The relative effects of interspecific and intraspecific diversity on microplastic trapping in coastal biogenic habitats

Lorenzo Cozzolino a,⁎, Katy R. Nicastro a,b,c, Laurent Seuront b,c,d, Christopher D. McQuaid c, Gerardo I. Zardi c,e

a CCMAR—Centro de Ciências do Mar, CIMAR Laboratório Associado, Universidade do Algarve, Campus de Gambelas, Faro 8005-139, Portugal
b Univ. Lille, CNRS, Univ. Littoral Côte d’Opale, UMR 8187 – LOG – Laboratoire d’Océanologie et de Géosciences, F-59000 Lille, France
c Department of Zoology and Entomology, Rhodes University, Grahamstown 6140, South Africa
d Department of Marine Resources and Energy, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan
e Normandie Université, UNICAEN, Laboratoire Biologie des Organismes et Ecosystèmes Aquatiques, UMR 8067 BOREA (CNRS, MNHN, UPMC, UCBN, BRD-207), CS 14032, 14000 Caen, France

ABSTRACT

Our understanding of how anthropogenic stressors such as climate change and plastic pollution interact with biodiversity is being widened to include diversity below the species level, i.e., intraspecific variation. The emerging appreciation of the key ecological importance of intraspecific diversity and its potential loss in the Anthropocene, further highlights the need to assess the relative importance of intraspecific versus interspecific diversity. One such issue is whether a species responds as a homogenous whole to plastic pollution. Using manipulative field transplant experiments and laboratory-controlled hydrodynamic simulations, we assessed the relative effects of intraspecific and interspecific diversity on microplastic trapping in coastal biogenic habitats dominated by two key bioengineers, the brown intertidal macroalgae Fucus vesiculosus and F. guiryi. At the individual level, northern morphotypes of F. guiryi trapped more microplastics than southern individuals, and F. vesiculosus trapped more microplastics than F. guiryi. Canopy density varied among species, however, leading to reversed patterns of microplastic accumulation, with F. guiryi canopies accumulating more microplastics than those of F. vesiculosus, while no differences were observed between the canopies of F. guiryi morphotypes. We emphasize the importance of assessing the effects of intraspecific variation which, along with other crucial factors such as canopy density, flow velocity and polymer composition, modulates the extent of microplastic accumulation in coastal biogenic habitats. Our findings indicate that a realistic estimation of plastic accumulation in biogenic habitats requires an understanding of within- and between-species traits at both the individual and population levels.

⁎ Corresponding author.
E-mail address: Lorenzo_cozzolino@hotmail.it (L. Cozzolino).

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1. Introduction

The unprecedented rapidity of alterations to the environment triggered by anthropogenic activities is threatening biodiversity at increasing rates (Pawar, 2016; Hendry et al., 2017; WWF, 2020). Globally, ongoing biodiversity loss and reshuffling have significant deleterious effects on ecosystem systems and thus the goods and services they provide to humanity (Worm et al., 2006; Cardinale et al., 2012; Balvanera et al., 2014; Isbell et al., 2017). Over the last two decades, plastic litter has rapidly emerged as a new worldwide threat degrading ecosystems throughout the planet (Heidbreder et al., 2019; Thushari and Seneviratna, 2020). Growing attention has been given to microplastics (MPs; size <5 mm; Barnes et al., 2009; Arthur et al., 2009) which have become ubiquitous: from high-mountain lakes to deep-sea sediments (Woodall et al., 2014; Rowlands et al., 2021; Pastorino et al., 2022). There is now overwhelming evidence that plastic contamination has major effects on biodiversity, contributing to the effects of other forms of global anthropogenic disturbance (Deudero and Alomar, 2015; Thushari and Seneviratna, 2020; Azevedo-Santos et al., 2021).

The distinct responses of different species to plastic pollution have been emphasized in several studies (Green, 2016; Law, 2017; Seuront et al., 2021). However, such studies have mostly been conducted with the assumption that species respond as ecologically and physiologically homogenous entities. This assumption potentially underestimates and overgeneralises the effects of plastics in the environment. Indeed, it is now well established that genotypic and phenotypic variation within a single species can have important ecological implications for processes such as primary productivity (Crutsinger et al., 2006; Zhu et al., 2000), nutrient cycling (Lecerf and Chauvet, 2008), species coexistence (Siefert, 2012; Spasojevic and Suding, 2012) and ecosystem resilience and resistance (Hughes and Stachowicz, 2004; Reusch et al., 2005; Des Roches et al., 2018; Raffard et al., 2018). Importantly, the few studies directly examining the ecological importance of intraspecific diversity have shown that it can have even greater implications for ecosystem function and services than differences among species (i.e., Cardinale et al., 2012; Raffard et al., 2018).

The biodiversity of coastal biogenic habitats (i.e., habitats created by living organisms) has been particularly affected by the excessive use and mismanagement of plastics (Agardy and Alder, 2005; Duarte et al., 2015; Perillo et al., 2018; Williams et al., 2021). Indeed, the accumulation of both micro- and macroplastic has been highlighted for a wide variety of species, including mangroves (Martin et al., 2020), seagrasses (Bonanno and Orlando-Bonaca, 2020; Cozzolino et al., 2020; Carmen et al., 2021; de Smit et al., 2021; Kreisberg et al., 2021) and marine macroalgae (Cozzolino et al., 2020; de Smit et al., 2021; Li et al., 2021), as well as rhodoliths (Teichert et al., 2021). In freshwater systems, plastics accumulate in riparian vegetation (Cesarine and Scali, 2022) and floating aquatic vegetation (Schreyers et al., 2021), but the phenomenon is not limited to primary producers. Significant plastic accumulation occurs in biogenic habitats formed by corals (de Smit et al., 2021; Reichert et al., 2022), oysters (Li et al., 2018; Lozano-Hernández et al., 2021; Han et al., 2022), and mussels (Santana et al., 2016; Khan and Prezant, 2018; Lim et al., 2020; Nicoastro et al., 2022). This suggests potential risks to the organisms associated with these habitats, such as ingestion of microplastics by grazers (Gutow et al., 2016, 2019; Seuront, 2018; Jones et al., 2020), and further consequences of MP accumulation in coastal habitats (Pendleton et al., 2012; Mehvar et al., 2018). Macroalgae are important habitat providers and differences in their ability to trap plastics have been attributed to properties of the canopies they produce, with higher architectural complexity and surface roughness increasing the trapping of both macro and microplastics (Cozzolino et al., 2020; de Smit et al., 2021; Nicoastro et al., 2022).

Here, we assess the relative contributions of between- and within-species diversity of macroalgae to the level of plastic trapping and retention within the habitats they create. Specifically, through manipulative field transplant experiments and laboratory-controlled hydrodynamic simulations, we investigated the effects of intraspecific versus interspecific diversity on microplastic (MP) accumulation in coastal biogenic habitats dominated by two key bioengineers. We focused on the intertidal brown alga *Fucus vesiculosus* and *Fucus guiryi*, the latter represented by two distinct genetic lineages. Previous studies have not only shown spatial segregation between *F. vesiculosus* and *F. guiryi* but have also highlighted fundamental differences in the morphologies of distinct genetic lineages of *F. guiryi*, including frond length, bushiness, and biomass (Zardi et al., 2011). These lineages (referred to as north and south hereafter) also differ in traits known to affect survival along large- and small-scale environmental gradients (Zardi et al., 2015) in their population densities, leading to microclimate differences within their canopies that affect their role as bioengineers (Montero et al., 2019). Our hypotheses are based on the knowledge that the structural properties of the bioengineer (i.e., habitat complexity, canopy height, density and stiffness), its surrounding hydrodynamics (i.e., flow velocity, currents and wave action) and the physical properties of MP particles (e.g., polymer composition) are all determinant factors of MP accumulation in coastal habitats (Hendriks et al., 2008; Wilkie et al., 2012; Chubarenko et al., 2016; Zhang, 2017; Meysick et al., 2019). On this basis, we hypothesised, first, that the effects of intraspecific diversity (i.e., between genetic lineages) on MP trapping capacity would be greater than those of interspecific diversity (i.e., between *F. vesiculosus* and *F. guiryi*). We tested this hypothesis at two levels, comparing MP trapping per individual and per canopy. Second, we hypothesised that any differences in the relative effects of inter- and intraspecific diversity on MP trapping would not be consistent under different flow velocities (by comparing high and low flow conditions) or MP composition (by comparing different polymers).

2. Material and methods

2.1. Model species and study area

*Fucus* species are canopy-forming, externally fertilizing perennial brown macroalgae (Pearson and Brawley, 1996; Serraio et al., 1996). They are dominant bioengineers on boreal intertidal semi-exposed and sheltered rocky shores where they exhibit a positive effect on biodiversity, enhancing species richness by modifying habitats, increasing spatial complexity, and providing feeding areas and refuge to other species (Haage, 1975; Seed and O’Connor, 1981; Kautsky and Kautsky, 1989). They occur widely at temperate latitudes along northern hemisphere shores where, depending on local environmental conditions, they can exhibit patchy or continuous belt-like distributions.

This study was performed along the southern and northern coast of Portugal and focused on two *Fucus* spp., i.e., *F. guiryi* and *F. vesiculosus* Linnaeus. The two species have clear morphological differences such as the occurrence of sterile margins or rims around the receptacles in *F. guiryi* and the presence of air bladders in *F. vesiculosus* (Zardi et al., 2011). Despite being morphologically different, they share similar distributions, occasionally occurring in sympathy in northern Portugal and farther north. *F. guiryi* (previously referred to as *F. spiralis var. platycarpus* or *F. spiralis Low*) inhabits the temperate Atlantic coasts of Europe and North Africa (Zardi et al., 2015; Lourenço et al., 2016; Melero-Jiménez et al., 2017). In Iberia, *F. guiryi* exhibits two distinct genetic lineages (also referred to as morphotypes) distributed according to local latitudinal environmental gradients of air and sea temperature (Zardi et al., 2011, 2015). The southern lineage (hereafter referred to as “*Fucus guiryi* S”) occurs in allopatry in southern marginal populations (south of central Portugal) and has a compact, bushy short and wide appearance, whereas the northern lineage (“*Fucus guiryi* N”) inhabits northern sites where its genetic structure is
influenced by hybridization with sympatric sister species (Zardi et al., 2011, 2015). The northern lineage is characterised by less bushy individuals with longer fronds (Zardi et al., 2015). It is still unclear whether the unique morphologies and densities displayed at the rear edges of species distributions are the result of adaption or phenotypic plasticity. *F. vesiculosus* occurs on the coasts of the North Sea and the western Baltic Sea, on the Atlantic shores of Europe, in the Azores, Madeira and the Canary Islands (Pozzari and Skyta, 2018) and is widely distributed in the Arctic and cold temperate regions throughout northern Russia and Greenland (Linning, 1990). It is absent from the Bay of Biscay and its southern distributional limit lies in Iberia in the Tejo estuary (NICASTRO et al., 2013).

2.2. Sampling

A preliminary morphological assessment (April 2021) was conducted among distinct sites within the study area on *F. guiryi S* (*n* = 2 sites; S1: Praia de Monte Clérigo and S2: Praia de Arrifana, Aljezur), *F. guiryi N* and *F. vesiculosus* (*n* = 2 sites; both at Praia Norte, Viana do Castelo). At each sampling site, quadrats (30 cm × 30 cm; *n* = 4) were haphazardly placed in the middle of each species’ intertidal range. For each quadrat, adults and recruits were counted separately and a subsample (*n* = 5) of intact adult individuals showing no visible damage due to wave action or grazing was collected. Specimen morphology was assessed (Tables 1Sa and 2S) following Zardi et al. (2011). Specifically, individual maximum length, maximum circumference (measured with a tape measure around the widest part of the frond) and total biomass (i.e., dry weight, or *dw*, in g), individual bushiness (i.e., maximum circumference-to-length ratio) and number and biomass of receptacles (*dw*, *g*) were determined. Algal body and receptacles were dried separately in an oven at 60 °C for 72 h. Values of each morphological trait were calculated as averages for five individuals per quadrat and means for the maximum number of individuals counted in the quadrat (when *n* < 5).

One-way ANOVA, performed on each species using the morphological traits (e.g., length and number of receptacles) as response variables, did not identify significant morphological differences between sites (Table 1Sb). Therefore, collection of each species/morphotype was restricted to only one of the two sites previously explored.

2.3. Laboratory plastic trapping experiments

Plastic trapping experiments were conducted in the laboratory using intact adult reproductive individuals of *F. guiryi N*, *F. guiryi S* and *F. vesiculosus* collected in May 2021 from Praia de Monte Clérigo, Aljezur (37° 20' 29.16" N; 8° 51' 16.16" W; *F. guiryi S*) and Praia Norte, Viana do Castelo (41° 41' 29.93" N, 8° 50' 57.99" W; *F. guiryi N* and *F. vesiculosus*). The two sites are approximately 280 km apart. The algae were maintained in cool boxes and transported to the laboratory within 48 h. In the laboratory, they were kept at a temperature of 12 °C, 100 % humidity and in darkness.

2.3.1. Flow generation

The experiments were performed using a recirculating circular flume (Fig. 1S) that allowed the creation of a steady unidirectional flow following NICASTRO et al. (2022). The device was 2 m long by 1 m wide, with 30-cm wide smooth-wall (Fig. S1). The bottom and lateral walls of the tank were composed of dark grey PVC and transparent acrylic, respectively. Flow was generated through surface friction by the rotation of a set of 10 parallel PVC disks 5 mm thick and 0.6 m in diameter. The rotation frequency of the disks was controlled using an electronic control board connected to an electric engine. The flume was calibrated using the ensemble average velocity of a drifter that travelled between distinct locations in the linear section of the flume. This surface velocity highly significantly (*p* < 0.01, *r*^2^ = 0.99) correlated linearly with the rotation speed of the wheels. The velocity of the free-stream flow, *V*\(_{0}\), and the flow observed behind the vegetated structure, *V*\(_{w}\), were measured at 100 Hz for the duration of 1 min (i.e., *N* = 6000 velocity measurements) with an Acoustic Doppler Velocimeter (ADV, Nortek Vectrino Cable Probe) respectively positioned 25 cm upstream and 25 cm downstream of the sample. These measurements were consistently triplicated. Velocity was measured at two different depths, *z*\(_{1}\) = *Top* (within 5 cm of the surface) and *z*\(_{2}\) = *Bottom* (within 5 cm of the bottom). Two free-stream velocities respectively representative of peak tidal flow of microtidal systems (de Smit et al., 2021); i.e., *V*\(_{0}\) (low flow) = 0.15 m s\(^{-1}\) and average maximum flow velocity in wave-exposed locations (Gaylord, 1997; i.e., *V*\(_{0}\) (high flow) = 0.45 m s\(^{-1}\)) were considered. For each flow velocity, inter- vs intraspecific effects on water flow were quantified using the velocity ratio *d* = *V*\(_{0}\)/*V*\(_{w}\) as a proxy of the dissipation of the flow by the algae. The comparison was made between species (*Fucus vesiculosus* vs *F. guiryi*) for the interspecific effect (*n* = 3) and within species (*F. guiryi* Southern vs *F. guiryi* Northern) for the intraspecific effect (*n* = 3).

*F. guiryi* S individuals were consistently smaller than the other two species/morphotypes (i.e., 3-fold smaller). Hence, to consider the difference in size of the Fucus spp. used in the experiments, the flume was filled with 300 l and 175 l of seawater (salinity 33 ppt) for experiments on northern (*F. guiryi N* and *F. vesiculosus*) and southern (*F. guiryi S*) species/morphotypes respectively. This approach has specifically been chosen to ensure that all individuals experienced similar flow conditions, with the water surface about 2–5 cm above the specimen’s tip, simulating an incoming high tide with the alga just submerged.

2.3.2. Microplastic trapping

Microplastic trapping was consistently tested on solitary individuals of the three species/morphotypes under both low (*V*\(_{0}\) (low flow) = 15 cm s\(^{-1}\)) and high flow (*V*\(_{0}\) (high flow) = 45 cm s\(^{-1}\)) with *n* = 4 for each velocity and species/morphotype combination.

Under each tested velocity, a mixture of MPs was added to the flume ca. 50 cm behind the rotating wheel and allowed to circulate for 2 min. The microplastics consisted of polyamide (PA) and polypropylene (PP) fibres (3 mm in diameter, and length variable between 0.05 mm and 5 mm) and polyethylene pellets in a combination of both low-density (LDPE) and high-density polyethylene (HDPE; length 1 mm). Each polymer type was added at a fixed concentration (0.0265 g l\(^{-1}\)) to the microplastic mixture following the concentration proposed by de Smit et al. (2021). These polymers were specifically chosen because: (1) they are among the most common polymers found in the marine environment (Gallo et al., 2018), and (2) because they have different densities, some tend to float to the surface (i.e., PP and LDPE) while others tend to sink (i.e., PA and HDPE), providing the opportunity to investigate MP trapping through the entire water column. To easily distinguish the microplastics from potential external contamination, bright green polypropylene and light green polyamide fibres were used.

Each run consisted of one individual alga glued to a flat rock using a two-part epoxy compound glue (Splash Zone A-788) and placed in the middle of the linear section of the flume. Specimens were surrounded by similar rocks (10–15 cm in length each), simulating the heterogenous substrate of rocky shores. After two minutes, a net (mesh size 0.05 mm) was dropped ca. 50 cm on the vegetative structure to block the microplastics stream. Subsequently, the alga was collected and rinsed with filtered seawater. All rinsing water was filtered through glass microfiber Whatman GF/C filters (47 mm diameter) using a vacuum pump (VWR VP-86; 100 mbar). The filters were dried and observed under a stereomicroscope (Olympus, SZX16). All microplastics resembling those added to the flume were counted.

2.4. Structural complexity

The effect of the two experimental flows on the structural complexity of northern (*F. guiryi N* and *F. vesiculosus*) and southern (*F. guiryi S*) species/morphotypes was assessed using fractal geometry (Mandelbrot, 1983). Fractals have the desirable properties of being independent of the observation scales and more sensitive to even slight variation in structural complexity than traditional Euclidean descriptors (Sugihara and May, 1990; Seuront, 2009). Noticeably, fractals have been successfully applied...
to describe architectural complexity in a range of marine organisms such as coral reefs (Basililais, 1997), sponges (Kaandorp, 1991; Kaandorp and de Kluiver, 1992; Abraham, 2001), gorgonians (Burlando et al., 1991; Mistri and Ceccherelli, 1993), crabs burrows (Katrak et al., 2008), mussel beds (Erlandsson et al., 2011; Zardi et al., 2021), and seaweeds (Corbit and Garbary, 1995; Kübler and Dudgeon, 1996; Davenport et al., 1996; Ndhlou et al., 2021). Here, for each flow velocity and for each species and morphotype, we took high-resolution digital pictures of each experimental algae (n = 3) from the side and the top of the linear section of the flume. We subsequently assessed the complexity of the contour of each individual algae using the dividers procedure, which uses a set of dividers of fixed length l along the algae edge. The length of the algae’s edge was subsequently estimated as the product of individual algae using the dividers procedure, which uses a set of dividers of maximum length (i.e., from the holdfast to the furthest apex) measured. The number of dividers necessary to cover the algae’s edge increases with decreasing measurement scale as \( N(l) = k \times l^D \), where l is the measurement scale, D the so-called fractal dimension estimated as the slope of the log-log plot of \( N(l) \) vs l, and k an empirical constant. The fractal dimension D is theoretically bounded between 1 and 2. D = 1 when the edge is linear, and in the opposite instance of curviness, D = 2 when the edge geometry is so complex that it virtually fills the whole available space. Because the value of N(l) may vary depending on the starting position along the algae’s edge, we systematically ran our analyses by starting the dividers procedure at 10 different, randomly chosen location along the edge, walking forwards and backwards, and using the distribution of the resulting divider dimensions as an estimate of D (Seuront, 2009). The potential presence of anisotropy in the structural edge complexity was assessed for each flow velocity and for each species and morphotype through a comparison of the fractal dimensions \( D_l \) of the algae’s edge returned from the analysis of the side (\( D_{side} \)) and top (\( D_{top} \)) view of individual algae using a t-test (Zar, 1999). If the null hypothesis of non-significant differences between \( D_{side} \) and top \( D_{top} \) was rejected, the edge complexity was subsequently estimated as D = \( D_{side} + D_{top} / 2 \) (Zar, 1999; Seuront, 2009). Interspecific differences were assessed through comparisons of the structural complexity of F. vesiculosus and F. guiryi (Northern and Southern combined) whereas intraspecific differences were inferred by comparing the fractal dimensions of individual F. guiryi N and F. guiryi S. The presence of significant differences in algae’s edge complexity at the inter- and intraspecific levels were assessed using an analysis of covariance, and a subsequent multiple comparison procedure based on the Tukey test was used to identify distinct groups of measurements (Zar, 1999).

2.5. Field plastic trapping experiments

A field experiment on plastic trapping was conducted using intact adult reproductive individuals collected in January 2020 from Praia de Monte Clérigo (37° 20′ 29.16″ N; 8° 51′ 16.16″ W; F. guiryi S) and from Viana do Castelo (41° 41′ 29.93″ N; 8° 50′ 57.99″ W; F. guiryi N and F. vesiculosus). The algae were maintained in cool boxes and transported to the laboratory within 72 h. In the laboratory, all individuals were cleaned of epibionts and carefully rinsed with filtered seawater (GF/C Whatman filter, 47 mm of diameter, 1.2 μm of pore size). A visual examination under a stereomicroscope (Leica S8 APO, magnification ×40) was also done for each individual before it was deployed in the field. A small portion of the substratum to which each individual was attached was carefully removed using a chisel and was then used to affixed to solid substratum at the host site (Vilamura; 37° 4′ 15.00″ N; 8° 7′ 22.43″ W) using a fast-curing epoxy compound (Zap Spar Splash Zone Compound, Underwater Epoxy Putty, A-788). The host substratum was cleaned of natural biological cover and mud prior to gluing. Individuals were deployed in the field for 14 days after which each one was cut at the base and carefully rinsed with filtered seawater. All rinsing water was filtered as above. To prevent loss of microplastics, each container was further rinsed and each individual visually inspected under the stereomicroscope. For each alga, the dry biomass was calculated and the initial maximum length (i.e., from the holdfast to the furthest apex) measured. MP counts were expressed as Total MPs/individual.

2.6. Quality control

To eliminate post-sampling contamination (e.g., contamination by MPs through sample handling) several measures were adopted while handling and processing the samples. Gloves and 100 % cotton laboratory coats were worn during laboratory processing. In addition, all equipment used was non-plastic (i.e., glass or metal), and was rinsed twice with pre-filtered ultrapure water between each sample extraction. Aluminium foil was used to cover material before use and to cover glass jars during settling intervals and while filtering.

To obtain information on polymer composition and to validate MP identification, Fourier transform infrared micro-spectroscopy was performed on a subsample of microplastics trapped in the field (50 % representativeness of total number of MPs). Infra-red Attenuated Total Reflection (IR-ATR) spectra between 500 and 3500 cm⁻¹ were collected using a Nicotel IN10 Fourier infrared micro-spectroscopy (Thermal Fisher Scientific Co., USA). Micro Tip ATR was placed in contact with the sample to record the spectra, with air as the background spectrum. Recorded spectra were compared against commercial FT-IR spectral libraries (Hummel Polymer and Additives Library and FBI fibre library).

2.7. Data analyses

Analyses were performed for both laboratory and field plastic trapping experiments using data expressed as total number of MP per individual. In addition, estimates of MP trapping at the canopy level were calculated by standardising the field MP trapping to the canopy density occurring at the sites where the species/morphotypes were collected. The density of each species/morphotype was expressed as the number of adult individuals counted in a 30 × 30cm quadrat. The total number of MP per canopy was standardised as the average number of MP trapped by an individual in the field (sample size n = 12) multiplied by the total number of individuals present in each quadrat (30 × 30 cm, sample size n = 8). The comparison of inter- vs intraspecific effects was made by comparing the strengths of two contrasts. The effect of interspecific variation (Contrast 1) was assessed by comparing all Fucus guiryi treatments (Southern and Northern combined) versus the Fucus vesiculosus treatment. The effect of intraspecific variation (Contrast 2) was derived by comparing F. guiryi S versus F. guiryi N.

The effect of inter and intraspecific variation on MP retention was assessed using three statistical procedures. First, orthogonal contrasts were used for each dataset for both laboratory and field experiments. For the laboratory experiment, each velocity was treated separately. Second, the effect size was calculated for each orthogonal contrast analysed and used to compare the magnitudes of the effects between inter-(Contrast 1) and intraspecific variation (Contrast 2). Given the sample sizes (~20), Hedges’ g was chosen over Cohen’s d (Grissom and Kim, 2005). Descriptors for magnitudes based on Cohen (1992) and adapted according to Sawilowsky (2009) were used. The 95 % confidence interval for the effect size was calculated (Hedges and Olkin, 2014). Third, Permutational Multivariate Analyses of Variance (PERMANOVA; Anderson, 2001) were performed to test the effects of inter- and intraspecific variability (i.e., the two contrasts) on MP polymer composition (i.e., polyamide, polypropylene or polyethylene). This analysis was performed only for data from laboratory experiments as 90 % of MPs trapped in the field were fibres. The tests were performed with either Contrast 1 (effect of interspecific variability) or Contrast 2 (effect of intraspecific variability) and velocity (\( V_{flow \; flow} = 15 \text{ cm s}^{-1} \text{ and } V_{high \; flow} = 45 \text{ cm s}^{-1} \)) as fixed factors and MP polymer abundance (n) as the dependent variable. For each PERMANOVA analysis, a Bray-Curtis dissimilarity matrix for square root transformed multivariate data was used. Significance of F-ratios was determined from 9999 randomizations of the data (Anderson, 2005). When only a restricted number of permutations was possible, Monte Carlo p-values (p(mcc)) were preferred to permutational p-values (p(perm)). A similarity percentage analysis (SIMPER) was used to determine the percentage contribution that each variable (MP polymer type abundance) made to the Bray-Curtis dissimilarities.
Differences between *Fucus* species/morphotypes were visualized using non-metric multidimensional scaling (nMDS) ordinations based on Bray-Curtis similarity matrices.

The ADV statistical analysis was carried out for each flow velocity separately. A two-way ANOVA was performed with the effect of intraspecific or interspecific variation and distance from the bottom (Z1 = *Top* and Z2 = *Bottom*) as fixed factors, and dissipation (d) as the dependent variable. When data did not follow a normal distribution or exhibit homoscedasticity, a non-parametric Kruskal-Wallis test was used.

3. Results

3.1. Structural complexity

For each species and morphotype, no significant differences were found between the structural complexity obtained from the analysis of the side and top view of the individual algae (p > 0.05). As a consequence, the corresponding fractal dimensions Dside and Dtop were pooled for further analysis.

The fractal analysis revealed that both *F. vesiculosus* and *F. guiryi* species/morphotypes significantly change in structural complexity with flow conditions (DNoFlow = DLowFlow > DHighFlow; Tukey test; p < 0.05; Figs. 1 and 2). These results indicate a significant effect of high flow velocity on the structural complexity observed between and among species.

Both inter- and intraspecific differences were detected in *Fucus* spp. structural complexity (Fig. 2). Specifically, at the interspecific level, *F. vesiculosus* was structurally more complex than *F. guiryi* in the absence of flow and under high flow conditions (p < 0.05) but the two species showed similar structural complexity at low flow velocity (p > 0.05; Fig. 2A). In contrast, at the intraspecific level, *F. guiryi* N was always structurally more complex than *F. guiryi* S (i.e., D*guiryi* N > D*guiryi* S), irrespectively of flow conditions (Fig. 2B).

3.2. Individual plastic trapping under laboratory conditions

The Acoustic Doppler Velocimeter (ADV) revealed no significant intra-specific or interspecific differences in flow dissipation at low flow velocity (V*ADV*low flow = 0.15 m s$^{-1}$), regardless of the depth (Fig. 3S-A and C). At higher flow velocity (V*ADV*high flow = 0.45 m s$^{-1}$), no significant interspecific effects on water flow dissipation were observed (Fig. 3S-D) regardless of the depth. There was, however, significant intraspecific variation in surface flow dissipation, with the northern lineage of *Fucus guiryi* being more effective at dissipating flux than the southern lineage (Fig. 3S-Btop Tukey test: p = 0.0003). In contrast, flow dissipation did not differ significantly between the two lineages at the bottom of the water column (Fig. 3S-Bbottom; Tukey test: p = 0.8315).

In general, the orthogonal contrast analyses highlighted significant inter- and intraspecific effects on the number of microplastic particles trapped under laboratory conditions. Data were expressed as total number of MP per individual (hereafter Total MPs/individual (n)). Effect sizes were always relatively higher between morphotypes than between species (Huge vs Small/Medium; Table 1). Specifically, northern individuals of *F. guiryi* trapped significantly more microplastics than southern individuals at both low (t = −6.693, p = 0.001; Fig. 3A) and high flow velocities (t = −6.682, p = 0.001; Fig. 3B). *F. vesiculosus* trapped significantly more MPs than *F. guiryi* at high flow velocity (t = 2.977, p = 0.016, effect size = Medium; Table 1) while no interspecific effect was detected at low velocity.
When data expressed as Total MPs polymer/individual, there was a significant intraspecific (PERMANOVA, $p = 0.001$), but no interspecific effect on MP polymer composition (i.e., polyamide, polypropylene and polyethylene; n/individual; Table 3SM). The nMDS plots mirrored the PERMANOVA results, revealing clear separation between the two lineages (Fig. 4B). Polyamide and polypropylene fibres contributed the most to the observed difference (65 % and 26.7 % respectively; SIMPER).

### 3.3. Individual plastic trapping in the field

The orthogonal contrast analyses highlighted significant interspecific and intraspecific effects on the numbers of microplastics trapped in the field. When data were expressed as Total MPs/individual (n; Fig. 5A), the effect sizes between lineage were always higher than between species (Huge vs Medium respectively). Northern individuals of *F. guiryi* accumulated on average 3.5 times more MP than southern individuals ($t = 9.466$, $p = 0.001$), while *F. vesiculosus* trapped 1.4 times more MP than *F. guiryi* ($t = 4.251$, $p = 0.001$ Table 2).

### 3.4. FT-IR spectroscopy characterization in the field

Overall, 90 % of MPs found were fibres while the remainder consisted of polymer fragments. Fibres were mostly polyethylene terephthalate (PET; 55 %) or polyethylene (PE; 21 %), whereas fragments were mainly made up of polystyrene (PS; 50 %) or polyethylene (PE; 29 %).
accumulating significantly more within northern than southern morphotypes of *F. guiryi*. The results differed slightly under field conditions, where we found differences in MP retention between the morphotypes of *F. guiryi* at the individual level, but not at the canopy level.

The number of studies assessing plastic contamination in biogenic habitats has grown substantially in recent years and biogenic habitats are often described as sinks for plastic litter (Garcés-Ordóñez et al., 2019; Huang et al., 2020; Jones et al., 2020; Cozzolino et al., 2020; Stead et al., 2020; Unsworth et al., 2021). Importantly, experimental evidence from laboratory and field flume tests has shown how the individual structural complexity of the organisms forming the biogenic habitat is a key determinant of the magnitude of plastic trapping (de Smit et al., 2021; Carmen et al., 2021; Nicastro et al., 2022). The relevance of structural features has been confirmed by field assessments of architectural complexity (i.e., total surface area and surface area to volume ratio) and physical properties such as stiffness/ flexibility and surface characteristics. Specifically, benthic structures with a rough surface and more complex architecture provide larger exposed surface areas resulting in greater retention of plastic particles (Cozzolino et al., 2020; de Smit et al., 2021; Nicastro et al., 2022). Our laboratory experiments provide further evidence that morphological variation within a species can have even stronger effects than variation between species. In other words, what matters the most is morphology, not species identity sensu stricto. This was confirmed by the patterns observed in the field transplant experiment. The consistency of field and laboratory findings, also maintained under variable flow velocities, clearly indicate that phenotypic variation between northern and southern individuals of *F. guiryi* is crucial in determining differences in the efficiency with which they accumulate microplastics.

The ADV did not reveal intra- or interspecific effects on flow dissipation at low velocities. At higher flows, however, interspecific morphological differences affected surface flow dissipation. In the natural environment, *Fucus* spp. are subjected to high hydrodynamic forces including lift, drag and impingement forces due to wave breakage in the rocky intertidal (Gaylord, 1997; Gaylord, 2000; Denny and Gaylord, 2002). It has been suggested that the structural properties of organisms are correlated with hydrodynamic performance through velocity-dependent relations (Boller and Carrington, 2007). In response to increased velocity, flexible macroalgae such as *Fucus* spp. tend to bend passively by changing their shape and profile, allowing an exponential reduction in drag relative to their canopies (Denny and Gaylord, 2002; Harder et al., 2004). This phenomenon, known as reconfiguration, has been quantified as a measure of the relationship between velocity and drag (Vogel, 1994; Harder et al., 2004). It occurs through two mechanisms: (i) stem deflection at low velocities (<20 cm s$^{-1}$) and (ii) canopy compaction, consisting of a reduction in frontal area and a change in shape, at higher velocities (Boller and Carrington, 2006). Thus, it is likely that at higher flow velocities the phenotypical variation within *F. guiryi* morphotypes results in different plant reconfigurations — in agreement with the observed change in structural complexity under high flow.

### Table 2

Data from the field transplant experiment. P-value, t, Common Language Effect Size (CLES), Mean and 95 % Confidence Intervals (CI) of effect sizes (g Hedges) of Total MPs/individual (n) for interspecific and intraspecific variation. Asterisk indicates approaches where the relevant 95 % CI of effect size overlaps with zero. Descriptors for magnitudes are based on Cohen (1992) and expanded by Sawilowsky (2009).

<table>
<thead>
<tr>
<th>Variable</th>
<th>p-Value</th>
<th>t</th>
<th>CLES</th>
<th>(lower CI-upper CI)</th>
<th>Descriptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interspecific variation</td>
<td>0.001</td>
<td>4.251</td>
<td>0.688</td>
<td>0.692 (−0.019; 1.403)$^\dagger$</td>
<td>Medium</td>
</tr>
<tr>
<td>Intraspecific variation</td>
<td>0.001</td>
<td>9.466</td>
<td>0.994</td>
<td>3.584 (2.293; 4.876)</td>
<td>Huge</td>
</tr>
</tbody>
</table>

### 3.5. Estimated canopy microplastic trapping in the field

Orthogonal contrast tests using data expressed as Total MPs/canopy (mean n) revealed a significant interspecific effect (Fig 5B) on the number of microplastics trapped, with *Fucus guiryi* trapping more MP particles than *F. vesiculosus* (p = 0.002) resulting in a very large effect size (Table 3). No intraspecific effect was detected.

### 4. Discussion

We assessed the relative effects of intraspecific versus interspecific diversity on plastic trapping in coastal biogenic habitats. While *F. vesiculosus* trapped more plastic than *F. guiryi* at higher flow velocity in the laboratory, there was also an intraspecific effect, with plastic trapping significantly more within northern than southern morphotypes of *F. guiryi*. The results differed slightly under field conditions, where we found differences in MP retention between the morphotypes of *F. guiryi* at the individual level, but not at the canopy level.

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### Table 3

Data adapted from the field transplant experiment and the preliminary field assessment. P-value, t, Common Language Effect Size (CLES), Mean and 95 % Confidence Intervals (CI) of effect sizes (g Hedges) of Total MPs/canopy (mean n) for interspecific and intraspecific variation. Asterisk indicates approaches where the relevant 95 % CI of effect size overlaps with zero. Descriptors for magnitudes are based on Cohen (1992) and expanded by Sawilowsky (2009).

<table>
<thead>
<tr>
<th>Variable</th>
<th>p-Value</th>
<th>t</th>
<th>CLES</th>
<th>(lower CI-upper CI)</th>
<th>Descriptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interspecific variation</td>
<td>0.002</td>
<td>−3.948</td>
<td>0.804</td>
<td>1.211 (0.296; 2.126)</td>
<td>Very large</td>
</tr>
<tr>
<td>Intraspecific variation</td>
<td>0.123</td>
<td>−1.708</td>
<td>0.727</td>
<td>0.854 (−0.17; 1.877)$^\dagger$</td>
<td>Large</td>
</tr>
</tbody>
</table>
conditions (Fig. 2) — which in turn modulate surface flow dissipation. This underlies a strict correlation between the fractal dimension of the alga, its reconfiguration shape and the amount of microplastic trapped. Importantly, the interspecific difference in structural complexity observed in still water conditions between *F. vesiculosus* and *F. guiryi* is lost under conditions of low flow velocity (Figs. 1 and 2A). In contrast, these differences are maintained at the intraspecific level between *F. guiryi* northern and southern lineages (Fig. 2B), further highlighting the importance of intraspecific differences in morphology.

Together with flow velocity, polymer type affects the probability of microplastics being retained in marine vegetated canopies (Carmen et al., 2021). Previous studies have reported that microplastics made up of denser polymers (e.g., polyamide and polyethylene terephthalate pellets) are more likely to be retained in seagrass beds than less dense MPs (e.g., polypropylene pellets). Particularly, the trapping of floating MPs has been observed at low velocities (<12 cm s⁻¹) presumably due to the barrier created at the canopy/surface-water interface (Carmen et al., 2021). These results are in contrast to our observations of higher occurrence of polyamide fibres than floating microplastics (i.e., polypropylene), that might be explained by our simulation of an incoming high-tide during the laboratory-controlled hydrodynamic experiment using a stronger flow regime (i.e., 15 and 45 cm s⁻¹). In addition to flow-induced algal reconfiguration at higher flow velocity and the related significant decrease in structural complexity, the fact that the water surface just covered the algal would have hindered the formation of the canopy/surface-water interface, thus minimising the likelihood of trapping for less dense MP polymers. We observed similar patterns in the field with higher density polymers (i.e., PET and PE) being the most common MPs. Polymer-specific density influences particle position in the water column (i.e., the tendency to float at the surface or sink to the bottom) consequently driving the tendency of particles to settle, disperse or be retained.

Our results further show that, in combination with individual architectural complexity, the density of the biogenic structure plays a crucial role in the extent of plastic trapping. This is in line with recent evidence highlighting that microplastic retention within canopies of the seagrass *Zostera marina* increases with increasing shoot density (Carmen et al., 2021). Here, we found that canopies of *F. guiryi* accumulated more microplastics than those of *F. vesiculosus*, thus reversing the trend observed at the individual level (*F. vesiculosus* > *F. guiryi*). Plant density differs significantly but gradually across the north–south distribution of *F. guiryi* (Zardi et al., 2015); southern populations of *F. guiryi* are characterised by higher densities and provide more uniform cover than those of the northern morphotype. At the southern edge, inter-individual distances are reduced but patches are more scattered (Zardi et al., 2015). Hence, southern patches of *F. guiryi* would be expected to concentrate higher amount of microplastics. Despite marked differences in the structure and plant densities between northern and southern canopies of *F. guiryi* (Zardi et al., 2015), we observed, however, no difference in MP trapping at the canopy level. It is known that aggregations of individuals can strongly modify environmental factors. For instance, laboratory flume experiments with the blue mussel *Mytilus edulis* have shown that density and spatial arrangement of the individuals comprising a biogenic reef reduce boundary layer velocities, and increase turbulence and boundary layer thickness, eventually leading to higher risk of microplastic bioaccumulation due to plastic retention over reef surfaces (Lim et al., 2020). Similarly, a recent study showed that the increase in surface complexity of a mussel bed induced by endolithic infestation of the shell affects the structure of the overlying flow and increases plastic retention (Nicastro et al., 2022). In the context of macroalgae, previous research on *F. guiryi* showed a tempering effect of the canopy on local conditions of humidity, light intensity and temperature, attenuating stress level within the group (Monteiro et al., 2019). However, along the species’ distributional range, the habitat-modifying abilities of the southern and northern *F. guiryi* morphotypes do not differ significantly and do not result in distinct microclimates (Monteiro et al., 2019), which is in agreement with the lack of intraspecific effects at the canopy level found in the present study.

5. Conclusion

Our results demonstrate that intraspecific phenotypic variation not only modulates the extent of plastic trapping between morphotypes of the same species, but that its effects can be larger than those due to interspecific differences. Our findings also emphasize the crucial contribution of flow velocity, polymer composition and canopy density in triggering the patterns of microplastic accumulation observed. We thus argue that assessing the relative effects of intra- and interspecific diversity on microplastic accumulation in key ecosystems is essential to developing mitigation strategies.

CRediT authorship contribution statement

**Lorenzo Cozzolino:** Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Katy R. Nicastro:** Conceptualization, Investigation, Formal analysis, Resources, Writing – review & editing, Funding acquisition, Project administration, Supervision. **Laurent Seuront:** Investigation, Formal analysis, Resources, Writing – review & editing. **Christopher D. McQuaid:** Investigation, Resources, Writing – review & editing, Supervision. **Gerardo I. Zardi:** Conceptualization, Investigation, Formal analysis, Resources, Writing – review & editing, Supervision.

Data availability

Data will be made available on request.

Declaration of competing interest

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

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Appendix A. Supplementary data

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References


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