



## Mussel power: Scoping a nature-based solution to microplastic debris

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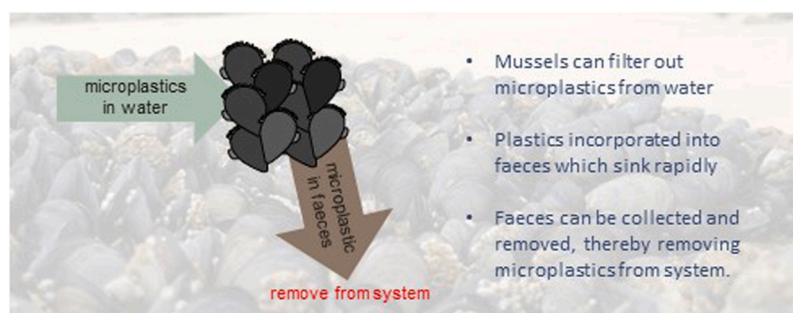
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### HIGHLIGHTS

- Experiments, modelling and field trials used to demonstrate capacity of mussels to remove microplastic from flowing water.
- Under lab conditions, 1 kg of mussels demonstrated capacity to filter out 40,146 microplastics h<sup>-1</sup> from flowing water.
- Mussel faeces sink irrespective of microplastic content, with mean sinking rates of 223–266 m day<sup>-1</sup>.
- Models predict mussels sited at the mouths of estuaries could remove 4% of microplastics emanating from nearby rivers.
- In field trials, 5 kg mussels removed 239.9 ± 145.9 microplastics and anthropogenic particles day<sup>-1</sup>.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Microplastics are a prolific environmental contaminant. Curbing microplastic pollution requires an array of globally relevant interventions, including source-reduction and curative measures. A novel, nature-based solution to microplastics is proposed, in which mussels are deployed in aquatic ecosystems to act as microplastic biofilters, removing waterborne microplastics and repackaging them into biodeposits that are subsequently captured and removed. Blue mussels (*Mytilus edulis*) were used to establish the feasibility of such an approach. In the laboratory, mussels were exposed to representative microplastics in a flume tank; at an initial concentration of 1000 microplastics L<sup>-1</sup>, mussels reduced waterborne microplastic concentrations at an average rate of 40,146 microplastics kg<sup>-1</sup> h<sup>-1</sup>. Mussel faeces sank irrespective of microplastic content, with average sinking velocities of 223–266 m day<sup>-1</sup>. Modelling predicts ~3 × 10<sup>9</sup> mussels deployed on ropes at the mouths of estuaries could remove 4% of waterborne microplastics discharged from rivers. Mussels were successfully deployed in a prototype biodeposit collection system in an urban marina, with 5.0 kg of mussels removing and repackaging 239.9 ± 145.9 microplastics and anthropogenic particles day<sup>-1</sup> into their faeces. These results provide impetus for further development of nature-based solutions targeting plastic debris.

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## 1. Introduction

Microplastic debris is a widespread environmental contaminant, polluting terrestrial, freshwater and marine ecosystems across the globe [1,2]. Microplastics describe plastic particles and fibres, 0.1  $\mu\text{m}$ –1 mm in size, that are either manufactured directly (e.g. exfoliates in personal care products) or derived from the fragmentation of larger plastics (e.g. microfibrils from textiles, tyre-wear particles) [3]. Recent estimates suggest there is in the order of 12.5–125 trillion microplastics floating in the oceans [4], with even higher concentrations accumulating in underlying sediments [5]. Given continued growth in global plastic production and the persistence of plastic in the environment, the concentration of microplastics in natural ecosystems is expected to increase over time [6,7]. Microplastics can be ingested by a wide array of organisms, and have the potential to cause adverse health effects, including reductions in fecundity, growth and survival [8,9] and negatively impact on ecological functioning (e.g. nutrient cycling, water filtration) [10,11]. To achieve a long-term decline in global marine plastic, substantial shifts in societal behaviour and product design, tracking towards a sustainable, circular economy approach is required [12]. In the interim, there is an opportunity for the development and optimisation of novel technologies, guided by the available science, to concurrently limit plastic emissions and mitigate the impacts of marine plastic [13]. Such interventions are expected to be diverse, including source reduction and preventative measures, for example schemes to prevent the industrial loss of pre-production pellets [14] and technologies to capture microfibrils released during laundry cycles [15]; and curative measures whereby plastic is removed from the environment [16]. In recent years, an array of technologies that redirect floating macroplastic litter into waste disposal units have been successfully deployed in harbours, rivers and the open ocean [17–19], but there remains a clear need for solutions targeting the removal of microplastics too.

In this project, we investigate whether mussels could be deployed as part of a Nature-based Solution for microplastics debris. Nature-based solutions are evidence-based, large-scale, innovative environmental interventions inspired or supported by ecological processes, that address globally relevant societal challenges (e.g. climate change, marine plastic) [20]. Such interventions should be devised so as to protect, restore or sustainably manage natural or modified ecosystems, with the scope to improve biodiversity and ecosystem services [21,22]. Mussels are suspension-feeding bivalves that filter out particles by continuously drawing water into their branchial chamber via the inhalant siphon. Captured particles are subsequently processed at the labial palps and either rejected within mucous-laden pseudofaeces (typically associated with high food availability), or ingested [23]. As natural biofilters, mussels can reduce algal blooms, suspended particulates and bacterial concentrations in lakes and estuaries, reducing the risk of eutrophication and improving water quality [24,25]. A range of mussel species, including blue mussels (*Mytilus edulis*), Mediterranean mussels (*Mytilus galloprovincialis*), brown mussels (*Perna perna*) and zebra mussels (*Dreissena polymorpha*), have been demonstrated to ingest microplastics [26]. While there is some evidence that nanoplastic and very small microplastics can be phagocytosed into epithelial tissues or even translocated into the circulatory system [27,28], the vast majority of research papers have observed microplastics readily transition through the gastrointestinal system and are subsequently egested within faecal pellets [29–37]. Given their ecological niche, mussels are well-adapted to handling particulates and toxicants, and are considered to be relatively pollution tolerant [38]. In bivalve shellfish, exposure to microplastics (at concentrations typically exceeding those observed in the natural environment) can result in sub-lethal health effects (e.g. histopathological alterations, oxidative stress, genotoxic damage), however there is limited evidence of impact on higher levels of biological hierarchy [39]. Based on these findings, we propose that mussels could be deployed in native rivers, estuaries or coastal waters to act as

microplastic biofilters, removing waterborne microplastics and repackaging them into biodeposits (i.e. faeces, pseudofaeces) that can be collected and removed.

Here, we establish the feasibility of deploying mussels to remove waterborne microplastics. Focussing upon the blue mussel (*Mytilus* spp.), we use laboratory studies, a coupled hydrodynamic-biogeochemical-shellfish model and field experiments to evaluate: (1) microplastic removal rates by mussels under continuous flow (laboratory); (2) fate of microplastics in mussel faeces (laboratory); (3) microplastic removal by mussels deployed in a coastal system (modelled); and (4) microplastic removal rates by mussels in an urban marina (field). The study provides evidence that mussels can effectively remove microplastics which are subsequently repackaged into sinking faeces and consider the benefits and challenges of deploying mussels as a nature-based solution to microplastic debris in the real world.

## 2. Materials and methods

### 2.1. Mussels

For this project, we used blue mussels (*Mytilus edulis*) which are filter-feeding bivalve shellfish native to the UK and prevalent in temperate coastal waters of the North Atlantic. *M. edulis* were taken from two populations: (1) to calculate microplastic removal rates in both the laboratory and field, fresh, unprocessed mussels were purchased from a mussel farm in Cornwall, UK; (2) to explore the fate of microplastics in mussel biodeposits, mussels were hand collected from the Teign estuary, UK (50°32'41"N, 3°30'36"W). Smaller mussels (<2 cm shell width) were removed from stocks. Remaining mussels were depurated in aerated seawater for a minimum of 72 h, and epibionts and byssal threads carefully removed. Prior to experimental work, mussels were maintained in filtered natural seawater (FSW; 10  $\mu\text{m}$  and 1  $\mu\text{m}$  Hytrec-II filter; 35 PSU) under controlled laboratory conditions (15  $\pm$  1 °C; 16:8 light:dark regimen). Mussels were fed ad libitum with a commercial microalgal blend (Shellfish Diet 1800®, Reed Mariculture); typically feeding was conducted twice weekly, adding sufficient microalgae to tinge the water a green-brown colour, following a partial water-change.

### 2.2. Microplastics

A suite of microplastics, varying in polymer, shape and size, representative of the diverse range of microplastics identified within marine environments, were used within this study (Table 1). Where necessary, fluorescent microplastics were used in laboratory experiments to improve particle visualisation. Plastic stocks were prepared as follows: (1) Polystyrene (PS) and polyethylene (PE-S) fluorescent microplastic

**Table 1**  
Microplastics used within the experiments.  $\emptyset$  = diameter.

Code	Polymer	Shape	Mean size ( $\mu\text{m}$ )	Density (g $\text{cm}^{-3}$ )	Supplier
PS	Polystyrene	Sphere	30 $\emptyset$	0.96–1.05	Spherotech (FP30052)
PA-S	Polyamide (Nylon 6,6)	Fibre	10 $\emptyset$ x 40	1.14	Goodfellow (AM325705)
PA-L	Polyamide (Nylon 6,6)	Fibre	10 $\emptyset$ x 100	1.14	Goodfellow (AM325705)
PE-S	Polyethylene	Spheres	30 $\emptyset$	0.93	Cospheric (UVPMS-BG-1.01)
PE-L	Polyethylene	Granule	70 $\emptyset$	0.93	Two H Chem Ltd (HM080/191016S)
PP	Polypropylene	Granule	20 $\emptyset$	0.90	Two H Chem Ltd (FPP4020/190808)

spheres (aq) were repeatedly centrifuged and resuspended in ultrapure water to remove antimicrobial agents, per the method of Cole & Galloway (2015) [40]. (2) Polyethylene (PE-L) and polypropylene (PP) microplastic powders (s) were size fractionated using stainless steel sieves and rinsed with 70% ethanol and flushed with ultrapure water to remove labile additives; these hydrophobic, buoyant microplastics were resuspended in 0.01% Tween20 surfactant to aid dispersal in seawater. (3) Nylon microfibrils (PA-S, PA-L) were prepared from nylon polyfilament (s) using a cryogenic microtome (Thermo Fisher Scientific, HM525 NX Cryostat), and PA-L fluorescently dyed using Nile Red, per the protocol of Cole (2016) [41]; microfibrils were subsequently resuspended in ultrapure water. Particle size and polymer type were corroborated using microscopy (Olympus SZX15, CellSens software) and Fourier-transform infrared (FTIR) spectroscopy (Perkin Elmer, Spotlight 400). Stock concentrations were ascertained using a Sedgewick Rafter counting chamber in conjunction with a light microscope (Olympus, SZX16). Stocks were maintained in the dark at  $-20^{\circ}\text{C}$  to prevent microbial growth.

### 2.3. Microplastic removal rates by mussels under continuous flow (laboratory)

This laboratory experiment was designed as a proof-of-principle test to establish the capacity for mussels to effectively remove waterborne microplastics under continuous flow and repackage these microplastics into their biodeposits. Here, blue mussels were exposed to representative microplastic particles (PS) and fibres (PA-L) at a nominal total microplastic concentration of 1000 microplastics  $\text{mL}^{-1}$ , alongside microalgal prey, in a flume tank under continuous flow ( $2.5\text{ cm s}^{-1}$ ) for a nominal period of 4 h.

#### 2.3.1. Flume tank

Experiments were conducted using a stainless-steel flume tank comprising a primary reservoir, a 2 m long x 0.75 m wide open channel with a slope of  $\sim 3^{\circ}$ , a secondary reservoir and a 63 mm  $\varnothing$  pipe returning water to the primary reservoir (Fig. 1 A; SI, Fig. S1). A 1.5 HP pump (Certikin, Swimflo Plus) provided a continuous flow rate ( $\sim 2.5\text{ cm s}^{-1}$ ). A curved baffle plate and a laminar grid was installed at the entrance to the open channel to smooth the flow, and a corrugated cover added to the tank to avoid fluctuations in surface turbidity and limit airborne contamination. Two in-line pumps (Qmax, 1500  $\text{L h}^{-1}$ ) were positioned at both the water surface and base of the secondary reservoir to keep microplastics in suspension. Additionally, four in-line pumps connected to 12 mm  $\varnothing$  polyvinyl chloride pipe (spanning the breadth of the channel) with 1 mm holes (2 cm spacings) drilled along one side, were positioned at 0.4 m intervals along the latter half of the open channel; these pump-pipe systems forced seawater through the holes at a  $\sim 45^{\circ}$  angle to resuspend any microplastics settling on the base of the open channel (SI, Fig. S1, D-E).

#### 2.3.2. Experimental set-up

Prior to experimental runs, the flume tank was filled with 925 L of temperature acclimated FSW, with a mean water depth of 0.15 m in the open channel. The flume tank was maintained in a controlled-temperature laboratory at  $15^{\circ}\text{C}$  and a water chiller (Boyu, L-350, 1200–3000  $\text{L h}^{-1}$ ) used to limit temperature increases stemming from continuous use of the pump. To facilitate thorough mixing, microalgae (Shellfish Diet 1800®, Reed Mariculture;  $\sim 50\text{ }\mu\text{g Chla L}^{-1}$ ) and PS and PA-L stocks were added to the tank whilst filling. Fluorescently labelled microplastics were used to improve visualisation of particles in subsamples. Microplastic and algal concentrations within the tank were ascertained by taking dip-samples every 30 min from the head of the primary reservoir. Microplastic samples (500 mL) were vacuum filtered sequentially onto 18  $\mu\text{m}$  polycarbonate filters and 1.2  $\mu\text{m}$  GFFs (Whatman), and fluorescent PS and PA-L present on the filters systematically enumerated using an Olympus SZX16 microscope (GFPA and RFP2 filter blocks). Algal samples (25 mL) were vacuum filtered onto 1.2  $\mu\text{m}$  GFFs

(Whatman), and filters transferred into a 15 mL Falcon tube with 10 mL of 90% Acetone and kept in the dark at  $-20^{\circ}\text{C}$  for a minimum of 24 h; absorbance levels of triplicate  $\sim 2\text{ mL}$  aliquots of acetone were ascertained using a fluorometer (Turner Trilogy, Model 7200) with data compared against analytical standards to determine chlorophyll concentrations ( $\text{mg Chla m}^{-3}$ ) [42]. An electromagnetic flowmeter (Valeport, 801 EM with flat sensor) was used to systematically measure flow ( $\text{cm s}^{-1}$ ; mean flow rate across 10 s period) at five intervals across the width of the open channel, at incremental depths 0.1 and 0.6 m along the open channel, both in the presence and absence of mussels.

#### 2.3.3. Exposures

Control runs, absent of mussels, using two microplastic concentrations (250 microplastics  $\text{L}^{-1}$ ; 125 microplastics  $\text{L}^{-1}$ ) were conducted for both PS and PA-L to verify that concentrations remained stable over time. For mussel exposures, 5 kg of mussels ( $\sim 300$  mussels) were transferred to 0.20  $\text{m}^2$  stainless steel grids (0.5  $\text{mm}^2$  mesh size), suspended at a height of 6 cm to permit deposition of faeces (SI, Fig. S1C). Baseline microplastic (500 PS  $\text{L}^{-1}$ ; 500 PA-L  $\text{L}^{-1}$ ) and algal concentrations in the flume tank were ascertained over a 1-hour period (as in 2.3.2), before carefully placing cohorts of mussels ( $N = 5$ ) in the open channel of the tank. Mussels were exposed for 4 h, with microplastic and algal samples taken at regular intervals. During exposures, mussels were observed via a polycarbonate window in the open channel. After the 4-hour exposure period, mussels were removed from the flume tank and the number of individuals per cohort, plus the shell length of a subsample of 500 g of mussels per cohort, was recorded. Mean microplastic removal rates (normalised to 5.0 kg mussels) were calculated for the first three hours of the exposure where microplastic concentrations followed a negative linear relationship.

### 2.4. Fate of microplastics in mussel biodeposits (laboratory)

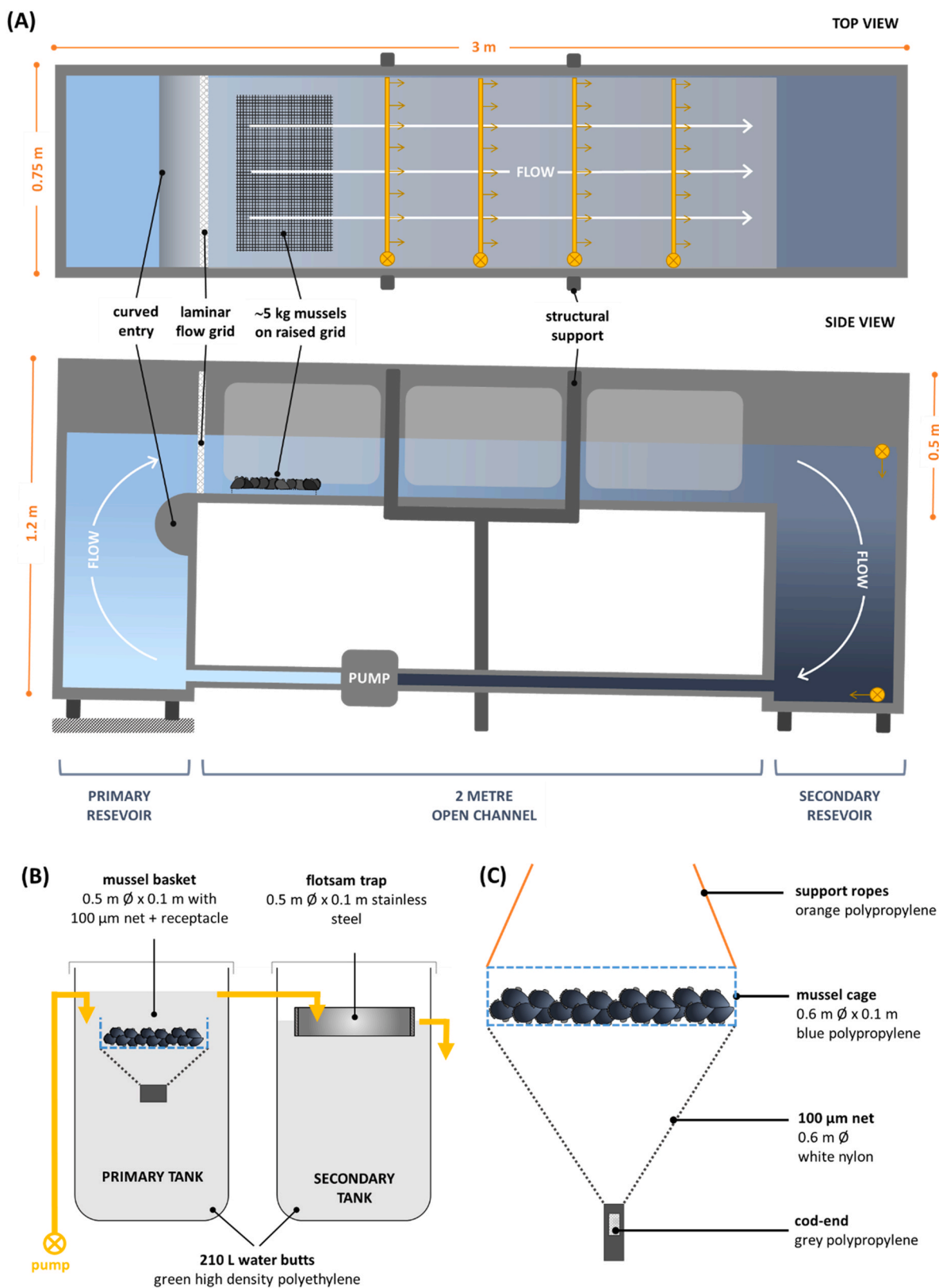
This laboratory experiment was designed as a proof-of-principle test to establish whether incorporation of microplastics into mussel faeces affects their sinking velocity. Here, mussels were exposed to an array of microplastics, ranging in shape, size and polymer, at a nominal concentration of 100,000 microplastics  $\text{L}^{-1}$  to ensure mussel-microplastic interaction, for a nominal duration of 16 h, and the sinking rates of microplastic-laden biodeposits determined under controlled conditions.

#### 2.4.1. Exposures

Glass beakers were filled with 2 L of lightly aerated FSW containing microalgae (Shellfish Diet 1800®, Reed Mariculture) provided ad libitum ( $\sim 100\text{ }\mu\text{g Chla L}^{-1}$ ) and 100,000 microplastics  $\text{L}^{-1}$  of either PS, PA-S or PA-L, or PE-S, PE-L or PP with 0.01% Tween surfactant to promote particle mixing (Table 1). Individual mussels (45–65 mm shell lengths) were affixed to wooden lollipop sticks using superglue ( $N = 5$  per treatment) and each stick suspended within the centre of a beaker (SI, Fig. S2 A-B). Controls comprised beakers containing: FSW with microalgae (Control); and FSW with microalgae and 0.01% Tween 20 (Tween Control). Mussels were exposed for 16 h ( $15 \pm 1^{\circ}\text{C}$ ) in the dark. Following exposures, mussel pseudofaeces were carefully removed using a 2 mL pipette, and mussel faeces removed by gently pouring the contents of the beaker through a 50  $\mu\text{m}$  mesh suspended in FSW; mussel egests were subsequently washed into a Petri dish prior to examination.

#### 2.4.2. Mussel biodeposits

Pseudofaeces production was minimal, therefore subsequent measurements were conducted on mussel faeces only. Intestinal faecal pellets ( $N \leq 15$  pellets per individual), identified by their characteristic ribbon-shape, were individually transferred via micropipette to a grid-dish with  $\sim 100\text{ }\mu\text{L}$  FSW, and visualised (Olympus SZX16 microscope), photographed (Olympus DP74 camera), and latterly sized using CellSens® software (Olympus). Sinking velocities ( $\text{cm s}^{-1}$ ) of faecal pellets were calculated using established protocols [43–45]. In brief: a



**Fig. 1.** Schematics for the flume tank (laboratory experiment) and mussel deployment systems (field trials). (A) The flume tank housed mussels in an open channel with continuous  $\sim 0.2 \text{ m s}^{-1}$  flow generated by a 1.5 HP pump. Smaller in-line pumps (yellow circles; yellow arrows denote direction of flow) were positioned within the tank to prevent microplastics settling out; along open channel, the in-line pumps were connected to piping to direct water flow evenly across the base of the chamber. Three polycarbonate windows in the side of the 2 m long open channel permitted observation of mussels and biodeposit formation. (B) Flowthrough system in which natural seawater is pumped into the primary tank containing a basket of mussels; a 100  $\mu$ m mesh net directs sinking faeces into a receptacle, while floating pseudofaeces, are directed into a flotsam trap in the secondary tank. Yellow arrows indicate water flow via piping. (C) Sub-surface system in which caged mussels are suspended in water-column, with a 100  $\mu$ m mesh net and cod-end positioned beneath to capture microplastic-laden faeces.



2 L glass measuring cylinder was filled with temperature-acclimated FSW and maintained at  $15 \pm 1$  °C. Faecal pellets were carefully transferred to the head of the sinking column, and once a constant velocity was observed, the time taken for the pellet to travel between markers, 33 mm apart, was recorded (SI, Fig. S2C).

## 2.5. Microplastic removal by mussels deployed in a coastal system (modelled)

A coupled modelling system was used to simulate the effectiveness of deploying rope-grown mussels at the mouths of estuaries in a model of Lyme Bay (UK). The model system was built of three main components: ShellSIM [46] was used to estimate shellfish growth and their filtration capacity; ERSEM [47] provided the description of the biogeochemical processes and in particular the estimates of the primary productivity and subsequent organic detritus that were used as food source for the shellfish in ShellSIM as well as the sedimentation of the microplastics and FVCOM [48] simulated the physical environment (temperature, salinity, currents) and the transport of the different tracers (i.e. phytoplankton, microplastics) in the domain. The three models are coupled through the Framework for Aquatic Biogeochemical Models [49,50].

### 2.5.1. ShellSIM

ShellSIM is a coupled individual growth/population model for bivalves that simulates the growth of individual bivalves in response to food availability and environmental parameters [46]; individual growth dynamics are subsequently upscaled to the population level, with application in modelling shellfish aquaculture [51,52]. Here, ShellSIM was extended to account for the filtration, ingestion and egestion of microplastics by mussels. Microplastic filtration rates ( $FR$  expressed as microplastic  $L^{-1} \text{ ind}^{-1} \text{ d}^{-1}$ ) were calculated using the formula

$$FR = FR_{\max} (1 - e^{-\kappa \cdot MP})$$

where  $FR_{\max}$  is the maximum filtration rate (in microplastic  $L^{-1} \text{ ind}^{-1} \text{ d}^{-1}$ ),  $\kappa$  ( $L \text{ microplastic}^{-1}$ ) is the parameter characterising the initial slope of the relation between the filtration rate and the environmental concentration of microplastic  $MP$  (in microplastic  $L^{-1}$ ). Parameters for the filtration rate were taken from Woods, Stack, Fields, Shaw and Matrai [30] and are primarily affected by microplastic concentrations with saturation reached at very high microplastic concentrations ( $\sim 10^3$  microplastics  $L^{-1}$ ). The calculated filtration rate is then modified by applying standard environmental modulating factors (e.g. temperature, oxygen, salinity) according to the standard ShellSIM parameterisation for blue mussels [46]. Filtered microplastics are then removed within biodeposits (faeces, pseudofaeces) that rapidly sink and for the scope of this study they are considered removed from the system.

### 2.5.2. ERSEM

ERSEM (originally European Regional Sea Ecosystem Model) is a marine ecosystem model that simulates the dynamics of lower trophic levels and their consequences on the biogeochemical cycling of key elements (carbon, nitrogen, phosphorus and silicon) [47]. ERSEM has been used extensively to study a wide range of issues including climate change, eutrophication and Harmful Algal Blooms [53,54]. For this study, a new module was developed with the purpose of representing microplastic behaviour in the marine environment. In this application, it has been assumed that microplastics do not undergo physico-chemical degradation and that they are only affected by sedimentation. This process has been parameterised using equation 10 of Kaiser et al. (2019) [55], where the sedimentation rate of microplastics depends on the size of the particles (in particular the Equivalent Spherical Diameter) and the difference between the density of plastic and seawater.

### 2.5.3. FVCOM

The Finite Volume Coastal Ocean Model (FVCOM) is an

unstructured-grid ocean model that simulates the hydrodynamics of the coastal ocean [56]. With a triangular mesh of variable size, FVCOM is well suited to simulating irregular coastlines and providing high spatial resolution at sites of interest. In order to adequately simulate the small-scale features of the environment in the locations where the mussels are deployed in the model domain, we need resolving sub-km scale dynamics requiring a nested modelling approach of increasing model resolution. The atmospheric forcing is provided by a 3-step downscaling of Global Forecast System datasets (U.S. National Weather Service) using the weather-research-and-forecasting model to reach the 3 km of the final model domain. For the coupled model we use a parent domain of 1.5–10 km resolution to drive our 350 m–5 km high-resolution Lyme Bay model domain. The Lyme Bay domain and mesh are defined by the initial coastline sampled at resolutions of 700 m (Fig. 4 A). The model grid is constructed using an automatic mesh generator known as ADMESH+ [57] such that the resolution in the interior is controlled by the water depth, bathymetry gradient, coastline curvature and coastline resolution using a size function to build spatially varying element sizes to satisfy the hydrodynamic requirements. Final manual adjustment of the grid ensures the quality criteria in the FVCOM manual [56] are met. The final model grid contains 7996 elements constructed from 4136 nodes; element sizes range in size from 3500 m at the open boundaries to 350 m along parts of the area where the Lyme Bay Fisheries and Conservation Reserve is defined. The vertical discretisation of the water column uses a sigma level distribution of 24 vertical levels.

### 2.5.4. Model setup

The model set-up was used to simulate how mussels deployed at the mouths of rivers affect waterborne microplastic concentrations in coastal waters. To reduce the computational cost of the simulation, models incorporated only two types of microplastics, PS and PA-L as used in laboratory experiments, as representative microplastics. Microplastics were seeded in rivers with a constant concentration of 100 microplastics  $m^{-3}$ , with reference to literature-derived values [4]. An initial run without any mussels was used to simulate how microplastics discharged from local rivers were redistributed within the domain. Subsequently, we simulated the deployment of rope-grown mussels, in five areas close to the mouths of four estuaries discharging into the bay (Dart, Teign, Exe, Brit; Fig. 4 A) where the bathymetry was deeper than 5 m. The total area where mussels were deployed covered approximately 44  $km^2$  (equivalent to 192 nodes of the grid), with an average rope density of 0.01 ropes  $m^{-2}$  and each rope was seeded with 200 juvenile mussels  $m^{-1}$  (3.5 cm initial shell length) for a total of approximately 3 billion individuals. Ropes were seeded on May 1st 2005, about one week into the modelled spring bloom. All subsequent analysis of model results uses daily averaged fields calculated at runtime. Any further manipulation of the model results was performed with the python package PyFVCOM [48]. Where appropriate, the variables have been depth or domain integrated, accounting for the differences in volume that results from using a free surface and the unstructured horizontal grid and vertical sigma coordinates of FVCOM.

## 2.6. Microplastic removal rates by mussels in an urban marina (field)

Two complimentary mussel deployment systems with the capacity to capture mussel biodeposits were trialled in an urban marina, with the aim of: (1) determining the comparative role of mussel faeces and pseudofaeces in “capturing” microplastics under environmental conditions; and (2) testing the suitability of a prototype system for capturing microplastics in a real-world setting.

### 2.6.1. Flowthrough mussel deployment system

A flowthrough mussel deployment system was designed to compare the amount of microplastics encapsulated within mussel faeces and pseudofaeces under environmental conditions (Fig. 1B; SI, Fig. S3A).

This system uses a submersible pump (Marine DC, DCW-3500) to direct seawater via 20 mm Ø silicone piping into a primary tank (210 L capacity; dark green high-density polyethylene) containing mussels housed in a basket (blue polypropylene; 0.5 m Ø x 0.1 m height; 10 mm holes; suspended ~0.2 m below water surface using steel chains), with a 100 µm mesh net (white nylon; 0.5 m Ø aperture) and receptacle (600 mL glass jar “cod-end”) secured beneath. An outlet positioned at the top of the primary tank directs seawater via 43 mm Ø (grey polypropylene) into a “flotsam trap” (stainless steel ring; 0.5 m Ø x 0.1 m height; suspended at the water’s surface with steel chains) in the secondary tank (210 L capacity; dark green high-density polyethylene). Finally, an outlet in the secondary tank allows seawater to flow out of the system. Tanks were insulated with thermal foil (aluminium) to limit temperature change in seawater and wrapped in hessian to prevent gulls interfering with experimental set-up. The principle of this system is that any microplastics consumed by mussels will be egested, with sinking microplastic-laden faeces collecting in the cod-end, while any microplastics in floating pseudofaeces will be caught in the flotsam trap. To compare the proportion of microplastics in biodeposits, the number of microplastics identified in the cod-end and flotsam trap was adjusted to account for the quantity of microplastics identified in equivalent control samples.

#### 2.6.2. Prototype sub-surface mussel deployment system

A sub-surface mussel deployment system, enabling mussels to continuously filter out microplastics whilst submerged, was developed and optimised (Fig. 1 C; SI, Fig. S3 B-C). The prototype comprised a cylindrical cage (blue polypropylene; 0.6 m Ø x 0.1 m height; 10 mm Ø apertures; suspended at 1–2 m depth by orange polypropylene rope) in which the mussels could be exposed to a continuous flow of seawater. A 100 µm mesh net (white nylon; 55 cm Ø aperture) was secured beneath the cage, with the purpose of directing sinking mussel faeces into a cod-end (grey polypropylene; 1.5 L capacity; 100 µm mesh window for drainage during sample procurement).

#### 2.6.3. Field deployment

Mussel deployment systems were set-up near the inlet to an urban marina in Plymouth Sound, UK (50°21'46.97"N, 4°9'7.65"W) across July–August 2021 (seawater temperature 16.5–18°C). The marina receives anthropogenic inputs from run-off, drainage, recreational boats and commercial ships; nevertheless, an abundance of fish, mussels, sponges and macroalgae were observed within the marina, demonstrating suitability for mussel deployment. Cohorts of 5.0 kg of mussels (~300 mussels) were acclimated in cages maintained at 1–2 m depth within the marina for a minimum of 2 weeks prior to experimental use. Mussel cohorts were weighed (Hyindoor digital travel scale) and carefully transferred to the relevant deployment system; each system was deployed for 24 h, with a control system (with no mussels; located ~10 m apart) run in parallel (n = 4 per treatment). Following exposure, mussels were removed and samples procured: net samples from the flowthrough system were retained in the glass jar receptacle; particles in the flotsam trap were siphoned off using an in-line pump (Qmax) with 12 mm Ø tubing onto 100 µm nylon mesh and mesh subsequently folded-in on itself and sealed. Cod-end samples from the sub-surface system were poured into 2 L containers, with ultrapure water used to rinse out the cod-end to maximise sample collection. All samples were stored at 2–4 °C prior to analysis.

#### 2.6.4. Microplastic analysis

To limit contamination of samples with airborne or clothing-derived microplastics, all sample processing and analysis was conducted in Plymouth Marine Laboratory’s ultraclean laboratory, equipped with positive-pressure, air-filtration system (HEPA filters), controlled access double-door entry system, cotton labcoats and tac mats. Furthermore, all equipment was double-rinsed with ultrapure water and covered with aluminium foil between processing, and all filtration steps were

conducted in a laminar flow hood (Bassaire). To account for any potential contamination during sample processing, procedural blanks were treated in the same way as environmental samples. Field samples were filtered through a 100 µm nylon mesh and subsequently incubated with 0.2 µm filtered 10% KOH and 0.01% Tween 20 surfactant at 50 °C on an orbital shaker (Stuart Scientific SI50; 125 rpm) to breakdown organics and aid particle dispersion. Digested samples were then filtered through 50 µm nylon mesh discs (47 mm Ø), and mesh filters systematically analysed under a microscope for suspected microplastics (Olympus SZX16; x25 magnification). Organic-rich samples were filtered across multiple mesh discs and samples teased apart with a needle or fine forceps to improve visualisation of microplastics. Suspected microplastics were selected based on morphology, texture and hardness, recording particle shape, colour and size, with shortest and longest dimension measured using CellSens® software (Olympus). Suspected microplastics were selected for polymeric analysis using Fourier-transform infrared (FT-IR) spectroscopy. These particles were carefully transferred onto slides covered with aluminium foil, and analysed in reflectance mode (4000–1250 cm<sup>-1</sup>, 10–20 scans) using a Spotlight 400 imaging system (Perkin Elmer). Resultant spectra were compared against in-house, published [58] and commercially available spectral databases using SpectrumIR software (Perkin Elmer, 2017, version 10.6.0.893). The spectra of natural and semi-synthetic cellulosic microfibrils cannot be readily distinguished using FT-IR, therefore particles with spectra matching that of cellophane or cellulose were allocated as ‘semi-synthetic’ if red, blue or any other bright colour, and categorised as ‘natural’ if brown, clear, black or white. In total, spectral matches were ascertained for 737 particles (35%); of these particles 683 (93%) were identified as anthropogenic (i.e., synthetic, semi-synthetic, cotton).

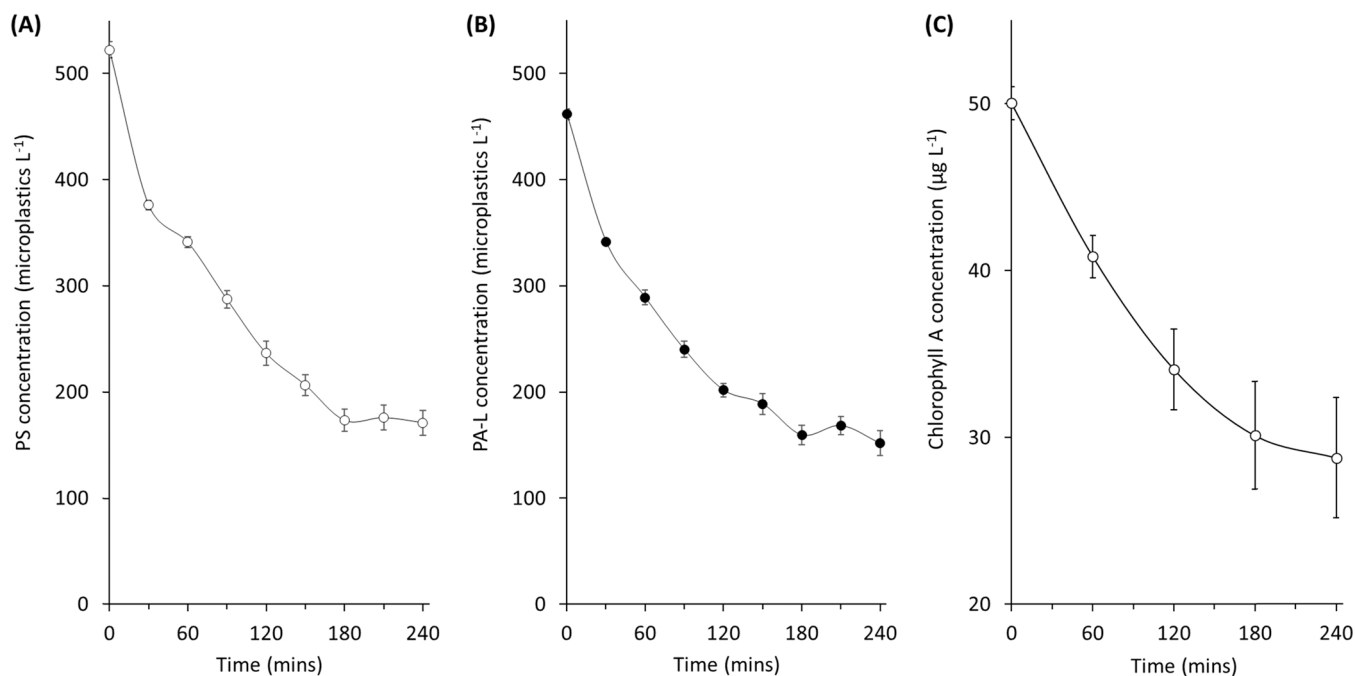
#### 2.7. Data analysis

All data were analysed using R statistical software (R Core Team, v3.4.1) [59]. Flume tank flow data conformed to *a priori* requisites and was therefore compared using ANOVA. Faecal pellet sinking rate data did not satisfy *a priori* requisites for parametric testing, therefore Kruskal Wallis tests with Dunn Test pairwise comparisons were used to compare between treatments. To calculate total microplastic abundance in each sample, the 93% microplastic identification rate was applied to all unidentified particles (i.e. particles not selected for FT-IR, particles lost during transfer to the FT-IR, or particles that did not achieve clear spectral data). Data were normalised to 5.0 kg mussels. For the sub-surface system, microplastic data were logarithmically transformed to satisfy *a priori* requisites for parametric testing and a Welch Two Sample t-test used to compare between treatments.

### 3. Results

#### 3.1. Microplastic removal rates by mussels under continuous flow (laboratory)

Average flow rates in the open channel of the flume tank were 2.6 ± 0.4 cm s<sup>-1</sup> without mussels (control) and 2.8 ± 0.4 cm s<sup>-1</sup> with mussels (ANOVA, F=1.388, p = 0.255; SI, Fig. S4). In the absence of mussels, microplastic concentrations remained stable over time (SI, Fig. S5). With mussels present, a rapid decrease in waterborne microplastic concentrations was observed. Mussels reduced waterborne microplastic concentrations in the tank by 66% over a three-hour period, removing microplastics at an average rate of 40,146 microplastics kg<sup>-1</sup> mussels h<sup>-1</sup>; accounting for the mean number of mussels per cohort (~300 individuals), this equates to an average removal rate of ~669 microplastics individual<sup>-1</sup> h<sup>-1</sup>. Mussels removed polystyrene microplastics at an average rate of 21,521 microplastics kg<sup>-1</sup> h<sup>-1</sup> (Fig. 2A) and nylon microfibrils at an average rate of 18,625 microplastics kg<sup>-1</sup> h<sup>-1</sup> (Fig. 2B). Microplastic removal rates closely paralleled algal ingestion rates



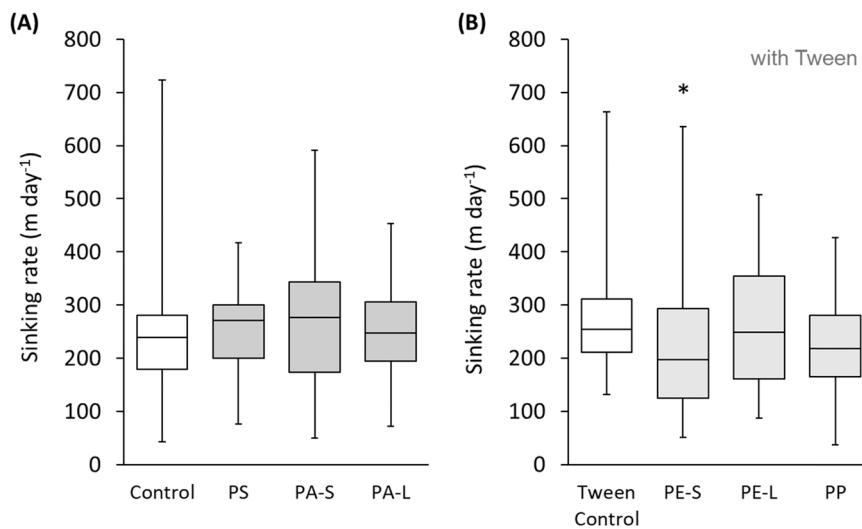
**Fig. 2.** Concentrations of (A) PS and (B) PA-L (microplastics L<sup>-1</sup>), and (C) Chlorophyll A (µg L<sup>-1</sup>) in flume tank over a 4-hour period following exposure to mussels (*Mytilus edulis*). Data shown as mean ± standard error, normalised to 5.0 kg mussels.

(Fig. 2C), with a sharp decline in algal and microplastic consumption observed after 3 h. During the exposure, mussels were observed feeding (i.e. open valves with visible mantle) and producing biodeposits; notably, we observed pseudofaeces (mucus-strings) either anchored to the mussels or floating at the water's surface, and faecal pellets collecting directly underneath the tray of mussels.

### 3.2. Fate of microplastics in mussel biodeposits (laboratory)

Mussels produced both larger, ribbon-shaped intestinal faeces and darker, irregularly shaped glandular faeces (SI, Fig. S6). Pseudofaeces production was infrequent within the static exposure systems, with only a handful of mussels producing pseudofaeces across all treatments. Owing to their high abundance, sinking rate experiments were

conducted on intestinal faeces only. Faecal sinking rates averaged 223–278 m day<sup>-1</sup> across treatments, demonstrating microplastic-laden mussel faeces sink rapidly (Fig. 3). Lowest sinking velocities were associated PE-S (223 ± 19 m day<sup>-1</sup>) and PP (231 ± 9 m day<sup>-1</sup>), and the highest sinking velocities observed with faeces laden with PA-S (266 ± 25 m day<sup>-1</sup>). However, only faeces contaminated with PE-S had sinking rates significantly different from controls (Kruskal Wallis, P = 0.04; SI, Table S2). Faecal volumes averaged 0.24 ± 0.03 mm<sup>3</sup> in the control and 0.19 ± 0.02 mm<sup>3</sup> in the Tween control. Faeces containing PA-L (0.16 ± 0.01 mm<sup>3</sup>) and PP (0.13 ± 0.01 mm<sup>3</sup>) were significantly smaller than their respective controls (PA-L: Kruskal Wallis, P = 0.02; PP: Kruskal Wallis, P = 0.01; SI, Fig. S7). Faeces containing polyethylene were on average 17–37% larger than controls (PE-S: 0.26 ± 0.04 mm<sup>3</sup>; PE-L 0.22 ± 0.02 mm<sup>3</sup>), however this result was not



**Fig. 3.** Box-and-whisker plot showing sinking rates (m day<sup>-1</sup>) of mussel faeces, with and without microplastics, in seawater: (A) Exposure to neutrally buoyant PS, PA-S and PA-L; (B) Exposure to low density PE-S, PE-L and PP with 0.01% Tween surfactant to facilitate immersion in seawater. \* denotes statistical significance from relevant control (Kruskal-Wallis, P = 0.04).

significantly different (Kruskal Wallis,  $P = 0.07$ ; SI, Table S2).

### 3.3. Microplastic removal by mussels deployed in a coastal system (modelled)

Modelled waterborne microplastic content in Lyme Bay correlated with river discharge (SI, Table S1), with highest microplastic loading in winter months when peak flows arise from higher seasonal rainfall (SI, Fig. S8A). Mussel growth followed a seasonal cycle mirroring the availability of food supplied by planktonic groups within ERSEM: mean shell length increased from 3.5 cm in May 2005–4.75 cm in September, then stays constant throughout winter to start growing again after the following spring bloom reaching 5.8 cm by the end of the simulation in most of the areas (SI, Fig. S8B). In the absence of mussels, the model showed microplastics redistributing throughout the Lyme Bay domain, with highest microplastic concentrations located close to riverine inputs and along the coast (SI, Fig. S8C). In total  $2 \times 10^{10}$  waterborne microplastics were removed by the mussels across the 20-month model run, representing close to 4% of the total microplastics discharged by the rivers in the same period. The temporal evolution of the removal efficiency (defined as the amount of microplastics the mussels filter compared to those discharged by the river at the same time) is noisy (Fig. 4B), following short term variability of the environmental conditions. However, a small seasonal cycle can be observed, with higher values (>9% removal) in late autumn and winter, and lower in early spring (<3% removal). While mussel growth is reduced during the winter due to insufficient food availability, the proportion of filtered microplastics (and subsequent packaging in biodeposits) is higher, resulting in higher removal efficiency. While the removal of microplastics occurs in the proximity of river mouths, the effect is visible in most of the coastal zone (Fig. 4 C).

### 3.4. Microplastic removal rates by mussels in an urban marina (field)

Mussels were successfully deployed in both the flowthrough and sub-surface systems. Samples contained anthropogenic particles comprising plastics, semi-synthetics (e.g. cellulosic bioplastic) and cotton in fibrous and particulate forms. An average of 1.5 semi-synthetic fibres sample<sup>-1</sup> were found in procedural blanks, indicating low levels of contamination. In the flowthrough system, after 24 h exposure: cod-ends contained  $14.2 \pm 2.7$  anthropogenic particles in the control system and  $48.9 \pm 12.7$  anthropogenic particles with mussels; flotsam traps contained  $18.5 \pm 6.0$  anthropogenic particles in the control system and  $13.4 \pm 2.5$  anthropogenic particles with mussels. Accounting for control data, we calculate mussel faeces encapsulated  $34.6 \pm 14.1$  anthropogenic particles day<sup>-1</sup> and floating pseudofaeces encapsulated  $-5.1 \pm 7.0$  anthropogenic particles day<sup>-1</sup> (t-test,  $P = 0.06$ ; Fig. 5A). In the sub-surface system, mussels were demonstrated to capture significantly more anthropogenic particles than in control systems: following a 24 h exposure, cod-ends contained  $72.5 \pm 20.5$  anthropogenic particles in the control system, and  $320 \pm 140.1$  anthropogenic particles with mussels (t-test,  $P = 0.04$ ; Fig. 5B). Accounting for control data, we calculate 5.0 kg mussels captured  $239.9 \pm 145.9$  anthropogenic particles day<sup>-1</sup>. The polymer, shape and colour of anthropogenic particles identified in the sub-surface system were similar for control and mussel treatments (SI, Fig. S9). However, the size of particles identified in the mussel treatments (mean fibre length: 817  $\mu\text{m}$ ; mean fibre diameter: 25.8  $\mu\text{m}$ ; mean fragment diameter: 148  $\mu\text{m}$ ) were on average smaller than in controls (mean fibre length: 1394  $\mu\text{m}$ ; mean fibre diameter: 32.9  $\mu\text{m}$ ; mean fragment diameter: 263  $\mu\text{m}$ ).

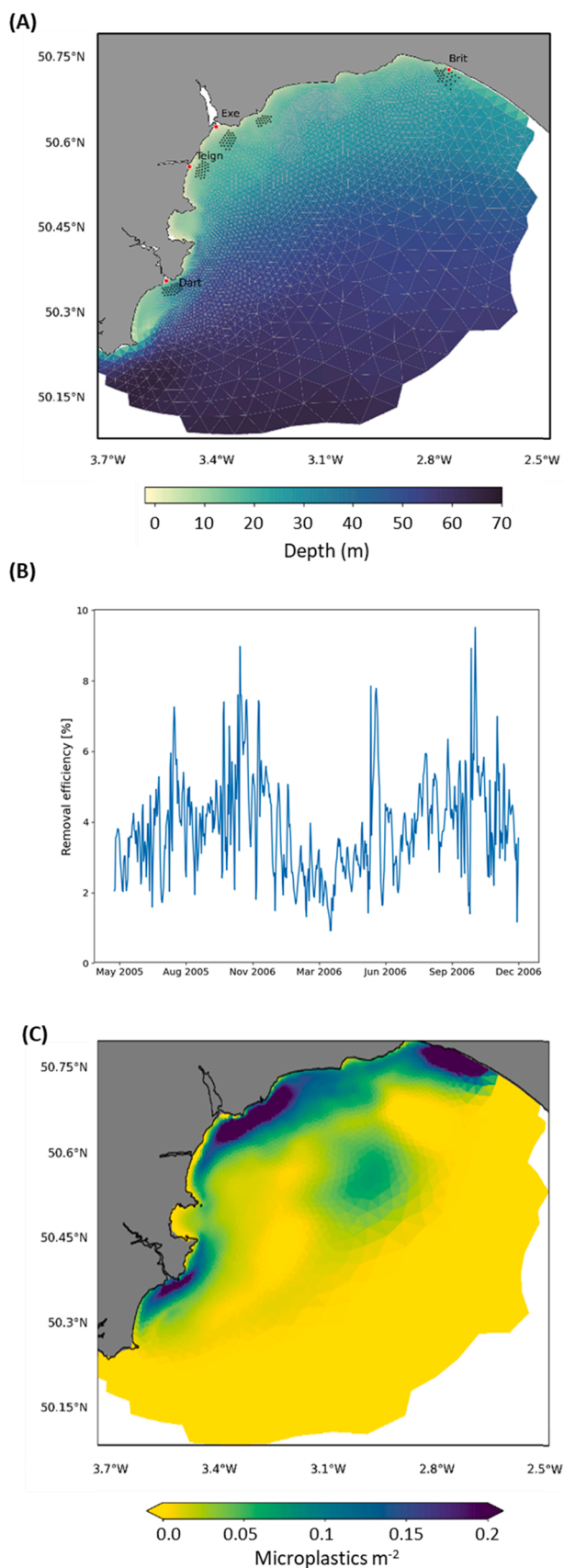
## 4. Discussion

Our flume experiments ascertained mussels are effective at removing waterborne microplastics under continuous flow (2.5 cm s<sup>-1</sup>). Exposed to 1000 microplastics L<sup>-1</sup>, *M. edulis* (57.2  $\pm$  0.6 mm shell length)

demonstrated capacity to filter out microplastics at a rate of 40,146 microplastics kg<sup>-1</sup> h<sup>-1</sup> or  $\sim 669$  microplastics mussel<sup>-1</sup> h<sup>-1</sup>. Laboratory exposures using 1 L static systems provide evidence of even higher microplastic removal rates by mussels exposed to higher microplastic concentrations. For example, *M. edulis* exposed to 3000–30,000 polyethylene terephthalate microfibrils L<sup>-1</sup> (20  $\mu\text{m}$   $\times$  450  $\mu\text{m}$ ) exhibited maximal microplastic removal rates of  $\sim 5200$  microfibrils mussel<sup>-1</sup> h<sup>-1</sup> [30], and *M. galloprovincialis* exposed to 10,000–1,000,000 polystyrene microbeads L<sup>-1</sup> (2–10  $\mu\text{m}$ ) reduced waterborne microplastics concentrations by  $\sim 80\%$  in 1 h at a rate of  $\sim 260,000$  microplastics mussel<sup>-1</sup> h<sup>-1</sup> [32]. Mussel filtration rates are affected by the age, size and health of the population, and environmental parameters including temperature, salinity, pH and food availability [60]. In *M. edulis*, microalgal concentrations exceeding 10.0  $\mu\text{g}$  Chla L<sup>-1</sup> have been shown to trigger reductions in filtration activity to prevent saturation of the alimentary canal [23]. This physiological response is evident in our flume experiments, where high concentrations of microalgal prey ( $\sim 50$   $\mu\text{g}$  Chla L<sup>-1</sup>) can explain the observed decline in Chla, PS and PA-L removal after 3–4 h exposure. Microplastic removal rates can be further affected by the size, shape, surface charge and concentration of waterborne microplastics [29,61], and the structural complexity of mussel populations and flow velocity in overlying waters [62]. Using a flume, Lim et al. [62] demonstrated mussels can reduce near-bed flow velocities through drag and formation of turbulent eddies, with resulting energy dissipation associated with microplastic deposition; the authors further observed mussels captured significantly greater amounts of microplastic at higher flow velocities (48 cm s<sup>-1</sup> vs 8 cm s<sup>-1</sup>,  $P < 0.001$ ). Given our flume experiments used a mean flow velocity of  $\sim 2.5$  cm s<sup>-1</sup>, we surmise that microplastic removal rates of mussels presented in this study are likely an underestimation of their full potential.

Mussels produce two main types of biodeposit: pseudofaeces, describing strings of mucus that entrap and remove particles from the labial palps (i.e. does not pass through gut) in response to the presence of inorganic particles (e.g. silt, kaolin) and high food concentrations (>15  $\mu\text{g}$  Chla L<sup>-1</sup>) [23]; and faeces, comprising intestinal faeces, containing material partially processed in the hindgut, and glandular faeces, composed of material processed within the midgut gland [23]. Laboratory studies suggest microplastic shape, size and concentration are determining factors in whether plastic particles are selectively rejected or ingested by mussels [29]. For example, *M. galloprovincialis* exposed to 2–10  $\mu\text{m}$  polystyrene and 1–22  $\mu\text{m}$  high density polyethylene microplastics eliminated all microplastics via their faeces [32,63], whereas *M. edulis* rejected  $\sim 70\%$  of the 20  $\mu\text{m}$   $\times$  450  $\mu\text{m}$  polyethylene terephthalate microfibrils to which they were exposed via pseudofaeces (Woods, Stack et al., 2018). We postulate pseudofaeces production observed in the flume tank resulted from continuous exposure to high microalgal concentrations ( $\sim 50$   $\mu\text{g}$  Chla L<sup>-1</sup>), which was similarly linked to observed reductions in filtration activity after 3–4 h exposure. As such, we recommend future flume experiments compare microplastic removal rates and pseudofaeces production by mussels exposed to anthropogenic particles of varying size, shape and concentration with a range of microalgal concentrations and flow rates. Mussel biodeposits have been observed and modelled to rapidly settle out of the water column, enriching underlying sediments with organic matter and nutrients [64,65], and potentially redistributing waterborne microplastics to the benthos [33,35,66]. In our experiments, mean sinking rates of intestinal faeces were 230–278 m day<sup>-1</sup> across treatments, with faeces containing polyethylene (PE-S) exhibiting significantly reduced sinking rates. Comparatively, *M. galloprovincialis* faeces containing 41–129  $\mu\text{m}$  polyamide or 100–500  $\mu\text{m}$  polypropylene microplastics had mean sinking rates of 352–422 m day<sup>-1</sup>, with all microplastic treatments sinking at significantly reduced rates compared to controls [33]; similarly, *M. trossulus* faeces containing 32–38  $\mu\text{m}$  polyethylene microplastics had median sinking rates of  $\sim 700$  m day<sup>-1</sup>, with significantly reduced sinking rates compared to controls [45]. In the flume tank, we observed that pseudofaeces were either anchored to the mussels or



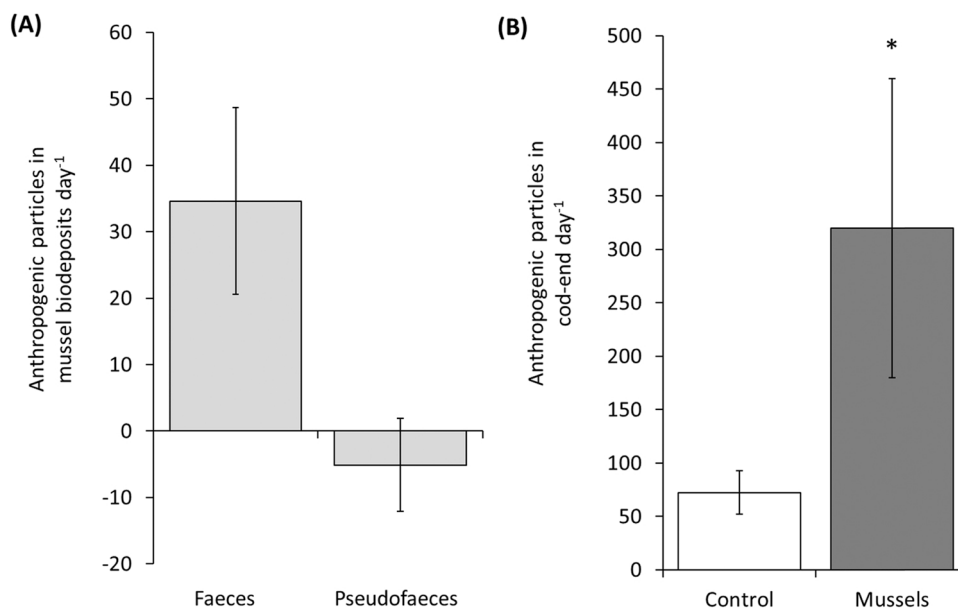


(caption on next column)

**Fig. 4.** Modelled mussel deployment in Lyme Bay domain. (A) High-resolution model of Lyme Bay domain with triangular mesh; red dots (●) denote river outflows (Dart, Teign, Exe and Brit) and black dots (●) show deployment of mussels on ropes. (B) Temporal variability of the removal efficiency, defined as total microplastic filtered by mussel each day relative to those discharged by the rivers. (C) Average decrease in waterborne microplastic concentrations in Lyme Bay domain following deployment of mussels at the mouths of local rivers, averaged across 1-year timescale.

floating at the water surface, suggesting they are buoyant. However, this contradicts the results of Harris et al. [45] who observed *M. trossulus* pseudofaeces containing 32–38  $\mu\text{m}$  polyethylene microplastics had median sinking rates of  $\sim 370 \text{ m day}^{-1}$ ; such differences may stem from the size and consistency of the pseudofaeces and the prevalence of higher-density biogenic particles (e.g. silicate diatoms) present in the mucosal matrix. To establish the fate of microplastics filtered out by *M. edulis* under environmental conditions, we designed the flowthrough mussel deployment system. As with our static exposures, our field data suggests microplastics are typically incorporated into mussel faeces, with minimal evidence of floating pseudofaeces production.

To evaluate the capacity for mussels to remove microplastics in real-world scenarios, we developed a coupled FVCOM-ERSEM-ShellSIM model and conducted field-trials with a prototype deployment system. Microplastic dispersion is typically simulated using particle tracking models (i.e. a Lagrangian approach tracking all particle trajectories); these models can simulate vast numbers of particles, each capable of random behaviours to better represent the stochasticity of the real environment [67–69]. However, here we adopted an Eulerian framework, in which we modelled the evolution of microplastic concentrations at every single point in the domain over time. This approach allowed for seamless integration with other models that work in the same Eulerian framework (i.e. FVCOM, ERSEM and ShellSIM), and is considered particularly well suited to modelling regions with high microplastic concentrations [70]. In the model, mussels were deployed from long-lines, mimicking mussel deployment in aquaculture, close to the mouths of estuaries; placing mussels in such locations in the real-world would interfere with other maritime activity (i.e. shipping, fishing), but was used in the model to provide an indication of how mussels handle microplastic emitted from nearby outflows. Mussel growth estimated by ShellSIM is in line with growth rates observed in aquaculture farms in the area, with a time to market of about 12–15 months (SI, Fig. S8B). In the absence of intervention, the spatial distribution of waterborne microplastics along the coast is associated with coastal tidal excursion (3–7 km) which takes place parallel to the coast, with microplastic sedimentation most prevalent within 3 km of their input into the model domain (SI, Fig. S8C). The simulated specific filtration rate was on average  $0.75 \pm 0.72 \text{ microplastics kg}^{-1} \text{ d}^{-1}$ , with peaks of  $5.5 \text{ microplastics kg}^{-1} \text{ d}^{-1}$ . Filtration rates were dependent on the ambient concentration of microplastics, which in the model domain were  $< 1 \text{ particle m}^{-3}$ . Comparatively, a recent study in the Western English Channel estimates microplastics concentrations for  $< 100 \mu\text{m}$  microplastics to be  $\sim 20 \text{ particles m}^{-3}$  [4]. If model data is scaled to match these ambient concentrations, the modelled filtration rates would only be a factor of two different from the 48 anthropogenic particles  $\text{kg}^{-1} \text{ d}^{-1}$  measured in the field. Differences in microplastic capture rates can be further explained by differences in mussel size, stocking density, position in the water column, as well as potential underestimations of microplastic removal rates in the model. The seasonal variability of the removal efficiency follows closely the dynamics of the inputs from rivers: in late autumn and winter, when the high river flow discharges a large amount of microplastics, their concentration in the bay is higher and mussels are more effective at removing them, defined here as microplastic particles removed per unit of time (e.g. day). The effect of mussels in filtering microplastic was mostly evident parallel to the coast where microplastic are more abundant (Fig. 4B). However, it is noticeable how, by removing the microplastics close to the coastline, the



**Fig. 5.** Microplastics collected via mussel deployment systems in an urban marina. (A) Comparison of microplastic concentrations in mussel faeces and pseudofaeces in flowthrough systems. (B) Microplastics samples from cod-end of sub-surface system for control and mussel treatments. Data shown as mean  $\pm$  standard error, normalised to 5.0 kg mussels; \* denotes statistical significance from the control (t-test,  $P < 0.05$ ).

benefits can also be observed offshore as less microplastic are advected with currents.

The design of our prototype sub-surface mussel deployment system was informed by experimental results, with a focus on the collection of microplastic-laden mussel faeces to facilitate the removal of microplastics from estuarine and coastal environments. The lightweight, modular prototype system was constructed from plastic materials, reducing manufacturing costs and facilitating rapid design modifications during method optimisation. A limitation of using plastic in the design, is that there is the potential these materials might shed and contaminate the samples. Of the 1955 anthropogenic particles in our samples, we found no orange polypropylene fibres (support lines), white nylon fibres (net) or grey polypropylene (cod-end), however we did observe 18 blue polypropylene fragments (7 control; 11 mussel treatment) that may have derived from the mussel cages; as such, we recommend future trials use deployment systems constructed from metal (e.g. stainless steel). A peristaltic pump was used to continuously sample marina water to ascertain average microplastic concentrations, however flotsam (e.g. jellyfish, salps, seaweed) repeatedly blocked the inlet. Based on recent fieldwork in the Plymouth Sound, we estimate waterborne concentrations of  $> 100 \mu\text{m}$  microplastics to be  $\sim 20$  microplastics  $\text{m}^{-3}$  [4]. In our field trial, *M. edulis* removed 4.5-fold more anthropogenic particles than controls, at a rate of 48 anthropogenic particles  $\text{kg}^{-1} \text{day}^{-1}$ . Our results closely align with those of van Colen et al. [35], who deployed *M. edulis* at 0.5 m depth in an urban port in Belgium, and identified the mussels removed 4.3-fold more microplastics than controls, at a rate of 11 microplastics  $\text{kg}^{-1} \text{day}^{-1}$ . Here, mussel faeces were shown to contain microplastics, semi-synthetic and cotton fibres representative of the diverse array of anthropogenic particles contaminating aquatic ecosystems [3,71]. Anthropogenic particles in mussel faeces ranged 29–515  $\mu\text{m}$   $\text{Ø}$ , while fibres ranged 8–32  $\mu\text{m}$   $\text{Ø}$  and up to 6.9 mm in length. This corresponds with laboratory data showing *M. edulis* can filter out  $< 100 \mu\text{m}$   $\text{Ø}$  microplastics with high efficiency, and, although mussels can capture 500–1000  $\mu\text{m}$   $\text{Ø}$  microplastics, physiological constraints (i.e. gape size) preclude ingestion of microplastics larger than  $\sim 500 \mu\text{m}$  [29,72].

This study provides clear evidence that mussels can be used to remove anthropogenic particles from aquatic ecosystems. While we advocate that blue mussels (*M. edulis*) are well-adapted to removing

microplastics from temperate coastal and estuarine waters, other pollution-tolerant filter-feeding bivalves, including zebra mussels (*Dreissena polymorpha*), Asian clams (*Corbicula fluminea*) and ribbed mussels (*Geukensia demissa*), could provide similar functionality in native freshwater and brackish habitats [73–75]. Prior to deployment, it is imperative to ensure environmental conditions (e.g. temperature, salinity, food availability) are suitable [23], the species of concern will not pose a biosecurity risk (e.g. biofouling, invasive species), and that protocols for recovering and processing microplastic-laden biodeposits are established. To maximise microplastic removal efficacy, mussels would ideally be situated near point sources of pollution (e.g. marinas, wastewater outflows) to capture anthropogenic particles prior to dilution. However, it may be necessary to site mussels further downstream to avoid health deterioration in response to high concentrations of anthropogenic contaminants (e.g. pesticides, pharmaceuticals, endocrine disruptors, pathogenic microbes, surfactants, metals, polycyclic aromatic hydrocarbons) stemming from wastewater and industry [76]. Exposure studies with mussels have identified microplastics can affect sub-cellular and cellular function (e.g. genotoxic damage, antioxidation pathway activation, heightened immune response) [32,77–80] and physiology (e.g., byssal strength, metabolic rate) [30,81–83]. While these results may sound alarming, it is important to note that microplastic concentrations used in mussel exposure studies ( $42, 900\text{--}73 \times 10^{12}$  microplastics  $\text{L}^{-1}$ ) typically far exceed waterborne microplastic concentrations *in natura* ( $0.28 \times 10^6\text{--}1770$  microplastics  $\text{L}^{-1}$ ) [84,85]. Based on the current literature, at environmentally relevant microplastic concentrations, chronic exposures studies have evidenced sub-lethal effects reduced feeding and growth in mussels [86,87], but have not indicated any increase in mussel morbidity or mortality. However, combined effects of microplastics and other anthropogenic stressors (e.g. chemicals, metals) on aquatic biota, especially over chronic timescales, are currently unclear [88–90]. We therefore advise future studies considering the efficacy of mussels in capturing microplastics should closely monitor the health of the mussel population. Given the ecosystem services offered by filter-feeding bivalves, their deployment may have additional environmental benefits, including pollution abatement, water purification, nutrient cycling, biodiversity gains, wave attenuation and opportunities for educational outreach [91, 92]. Indeed, systems incorporating mussels, or mussels in combination

with macroalgae (e.g. seaweed, kelp), have been suggested as eco-engineering solutions for improving water quality, through reductions in ammonium, nitrogen, bacteria and suspended particulates [25,93–95], and coastal protection [96,97]. We conclude that deploying mussels in apt locations could provide holistic environmental benefits to freshwater, estuarine and coastal habitats, warranting further testing through comprehensive trials in real-world scenarios.

### Environmental Implication

Anthropogenic particles, including microplastics, microfibrils and other manmade materials, are a prolific environmental contaminant. Over the past fifteen years there has been growing evidence that these particles can be readily ingested by a wide array of organisms and can cause adverse health effects across levels of biological organisation. This study provides clear evidence that mussels can be used to remove anthropogenic particles from aquatic ecosystems, encapsulating them into their biodeposits which can be subsequently captured and removed. This novel and timely methodology is part of a Nature-based Solutions approach to tackling the growing threat plastic debris poses to natural ecosystems.

### CRedit authorship contribution statement

MC & PKL undertook Project conceptualization, Funding acquisition, Project administration. All authors contributed to Methodology and Investigation. YA, GG & RT undertook modelling and relevant validation and analysis. MC, RC, PKL, RS & TV contributed to labwork and fieldwork. MC, RC, YA, GG & RT undertook Formal analysis. MC, RC, YA & RT prepared original draft. All authors reviewed and edited manuscript.

### Declaration of Competing Interest

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

### Data availability

Data will be made available on request.

### Acknowledgements

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2023.131392](https://doi.org/10.1016/j.jhazmat.2023.131392).

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