183	24	35	9	35	2	500	75	63	500	500	18	500
AVERAGE	157.5	239	198	28.5	40.75	9	35	2	500	78.25	62.75	500	500	18	500
4	207	29	143	106	29	1	60	5	500	232	500	500	500	17	500
4	199	29	138	98	35	1	60	5	500	244	500	500	500	17	500
4	203	29	141	113	40	1	60	4	500	265	500	500	500	17	500
4	190	29	143	59	42	1	60	4	500	221	500	500	500	17	500
AVERAGE	199.75	29	141.25	94	36.5	1	60	4.5	500	240.5	500	500	500	17	500
5	500	500	54	110	47	35	71	2	168	20	55	500	500	500	500
5	500	500	45	96	45	35	71	2	172	20	62	500	500	500	500
5	500	500	56	74	45	39	71	2	163	20	72	500	500	500	500
5	500	500	56	47	46	30	71	2	164	20	60	500	500	500	500
AVERAGE	500	500	52.75	81.75	45.75	34.75	71	2	166.75	20	62.25	500	500	500	500
6	166	274	139	73	64	11	42	78	17	22	68	87	500	142	500
6	176	278	137	74	48	11	42	77	17	22	72	83	500	149	500
6	183	271	120	89	76	14	42	88	17	22	81	84	500	132	500
6	187	284	136	72	55	19	42	88	17	22	73	84	500	137	500
AVERAGE	178	276.75	133	77	60.75	13.75	42	82.75	17	22	73.5	84.5	500	140	500
7	201	500	60	128	8	22	80	2	70	117	71	500	46	500	91
7	213	500	48	112	7	21	80	2	63	122	71	500	46	500	91
7	210	500	69	138	11	25	80	2	76	119	74	500	53	500	98
7	215	500	66	127	11	21	76	2	83	119	74	500	44	500	98
AVERAGE	209.75	500	60.75	126.25	9.25	22.25	79	2	73	119.25	72.5	500	47.25	500	94.5
8	N/A	9	500	80	2	8	118	500	500	25	120	500	500	500	500
8	N/A	9	500	87	3	8	109	500	500	25	127	500	500	500	500
8	N/A	9	500	25	3	8	128	500	500	25	143	500	500	500	500
8	N/A	9	500	23	3	13	115	500	500	25	136	500	500	500	500
AVERAGE	N/A	9	500	53.75	2.75	9.25	117.5	500	500	25	131.5	500	500	500	500
9	N/A	N/A	N/A	N/A	N/A	N/A	181	500	143	500	500	18	83	213	88
9	N/A	N/A	N/A	N/A	N/A	N/A	209	500	150	500	500	18	83	228	88
9	N/A	N/A	N/A	N/A	N/A	N/A	208	500	137	500	500	18	83	233	88
9	N/A	N/A	N/A	N/A	N/A	N/A	199	500	142	500	500	18	83	209	88
AVERAGE							199.25	500	143	500	500	18	83	220.75	88
10	N/A	N/A	N/A	N/A	N/A	N/A	62	35	54	500	60	500	500	500	281
10	N/A	N/A	N/A	N/A	N/A	N/A	62	28	54	500	60	500	500	500	303
10	N/A	N/A	N/A	N/A	N/A	N/A	62	39	55	500	63	500	500	500	275
10	N/A	N/A	N/A	N/A	N/A	N/A	62	38	56	500	62	500	500	500	273
AVERAGE							62	35	54.75	500	61.25	500	500	500	283
11	N/A	N/A	N/A	N/A	N/A	N/A	38	46	98	500	54	22	500	500	500
11	N/A	N/A	N/A	N/A	N/A	N/A	38	41	114	500	54	22	500	500	500
11	N/A	N/A	N/A	N/A	N/A	N/A	38	50	109	500	54	22	500	500	500
11	N/A	N/A	N/A	N/A	N/A	N/A	38	51	101	500	54	22	500	500	500
AVERAGE							38	47	105.5	500	54	22	500	500	500
12	N/A	N/A	N/A	N/A	N/A	N/A	145	500	203	500	52	500	24	500	500
12	N/A	N/A	N/A	N/A	N/A	N/A	144	500	218	500	52	500	24	500	500
12	N/A	N/A	N/A	N/A	N/A	N/A	151	500	199	500	52	500	24	500	500
12	N/A	N/A	N/A	N/A	N/A	N/A	138	500	194	500	46	500	24	500	500
AVERAGE							144.5	500	203.5	500	50.5	500	24	500	500

Table 5. Kruskal-Wallis Pairwise comparison comparing overall significant difference in average microplastics L^{-1} over the course of the study, featuring Test statistic, Standard error and Significance.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Wetland-Pebbles	131.520	15.129	.000
Wetland-Water	155.751	11.611	.000
Pebbles-Water	24.231	14.373	.092

Table 6. Kruskal-Wallis Pairwise Comparisons of average number of microplastics L^{-1} between each sampling day for water treatment, featuring Test Statistic, Standard Error and Significance.

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.
6.00-5.00	21.625	21.937	.986	.324
6.00-8.00	-42.646	20.026	-2.130	.033
6.00-7.00	-43.646	20.026	-2.179	.029
6.00-11.00	-58.937	20.026	-2.943	.003
6.00-4.00	61.938	21.937	2.823	.005
6.00-3.00	63.375	21.937	2.889	.004
6.00-2.00	67.813	21.937	3.091	.002
6.00-9.00	-71.354	20.026	-3.563	.000
6.00-10.00	-73.437	20.026	-3.667	.000
6.00-1.00	81.634	22.707	3.595	.000
6.00-14.00	-83.062	20.026	-4.148	.000
6.00-12.00	-85.479	20.026	-4.268	.000
6.00-13.00	-87.896	20.026	-4.389	.000
6.00-15.00	-103.146	20.026	-5.151	.000
5.00-8.00	-21.021	20.026	-1.050	.294
5.00-7.00	-22.021	20.026	-1.100	.271
5.00-11.00	-37.312	20.026	-1.863	.062
5.00-4.00	40.313	21.937	1.838	.066
5.00-3.00	41.750	21.937	1.903	.057
5.00-2.00	46.188	21.937	2.105	.035
5.00-9.00	-49.729	20.026	-2.483	.013

5.00-10.00	-51.812	20.026	-2.587	.010
5.00-1.00	60.009	22.707	2.643	.008
5.00-14.00	-61.437	20.026	-3.068	.002
5.00-12.00	-63.854	20.026	-3.189	.001
5.00-13.00	-66.271	20.026	-3.309	.001
5.00-15.00	-81.521	20.026	-4.071	.000
8.00-7.00	1.000	17.912	.056	.955
8.00-11.00	-16.292	17.912	-.910	.363
8.00-4.00	19.292	20.026	.963	.335
8.00-3.00	20.729	20.026	1.035	.301
8.00-2.00	25.167	20.026	1.257	.209
8.00-9.00	-28.708	17.912	-1.603	.109
8.00-10.00	-30.792	17.912	-1.719	.086
8.00-1.00	38.988	20.866	1.868	.062
8.00-14.00	-40.417	17.912	-2.256	.024
8.00-12.00	-42.833	17.912	-2.391	.017
8.00-13.00	-45.250	17.912	-2.526	.012
8.00-15.00	-60.500	17.912	-3.378	.001
7.00-11.00	-15.292	17.912	-.854	.393
7.00-4.00	18.292	20.026	.913	.361
7.00-3.00	19.729	20.026	.985	.325
7.00-2.00	24.167	20.026	1.207	.228
7.00-9.00	-27.708	17.912	-1.547	.122
7.00-10.00	-29.792	17.912	-1.663	.096
7.00-1.00	37.988	20.866	1.821	.069
7.00-14.00	-39.417	17.912	-2.201	.028
7.00-12.00	-41.833	17.912	-2.336	.020
7.00-13.00	-44.250	17.912	-2.470	.013
7.00-15.00	-59.500	17.912	-3.322	.001
11.00-4.00	3.000	20.026	.150	.881
11.00-3.00	4.438	20.026	.222	.825
11.00-2.00	8.875	20.026	.443	.658
11.00-9.00	12.417	17.912	.693	.488
11.00-10.00	14.500	17.912	.810	.418
11.00-1.00	22.696	20.866	1.088	.277
11.00-14.00	-24.125	17.912	-1.347	.178
11.00-12.00	-26.542	17.912	-1.482	.138
11.00-13.00	-28.958	17.912	-1.617	.106
11.00-15.00	-44.208	17.912	-2.468	.014
4.00-3.00	1.438	21.937	.066	.948
4.00-2.00	5.875	21.937	.268	.789
4.00-9.00	-9.417	20.026	-.470	.638
4.00-10.00	-11.500	20.026	-.574	.566
4.00-1.00	19.696	22.707	.867	.386
4.00-14.00	-21.125	20.026	-1.055	.291
4.00-12.00	-23.542	20.026	-1.176	.240
4.00-13.00	-25.958	20.026	-1.296	.195
4.00-15.00	-41.208	20.026	-2.058	.040

3.00-2.00	4.438	21.937	.202	.840
3.00-9.00	-7.979	20.026	-.398	.690
3.00-10.00	-10.062	20.026	-.502	.615
3.00-1.00	18.259	22.707	.804	.421
3.00-14.00	-19.687	20.026	-.983	.326
3.00-12.00	-22.104	20.026	-1.104	.270
3.00-13.00	-24.521	20.026	-1.224	.221
3.00-15.00	-39.771	20.026	-1.986	.047
2.00-9.00	-3.542	20.026	-.177	.860
2.00-10.00	-5.625	20.026	-.281	.779
2.00-1.00	13.821	22.707	.609	.543
2.00-14.00	-15.250	20.026	-.762	.446
2.00-12.00	-17.667	20.026	-.882	.378
2.00-13.00	-20.083	20.026	-1.003	.316
2.00-15.00	-35.333	20.026	-1.764	.078
9.00-10.00	-2.083	17.912	-.116	.907
9.00-1.00	10.280	20.866	.493	.622
9.00-14.00	-11.708	17.912	-.654	.513
9.00-12.00	-14.125	17.912	-.789	.430
9.00-13.00	-16.542	17.912	-.924	.356
9.00-15.00	-31.792	17.912	-1.775	.076
10.00-1.00	8.196	20.866	.393	.694
10.00-14.00	-9.625	17.912	-.537	.591
10.00-12.00	-12.042	17.912	-.672	.501
10.00-13.00	-14.458	17.912	-.807	.420
10.00-15.00	-29.708	17.912	-1.659	.097
1.00-14.00	-1.429	20.866	-.068	.945
1.00-12.00	-3.845	20.866	-.184	.854
1.00-13.00	-6.262	20.866	-.300	.764
1.00-15.00	-21.512	20.866	-1.031	.303
14.00-12.00	2.417	17.912	.135	.893
14.00-13.00	4.833	17.912	.270	.787
14.00-15.00	-20.083	17.912	-1.121	.262
12.00-13.00	-2.417	17.912	-.135	.893
12.00-15.00	-17.667	17.912	-.986	.324
13.00-15.00	-15.250	17.912	-.851	.395

Table 7. Kruskal-Wallis Pairwise Comparisons of average number of microplastics L^{-1} between each sampling day for pebbles treatment, featuring Test Statistic, Standard Error and Significance.

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.
2.00-1.00	2.500	11.941	.209	.834
2.00-3.00	-12.250	11.941	-1.026	.305
2.00-13.00	-15.000	11.941	-1.256	.209
2.00-7.00	-19.000	11.941	-1.591	.112
2.00-4.00	-20.583	12.898	-1.596	.111
2.00-14.00	-23.750	11.941	-1.989	.047
2.00-6.00	-24.500	11.941	-2.052	.040
2.00-15.00	-28.500	11.941	-2.387	.017
2.00-9.00	-29.750	11.941	-2.491	.013
2.00-8.00	-31.750	11.941	-2.659	.008
2.00-10.00	-40.500	11.941	-3.392	.001
2.00-5.00	-43.250	12.898	-3.353	.001
2.00-12.00	-47.500	11.941	-3.978	.000
2.00-11.00	-50.500	11.941	-4.229	.000
1.00-3.00	-9.750	11.941	-.817	.414
1.00-13.00	-12.500	11.941	-1.047	.295
1.00-7.00	-16.500	11.941	-1.382	.167
1.00-4.00	-18.083	12.898	-1.402	.161
1.00-14.00	-21.250	11.941	-1.780	.075
1.00-6.00	-22.000	11.941	-1.842	.065
1.00-15.00	-26.000	11.941	-2.177	.029
1.00-9.00	-27.250	11.941	-2.282	.022
1.00-8.00	-29.250	11.941	-2.450	.014
1.00-10.00	-38.000	11.941	-3.182	.001
1.00-5.00	-40.750	12.898	-3.160	.002
1.00-12.00	-45.000	11.941	-3.769	.000
1.00-11.00	-48.000	11.941	-4.020	.000
3.00-13.00	-2.750	11.941	-.230	.818
3.00-7.00	-6.750	11.941	-.565	.572
3.00-4.00	-8.333	12.898	-.646	.518
3.00-14.00	-11.500	11.941	-.963	.336
3.00-6.00	-12.250	11.941	-1.026	.305
3.00-15.00	-16.250	11.941	-1.361	.174
3.00-9.00	-17.500	11.941	-1.466	.143
3.00-8.00	-19.500	11.941	-1.633	.102
3.00-10.00	-28.250	11.941	-2.366	.018
3.00-5.00	-31.000	12.898	-2.404	.016
3.00-12.00	-35.250	11.941	-2.952	.003
3.00-11.00	-38.250	11.941	-3.203	.001
13.00-7.00	4.000	11.941	.335	.738
13.00-4.00	5.583	12.898	.433	.665

13.00-14.00	-8.750	11.941	-.733	.464
13.00-6.00	9.500	11.941	.796	.426
13.00-15.00	-13.500	11.941	-1.131	.258
13.00-9.00	14.750	11.941	1.235	.217
13.00-8.00	16.750	11.941	1.403	.161
13.00-10.00	25.500	11.941	2.136	.033
13.00-5.00	28.250	12.898	2.190	.028
13.00-12.00	32.500	11.941	2.722	.006
13.00-11.00	35.500	11.941	2.973	.003
7.00-4.00	1.583	12.898	.123	.902
7.00-14.00	-4.750	11.941	-.398	.691
7.00-6.00	5.500	11.941	.461	.645
7.00-15.00	-9.500	11.941	-.796	.426
7.00-9.00	-10.750	11.941	-.900	.368
7.00-8.00	-12.750	11.941	-1.068	.286
7.00-10.00	-21.500	11.941	-1.801	.072
7.00-5.00	24.250	12.898	1.880	.060
7.00-12.00	-28.500	11.941	-2.387	.017
7.00-11.00	-31.500	11.941	-2.638	.008
4.00-14.00	-3.167	12.898	-.246	.806
4.00-6.00	-3.917	12.898	-.304	.761
4.00-15.00	-7.917	12.898	-.614	.539
4.00-9.00	-9.167	12.898	-.711	.477
4.00-8.00	-11.167	12.898	-.866	.387
4.00-10.00	-19.917	12.898	-1.544	.123
4.00-5.00	-22.667	13.788	-1.644	.100
4.00-12.00	-26.917	12.898	-2.087	.037
4.00-11.00	-29.917	12.898	-2.320	.020
14.00-6.00	.750	11.941	.063	.950
14.00-15.00	-4.750	11.941	-.398	.691
14.00-9.00	6.000	11.941	.502	.615
14.00-8.00	8.000	11.941	.670	.503
14.00-10.00	16.750	11.941	1.403	.161
14.00-5.00	19.500	12.898	1.512	.131
14.00-12.00	23.750	11.941	1.989	.047
14.00-11.00	26.750	11.941	2.240	.025
6.00-15.00	-4.000	11.941	-.335	.738
6.00-9.00	-5.250	11.941	-.440	.660
6.00-8.00	-7.250	11.941	-.607	.544
6.00-10.00	-16.000	11.941	-1.340	.180
6.00-5.00	18.750	12.898	1.454	.146
6.00-12.00	-23.000	11.941	-1.926	.054
6.00-11.00	-26.000	11.941	-2.177	.029
15.00-9.00	1.250	11.941	.105	.917
15.00-8.00	3.250	11.941	.272	.785
15.00-10.00	12.000	11.941	1.005	.315
15.00-5.00	14.750	12.898	1.144	.253
15.00-12.00	19.000	11.941	1.591	.112

15.00-11.00	22.000	11.941	1.842	.065
9.00-8.00	2.000	11.941	.167	.867
9.00-10.00	-10.750	11.941	-.900	.368
9.00-5.00	13.500	12.898	1.047	.295
9.00-12.00	-17.750	11.941	-1.486	.137
9.00-11.00	-20.750	11.941	-1.738	.082
8.00-10.00	-8.750	11.941	-.733	.464
8.00-5.00	11.500	12.898	.892	.373
8.00-12.00	-15.750	11.941	-1.319	.187
8.00-11.00	-18.750	11.941	-1.570	.116
10.00-5.00	2.750	12.898	.213	.831
10.00-12.00	-7.000	11.941	-.586	.558
10.00-11.00	-10.000	11.941	-.837	.402
5.00-12.00	-4.250	12.898	-.330	.742
5.00-11.00	-7.250	12.898	-.562	.574
12.00-11.00	3.000	11.941	.251	.802

Table 8. Kruskal-Wallis Pairwise Comparisons of average number of microplastics L^{-1} between each sampling day for wetland treatment, featuring Test Statistic, Standard Error and Significance.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
2.00-6.00	-4.875	17.076	.775
2.00-4.00	-10.600	19.146	.580
2.00-8.00	-14.143	17.591	.421
2.00-1.00	14.688	17.076	.390
2.00-15.00	-33.000	17.076	.053
2.00-3.00	-36.167	18.255	.048
2.00-13.00	-37.375	17.076	.029
2.00-5.00	-38.312	17.076	.025
2.00-7.00	-47.187	17.076	.006
2.00-12.00	-49.687	17.076	.004
2.00-14.00	-56.562	17.076	.001
2.00-11.00	-57.643	17.591	.001
2.00-9.00	-67.562	17.076	.000
2.00-10.00	-74.500	17.076	.000
6.00-4.00	5.725	18.025	.751
6.00-8.00	-9.268	16.364	.571
6.00-1.00	9.813	15.809	.535
6.00-15.00	-28.125	15.809	.075
6.00-3.00	31.292	17.076	.067
6.00-13.00	-32.500	15.809	.040

6.00-5.00	33.438	15.809	.034
6.00-7.00	-42.312	15.809	.007
6.00-12.00	-44.812	15.809	.005
6.00-14.00	-51.687	15.809	.001
6.00-11.00	-52.768	16.364	.001
6.00-9.00	-62.687	15.809	.000
6.00-10.00	-69.625	15.809	.000
4.00-8.00	-3.543	18.514	.848
4.00-1.00	4.088	18.025	.821
4.00-15.00	-22.400	18.025	.214
4.00-3.00	25.567	19.146	.182
4.00-13.00	-26.775	18.025	.137
4.00-5.00	-27.712	18.025	.124
4.00-7.00	-36.587	18.025	.042
4.00-12.00	-39.087	18.025	.030
4.00-14.00	-45.962	18.025	.011
4.00-11.00	-47.043	18.514	.011
4.00-9.00	-56.962	18.025	.002
4.00-10.00	-63.900	18.025	.000
8.00-1.00	.545	16.364	.973
8.00-15.00	-18.857	16.364	.249
8.00-3.00	22.024	17.591	.211
8.00-13.00	-23.232	16.364	.156
8.00-5.00	24.170	16.364	.140
8.00-7.00	33.045	16.364	.043
8.00-12.00	-35.545	16.364	.030
8.00-14.00	-42.420	16.364	.010
8.00-11.00	-43.500	16.901	.010
8.00-9.00	-53.420	16.364	.001
8.00-10.00	-60.357	16.364	.000
1.00-15.00	-18.312	15.809	.247
1.00-3.00	-21.479	17.076	.208
1.00-13.00	-22.687	15.809	.151
1.00-5.00	-23.625	15.809	.135
1.00-7.00	-32.500	15.809	.040
1.00-12.00	-35.000	15.809	.027
1.00-14.00	-41.875	15.809	.008
1.00-11.00	-42.955	16.364	.009
1.00-9.00	-52.875	15.809	.001
1.00-10.00	-59.812	15.809	.000
15.00-3.00	3.167	17.076	.853
15.00-13.00	4.375	15.809	.782
15.00-5.00	5.313	15.809	.737
15.00-7.00	14.188	15.809	.369
15.00-12.00	16.688	15.809	.291
15.00-14.00	23.563	15.809	.136
15.00-11.00	24.643	16.364	.132
15.00-9.00	34.563	15.809	.029

15.00-10.00	41.500	15.809	.009
3.00-13.00	-1.208	17.076	.944
3.00-5.00	-2.146	17.076	.900
3.00-7.00	-11.021	17.076	.519
3.00-12.00	-13.521	17.076	.428
3.00-14.00	-20.396	17.076	.232
3.00-11.00	-21.476	17.591	.222
3.00-9.00	-31.396	17.076	.066
3.00-10.00	-38.333	17.076	.025
13.00-5.00	.938	15.809	.953
13.00-7.00	9.813	15.809	.535
13.00-12.00	12.313	15.809	.436
13.00-14.00	-19.187	15.809	.225
13.00-11.00	20.268	16.364	.216
13.00-9.00	30.188	15.809	.056
13.00-10.00	37.125	15.809	.019
5.00-7.00	-8.875	15.809	.575
5.00-12.00	-11.375	15.809	.472
5.00-14.00	-18.250	15.809	.248
5.00-11.00	-19.330	16.364	.238
5.00-9.00	-29.250	15.809	.064
5.00-10.00	-36.187	15.809	.022
7.00-12.00	-2.500	15.809	.874
7.00-14.00	-9.375	15.809	.553
7.00-11.00	-10.455	16.364	.523
7.00-9.00	-20.375	15.809	.197
7.00-10.00	-27.312	15.809	.084
12.00-14.00	-6.875	15.809	.664
12.00-11.00	7.955	16.364	.627
12.00-9.00	17.875	15.809	.258
12.00-10.00	24.813	15.809	.117
14.00-11.00	1.080	16.364	.947
14.00-9.00	11.000	15.809	.487
14.00-10.00	17.938	15.809	.257
11.00-9.00	9.920	16.364	.544
11.00-10.00	16.857	16.364	.303
9.00-10.00	-6.937	15.809	.661

Table 9. Independent Samples Kruskal-Wallis test for significant difference in average number of microplastics L^{-1} sampled from each treatment on Day 1, featuring Test Statistic, Standard Error and Significance.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Wetland-Pebbles	6.000	3.437	.081
Wetland-Water	11.500	2.905	.000
Pebbles-Water	5.500	3.518	.118

Table 10. Independent Samples Kruskal-Wallis test for significant difference in average number of microplastics L^{-1} sampled from each treatment on Day 2, featuring Test Statistic, Standard Error and Significance.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Wetland-Pebbles	6.500	3.376	.054
Wetland-Water	10.250	2.825	.000
Pebbles-Water	3.750	3.203	.242

Table 11. Independent Samples Kruskal-Wallis test for significant difference in average number of microplastics L^{-1} sampled from each treatment on Day 3, featuring Test Statistic, Standard Error and Significance.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Wetland-Pebbles	5.000	3.444	.147
Wetland-Water	11.000	2.882	.000
Pebbles-Water	6.000	3.267	.066

Table 12. Independent Samples Kruskal-Wallis test for significant difference in average number of microplastics L^{-1} sampled from each treatment on Day 4, featuring Test Statistic, Standard Error and Significance.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Wetland-Pebbles	6.000	3.464	.083
Wetland-Water	8.750	2.704	.001
Pebbles-Water	2.750	3.211	.392

Table 13. Independent Samples Kruskal-Wallis test for significant difference in average number of microplastics L^{-1} sampled from each treatment on Day 5, featuring Test Statistic, Standard Error and Significance.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Wetland-Water	6.750	2.807	.016
Wetland-Pebbles	12.875	3.801	.001
Water-Pebbles	-6.125	3.801	.107

Table 14. Independent Samples Kruskal-Wallis test for significant difference in average number of microplastics L^{-1} sampled from each treatment on Day 6, featuring Test Statistic, Standard Error and Significance.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Wetland-Water	8.000	2.919	.006
Wetland-Pebbles	14.000	3.575	.000
Water-Pebbles	-6.000	3.575	.093

Table 15. Independent Samples Kruskal-Wallis test for significant difference in average number of microplastics L^{-1} sampled from each treatment on Day 7, featuring Test Statistic, Standard Error and Significance.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Wetland-Pebbles	10.500	4.329	.015
Wetland-Water	12.500	3.227	.000
Pebbles-Water	2.000	4.082	.624

Table 16. Independent Samples Kruskal-Wallis test for significant difference in average number of microplastics L^{-1} sampled from each treatment on Day 8, featuring Test Statistic, Standard Error and Significance.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Wetland-Water	10.125	3.198	.002
Wetland-Pebbles	12.750	4.215	.002
Water-Pebbles	-2.625	3.883	.499

Table 17. Independent Samples Kruskal-Wallis test for significant difference in average number of microplastics L^{-1} sampled from each treatment on Day 9, featuring Test Statistic, Standard Error and Significance.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Wetland-Pebbles	9.000	4.320	.037
Wetland-Water	11.500	3.220	.000
Pebbles-Water	2.500	4.073	.539

Table 18. Independent Samples Kruskal-Wallis test for significant difference in average number of microplastics L^{-1} sampled from each treatment on Day 10, featuring Test Statistic, Standard Error and Significance.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Wetland-Pebbles	9.500	4.311	.028
Wetland-Water	9.833	3.213	.002
Pebbles-Water	.333	4.065	.935

Table 19. Independent Samples Kruskal-Wallis test for significant difference in average number of microplastics L^{-1} sampled from each treatment on Day 11, featuring Test Statistic, Standard Error and Significance.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Wetland-Water	10.500	3.222	.001
Wetland-Pebbles	14.500	4.247	.001
Water-Pebbles	-4.000	3.912	.307

Table 20. Independent Samples Kruskal-Wallis test for significant difference in average number of microplastics L^{-1} sampled from each treatment on Day 12, featuring Test Statistic, Standard Error and Significance.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Wetland-Pebbles	10.000	4.249	.019
Wetland-Water	12.667	3.167	.000
Pebbles-Water	2.667	4.006	.506

Table 21. Independent Samples Kruskal-Wallis test for significant difference in average number of microplastics L^{-1} sampled from each treatment on Day 13, featuring Test Statistic, Standard Error and Significance.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Wetland-Pebbles	7.625	4.246	.072
Wetland-Water	13.208	3.164	.000
Pebbles-Water	5.583	4.003	.163

Table 22. Independent Samples Kruskal-Wallis test for significant difference in average number of microplastics L^{-1} sampled from each treatment on Day 14, featuring Test Statistic, Standard Error and Significance.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Wetland-Pebbles	8.125	4.276	.057
Wetland-Water	12.042	3.187	.000
Pebbles-Water	3.917	4.032	.331

Table 23. Independent Samples Kruskal-Wallis test for significant difference in average number of microplastics L^{-1} sampled from each treatment on Day 15, featuring Test Statistic, Standard Error and Significance.

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Wetland-Pebbles	6.750	4.211	.109
Wetland-Water	13.750	3.139	.000
Pebbles-Water	7.000	3.970	.078

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7. Conclusions

7a. Overall Findings

The initial study into utilising fluorescence excitation in order to identify microplastics in freshwater samples (Fears, 2021c) was somewhat successful, with significantly more (p <0.05) microplastics identified utilising fluorescence microscopy than standard dissection microscopy for 50% of sites analysed, with fragment-type microplastics generally being the most prolific, followed by fibre types (though it is thought that sites with no significant difference between analysis methods is due to low microplastic populations, or issues with statistical tests). Although some issues were encountered with the more sediment-polluted samples (such as the River Thames) taking much longer to filter, this method proved a lot quicker than the widely-used NOAA method by Masura et al. (2015) by at least two days, and required far less steps and equipment used, thus running at a lower cost. With the success of this fluorescence microscopy method, it was utilised in the studies that followed, barring the final wetland microplastic-retention study where its use was not necessary. As such, fluorescence microscopy holds great potential in being utilised globally as a premier method of accurately and effortlessly surveying microplastic pollution in water. Currently, microplastic pollution is not a factor which is standard in examining water sources for pollution indicators (such as biological and chemical contaminants), but with the ease of fluorescence microscopy, microplastic analysis could near-effortlessly be introduced as a standard measurement for pollution sampling on a global scale, helping to paint a broader and more accurate picture of microplastic pollution both worldwide and on a local basis. Furthermore, with previous studies of microplastic pollution in the natural environment not having used fluorescence microscopy, which in turn reveals greater numbers of microplastics, the actual levels of microplastic pollution across the global environment could be much higher than initially anticipated, and thus addressing the issues of microplastic pollution may be much more urgent than we realise. However, it most be noted that fluorescence microscopy still relies on visual identification of microplastics and is thus subject to human error and should not be utilised as a replacement for more accurate chemospectroscopy methods.

Fears (2021b) found significantly more (p <0.05) microplastics in samples taken from points along the coast of Anglesey, North Wales (and Bangor, Gwynedd) than from control samples, indicating the Anglesey coastline to be polluted by microplastics. Samples taken from the Bangor, Gwynedd side of the Menai Strait showed the highest levels of microplastics per litre ($7.958 \pm 0.52\ L^{-1}$), followed by the less-densely-populated Menai Bridge ($2 \pm 0.87\ L^{-1}$), indicating local population density and/ or size to be a significant factor, though the sparsely-populated Penmon Point showed

the third-highest levels (1.95 ± 0.27 L^{-1}). Other factors such as boat traffic, weather, tides, and distance from the shoreline were discussed, but further study is required to determine the effect of said factors. Furthermore, more sites around Anglesey require sampling to give a full picture of microplastic pollution around its coast. There have been no previous publicized levels of microplastics from these sites, and as such regularly monitoring is required to keep an up-to-date record of microplastic pollution, possible through the usage of the fluorescence microscopy method detailed by Fears (2021c).

Fears (2021a) successfully determined that CTWs can filter out microplastics from polluted water, as throughout each day of a 15-day trial with 1000 to 1500 microplastics being added to each treatment microcosm, the wetland treatment released a significantly (p <0.05) lower average number of microplastics than the standing water control microcosms. Furthermore, overall, the wetland treatments released a significantly (p <0.05) lower average number of microplastics than a treatment testing the effectiveness of just the wetland substrate consisting of pebbles (although on most days there was no significant difference between the two). This proves that CTWs are indeed effective at retaining microplastics, and that CTWs as a whole with their plant and biotic components are more effective than the inorganic substrate. The topic of the saturation point, or loading potential of the treatments was discussed, where the maximum possible retention of microplastics by the system is reached, leading to increasingly large flushes of microplastics being released. It is theorized that the loading potential of the pebbles microcosms was perhaps reached and flushed twice (days 5 and 11), with the loading potential increasing between the first peak and the second peak, and due to the consistently low output of microplastics by the wetland microcosms, it is thought that the loading potential was not ever reached for this system, demonstrating a high potential storage capacity. A number of issues afflicted the study, ranging from only two workable microcosms for the pebbles treatment, to weather and external conditions, but a valid data set was able to be gathered, although a repeat of this study of a longer period of time would be optimal to determine the loading potential of the wetland microcosms. As this study just used a typical example of a CTW consisting of pebbles, *Typha* and natural biota, this method of investigation could be utilised for models of various different wetland types and environments, for potential national and international usage in trapping microplastics before they reach larger bodies of water. The relatively small scale of this study allows for the easy replication with a huge number of variables to test CTW microplastic retention efficiency.

7b. References

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