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Spatial arrangement of biogenic reefs alters boundary layer characteristics to increase risk of microplastic bioaccumulation

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Abstract
Microplastics are now synonymous with human impacts on the environment and as a threat to marine organisms. Numerous taxa are at risk from microplastics including commercially valuable bivalves as seafood, which are also disproportionately important as biogenic reef-forming species that enhance biodiversity such that they are commonly protected under conservation actions. As a sessile filter-feeding organism, bivalves are highly susceptible to microplastic ingestion but despite their socio-economic and ecological importance, no research has been undertaken to assess how a reef’s structural arrangement might affect plastic ingestion. Here, using a series of flume experiments, we examined how change in spatial arrangement of the blue mussel, *Mytilus edulis*, interacts with different flow speeds to effect retention of microplastic over reef surfaces and ingestion risk by individual mussels. Our results show that clumped spatial arrangements reduce boundary layer velocities, and increase turbulence, boundary layer thickness and plastic retention over reef surfaces under faster flow conditions, increasing plastic ingestion by 3-fold. Our findings suggest that the structural arrangement and rugosity of natural reef structures may create natural sinks of anthropogenic pollution, and species like *Mytilus* that are also important species for human consumption, while disproportionately susceptible to microplastic pollution, may be useful bioindicators of microplastic pollution.

1. Introduction
In the Anthropocene, microplastics has been recognised as a threat to marine organisms due to their persistence and pervasiveness in the marine environment (Moore 2008, Thompson et al 2009, Brandon et al 2016). Plastic ingestion has been documented in over 300 species (Galloway et al 2017) across multiple feeding guilds from detritivores to deposit feeders, filter feeders and planktivores (Christaki et al 1998, Graham and Thompson 2009, Murray and Cowie 2011). Among these, suspension and filter feeders are argued to be the most susceptible to microplastic ingestion due to their large filtration capacities (Asmus et al 1992) and their sessile life-history preventing avoidance (Ward and Shumway 2004). Recently, Setälä et al (2016) demonstrated that bivalves ingest significantly more microplastics than any other taxa, perhaps because microplastics (i.e. particles between 0.1 µm to 5 mm diameter; Galloway et al 2017) are similar in size and density to phytoplankton and algae (Brillant and Macdonald 2000, Cole et al 2013). Some bivalves, such as mussels, can sort food particles (Kiørboe et al 1980), rejecting microplastics in their pseudofaeces (Woods et al 2018; see Ward and Shumway (2004) for review) but this selectivity may be compromised by the physical structure of the reef increasing an individual’s exposure to plastic pollution (D’Onghia et al 2017).

Reef-forming species are ecosystem engineers (sensu Jones et al 1994) that can form highly complex structures. Mussels, for example, create a reef matrix comprised of shell hash held together by byssal threads, underlying sediment and pseudofaeces (Suchanek 1985). These structures can serve as habitat for other organisms, provide support for commercial fishery species (Kent et al 2017), and are associated with conservation priorities including...
increased biodiversity, abundance and productivity (Ricciardi et al 1997, Koivisto and Westerbom 2010). The reef structure influences the physical environment by altering boundary layer flows near the bed (Butman et al 1994, Green et al 1998); an interaction shown to affect larval recruitment and sediment dynamics (Widows et al 1998, Lapointe and Bourget 1999). The structure of the reef slows water flow over its surface, dissipating energy in the form of turbulent eddies (van Donker 2013) resulting in turbulent mixing with the potential to resuspend particles, and increase uptake by filter-feeders. A larger reef surface area also has a higher probability of particles settling over them (Fuchs and Reidenbach 2013). These mechanisms may result in the increased susceptibility of reefs to microplastic ingestion and increase the likelihood of trophic-level transfer (Farrell and Nelson 2013, Khan and Prezant 2018) and biomagnification of microplastic-associated contaminants (Mato et al 2001) to higher trophic levels including humans (Seltenrich 2015). Furthermore, ingestion of microplastics by individuals may lead to reduced functioning of reef structures as biogenic habitats, although this effect is equivocal depending on the species (Green et al 2016). For instance, Green et al (2019) showed that blue mussels that ingested microplastics resulted in 50% reduction in byssal attachment strength potentially causing increased mortality through dislodgement by wave action, and/or reduce their ability to maintain or form reef structures with spill-over effects for their associated assemblages.

Despite the ecological importance of reefs and their potential for increased exposure to microplastic, little research has been undertaken to assess how the reef structures themselves interact with microplastics pollution despite considerable recent focus on plastic pollution. While a positive correlation between reef structures and microplastic pollution has been shown, the underlying mechanism that promotes microplastic retention over reefs has not. For instance, D’Onghia et al (2017) found correlation between the abundance of macroplastics and the structure of cold-water corals and inferred a ‘trapping effect’. Nel and Froneman (2018) identified microplastics in the tube structures of the reef-forming polychaete Guinarea gaimardi and suggested that they could be a sink for microplastics, while Khan and Prezant (2018) demonstrated ingestion in ribbed marsh mussel beds and high abundances of microplastics in surrounding sediment, which they attributed to change in settling velocity.

To address this gap, here we examine how changes in the spatial arrangement of an important reef-forming organism, the blue mussel Mytilus edulis, interact with different current flow speeds characteristic of exposed and sheltered environments to affect the density of microplastic in the boundary layer over a reef and ingestion of microplastics in mussels.

2. Methods

Unpurified Mytilus edulis were obtained from the Fowey Mussel Farm (Cornwall, UK) and transferred into flow-through seawater tanks (salinity = 35, temperature = 15 °C) for 1 week. Mussels were separated, cleaned of epibionts and byssal threads removed before spatial arrangement in a (i) clustered or (ii) random configuration (Plate 1) on square acrylic tiles (25 × 25 cm) at a density of 32 individuals per tile (equivalent to 512 individuals m⁻² or ~ 5 kg m⁻² based on aggregation densities as described in van de Koppel et al 2008). An acrylic tile had 16 × 16 evenly-spaced holes (loci), with each loci assigned a xy coordinate (e.g. x(y1)). Sixteen clustered and 32 random coordinates (loci) for each tile were generated in R (R Development Core Team 2017) that specified the placement of each mussel/pair of mussels on the tile. At each coordinate, either one (random) or two (clustered) mussels were attached to the tile in an upright position using metal wire passing through a small 1.5 mm hole drilled through its hinge (anterior end) (after Denny 1987). Following attachment, tiles were returned to flow-through tanks for 48 h to allow time for byssal thread attachment and recovery from stress.

2.1. Surface rugosity of patches

Rugosity index has long been used to examine habitat complexity at a range of scales from centimetres to kilometres, and is a common metric used to examine structural changes among reefs (see Dustan et al 2013 and references therein). A simple 2-D measure of rugosity/roughness was calculated for each tile. Rugosity index (RI) was calculated as a ratio of the length of the reef contour to the length of a straight line over the reef in one plane (Friedman, 2012), estimated from a 2D image in Imagel (Harwell et al 2010, Schneider et al 2012).

2.2. Flume experiment

Tiles were sequentially placed in a 20 m recirculating flume measuring 0.6 m width and 1 m depth and subjected to one of two advective flow speed regimes: (1) low flow = 8 cm s⁻¹, and (2) high flow = 48 cm s⁻¹. In extreme wave-exposed locations, flow velocities can exceed 1500 cm s⁻¹, although average maximum velocities are typically ~50 cm s⁻¹ (Bell and Denny 1994, Gaylord 1997). Surface flow characteristics and free-stream laminar flow velocities over the mussel models were measured using a Vectrino laboratory Acoustic Doppler Velocimeter (Nortek International, Norway). Water was seeded with a neutrally buoyant synthetic copolymer powder (Talisman 20, Plascoat®) comprised of non-uniformly shaped grains between 65–180 µm diameter. Microplastics were added to the flume at an approximate density of ~500...
particles cm\(^{-3}\), mixed well and allowed to circulate for 90 s prior to each tile being placed in the flume.

Two-dimensional Particle Tracking Velocimetry (PTV) was used to track the movement of individual plastic particles over time and generate time-averaged estimates of microplastic density, flow speed, turbulent kinetic energy (TKE) across the vertical (from the bed to the surface). Experiments were conducted in the dark and microplastics were illuminated using a custom-made light box comprising of a row of 50 white LEDs between two sheets of black acrylic to create a thin light sheet (~1 cm thick; 1 m length). A high-speed camera (Photron Fastcam SA4, USA) was used to record 60 frames sec\(^{-1}\) at a shutter speed of 1/100th of a second at 1024 × 1024 pixel resolution with the camera positioned horizontally perpendicular to the direction of flow (supplementary figure 1 (https://stacks.iop.org/ERL/15/064024/mmedia)).

For every tile \((n = 3\) per flow speed and spatial arrangement; \(N = 12\)), 60 s of footage for PTV analysis was recorded (3600 images). Each tile was placed in the flume for 40 min and mussels allowed to feed before removal and immediate euthanasi by freezing at \(-80^\circ\)C.

Camera images were processed using Streams 3.00 (Nokes, University of Canterbury, NZ). Images were first pre-processed using a range of brightness and movement algorithms to ensure that individual particles were easily identifiable and could be tracked in space and time. Individual still images were then stitched together to create a time series, and thus an animation of the movement of particles over a known distance (horizontal and vertical) with an arbitrary 3 mm\(^2\) grid overlaid. Pre-set algorithms within Streams 3.00 were then used to estimate Eulerian field velocity (mm s\(^{-1}\)), particle density and turbulent kinetic energy (TKE) dissipation rates (mm\(^2\) s\(^{-2}\)) within each grid cell, averaged over the 60 s of footage.

As the density of particles in the flume can naturally vary, the density of particles in each cell was standardised to a proportion of the total particle abundance within the field of view from the captured images allowing direct comparisons between tests, irrespective of differences in particle density.

2.3. Mussel plastic ingestion

Frozen mussels were first allowed to thaw to room temperature (21 °C) and the following morphometrics measured: umbo-dorsal length, width, wet whole weight (g), wet tissue weight (g). Biomass (dry mass per m\(^2\)) was then calculated using the equation described by Westerbom \(\text{et al} (2002)\):

\[
\text{Dry tissue mass (g)} = \text{shell length}^{2.307} \times 10^{-4.744}\]

From each tile, five mussels were chosen at random and their digestive gland and intestine removed by dissection, which was then freeze-dried in preparation for digestion. Standard methods (see Woodall \textit{et al} 2015) were used to minimise the risk of contamination. To avoid the cross-contamination of microplastics between mussels, all dissecting and measuring equipment were rinsed thoroughly with clean water between each dissection prior to microwave digestion. Microwave digestion was adopted instead of conventional digestion methods for its time-effectiveness and ability to digest difficult samples such as lipid-rich mussel tissue by creating a pressurised environment. Tissue samples were transferred into 20 ml Teflon tubes externally reinforced with composite sleeves. Since temperature and the sample mass determines the amount of pressure built up within the vessels, 3 ml working volume of 30%
hydrogen peroxide ($\text{H}_2\text{O}_2$) was added as per the manufacturer’s recommendations to a maximum mass of 0.07 g of freeze-dried mussel tissue (43 ml g$^{-1}$).

Microwave digestion was carried out using MARSXpress (CEM) and PEROX 16 40SM protocol outlined in table 1. This protocol was repeated twice to maximise digestion. Following microwave digestion, the samples were left to cool down to room temperature for 30 min. Each sample was then vacuum filtered onto Whatman Grade 541, Hardened Ashless Filter Paper ($D = 47 \mu m$, $22 \mu m$ pore size). For each sample, the digestion tubes and filter funnel were rinsed thrice with distilled water to ensure that all particles were collected and the beaker containing all rinse water also filtered to maximise recovery. Each filter paper was transferred to a sterile covered petri dishes and dried in an oven at 60 °C for 24 h.

2.4. Microscopic identification and counting
Each dried filter paper was examined under a compound microscope (Leica M205 C, Leica Microsystems) at a magnification of between 7.8x to 160x. Microplastics were distinguishable from residual organic debris as being roughly spherical shaped particles with a smooth, glossy appearance (Plate 2) and were manually counted using a grid method (see ‘Guide to microplastic identification’, MERI, available from www.meriresearch.org).

3. Data analysis
3.1. Morphometrics and surface rugosity data analysis
Permutational multivariate analysis of variance (PERMANOVA) using the adonis function in the vegan package (Oksanen et al. 2019) was used to compare morphometrics (see above) of mussels allocated to each tile, spatial arrangement and flow regime. An unpaired students t-test was performed to compare rugosity between tiles in clustered and random treatments.

3.2. Horizontal velocity, turbulent kinetic energy and particle densities
The particle tracking velocimetry (PTV) approach adopted here to generate spatially (by pixel) resolved estimates of horizontal velocity, turbulent kinetic energy (TKE) dissipation rate, and particle density are generated from a time series of 'stitched' still images. As such, data from each image are therefore both pseudo-replicated (Hurlbert 1984) and autocorrelated making formal hypothesis-driven statistical tests inappropriate, not least due to the risk of Type-II error. A meta-analysis approach using effect size to test for difference was therefore used to compare differences between the four experimental treatment combinations. Effect sizes were estimated based on standardised mean difference between pairs of experimental treatments and assessed using Cohen’s d (Cohen 1988; see Sawilowsky 2009 for a description of effect size magnitude and interpretation).

Height-over-reef ($y$-axis, cm) contours of flow velocities (the 5 cm s$^{-1}$ contour is shown for brevity) were plotted for the horizontal length of the reef. The $y$-axis heights of the contour for every value of x for each mean time-averaged plot (see figure 3) were then extracted and compared using a one-factor Kruskal-Wallis rank sum test. Differences among groups were then compared using a Nemenyi test.

3.3. Microplastic ingestion analysis
The number of plastic pieces ingested per individual per flow and spatial arrangement combination was assessed using a 2-factor generalised linear model (glm) with a quasipoisson error structure to account for over-dispersion. Differences between treatment groups were then identified using Tukey post-hoc pairwise comparisons.

4. Results
4.1. Morphometrics
There was no significant difference in biomass, valve length or width between the flow speeds and spatial arrangement ($F_{1,43} = 0.163, p = 0.73$), nor any significant interaction between spatial arrangement and flow velocity on mussel size and biomass ($F_{1,43} = 0.601, p = 0.45$). As such, any differences in hydrodynamic profiles and plastic ingestion are a result of aggregation and flow speed rather than the mussels themselves. Mussel biomass ranged from 73.2 to 226.8 g m$^{-2}$ ($\mu = 118.4 \pm 29.0$ g m$^{-2}$), length ranged from 4.9 to 8 cm ($\mu = 6 \pm 0.6$ cm), and width ranged from 2.9 to 4.6 cm ($\mu = 3.5 \pm 0.4$ cm).

4.2. Difference in horizontal velocity, TKE and plastic density profiles over reefs
There was a small effect size (Cohen’s $d = 0.05$ and 0.07) of spatial arrangement on horizontal velocities within flow speeds (i.e. SC vs. SR and FC vs. FR; figure 1), and a small to medium effect size (Cohen’s $d = 0.2–0.5$) of different flow speeds on horizontal velocities within spatial arrangement (i.e. SC vs. FC; figure 1). Mean horizontal advection velocities were ~1% slower in random (R) treatments than in clustered (C) treatments, and 20% faster in fast flow (F) treatments than slow (S) flow treatments despite a 500% increase in undisturbed flow velocities between flow treatments (8 cm s$^{-1}$ to
Figure 1. Forest plot and descriptive statistics of effect size of flow speed and spatial arrangement combinations (or Groups (G)) on the logarithm of turbulent kinetic energy (TKE). Estimates of standardised median difference (SMD), weight and Cohen’s d between pairwise group comparisons (S = slow, F = fast, C = clumped; R = random) are shown. Interpretation of Cohen’s d magnitudes are described in Sawilowsky (2009). FE Model = fixed effects global model.

Plate 2 Spherical microplastic (circled in red) shown relative to a 1 mm scale bar. The particle shown above is approximately 360 µm in diameter.

48 cm s\(^{-1}\)). Fastest instantaneous velocities occurred over randomly-arranged mussel reefs under fast flow conditions (114 cm s\(^{-1}\)), which were >3 \times\) faster than maximum velocities over clumped reefs (37 cm s\(^{-1}\)).
Maximum velocities reached 32 cm s$^{-1}$ over both clumped and randomly arranged reefs under slow flow conditions (figure 2(a)); a 4 × increase over free-stream velocities.

In general, there was a medium to very large effect (Cohen’s $d = 0.43–1.26$) of spatial arrangement and flow speed on mean log turbulent kinetic energy (TKE) dissipation rate over reef surfaces (figure 1), with the exception of between spatial arrangements in slow flow, which had only a small effect on TKE (SC vs. SR; Cohen’s $d = 0.07$). There were significant differences between 3 of 4 pairwise combinations (figure 2b). Greatest TKE occurred over clustered reefs under fast flow (FC; $\mu = 7.71$ mm$^2$ s$^{-2}$), which was 18% greater than over randomly-arranged mussels under the same flow regime (FR; $\mu = 6.52$ mm$^2$ s$^{-2}$). There was no difference in TKE between spatial arrangements under slow flow conditions (figure 2(b)). TKE under fast flow regimes ($\mu = 7.12$ mm$^2$ s$^{-2}$) was, on average, 36% higher than under slow flow regimes ($\mu = 5.24$ mm$^2$ s$^{-2}$). Turbulent wakes of higher TKE were localised on the lee of the leading edge and downstream of the mussel bed, in some instances, reaching up to 12.1 mm$^2$ s$^{-2}$ (figures 2(b) and 3) around clumped mussels under fast flow conditions.

There was a huge effect (Cohen’s $d > 2.0$, Sawilowsky, 2009) of flow rate on the density of plastic over reef surfaces (figure 1), but no effect of difference in spatial arrangement within flow speed (i.e. SC vs. SR and FC vs. FR; Cohen’s $d < 0.03$). Fast flow conditions led to a 2.7-fold decrease in the density of plastic over a reef ($\mu_{fast} = 272$ pieces per 500 cm$^3$; $\mu_{slow} = 745$ pieces per 500 cm$^3$; figure 2(c)). These plastic densities were 45% lower and 50% higher than in control images (no reef) under fast and slow flow conditions, respectively.

4.3. Differences in boundary layer thickness and microplastic ingestion

There were significant differences in boundary layer thickness (here, represented by the mean y-axis height of the 5 cm s$^{-1}$ isobar) among different flow speed and spatial arrangement combinations ($F_{2,410} = 160.9$, $p < 0.001$). The boundary layer...
thickness (BLT) was greatest over the randomly distributed mussels under slow flow conditions (mean height = 13 ± 0.34 cm). The BLT over clustered mussel reefs under both slow and fast flow conditions was not significantly different at 11 ± 0.49 cm and 11.2 ± 0.86 cm, respectively. BLT was lowest over the random reef under fast flow conditions (mean height = 10 ± 1.25 cm), but was also the most variable (figure 3).

There was significant difference in the number of microplastic pieces consumed by mussels depending on spatial arrangement ($z = -3.305$, $p < 0.001$) and flow speed ($z = 5.89$, $p < 0.001$), but there was no interaction between these factors ($t = -0.167$, $p = 0.87$). The median number of pieces of plastic consumed was $1.7 \times$ and $2.8 \times$ greater in clustered mussels than randomly arranged mussels in slow and fast flow conditions, respectively (figure 2(d)). Mussels in fast flow conditions consumed $2.7 \times$ (randomly arranged; max = 28 pieces) and $4.5 \times$ (clustered arrangement; max = 115 pieces) more plastic, respectively, than mussels in the same spatial arrangement in slow flow conditions (figure 2(d)). It therefore appears that feeding is less influenced by the abundance of particles and more by the creation of turbulence that leads to reduction in flow speeds and change in the boundary layer thickness over the reef surface.

5. Discussion

We tested the effect of spatial arrangement of mussel reefs and horizontal advection speeds on boundary layer dynamics and the passive delivery of microplastic to mussels for consumption. The structural complexity of biogenic reefs has been shown to alter boundary-layer flow dynamics (e.g. Green et al 1998).
by increasing drag, decelerating near-bed flow velocities, and creating turbulence in the bottom boundary layer (Butman et al 1994, van Duren et al 2006). This change in boundary layer conditions has been shown to affect the transport, deposition and resuspension of sediment, food particles and larvae to surfaces (Widdows et al 1998) but has not previously been linked directly to consumption. We hypothesised that the change in structural complexity associated with different spatial arrangement of mussels coupled with difference in flow speed would alter boundary layer dynamics, microplastic retention over reef structures, and ingestion rates by mussels.

The effect of structures on flow properties depends on their size, spacing, geometry and density (Wooding et al 1973, Stewart 2005, Frostick et al 2014). Here, controlling for density and biomass (size and geometry) revealed that spatial arrangement had no effect on mean instantaneous velocities over reef surfaces, although under fast flow conditions (48 cm s\(^{-1}\)), velocities over randomly arranged mussels were up to 3.1 \(\times\) faster than instantaneous velocities over clumped mussels; a difference not apparent under slow flow conditions. Increasing flow velocities can amplify energy dissipation (Saarenrinne and Piirto 2000) and the absence of high (> 20 cm s\(^{-1}\)) velocities over clumped mussels under fast flow is likely explained by the 12% increase in median TKE dissipation over the clumped surfaces than the randomly aggregated surfaces. The creation of turbulence is a function of flow speed (Saarenrinne and Piirto 2000) and surface roughness/complexity (e.g. Krogstad and Antonia 1999), the latter of which can be described using a measure of rugosity (\(R_0\)).

Rugosity can be measured in 2D (e.g. path length) or 3D (e.g. surface area). In this study, we a priori adopted a 2D measure of rugosity, which indicated that randomly distributed mussels displayed 11% higher rugosity than clustered mussel reef surfaces, but surprisingly, lower TKE dissipation rates. Rugosity as a metric has in the past received criticism (e.g. Commito and Rusignuolo 2000, Harwell et al 2010, Yanovski et al 2017). It cannot, for instance, distinguish between a protrusion and a depression with the same rugosity index, although these structural elements can have differential effects on the hydrodynamic processes and particle retention over a mussel bed. Fractal dimension, on the other hand, can distinguish between these features at different spatial scales. Commito and Rusignuolo (2000) using fractal dimensions showed that surface topography (i.e. vertical profile) is less complex than the spatial pattern of the reef itself (i.e. aerial view in the horizontal plane), suggesting the spatial distribution and its degree of fragmentation may be more important to boundary layer dynamics than the topography itself. Our results here would support that assertion, indicating a need to better understand the role of spatial arrangement and role of ‘gaps’ (sensu Yager et al 1993, Gacia and Duarte 2001, Granata et al 2001) in influencing near-bed hydrodynamics (which can modify bed roughness; Arcement and Schneider 1989) and its role in the generation of turbulence.

Somewhat paradoxically, despite increased TKE dissipation over clumped spatial aggregations of mussels, spatial arrangement had no effect on the number of pieces of plastic retained over a reef surface. Only decreasing flow speed from 48 cm s\(^{-1}\) to 8 cm s\(^{-1}\) led to a marked 2.7-fold reduction in plastic density over the reef surface. The absence of any difference in relative abundance of plastic pieces over reef surfaces between spatial arrangements is perhaps unsurprising given no difference in localised Eulerian flow speeds, especially under fast flow conditions. Beyond a critical velocity, flow can be sufficiently turbulent to perturb particles retained in areas of low velocity flow such as in the gaps that are hydrodynamically disconnected from the bulk flow. Darbha et al (2012) showed that surfaces with higher roughness resulted in greater particle retention until a critical velocity, beyond which, particles are displaced from a structure. Below this critical velocity, the surface protrusions impede particle displacement and the particles collect in low-velocity regions where eddies retain the particles in their circulation. This can greatly increase residence time, resulting in higher particle retention and accumulation of particles (McNair et al 1997).

In contrast, the number of plastic pieces ingested per individual differed markedly between spatial arrangements within flow speeds. Clustered mussels ingested between 1.7 \(\times\) and 2.8 \(\times\) more plastic than mussels in a random spatial arrangement in slow and fast flow conditions, respectively. Mussels in fast flow conditions consumed 2.7 \(\times\) (randomly arranged; max = 28 pieces) and 4.5 \(\times\) (clustered arrangement; max = 115 pieces) more plastic, respectively, than mussels in the same spatial arrangement in slow flow conditions. It therefore appears that feeding is less influenced by the abundance of particles and more by the creation of turbulence that leads to reduction in flow speeds and change in the boundary layer thickness over the reef surface.

Greater ingestion of plastic in clumped mussels could be explained by two possible mechanisms. First, increased turbulence over the surface of the reef structure and consequential reduction in flow velocities within the boundary layer facilitate increased capture efficiency by individual mussels. Increased flow velocities have previously been shown to significantly decrease filtration rates and ingestion in Mytilus edulis (Nielsen and Vismann 2014), although the negative effect of increased flow speed can in part be mitigated for by orientation of the inhalant siphon downstream of the flow direction or by clumping (Nielsen and Vismann 2014). Under higher flow speeds (48 cm s\(^{-1}\)), a significant thickening of the boundary layer over the surface of the clustered reefs could increase capture efficiency. Particles located
on the pseudo-bottom (within the boundary layer) and closer to the mussels’ feeding appendages require less energy for resuspension in comparison to those trapped in eddies in gaps between randomly spaced mussels and become hydrodynamically disconnected from the bulk flow such that they are more readily available to mussels (Nikora et al 2002). Alternatively, mussels as well as other species of bivalve, have been shown to preferentially select phytoplankton particles based on size and/or quality (see Shumway et al 1985, 1990) although there remains some debate over when this selection happens (see Ward and Shumway 2004 for review). If occurring at the filtration stage, increased turbulence could compromise the ability of an individual to preferentially select particles that lead to increased consumption of non-food particles, with ingestion determined by the movement of the fluid rather that an active capture process undertaken by the mussel (Jørgensen 1996).

Spatial arrangement clearly altered the likelihood of microplastic ingestion with the creation of gaps between clumps providing areas for particle accumulation and suspension (Yager et al 1993, García and Duarte 2001, Granata et al 2001). The geometric spacing distances appears crucial to this process, although the size of these ‘gaps’ will likely vary depending on physico-chemical properties of the fluid (e.g. temperature, salinity, density), physical forcing parameters (e.g. wind-driven kinetic energy), and the size of the reef structure itself. When structural elements are in close proximity to each other (e.g. when mussels occur at high densities), there is weaker flow penetration between mussels and wake formation is interrupted. Instead a coherent flow skims over the pseudo-bottom formed by connecting mussel edges (Wildish et al 2008, Folkard 2010) that can result in reduced roughness and lower drag coefficients (Nowell and Church 1979, Green et al 1998). In contrast, if roughness elements are spaced sufficiently distant from one another, while wake turbulence can develop more frequently, it may also dissipate before encountering the next element i.e. the creation of an isolated roughness flow. In the event of intermediate spacing distances, wakes and eddies can interact to produce an additive effect of greater turbulence (Folkard 2010) that may well promote particle aggregation.

Our findings highlight the important role of clumping to the feeding success of mussels that results from a change in the boundary layer hydrodynamics that occur over a reef. These results show that clumps promote the passive retention of particles over a reef surface by creating areas of turbulence kinetic energy dissipation that reduce flow speeds over reef surfaces and facilitates increased ingestion of suspended particles. For sedentary suspension-feeding species like *Mytilus edulis*, ordinarily this would be considered positive to their success. While previous studies have shown filter-feeding species like *Mytilus edulis* do ingest microplastic (e.g. Green et al 2019), these results suggest that healthy reef structures may disproportionately aggregate more microplastics over their surfaces as a result of their physical structure, placing individuals within these biogenic reefs habitats at greater risk of ingesting microplastic. Reef-forming genus like *Mytilus* and *Crassostrea* that are important species for human consumption (e.g. Lemasson et al 2017, 2018) and in particular, the structurally-complex reefs they form, may therefore act as natural sinks for microplastic (and potentially other particulate pollutants), with individual reef inhabitants especially susceptible to any negative consequences of anthropogenic pollution (Browne et al 2008).

**Authors’ contributions**

HSL and AMK devised the research and undertook flume experiments. HSL and AF devised and undertook microwave digestions. AMK undertook data analysis and all authors wrote the manuscript.

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**Data availability statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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