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Bacterial colonisation dynamics of household plastics in a coastal environment

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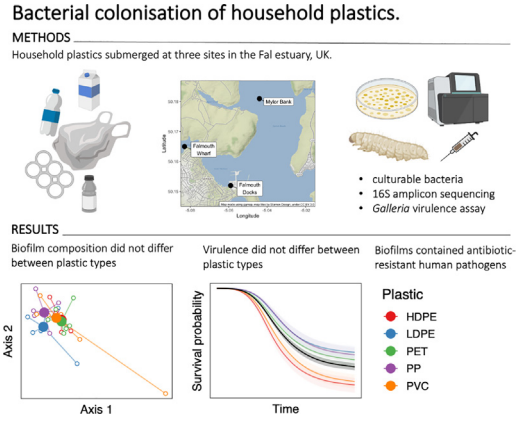
HIGHLIGHTS

- Bacterial colonisation of marine plastics may have potential risks to human health.
- There were no differences in bacterial biofilm composition between plastic types.
- The virulence of biofilms was similar across plastic types.
- Isolated potential human pathogens carried a range of antibiotic resistance genes.

GRAPHICAL ABSTRACT



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ABSTRACT

Accumulation of plastics in the marine environment has widespread detrimental consequences for ecosystems and wildlife. Marine plastics are rapidly colonised by a wide diversity of bacteria, including human pathogens, posing potential risks to health. Here, we investigate the effect of polymer type, residence time and estuarine location on bacterial colonisation of common household plastics, including pathogenic bacteria. We submerged five main household plastic types: low-density PE (LDPE), high-density PE (HDPE), polypropylene (PP), polyvinyl chloride (PVC) and polyethylene terephthalate (PET) at an estuarine site in Cornwall (U.K.) and tracked bacterial colonisation dynamics. Using both culture-dependent and culture-independent approaches, we found that bacteria rapidly colonised plastics irrespective of polymer type, reaching culturable densities of up to 1000 cells cm^3 after 7 weeks. Community composition of the biofilms changed over time, but not among polymer types. The presence of pathogenic bacteria, quantified using the insect model *Galleria mellonella*, increased dramatically over a five-week period, with *Galleria* mortality increasing from 4% in week one to 65% in week five. No consistent differences in virulence were observed between polymer types. Pathogens isolated from plastic biofilms using *Galleria* enrichment included *Serratia* and *Enterococcus* species and they harboured a wide range of antimicrobial resistance genes. Our findings show that plastics in coastal waters are rapidly colonised by a wide diversity of bacteria independent of polymer type. Further, our results show that marine plastic biofilms become increasingly associated with virulent bacteria over time.

1. Introduction

Plastic pollution has huge negative impacts on marine environments (Ritchie and Roser, 2018). In 2020, around 367 million tonnes of plastic

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were produced globally (PlasticsEurope, 2021). This figure is increasing year on year (Geyer, 2020), with a significant percentage of all plastics produced ending up in the marine environment (Hohn et al., 2020; Jambeck et al., 2015). Plastics have multiple adverse effects on a wide diversity of wildlife, including entanglement, ingestion and physical damage (e.g. (Duncan et al., 2017; Germanov et al., 2018; Lamb et al., 2018; Potocka et al., 2019; Solomando et al., 2022)). Moreover, it is increasingly recognised that there is potential for plastic pollution to pose a threat to human health (Waring et al., 2018), from the effects of ingestion of micro- and nano particles (e.g. via shellfish or drinking water (Van Cauwenberghe and Janssen, 2014)) followed by cellular uptake (Lehner et al., 2019), potentially accompanied by the transfer of sorbed pollutants (Fred-Ahmadu et al., 2020), to decreased psychological wellbeing through pollution of the natural environment (Wyles et al., 2016).

The surface of plastic fragments in aquatic environments is rapidly colonised by microorganisms, leading to diverse biofilm communities distinct from that of the surrounding seawater (Amaral-Zettler et al., 2020; Harrison et al., 2014; Lobelle and Cunliffe, 2011). Bacterial colonisation of marine plastics is dependent on a variety of abiotic factors, including season, location, colonisation time and polymer type (Amaral-Zettler et al., 2020; Oberbeckmann et al., 2014; Zettler et al., 2013). A growing number of studies have used 16S amplicon sequencing approaches to specifically investigate bacterial colonisation of marine plastics. Clear differences in bacterial community composition between plastic and other marine debris (e.g. wood or glass) have been reported (e.g. (Miao et al., 2019; Ogonowski et al., 2018)) (but see (Kesy et al., 2019)). Differences in plastic colonisation based on beta-diversity metrics have been mixed. Differences have been reported between polystyrene (PS) and polyethylene (PE) and polypropylene (PP) (Frère et al., 2018; Hansen et al., 2021), between PS and PP, PE and polyethylene terephthalate (PET) (Amaral-Zettler et al., 2015), between PE and PET (Muthukrishnan et al., 2019), between PE and PP and PS (Vaksmaa et al., 2021) and even between differently coloured PE microplastics (Wen et al., 2020). However, other studies have failed to find biofilm community differences between plastics, e.g. between polyethylene (PE), polypropylene (PP) and polystyrene (PS) (Ogonowski et al., 2018), between PE and PS (Kesy et al., 2019; Oberbeckmann et al., 2018) or between PE and PP (Miao et al., 2019). Inconsistent results between studies may have occurred because of other contributing factors that differ between experiments such as incubation time, location and season that also alter community composition. As differential plastic colonisation could affect ecological function and survival of biofilm communities (Miao et al., 2019) ultimately affecting biotransformation and bacterial dispersal (Amaral-Zettler et al., 2020), it is clear that between-plastic differences in bacterial colonisation deserve more attention.

Among bacterial colonisers of marine plastics are pathogenic species known to cause disease in animals (e.g. (Radisic et al., 2020; Viršek et al., 2017)) and humans (e.g. (Kirstein et al., 2016; Rodrigues et al., 2019; Silva et al., 2019; Wu et al., 2019)). For example, although *Vibrios* are generally sparse in the open ocean, they have been found in strikingly high numbers on marine microplastics (Amaral-Zettler et al., 2020; Zettler et al., 2013) and, in one study, *Vibrios* had colonised 13% of the marine microplastics tested (Kirstein et al., 2016). In this way, plastics could serve as potential vectors for pathogens (Kirstein et al., 2016) and/or bacteria carrying antimicrobial resistance genes (Rasool et al., 2021). Plastic pollution could also result in elevated horizontal gene transfer by facilitating close contact (Arias-Andres et al., 2018), and plastic-sorbed contaminants (Teuten et al., 2007) could potentially speed up antimicrobial resistance development (Vos, 2020). Therefore, in addition to direct threats related to ingestion, plastic-borne bacteria may also pose an infection risk to human and wildlife health (Bowley et al., 2020; Keswani et al., 2016; Parthasarathy et al., 2019).

To date, work investigating the pathogenicity of plastic biofilms has relied on the identification of potential pathogens using agar cultivation or amplicon sequencing (Kirstein et al., 2016; Wu et al., 2020). These methods cannot quantify the virulence or pathogenicity of bacteria, which is known to vary greatly even within a bacterial species. Here, we directly test for the presence of pathogens on plastic biofilms, employing a recently developed selective isolation method using the *Galleria mellonella* insect virulence model (Hernandez et al., 2019). The *Galleria* model is an accepted proxy for the mammalian innate immune system (Brennan et al., 2002; Wand et al., 2011), and allows the detection of pathogens that may cause human infection.

The aim of this study is to investigate the colonisation dynamics of bacterial pathogens on five of the most common types of household plastics (low-density PE (LDPE), high-density PE (HDPE)), polypropylene (PP), polyvinyl chloride (PVC) and polyethylene terephthalate (PET)), at multiple coastal sites, and over time. We focus on plastic colonisation in coastal waters where terrestrial inputs of plastics (Oberbeckmann et al., 2014) and human-, animal- and soil- associated pathogens is highest (Leonard et al., 2018). We investigate bacterial colonisation dynamics on plastics incubated in the marine environment by quantifying density for subsets of cultivable diversity and testing for differences in taxonomic diversity via 16S amplicon sequencing and test for the presence of pathogens using *Galleria* virulence assays. We performed two separate experiments: (i) we measured bacterial colonisation after seven weeks across three different locations, quantifying bacterial colonisation through cultivation-based approaches and measuring the presence of pathogens using the *Galleria* assay; (ii) we focused on one location close to shore and in addition to cultivation and the *Galleria* assay, used 16S rRNA amplicon sequencing to quantify bacterial colonisation over time.

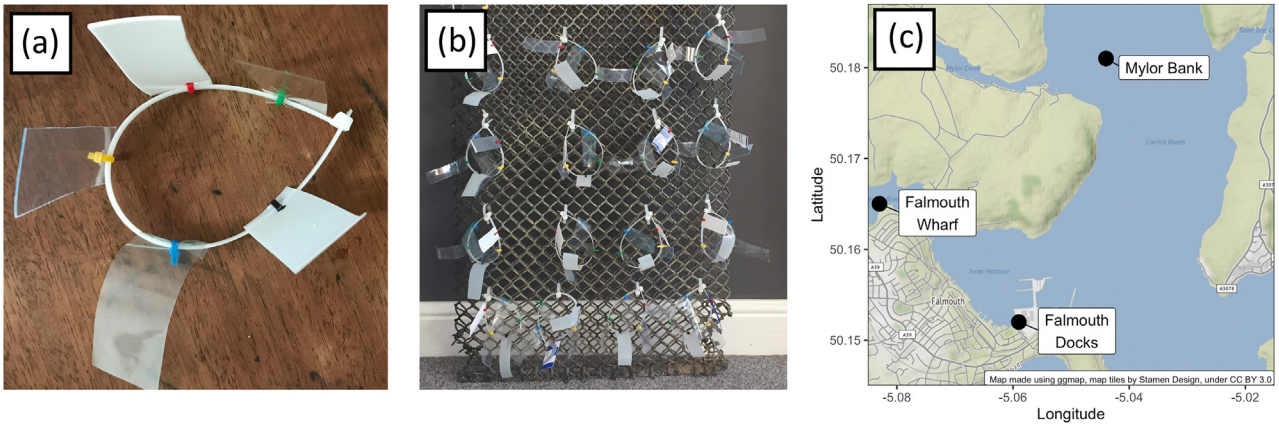

Fig. 1. Overview of the methods used to submerge household plastics in the Fal Estuary. (a) Strips of LDPE, HDPE, PP, PET and PVC strips were attached to a big cable tie ring using smaller, colour coded cable ties. (b) Each large ring was attached to a metal mesh bag using cable ties which was then submerged at each site (c) Sites used in this study: Falmouth Docks, Falmouth Wharves and Mylor Bank. All three sites were used in the first 'Location' experiment whereas the second 'Colonisation' experiment used only at the Falmouth Docks site.

2. Materials and methods

2.1. Plastics

Five plastic types were collected from household recycling: low-density polyethylene (LDPE) from sandwich bags, high-density polyethylene (HDPE) from milk bottles, polypropylene (PP) from peanut butter tubs, polyvinyl chloride (PVC) from roofing material and polyethylene terephthalate (PET) from drink bottles. Plastics were cut into 85 mm by 25 mm strips with a 5 mm diameter hole made at one end using a holepunch. One sample of each type of plastic was attached to a large cable tie via smaller, colour coded cable ties to form a ring (Fig. 1a). Rings were then attached to metal mesh bags using cable ties (Fig. 1b). Plastics were sterilised using 70% ethanol before environmental incubation.

2.2. Location experiment

A total of 18 rings (attached to three mesh bags) were deployed on 10th December 2019 at three sampling sites in the Fal Estuary (Falmouth, Cornwall, UK): Falmouth Docks (50.152, −5.059), Falmouth Wharves (50.165, −5.083) and Mylor Bank (50.181, −5.044) (Fig. 1c) with help from Cornwall Port Health Authority. Mylor Bank was further from the shore and was further away from human habitation (Fig. 1c). The bags were attached to pontoons at Falmouth Wharf and Falmouth Docks and to a buoy at Mylor Bank using a rope, ensuring the bags were fully submerged. Plastic rings were retrieved from each site on 28th January 2020 (after 7 weeks) and placed in a sterile plastic box (separately tied to a bar to avoid cross contamination) for transportation. Sample processing commenced within 1 hour (h) of collection.

2.3. Colonisation experiment

To measure differences in the bacterial colonisation of different plastic types through time, 28 rings of plastics (four timepoints, seven replicates) were attached to a single metal mesh bag and submerged as described above at the Falmouth Docks site on February 4th, 2020. One set of plastic rings was collected on February 11th, 18th and 24th and March 3rd (weeks 1, 2, 3 and 5) and brought back to the lab.

2.4. Biofilm processing

First, each plastic strip was cut in half using scissors that were sterilised between samples using 70% ethanol and placed in a 50 mL falcon tube (sealed and sterilised by the manufacturer) containing 10 mL NaCl buffer (9 g/L; Fisher Chemicals, Loughborough, UK) and five autoclaved glass beads to facilitate removal of biofilm (Millipore Colirollers Plating Beads, Billerica MA, USA). Each tube was vortexed for 60 seconds (s) at 2500 r.p.m. to remove and suspend plastic biofilms (Fig. S1). As our focus was on bacteria of terrestrial origin, specifically potential human pathogens, serial dilutions of biofilm suspensions were plated on LB agar (Fisher BioReagents, Loughborough, UK) and coliform agar (Millipore Sigma, Billerica MA, USA) and incubated at 37 °C. LB agar selects for relatively fast-growing bacteria; coliform agar was used to quantify both coliform bacteria and *E. coli*. Colony forming units (CFU) were counted after 24 h of incubation at 37 °C. All counts were standardised to CFU/cm^2. Biofilm suspensions were stored in glycerol (Fisher Chemicals, Loughborough, UK) (20% final concentration) at −70 °C.

2.5. Galleria mellonella virulence assay

G. mellonella larvae were purchased from Livefood U.K. (http://www.livefood.co.uk) and used within one week of purchase. A 100 μL Hamilton syringe (Sigma-Aldrich Ltd., Gillingham, UK) with 0.6 × 30 mm needles (BD Microlance 3, Becton Dickinson, Plymouth, UK) was used to inject the larvae with 10 μL of defrosted biofilm freezer stock into the last left proleg, using 20 larvae (location experiment) or 10 larvae (colonisation experiment) for each sample. The larvae were anaesthetised on ice for 30 minutes (min) before injection. Needles were sterilised between samples by flushing with 70% ethanol followed by NaCl buffer. Two negative controls for the experiment were used: a buffer control using 10 μL of sterile NaCl to control for the impact of physical trauma, and a no-injection control to account for background larvae mortality. After injection, larvae were incubated at 37 °C and inspected after 24, 48 and 72 h post-injection (location experiment) or 24 and 48 h post-injection (colonisation experiment) to record mortality. Larvae were scored as dead if they did not respond to touch stimuli (Hernandez et al., 2019).

2.6. Isolation of pathogenic bacteria

To isolate pathogens causing *G. mellonella* mortality, we reinjected 20 of the most virulent communities into ten larvae each as described above. Larvae demonstrating melanisation – a key indicator of infection – were anesthetised by placing on ice before their haemocoel was extracted. To extract haemocoel, 70% ethanol was used to sterilise the area around the last left proleg before the proleg was removed using sterile micro-scissors and the haemocoel collected using a pipette. This method is preferred over whole larvae destruction as it minimises contamination with skin and gut bacteria (Hernandez et al., 2019). Collected haemocoel (~5-15 μL) was diluted in 500 μL of buffer, plated onto LB agar and incubated for 24 h. Colonies were then picked and grown in 750 μL of LB at 37 °C for 24 h. Cultures were frozen at −80 °C in glycerol (at a final concentration of 25%). Defrosted stocks were diluted to (1×10^5 CFU/mL) and injected into ten larvae which were then incubated for 18 h; deceased larvae confirmed that isolated clones were pathogenic and not commensal gut or skin bacteria.

2.7. 16S rRNA amplicon sequencing

DNA was extracted from biofilms using a DNeasy UltraClean Microbial Kit (Qiagen, Hilden, Germany) according to the standard protocol with the only modification to increase the initial centrifugation speed and time (10 K x g for 10 min) to pellet bacteria suspended in the 20% glycerol stock. DNA concentrations were quantified using the Qubit HS DNA kit (Invitrogen), purity was assessed using nanodrop 260:280 ratios, and integrity was assessed using a 1% agarose gel. A 251 bp conserved fragment in the V4 hypervariable region was targeted using N515f and N806r primers (https://earthmicrobiome.org/protocols-and-standards/16s/) with phasing and a pool of indexed primers suitable for multiplex sequencing with Illumina technology. Sequencing was performed using an Illumina MiSeq V2 500 by the University of Exeter Sequencing Service. Sequencing adapters and any bases below a score of Q22 were removed, alongside any reads <150 bp using '*Cutadapt*' (Martin, 2011). We then processed and analysed the sequence data in R (v4.0.3) (Team, 2013) using the packages '*dada2*' (Callahan et al., 2016a) and '*phyloseq*' (McMurdie and Holmes, 2013). Following the standard full-stack workflow, we estimated error rates, inferred and merged sequences, constructed a sequence table, removed chimeric sequences and assigned taxonomy. During processing, the first 25 bp of forward and reverse reads were trimmed. Taxonomies were assigned to amplicon sequence variants (ASVs) using the SILVA database (Quast et al., 2012). We estimated phylogeny using '*fasttree*' (Price et al., 2010) to allow for the calculation of Unifrac distances (which take into account the phylogenetic distance between ASVs) between communities. We then removed any reads that had not been assigned to at least the phylum level (613 of 12,564 unique ASVs) and any sequences assigned to the phylum Cyanobacteria or classified as mitochondria. Processing and filtering steps resulted in three samples containing fewer than 8000 reads being removed (one replicate from week 1 LDPE, one replicate from week 1 HDPE, and one replicate from week 2 of PP), with the remaining samples having a maximum read number of 659,117, a minimum of 8007 and a median of 20,638.

2.8. Whole genome sequencing

DNA isolation, Illumina HiSeq sequencing and basic bioinformatics were performed through MicrobesNG, Birmingham, UK. Vials containing beads inoculated with liquid culture were washed with extraction buffer containing lysostaphin and RNase A, and incubated for 25 min at 37 °C. Proteinase K and RNaseA were added and vials were incubated for a further 5 min at 65 °C. Genomic DNA was purified using an equal volume of SPRI beads and resuspended in EB buffer. DNA was quantified in triplicate using the Quantit dsDNA HS assay in an Eppendorff AF2200 plate reader. Genomic DNA libraries were prepared using the Nextera XT Library Prep Kit (Illumina, San Diego, CA, USA) following the manufacturer's protocol with the following modifications: two nanograms of DNA instead of one were used as input, and PCR elongation time was increased to 1 min from 30 s. DNA quantification and library preparation were carried out on a Hamilton Microlab STAR automated liquid handling system. Pooled libraries were quantified using the Kapa Biosystems Library Quantification Kit for Illumina on a Roche LightCycler 96 qPCR machine. Libraries were sequenced on the Illumina HiSeq using a 250 bp paired end protocol.

Sequencing reads were adapter trimmed using '*Trimmomatic 0.39*' with a sliding window quality cutoff of Q15 (Bolger et al., 2014). De novo assembly was performed on samples using '*Unicycler v0.4.8*' (Wick et al., 2017), and summary stats of assemblies were calculated using CheckM v1.1.3 (Parks et al., 2015). Genome assemblies were visualised using Bandage (Wick et al., 2015) and manually split when there were two large non-contiguous assemblies which were then run separately through the other stages. The assemblies which were likely two or more species were identifiable by a high contamination value (~100%). Contigs were annotated using Prokka 1.14.6 (Seemann, 2014). Taxonomic classification of our assemblies was performed using '*MMSeqs2 13.45111*' (Steinegger and Söding, 2017) using the GTDB database (Chaumeil et al., 2020), and of 16S gene amplicons using '*dada2*' (Callahan et al., 2016b) using the SILVA reference database (Quast et al., 2012). Assemblies were screened for antimicrobial resistance genes and virulence factors using '*AMRFinderPlus v3.10.1*' (Feldgarden et al., 2021).

2.9. Statistical analyses

2.9.1. Analysing differences in culturable abundance between plastics, sites, and time

All analyses were conducted using the statistical programming language R v4.0.3 (Team, 2013) and all graphs were produced using the '*ggplot2*' package (Wickham and Wickham, 2007). Linear models were used to examine differences in culturable bacterial abundance between locations. The response variable was $\log_{10}$ abundance + 1 cm^{-2} plastic to normalise the residuals, and the predictors were location, plastic type and their interaction. Separate models were run for each category of culturable bacteria (bacteria culturable on LB, coliform and *E. coli*). Model selection was performed using likelihood ratio tests. Pairwise multiple comparisons to examine significant differences among locations or plastics were performed using the R package '*emmeans*' (Lenth et al., 2018). Changes in abundance through time during colonisation were analysed using linear models. The response variable was again $\log_{10}$ abundance + 1 cm^{-2}, and the predictors were week of sampling as a continuous predictor, plastic type and their interaction. Separate linear models were run for all three categories of culturable bacteria. Model selection and post-hoc multiple comparisons were performed as above.

2.10. Analysing virulence of plastisphere biofilms

Survival of *Galleria* inoculated with bacteria isolated from biofilms was quantified as a measure of virulence of the plastisphere communities. Survival curves were fitted using Bayesian regression in the package '*rstanarm*' (Brilleman et al., 2020) and parameters were estimated using '*tidybayes*' (Kay, 2019). Compared to the popular Cox model, Bayesian implementation can more easily visualise uncertainty and better handle random effects. For survival curves of *Galleria* injected with bacteria isolated from the location experiment, we fitted a proportional hazards model with an M-splines baseline hazard, with site, plastic type, and their interaction as fixed effects and a random effect of biofilm, as multiple *Galleria* were all inoculated with the same biofilm. For the survival curves through time at Falmouth Docks, we fitted the same model, but with week (as a categorical predictor), plastic type, and their interaction as fixed effects and a random effect of biofilm. Models were run for 3000 iterations and three chains were used with uninformative priors. Model convergence was assessed using Rhat values (all values were 1) and manual checking of chain mixing. For both models, log hazards were estimated for each plastic type in each site or week. To examine differences in virulence among sites and plastic types, hazard ratios were calculated as the exponential of the difference between two log hazards. A hazard ratio above one indicates an increase in virulence compared to the baseline treatment, with a value below one indicating a decrease in virulence compared to the baseline. We calculated median hazard ratios with 95% credible intervals and the probability that the given hazard ratio was above 1. Median hazard ratios with 95% credible intervals that do not cross 1 indicate a significant difference in virulence between two factors. For visualisation purposes, we show the hazard ratio of each location/polymer type where the baseline is the median log hazard in each experiment, allowing differences in virulence between sites/plastic types to be easily interpreted.

2.11. Analysing plastisphere community composition between different plastic types

To investigate how plastisphere bacterial communities changed, we examined changes in community composition, alpha and beta diversity through time. We used weighted Unifrac distance (Lozupone and Knight, 2005) as a measure of compositional dissimilarity between communities, which weights branches in a phylogeny based on the relative abundance of each ASV. As this distance requires a rooted phylogeny, we rooted the tree based on the longest tree branch terminating in a tip. Differences in composition between plastic communities were analysed using the R packages '*phyloseq*' (McMurdie and Holmes, 2013) and '*vegan*' (Oksanen et al., 2013). We tested whether week of colonisation and plastic type altered community composition using permutational ANOVAs. Permutational ANOVA tests were run using '*vegan::adonis*' with 9999 permutations and differences in group dispersion (which test for differences in beta diversity between treatments) were analysed using '*vegan::betadisper*'. An overall model including plastic type and colonisation week as interacting factors was performed first. We then split the data to examine an overall effect of plastic type by performing a separate PERMANOVA for each week and performing pairwise PERMANOVAs between each plastic type to assess differences. *P* values were corrected using the *fdr* method (Benjamini and Hochberg, 1995). When looking at differences in beta diversity, we calculated the distance from the centroid of each plastic type by week combination, and then ran a linear model where distance from the centroid was the response variable, and plastic type and week were potentially interacting factors. Model selection was performed as above.

To investigate differences in alpha diversity and evenness between communities, we rarefied data so that all samples had the same number of reads (8011). Richness (alpha diversity) was taken as the total number of ASVs and evenness was calculated as Pielou's evenness (Pielou, 1966). The amount of change and variation in the number of ASVs across weeks and across plastic types was tested using linear models. Total ASVs were $\log_{10}$-transformed to normalise residuals, with plastic type and week as potentially interacting predictors. As we did not expect alpha diversity to necessarily change linearly across weeks, we included it as a categorical predictor. For evenness, the analysis was the same, but without any transformation on the response. Model selection was performed as above.

3. Results

3.1. Bacterial abundance was lowest on plastics further away from human habitation

To determine the effects of location and polymer type on bacterial colonisation, five different plastics were incubated at one-meter depth at

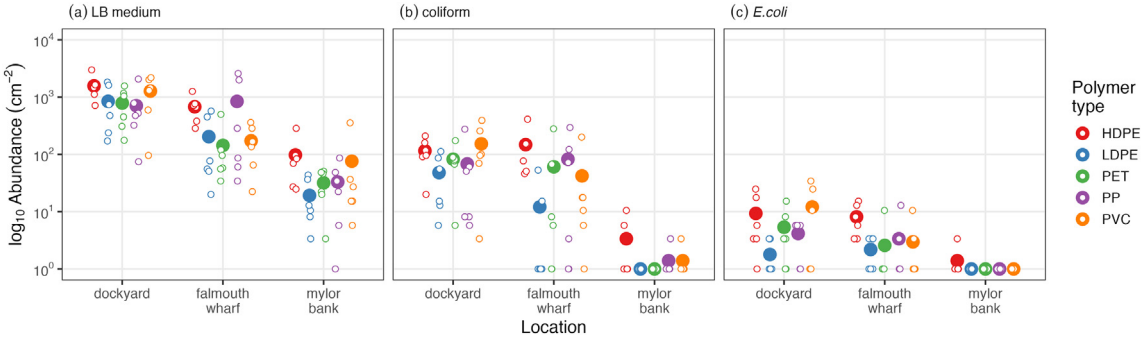

Fig. 2. Abundance of (a) bacteria on LB medium, (b) coliform bacteria and (c) *E. coli* found across polymer types and locations. Large points represent mean abundances of plastics at each site and small points represent individual replicates.

three estuarine locations near Falmouth, U.K. After being submerged for seven weeks, the abundance of bacteria culturable on LB, coliforms, and *E. coli*, significantly differed between locations (ANOVA between models with and without location as a predictor: bacteria culturable on LB: $F_{2,83} = 69.32, p < 0.001$; coliforms: $F_{2,83} = 54.34$, $p < 0.001$; *E. coli*: $F_{2,83} = 14.44$, $p < 0.001$) (Fig. 2). Across the three quantified groups, abundance was consistently highest at Falmouth Docks, which is located close to human habitation (bacteria culturable on LB: mean = 724 CFU cm^{-2}, 95%CI = 490 - 1096; coliform: mean = 50 CFU cm^{-2}, 95%CI = 30–83 *E. coli*: mean = 3.3 CFU cm^{-2}, 95%CI = 2.4–4.6) and lowest at Mylor Bank which is furthest from the shore and human habitation (bacteria culturable on LB: mean = 25 CFU cm^{-2}, 95%CI = 17–38; coliform: mean = 1.2 CFU cm^{-2}, 95%CI = 0.7–2.1; *E. coli*: mean = 1.04 CFU cm^{-2}, 95%CI = 0.76–1.4), although not all contrasts were significant (Table S1).

Significant differences in abundance were found between plastics (ANOVA between models with and without plastic as a predictor: bacteria culturable on LB: $F_{4,83} = 4.65$, $p = 0.002$; coliforms: $F_{4,83} = 4.05$, $p = 0.005$; *E. coli*: $F_{4,83} = 3.11$, $p = 0.019$), but there was no significant interaction between location and plastic (ANOVA between models with and without interaction between plastic and location: $p > 0.05$ for LB, coliform and *E. coli*). Abundance was consistently highest on HDPE, but this was only significant across all three quantified groups when compared to LDPE (Tukey comparison between HDPE and LDPE: bacteria culturable on LB: $t = 3.86$, $p = 0.002$; coliform: $t = 3.88$, $p = 0.0018$; *E. coli*: $t = 3.31$, $p = 0.0118$; Table S1).

3.2. Rate of bacterial colonisation did not differ between polymer types

To investigate bacterial colonisation of plastics through time, we performed a second experiment at the Falmouth Docks site only, and sampled plastics after one, two, three and five weeks of submergence. During this period, the abundance of bacteria growing on LB, coliforms, and *E. coli* all increased (slope of increase in abundance in best model: bacteria culturable on LB: 0.23, 95%CI = 0.17–0.29; coliform = 0.32, 95%CI = 0.25–0.39; *E. coli*: 0.17, 95%CI = 0.13–0.19) (Fig. 3). For both bacteria culturable on LB and coliforms, the increase in abundance occurred consistently across all types of plastic (ANOVA with and without plastic as a predictor: all p values >0.05). The only difference in colonisation rate between plastics was found for *E. coli* (ANOVA with and without the interaction between week and plastic: $F_{4,130} = 5.95$, $p = 0.0002$), where the rate of colonisation was lowest on LDPE (slope of increase in abundance in best model: *E. coli* on LDPE: 0.05, 95%CI = −0.02–0.12), but this was only significantly lower than PET (Tukey comparison between slope of LDPE and PET: $t = -4.12$, $p = 0.0005$) and PVC (Tukey comparison between slope of LDPE and PVC: $t = -3.92$, $p = 0.013$).

3.3. Plastic biofilm community composition changes through time, but does not differ between polymer types

In addition to culture-dependent approaches, we performed 16S amplicon sequencing across all four timepoints and plastic types for the colonisation experiment. Alpha diversity changed through time (ANOVA with and without week as a predictor: $F_{3,113} = 7.38$, $p = 0.00015$), increasing during the first two weeks of colonisation, after which the number of unique ASVs remained stable between weeks 2 and 5 (Fig. 4a, Table S2). A similar pattern was seen in the evenness of the biofilm communities (ANOVA with and without week as a predictor: $F_{3,113} = 3.54$, $p = 0.017$), with communities being most even at week one (Fig. 4b, Table S3), although observed differences in evenness were small across all weeks. Alpha diversity and evenness did not differ between polymer types (ANOVA with and without polymer type as a predictor: alpha diversity: $F_{4,112} = 0.0172$, $p = 0.999$; evenness: $F_{4,112} = 0.607$, $p = 0.66$).

Bacterial community composition changed significantly over the course of the experiment (PERMANOVA, effect of week: $F_{3,109} = 20.18$, $R^2 = 0.34$, $p = 0.001$) (Fig. 5). The first principal coordinate explained 20.3%

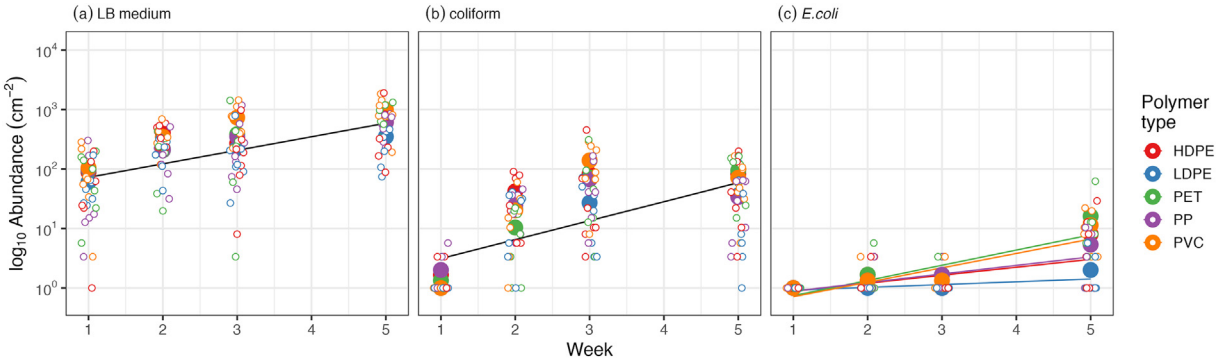

Fig. 3. Abundance of (a) bacteria on LB medium, (b) coliform bacteria and (c) *E. coli*, through five weeks of colonisation at the Falmouth Docks site. Small points represent individual replicates and large points represent mean abundances on each plastic at each week. Lines represent the predicted best fit from the model (see Methods), with the single black line in (a) and (b) indicating that there is no effect of plastic on abundance.

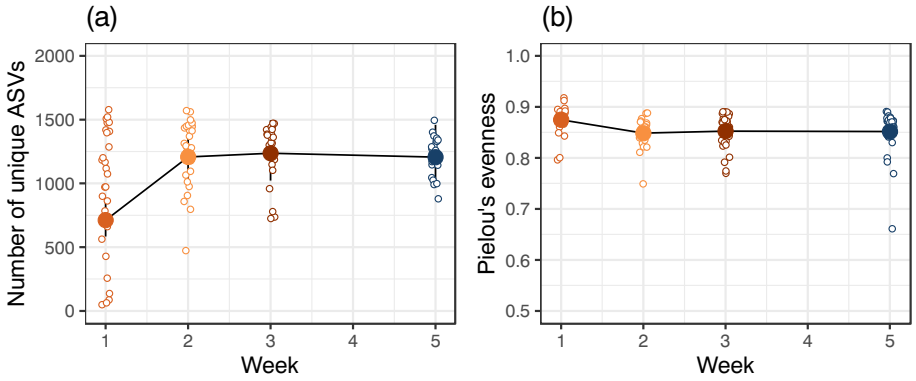

Fig. 4. Change in (a) alpha diversity and (b) evenness of plastic biofilm communities. (a) Alpha diversity increased from week 1 to week 2 and then remained stable. (b) At the same time, communities became more even, with the only difference in evenness occurring between week 1 and 2. In both panels, small points are individual replicates, large points and line bars represent estimates and 95% confidence intervals from the best model respectively (see Methods), and model estimates are joined by lines to help visualise differences between weeks.

of the total variation and separated the weeks, with the greatest difference between week 1 and week 5. While there was no interaction between plastic and week (PERMANOVA, interaction between week and polymer type: $F_{12,97} = 0.91$, $R^2 = 0.06$, $p = 0.68$), polymer type significantly altered community composition when added alongside week (PERMANOVA, $F_{4,109} = 1.89$, $R^2 = 0.04$, $p = 0.01$), although the amount of variation it accounted for was very small (~4%). To further examine this effect, we ran multiple pairwise permutational ANOVAs on the entire dataset and within each week. We found no significant differences between any two plastics on the whole dataset or within each week (PERMANOVAs: all $p_{adj} > 0.05$), indicating no consistent effect of plastic type. There were no overall differences in between-community diversity (beta-diversity) across weeks or plastics, with the simplest model being one with no predictor (Table S2).

We further explored the community composition of plastic biofilms by aggregating ASVs at different taxonomic ranks. These were plotted through time and across plastics to qualitatively look at differences in dominant taxa across samples. Across all plastics, the biofilm communities were dominated by three classes of bacteria: *Alphaproteobacteria*, *Bacteroidia* and *Gammaproteobacteria* which made up an average of 92% of the communities (Fig. S2). At the order level, we identified 5 different orders (*Burkholderiales*, *Enterobacterales*, *Flavobacteriales*, *Pseudomonadales*, and *Rhodobacterales*) which together made up an average of 68.8% of the communities (Fig. S3). Most orders remained stable in their relative abundance through time (Fig. S2), but *Burkholderiales* declined in relative abundance through time, going from an average of 5% in week 1 to 0.89% in week 5. At the family level, the two dominant families (*Flavobacteriaceae* and *Rhodobacteraceae*) increased in abundance

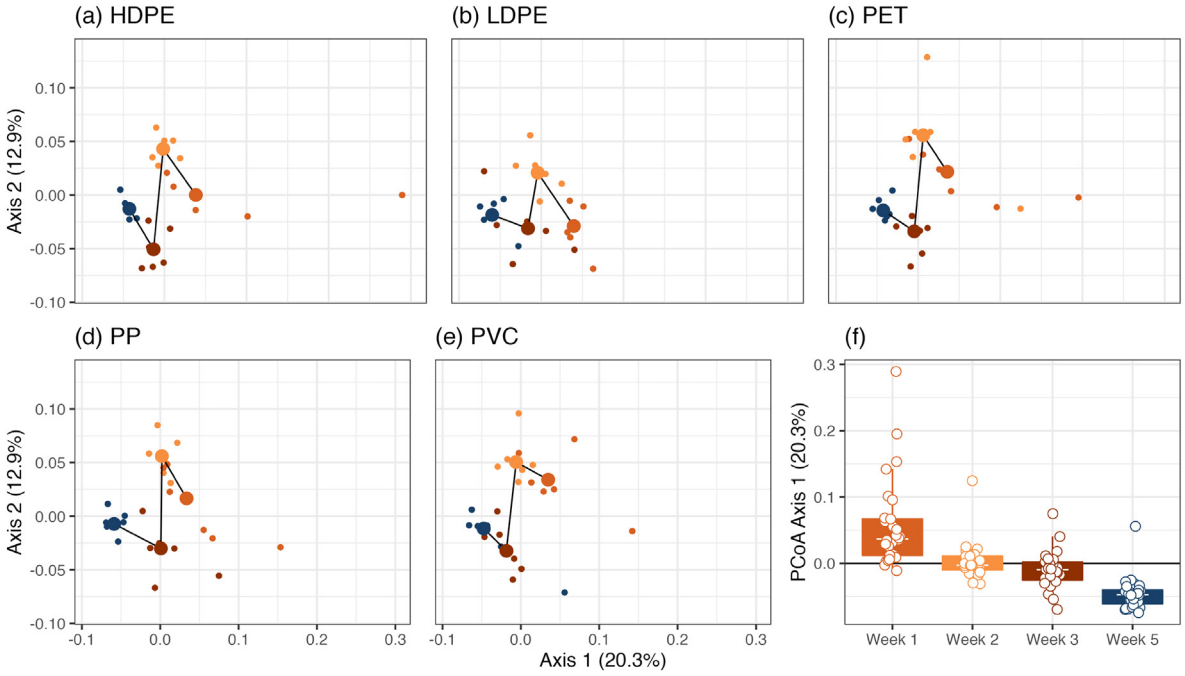

Fig. 5. Changes in community composition through time (different colours) and between plastics (panels a-e). (a-e) Principal Coordinate (PCoA) plot of communities based on weighted-Unifrac distance. Each panel represents biofilms from different polymer types and shows that community composition changes consistently through time irrespective of plastic type. The percentage of variation explained is shown on each axis (calculated from the relevant eigenvalues). (f) The separation of communities through time along PCoA axis 1 scores. In (a-e) small points are individual communities and large points are positions of centroids, with the black lines showing how centroid position changes through time. In (f) points are individual communities, tops and bottoms of the bars represent the 75th and 25th percentiles of the data. White lines are the medians, and the whiskers extend from the respective hinge to the smallest of largest value no further than 1.5*interquartile range.

through time (Fig. S4), and there was a marked decrease in *Weeksellaceae* between weeks 2 and 3.

3.4. Virulence of plastic biofilm communities

In the location experiment, all hazard ratios between the different sites had credible intervals that crossed one, indicating non-significance at a traditional 0.95 level. However, biofilms from Falmouth Dockyard (mean time to death = 28.5 h; proportion that died = 0.48) and Falmouth Wharf (mean time to death = 30.8 h; proportion that died = 0.48) were 2.5 times more virulent than biofilms from Mylor Bank (mean time to death = 34.4 h; proportion that died = 0.32) (Fig. 6a, Table S3). There was an 87% probability that the Docks and Wharf communities were more virulent than those from Mylor bank for both locations (Table S3).

In the colonisation experiment, biofilms from weeks 1, 2, and 3 killed only 4, 7, and 11% of *Galleria* respectively. In contrast, biofilms from week 5 were much more virulent, with 65% of *Galleria* dying during the assay. In both the location and colonisation experiments, we looked for differences in virulence between polymer types (Fig. 6a). To classify a polymer type as having more or less virulent communities than others, it would need to have a higher (or lower) hazard ratio than average in both experiments (Table S3). There were no hazard ratios between polymer types that had 95% credible intervals that did not overlap with 1 for both experiments meaning that there were no significant differences in virulence between polymer types. However, we found evidence that LDPE communities are less virulent than other polymer types, with all hazard ratios being above 1 when LDPE was the baseline polymer type (Table S3), with an 89% probability (on average) of the other polymer type being more virulent. The four other polymer types did not have consistent differences in their hazard ratios.

3.5. The identity of putative human pathogens

To obtain a more detailed picture of the taxonomic identity and antimicrobial resistance of individual pathogens in our plastic biofilm samples, we genome-sequenced 16 bacterial clones isolated from some of the most virulent biofilm communities spanning different locations and plastic types (Table 1). Eleven genome assemblies had high completion and low contamination and could be unambiguously assigned to a single bacterial species, but this was not the case for five remaining genome samples – which must have been due to the co-isolation of multiple clones. To retain as much information as possible, these assemblies were not discarded but were bioinformatically separated using the genome viewer Bandage, with assemblies split into non-contiguous sections (46). Using this approach, four contaminated genomes could be split into eight genomes (2 & 3, 6 & 7, 8 & 9, 15 & 16; Table 1), resulting in 20 sequenced genomes in total. One remaining assembly (17; Table 1) could not be split because it only had one contiguous genome. Retaining a large chimeric assembly was also the case for (split) sample 16 and so for both assemblies multiple identities are presented instead of one (Table 1).

The most prevalent pathogen genus isolated was *Serratia* (Table 1), with several clones identified as *S. liquefaciens*, and *S. fonticola*, which are known human pathogens (Mahlen, 2011) that have previously been reported to occur commonly in soils in Cornwall using the *Galleria* enrichment method (Ferraresso et al., 2020). Other species identified were *Enterococcus faecalis* which is known to infect humans (Elsner et al., 2000), and *Bacillus licheniformis* which can cause opportunistic infections (e.g. (Haydushka et al., 2012)). Surprisingly, no virulence genes were detected in any of the assemblies, although we used a relatively stringent sequence similarity cut-off of 95%. However, a host of antimicrobial resistance genes belonging to a variety of classes were uncovered (Table 2). These include genes conferring resistance to clinically relevant antibiotics such as cephalosporins, aminoglycosides and trimethoprim (in *E. faecalis*) (Table 2).

4. Discussion

To track bacterial colonisation and virulence as a function of plastic type, location, and time, we submerged common household plastics in the Fal Estuary (UK). Colonisation of plastics was largely independent of plastic type (LDPE, PE, PP, PVC and PET), with little or no effect on the number of culturable bacteria or 16S-based community composition across locations or time (Figs. 2–5). The exception was LDPE, which showed lower colonisation rates in both the location and colonisation experiments (Figs. 2 & 3), although these differences were only significant compared to HDPE for location, and compared to PET and PVC for *E. coli* in the colonisation experiment. However, as LDPE-derived biofilms exhibited lower virulence in the *Galleria* model (Fig. 6), LDPE may pose a lower risk of pathogen colonisation.

Approaches to determine the presence of pathogenic bacteria on plastics are usually based on the ability to detect specific taxa that are selected *a priori*, typically via agar-based isolation (e.g. (Kirstein et al., 2016)). A variety of bacteria associated with human infection such as *Vibrio* spp. and *E. coli* have been detected on plastic debris in the open ocean this way (Bowley et al., 2020), and there is increased interest in whether plastic surfaces are preferentially colonised by bacterial pathogens (Meng et al., 2021; Oberbeckmann and Labrenz, 2020). However, the virulence of plastic-associated isolates, or plastic biofilm communities more generally, remains mostly undetermined. To our knowledge, we are the first to experimentally measure the virulence of bacteria present in plastic biofilms (Fig. 6). In the colonisation experiment, biofilms with the highest bacterial density in week 5 resulted in much greater levels of *Galleria* death (65% mortality)

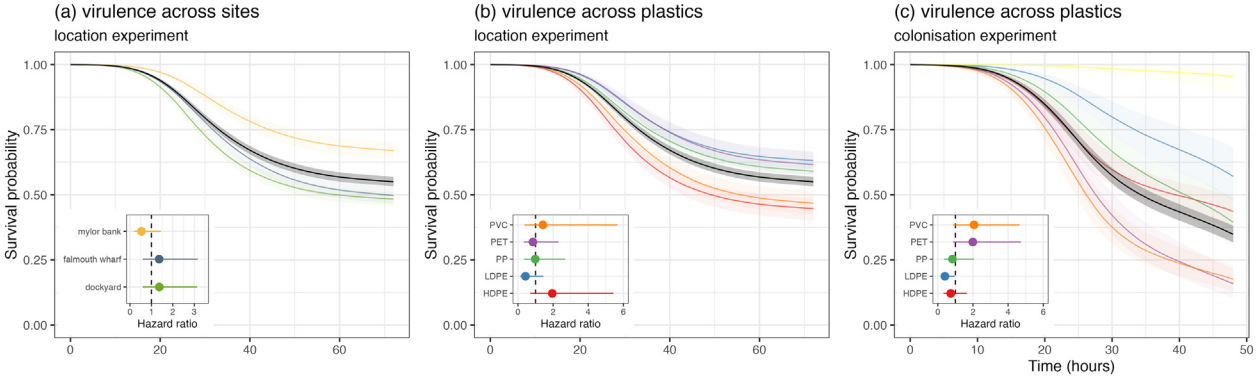

Fig. 6. Survival curves of *G. mellonella* inoculated with plastic biofilm communities from (a, b) the location experiment and (c) the colonisation experiment. Across all panels, the black line and shaded region depicts the median virulence of a biofilm community from that experiment, and the median log hazard was used as the baseline for calculating hazard ratios. (a) Effect of location on virulence of biofilm communities. (b) Effect of polymer type on virulence of biofilm communities from the location experiment. (c) Effect of polymer type on virulence of biofilm communities from the colonisation experiment. Lines represent the median prediction and shaded bands represent 95% credible intervals of those predictions. Inset plots are the hazard ratio where the median hazard of the experiment is the baseline. In (c) bright yellow is the survival curve for *G. mellonella* injected with water.

Table 1
Taxonomic assignment and genome characteristics of 20 sequenced genomes isolated from plastics using the *Galleria* enrichment method.

Isolate	Plastic	Site	Contig taxonomic assignment	16S taxonomic assignment	Completeness	Contamination	GC	Genome size (bp)	Number of contigs	N50	Number of AMR genes
1	HDPE	falmouth wharf	*Serratia liquefaciens*	*Serratia*	100.00	0.89	0.55	5,549,824	78	327,206	2
2	HDPE	falmouth wharf	*Serratia*	*Raoultella/Enterobacter/Serratia*	99.86	0.15	0.59	5,222,268	41	2,939,895	6
3	HDPE	falmouth wharf	*Bacillus licheniformis*	*Bacillus licheniformis*	98.82	0.00	0.46	4,254,100	20	2,234,065	1
4	HDPE	mylor bank	*Serratia*	*Serratia marcescens*	99.86	0.15	0.59	5,218,884	32	2,874,999	6
5	HDPE	dockyard	*Serratia liquefaciens*	*Serratia liquefaciens*	100.00	0.45	0.55	5,413,752	61	318,399	2
6	HDPE	dockyard	*Bacillus licheniformis*	*Bacillus licheniformis*	98.86	0.00	0.46	4,256,127	38	382,110	1
7	HDPE	dockyard	*Enterobacter/Enterobacteriaceae*	*Enterobacter/Cedecea*	98.78	0.60	0.56	5,014,388	240	160,006	3
8	LDPE	dockyard	*Bacillus/Enterobacter*	*Bacillus*	99.45	8.63	0.47	4,977,695	35	1,096,327	1
9	LDPE	dockyard	*Enterobacter/Enterobacteriaceae*	*Enterobacter/Cedecea*	98.78	0.60	0.56	5,014,653	245	160,006	3
10	LDPE	dockyard	*Serratia*	*Serratia marcescens*	99.86	0.15	0.59	5,218,582	30	2,874,999	6
11	LDPE	dockyard	*Serratia fonticola*	*Serratia*	99.94	0.29	0.54	5,756,538	113	185,130	1
12	LDPE	falmouth wharf	*Enterococcus faecalis*	*Enterococcus faecalis*	99.63	0.00	0.37	2,864,632	53	175,637	2
13	PET	dockyard	*Serratia*	*Serratia marcescens*	99.86	0.15	0.59	5,218,760	30	2,874,999	6
14	PP	dockyard	*Serratia*	*Serratia marcescens*	99.86	0.15	0.59	5,218,875	33	2,875,113	6
15	PP	dockyard	*Bacillus licheniformis*	*Bacillus licheniformis*	97.30	0.31	0.46	4,145,137	31	438,169	1
16	PP	dockyard	*Klebsiella/Serratia Enterobacter/Enterobacteriaceae*	*Enterobacter/Serratia*	97.93	188.43	0.57	15,957,883	573	182,752	10
17	PP	dockyard	*Serratia/Enterobacteriaceae*	–	99.66	95.89	0.56	11,471,120	188	336,395	7
18	PP	falmouth wharf	*Serratia*	*Serratia marcescens*	99.86	0.15	0.59	5,219,474	35	2,875,113	6
19	PVC	dockyard	*Serratia liquefaciens*	*Serratia liquefaciens*	100.00	0.45	0.55	5,571,931	37	398,855	2
20	PVC	falmouth wharf	*Serratia liquefaciens*	*Serratia*	100.00	0.45	0.56	5,131,311	33	531,913	2

compared to preceding timepoints (week 1: 4% mortality, week 2: 11% mortality, week 3: 7% mortality). Similarly, in the location experiment, mortality was lowest where bacterial density was lowest (32% mortality at Mylor bank). Together, these results indicate that total bacterial density is an important determinant of the virulence of plastic-associated biofilms. Although we detected high abundances of *E. coli* and coliforms using our plating-based assays (Figs. 2 and 3), none were found among the sequenced isolates after *Galleria* enrichment. It is possible that most *E. coli* encountered on marine plastics are relatively harmless, or that they form only a minor portion of pathogens present. Our relatively small sample size limits the power to differentiate between these alternatives, but raises a pertinent question to address in future studies.

In addition to measuring the virulence of whole biofilm communities, we selectively enriched pathogenic bacteria using the *Galleria* virulence model. Bacterial clones isolated included known human pathogens such as *Enterococcus faecalis* (Table 1). This species is known to be present in marine environments (Ferguson et al., 2005) and has been shown to successfully colonise LDPE in lab experiments (Hchaichi et al., 2020), but to our knowledge this is the first time that this important nosocomial pathogen has been isolated from marine plastics. The genomes we recovered from the most virulent plastic biofilm isolates harboured a wide variety of antimicrobial resistance genes, including a diversity of multi-drug efflux pumps and beta-lactams (Table 2). Thus, our findings highlight the potential importance of marine plastics as a reservoir for resistance genes, and is consistent with previous work suggesting that the plastisphere resistome differs from that of the surrounding water, and that it changes as a function of colonisation time (de Araújo et al., 2021; Yang et al., 2020).

Our cultivation methods were intentionally biased towards potential human pathogens able to grow at 37 °C. As expected, bacterial colonisation was more pronounced at the two locations closer to the shore and human habitation (Falmouth Docks and Falmouth Wharf), and are therefore likely to be associated with higher point and non-point source pollution. We suggest that bacterial plastic colonisation, including by pathogens, might be even more rapid in summer months when higher water temperatures favour bacterial growth and persistence (Garneau et al., 2008), although reduced rainfall during summer may result in lower input of terrestrial bacteria into the sea.

Bacterial colonisation of plastics occurred rapidly, with bacterial diversity plateauing after just two weeks (Fig. 4). In the colonisation experiment, bacterial density increased with time and was highest at our final timepoint of five weeks (Fig. 3). We found no effect of polymer type on community composition (Fig. 5), but we did see significant changes in composition through time. By aggregating ASVs at different levels of taxonomic rank, we were able to determine which organisms dominated our plastic biofilms. In common with previous studies (De Tender et al., 2015; Jiang et al., 2018; McCormick et al., 2014; Miao et al., 2019; Zettler et al., 2013), the biofilm communities were dominated by bacteria belonging to the *Alphaproteobacteria*, *Bacteroidia* and *Gammaproteobacteria* classes (Fig. S2). Moreover, as previously found in studies on the succession of plastic biofilm communities, we found that bacteria belonging to the orders *Rhodobacterales* and *Flavobacteriales* were present in high abundance (Fig. S3) (Vaksmaa et al., 2021; Xu et al., 2019; Zhang et al., 2022).

Based on our results, we identified several avenues for future research. Previous work has shown that plastic biofilms differ between distant locations in Northern European waters (De Tender et al., 2015; Oberbeckmann et al., 2014) and our location experiment revealed differences in bacterial density within a relatively small (kilometre-scale) area of the Fal estuary. Studies using replicated transects covering increasing distances from areas of human habitation through time could shed more light on the effect of coastal proximity and seasonality on plastic colonisation, especially in relation to potential human pathogens. Our 16S sequencing was not sufficient to identify which bacteria present in the community are pathogenic (only 195 of 2595 ASVs were assigned at the species level using the dada2 method). Experiments employing more in-depth meta-genomic sequencing or amplicon sequencing using more specific marker genes are needed to disentangle abundance effects of specific opportunistic pathogens. This could also help tease apart whether a change in density or a change in identity of the pathogens present causes increased virulence of plastic biofilm communities after prolonged exposure to the marine environment.

5. Conclusions

Despite an increase in research efforts into the colonisation of marine plastics by bacteria, our understanding of this process remains incomplete, and no previous studies have experimentally measured the virulence of plastic biofilms. We found that different plastic types accumulate similar bacterial communities, including a range of known human pathogens, and that they harbour a diversity of antibiotic resistance genes, increasing the potential risk they pose to human health. In combination with anticipated continued release of plastics into the marine environment and their high residence time, human exposure to marine plastics, and their associated 0.09pt? > microbial communities, is likely to increase. These effects are likely to be exacerbated through the effects of climate change, with increased flooding leading to more effluent being released into the marine

Table 2
Antimicrobial resistance genes present in 20 sequenced genomes isolated from plastics using the *Galleria* enrichment method.

Isolate	AMR gene
1	Multidrug efflux RND transporter permease subunit SdeB
	Class C beta-lactamase
2	Multidrug efflux RND transporter permease subunit SdeY
	Multidrug efflux RND transporter permease subunit SdeB
	Class C beta-lactamase SRT-3
	Tetracycline efflux MFS transporter Tet(41)
	Multidrug efflux MFS transporter SmfY
	Aminoglycoside 6'-*N*-acetyltransferase
3	Class A beta-lactamase BlaP
4	Multidrug efflux MFS transporter SmfY
	Tetracycline efflux MFS transporter Tet(41)
	Class C beta-lactamase SRT-3
	Multidrug efflux RND transporter permease subunit SdeB
	Multidrug efflux RND transporter permease subunit SdeY
	Aminoglycoside 6'-*N*-acetyltransferase
5	Multidrug efflux RND transporter permease subunit SdeB
	Class C beta-lactamase
6	Class A beta-lactamase BlaP
7	Cephalosporin-hydrolyzing class C beta-lactamase MIR-16
	Multidrug efflux RND transporter permease subunit OqxB9
	Fosfomycin resistance glutathione transferase FosA
8	Class A beta-lactamase BlaP
9	Cephalosporin-hydrolyzing class C beta-lactamase MIR-16
	Multidrug efflux RND transporter permease subunit OqxB9
	Fosfomycin resistance glutathione transferase FosA
10	Multidrug efflux MFS transporter SmfY
	Tetracycline efflux MFS transporter Tet(41)
	Class C beta-lactamase SRT-3
	Multidrug efflux RND transporter permease subunit SdeB
	Multidrug efflux RND transporter permease subunit SdeY
	Aminoglycoside 6'-*N*-acetyltransferase
11	Class A beta-lactamase FONA-4
12	Trimethoprim-resistant dihydrofolate reductase DfrE
	ABC-F type ribosomal protection protein Lsa(A)
13	Multidrug efflux MFS transporter SmfY
	Tetracycline efflux MFS transporter Tet(41)
	Class C beta-lactamase SRT-3
	Multidrug efflux RND transporter permease subunit SdeB
	Multidrug efflux RND transporter permease subunit SdeY
	Aminoglycoside 6'-*N*-acetyltransferase
14	Multidrug efflux RND transporter permease subunit SdeY
	Multidrug efflux RND transporter permease subunit SdeB
	Class C beta-lactamase SRT-3
	Tetracycline efflux MFS transporter Tet(41)
	Multidrug efflux MFS transporter SmfY
	Aminoglycoside 6'-*N*-acetyltransferase
15	Class A beta-lactamase BlaP
16	Multidrug efflux RND transporter permease subunit SdeB
	Multidrug efflux RND transporter permease subunit SdeY
	Multidrug efflux RND transporter permease subunit OqxB
	Phosphoethanolamine–lipid A transferase MCR-10.1
	Multidrug efflux MFS transporter SmfY
	Tetracycline efflux MFS transporter Tet(41)
	Class C beta-lactamase SRT-3
	Class A extended-spectrum beta-lactamase OXY-6-2
	Cephalosporin-hydrolyzing class C beta-lactamase MIR-12
	Aminoglycoside 6'-*N*-acetyltransferase
17	Multidrug efflux MFS transporter SmfY
	Tetracycline efflux MFS transporter Tet(41)
	Class C beta-lactamase SRT-3
	Multidrug efflux RND transporter permease subunit SdeB
	Multidrug efflux RND transporter permease subunit SdeY
	Aminoglycoside 6'-*N*-acetyltransferase
	Class A beta-lactamase FONA-6
18	Multidrug efflux MFS transporter SmfY
	Tetracycline efflux MFS transporter Tet(41)
	Class C beta-lactamase SRT-3
	Multidrug efflux RND transporter permease subunit SdeB
	Multidrug efflux RND transporter permease subunit SdeY
	Aminoglycoside 6'-*N*-acetyltransferase
19	Class C beta-lactamase
	Multidrug efflux RND transporter permease subunit SdeB
20	Class C beta-lactamase
	Multidrug efflux RND transporter permease subunit SdeB

environment and rising temperatures promoting bacterial growth and persistence. Thus, we suggest that further research into the virulence and antibiotic resistance potential of plastic-associated microbial communities is greatly needed.

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CRediT authorship contribution statement

LL: Conceptualisation, Methodology, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Supervision. **DP**: Formal analysis, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualisation. **TD**: Conceptualisation, Methodology, Investigation, Writing - Review & Editing. **MJ**: Conceptualisation, Methodology, Investigation, Writing - Review & Editing. **SK**: Investigation, Writing - Review & Editing. **AH**: Conceptualisation, Methodology, Writing - Review & Editing **MV**: Conceptualisation, Methodology, Writing - Original Draft, Writing - Review & Editing, Supervision.

Data availability

Scripts and final datasets are available on github (https://github.com/padpadpadpad/Lear_et_al_2022_stoten) and raw sequencing files (for the amplicon 16S) and genome assemblies (for the WGS) have been uploaded to the European Nucleotide Archive (Study accession number PRJEB5334).

Declaration of competing interest

All authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.156199.

References

Amaral-Zettler, L.A., Zettler, E.R., Slikas, B., Boyd, G.D., Melvin, D.W., Morrall, C.E., et al., 2015. The biogeography of the Plastisphere: implications for policy. Front. Ecol. Environ. 13, 541–546.
Amaral-Zettler, L.A., Zettler, E.R., Mincer, T.J., 2020. Ecology of the plastisphere. Nat. Rev. Microbiol. 18, 139–151.
Arias-Andres, M., Klümper, U., Rojas-Jimenez, K., Grossart, H.-P., 2018. Microplastic pollution increases gene exchange in aquatic ecosystems. Environ. Pollut. 237, 253–261.
Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B Methodol. 57, 289–300.
Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120.
Bowley, J., Baker-Austin, C., Porter, A., Hartnell, R., Lewis, C., 2020. Oceanic hitchhikers–assessing pathogen risks from marine microplastic. Trends Microbiol. 29 (2), 107–116.
Brennan, M., Thomas, D.Y., Whiteway, M., Kavanagh, K., 2002. Correlation between virulence of Candida albicans mutants in mice and Galleria mellonella larvae. FEMS Immunol.Med.Microbiol. 34, 153–157.
Brilleman, S.L., Elci, E.M., Novik, J.B., Wolfe, R., 2020. Bayesian Survival Analysis Using the rstanarm R Package arXiv preprint arXiv:2002.09633.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016a. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016b. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583.

Chaumeil, P.-A., Mussig, A.J., Hugenholtz, P., Parks, D.H., 2020. GTDB-Tk: A Toolkit to Classify Genomes With the Genome Taxonomy Database. Oxford University Press.

de Araújo, F.V., Castro, R.O., da Silva, M.L., Silva, M.M., 2021. Ecotoxicological effects of microplastics and associated pollutants. Aquaculture Toxicology. Elsevier, pp. 189–227.

De Tender, C.A., Devriese, L.I., Haegeman, A., Maes, S., Ruttink, T., Dawyndt, P., 2015. Bacterial community profiling of plastic litter in the Belgian part of the North Sea. Environ. Sci.Technol. 49, 9629–9638.

Duncan, E.M., Botterell, Z.L., Broderick, A.C., Galloway, T.S., Lindeque, P.K., Nuno, A., et al., 2017. A global review of marine turtle entanglement in anthropogenic debris: a baseline for further action. Endanger. Species Res. 34, 431–448.

Elsner, H.-A., Sobottka, I., Mack, D., Laufs, R., Claussen, M., Wirth, R., 2000. Virulence factors of Enterococcus faecalis and Enterococcus faecium blood culture isolates. Eur. J. Clin. Microbiol. Infect. Dis. 19, 39–42.

Feldgarden, M., Brover, V., Gonzalez-Escalona, N., Frye, J.G., Haendiges, J., Haft, D.H., et al., 2021. AMRFinderPlus and the Reference Gene Catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. Sci. Rep. 11, 1–9.

Ferguson, D., Moore, D., Getrich, M., Zhowandai, M., 2005. Enumeration and speciation of enterococci found in marine and intertidal sediments and coastal water in southern California. J. Appl. Microbiol. 99, 598–608.

Ferraresso, J., Lawton, B., Bayliss, S., Sheppard, S., Cardazzo, B., Gaze, W., et al., 2020. Determining the prevalence, identity and possible origin of bacterial pathogens in soil. Environ. Microbiol. 22, 5327–5340.

Fred-Ahmadu, O.H., Bhagwat, G., Oluyoye, I., Benson, N.U., Ayejuyo, O.O., Palanisami, T., 2020. Interaction of chemical contaminants with microplastics: principles and perspectives. Sci. Total Environ. 706, 135978.

Frère, L., Maignien, L., Chalopin, M., Huvet, A., Rinnert, E., Morrison, H., et al., 2018. Microplastic bacterial communities in the Bay of Brest: influence of polymer type and size. Environ. Pollut. 242, 614–625.

Garneau, M.È., Roy, S., Lovejoy, C., Gratton, Y., Vincent, W.F., 2008. Seasonal dynamics of bacterial biomass and production in a coastal arctic ecosystem: Franklin Bay, western Canadian Arctic. J.Geophys.Res.Oceans 113.

Germanov, E.S., Marshall, A.D., Bejder, L., Fossi, M.C., Loneragan, N.R., 2018. Microplastics: no small problem for filter-feeding megafauna. Trends Ecol. Evol. 33, 227–232.

Geyer, R., 2020. Production, use, and fate of synthetic polymers. Plastic Waste And Recycling. Elsevier, pp. 13–32.

Hansen, J., Melchiorsen, J., Ciacotich, N., Gram, L., Sonnenschein, E.C., 2021. Effect of polymer type on the colonization of plastic pellets by marine bacteria. FEMS Microbiol. Lett. 368, fnab026.

Harrison, J.P., Schratzberger, M., Sapp, M., Osborn, A.M., 2014. Rapid bacterial colonization of low-density polyethylene microplastics in coastal sediment microcosms. BMC Microbiol. 14, 1–15.

Haydushka, I.A., Markova, N., Kirina, V., Atanassova, M., 2012. Recurrent sepsis due to Bacillus licheniformis. J. Global Infect. Dis. 4, 82.

Hchaichi, I., Bandini, F., Spini, G., Banni, M., Cocconcelli, P.S., Puglisi, E., 2020. Enterococcus faecalis and Vibrio harveyi colonize low-density polyethylene and biodegradable plastics under marine conditions. FEMS Microbiol. Lett. 367, fnaa125.

Hernandez, R.J., Hesse, E., Dowling, A.J., Coyle, N.M., Feil, E.J., Gaze, W.H., et al., 2019. Using the wax moth larva Galleria mellonella infection model to detect emerging bacterial pathogens. PeerJ 7 e6150-e6150.

Hohn, S., Acevedo-Trejos, E., Abrams, J.F., de Moura, J.F., Spranz, R., Merico, A., 2020. The long-term legacy of plastic mass production. Sci. Total Environ. 746, 141115.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., et al., 2015. Plastic waste inputs from land into the ocean. Science 347, 768–771.

Jiang, P., Zhao, S., Zhu, L., Li, D., 2018. Microplastic-associated bacterial assemblages in the intertidal zone of the Yangtze Estuary. Sci. Total Environ. 624, 48–54.

Kay, M., 2019. tidybayes: Tidy Data And geoms for Bayesian Models. R Package Version. 1.

Keswani, A., Oliver, D.M., Gutierrez, T., Quilliam, R.S., 2016. Microbial hitchhikers on marine plastic debris: human exposure risks at bathing waters and beach environments. Mar. Environ. Res. 118, 10–19.

Kesy, K., Oberbeckmann, S., Kreikemeyer, B., Labrenz, M., 2019. Spatial environmental heterogeneity determines young biofilm assemblages on microplastics in Baltic Sea mesocosms. Front. Microbiol. 10, 1665.

Kirstein, I.V., Kirmizi, S., Wichels, A., Garin-Fernandez, A., Erler, R., Martin, L., et al., 2016. Dangerous hitchhikers? Evidence for potentially pathogenic Vibrio spp. on microplastic particles. Mar. Environ. Res. 120, 1–8.

Lamb, J.B., Willis, B.L., Fiorenza, E.A., Couch, C.S., Howard, R., Rader, D.N., et al., 2018. Plastic waste associated with disease on coral reefs. Science 359, 460–462.

Lehner, R., Weder, C., Petri-Fink, A., Rothen-Rutishauser, B., 2019. Emergence of nanoplastic in the environment and possible impact on human health. Environ.Sci.Technol. 53, 1748–1765.

Lenth, R., Singmann, H., Love, J., Buerkner, P., Herve, M., 2018. Emmeans: Estimated Marginal Means, aka Least-squares Means. R Package Version. 1, p. 3.

Leonard, A.F.C., Zhang, L., Balfour, A.J., Garside, R., Hawkey, P.M., Murray, A.K., et al., 2018. Exposure to and colonisation by antibiotic-resistant E. coli in UK coastal water users: environmental surveillance, exposure assessment, and epidemiological study (Beach Bum Survey). Environ. Int. 114, 326–333.

Lobelle, D., Cunliffe, M., 2011. Early microbial biofilm formation on marine plastic debris. Mar. Pollut. Bull. 62, 197–200.

Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71, 8228–8235.

Mahlen, S.D., 2011. Serratia infections: from military experiments to current practice. Clin. Microbiol. Rev. 24, 755–791.

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnetJ. 17, 10–12.

McCormick, A., Hoellein, T.J., Mason, S.A., Schluep, J., Kelly, J.J., 2014. Microplastic is an abundant and distinct microbial habitat in an urban river. Environ.Sci.Technol. 48, 11863–11871.

McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PloS one 8, e61217.

Meng, J., Zhang, Q., Zheng, Y., He, G., Shi, H., 2021. Plastic waste as the potential carriers of pathogens. Curr. Opin. Food Sci. 41, 224–230.

Miao, L., Wang, P., Hou, J., Yao, Y., Liu, Z., Liu, S., et al., 2019. Distinct community structure and microbial functions of biofilms colonizing microplastics. Sci. Total Environ. 650, 2395–2402.

Muthukrishnan, T., Al Khaburi, M., Abed, R.M., 2019. Fouling microbial communities on plastics compared with wood and steel: are they substrate-or location-specific? Microb. Ecol. 78, 361–374.

Oberbeckmann, S., Labrenz, M., 2020. Marine microbial assemblages on microplastics: diversity, adaptation, and role in degradation. Annu. Rev. Mar. Sci. 12, 209–232.

Oberbeckmann, S., Loeder, M.G., Gerdts, G., Osborn, A.M., 2014. Spatial and seasonal variation in diversity and structure of microbial biofilms on marine plastics in Northern European waters. FEMS Microbiol. Ecol. 90, 478–492.

Oberbeckmann, S., Kreikemeyer, B., Labrenz, M., 2018. Environmental factors support the formation of specific bacterial assemblages on microplastics. Front. Microbiol. 8, 2709.

Ogonowski, M., Motiei, A., Ininbergs, K., Hell, E., Gerdes, Z., Udekwu, K.I., et al., 2018. Evidence for selective bacterial community structuring on microplastics. Environ. Microbiol. 20, 2796–2808.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R., et al., 2013. Package 'vegan'. Community Ecology Package, Version. 2, pp. 1–295.

Parks, D.H., Imelfort, M., Skennerton, C.T., Hugenholtz, P., Tyson, G.W., 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. 25, 1043–1055.

Parthasarathy, A., Tyler, A.C., Hoffman, M.J., Savka, M.A., Hudson, A.O., 2019. Is Plastic Pollution in Aquatic And Terrestrial Environments a Driver for the Transmission of Pathogens And the Evolution of Antibiotic Resistance? ACS Publications

Pielou, E.C., 1966. Species-diversity and pattern-diversity in the study of ecological succession. J. Theor. Biol. 10, 370–383.

PlasticsEurope, 2021. Plastics - The Facts 2021: An Analysis of European Plastics Production, Demand And Waste Data.

Potocka, M., Bayer, R.C., Potocki, M., 2019. Plastic pollution affects American lobsters, Homarus americanus. Mar. Pollut. Bull. 138, 545–548.

Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2–approximately maximum-likelihood trees for large alignments. PloS one 5, e9490.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596.

Radisic, V., Nimje, P.S., Bienfait, A.M., Marathe, N.P., 2020. Marine plastics from Norwegian west coast carry potentially virulent fish pathogens and opportunistic human pathogens harboring new variants of antibiotic resistance genes. Microorganisms 8, 1200.

Rasool, F.N., Saavedra, M.A., Pamba, S., Perold, V., Mmochi, A.J., Maalim, M., et al., 2021. Isolation and characterization of human pathogenic multidrug resistant bacteria associated with plastic litter collected in Zanzibar. J. Hazard. Mater. 405, 124591.

Ritchie, H., Roser, M., 2018. Plastic pollution. Our World in Data.

Rodrigues, A., Oliver, D.M., McCarron, A., Quilliam, R.S., 2019. Colonisation of plastic pellets (nurdles) by E. coli at public bathing beaches. Mar. Pollut. Bull. 139, 376–380.

Seemann, T., 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30, 2068–2069.

Silva, M.M., Maldonado, G.C., Castro, R.O., de Sá, Felizardo J., Cardoso, R.P., Dos Anjos, R.M., et al., 2019. Dispersal of potentially pathogenic bacteria by plastic debris in Guanabara Bay, RJ,Brazil. Mar. Pollut. Bull. 141, 561–568.

Solomando, A., Pujol, F., Sureda, A., Pinya, S., 2022. Ingestion and characterization of plastic debris by loggerhead sea turtle, Caretta caretta, in the Balearic Islands. Sci. Total Environ. 826, 154159.

Steinegger, M., Söding, J., 2017. MMseqs2 enables sensitive protein sequence searching for the analysis of massive data sets. Nat. Biotechnol. 35, 1026–1028.

Team RC, 2013. R: A Language And Environment for Statistical Computing.

Teuten, E.L., Rowland, S.J., Galloway, T.S., Thompson, R.C., 2007. Potential for plastics to transport hydrophobic contaminants. Environ.Sci.Technol. 41, 7759–7764.

Vaksmaa, A., Knittel, K., Abdala Asbun, A., Goudriaan, M., Ellrott, A., Witte, H.J., et al., 2021. Microbial communities on plastic polymers in the Mediterranean Sea. Front. Microbiol. 12, 1021.

Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. Environ. Pollut. 193, 65–70.

Viršek, M.K., Lovšin, M.N., Koren, Š., Kržan, A., Peterlin, M., 2017. Microplastics as a vector for the transport of the bacterial fish pathogen species Aeromonas salmonicida. Mar. Pollut. Bull. 125, 301–309.

Vos, M., 2020. The evolution of bacterial pathogens in the Anthropocene. Infect. Genet. Evol. 86, 104611.

Wand, M.E., Müller, C.M., Titball, R.W., Michell, S.L., 2011. Macrophage and Galleria mellonella infection models reflect the virulence of naturally occurring isolates of B. pseudomallei, B. thailandensis and B. oklahomensis. BMC Microbiol. 11, 1–11.

Waring, R.H., Harris, R.M., Mitchell, S.C., 2018. Plastic contamination of the food chain: a threat to human health? Maturitas 115, 64–68.

Wen, B., Liu, J.-H., Zhang, Y., Zhang, H.-R., Gao, J.-Z., Chen, Z.-Z., 2020. Community structure and functional diversity of the plastisphere in aquaculture waters: does plastic color matter? Sci. Total Environ. 740, 140082.
Wick, R.R., Schultz, M.B., Zobel, J., Holt, K.E., 2015. Bandage: interactive visualization of de novo genome assemblies. Bioinformatics 31, 3350–3352.
Wick, R.R., Judd, L.M., Gorrie, C.L., Holt, K.E., 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput. Biol. 13, e1005595.
Wickham, H., Wickham, M.H., 2007. The ggplot Package. Google Scholar.
Wu, X., Pan, J., Li, M., Li, Y., Bartlam, M., Wang, Y., 2019. Selective enrichment of bacterial pathogens by microplastic biofilm. Water Res. 165, 114979.
Wu, N., Zhang, Y., Zhao, Z., He, J., Li, W., Li, J., et al., 2020. Colonization characteristics of bacterial communities on microplastics compared with ambient environments (water and sediment) in Haihe Estuary. Sci. Total Environ. 708, 134876.
Wyles, K.J., Pahl, S., Thomas, K., Thompson, R.C., 2016. Factors that can undermine the psychological benefits of coastal environments: exploring the effect of tidal state, presence, and type of litter. Environ. Behav. 48, 1095–1126.
Xu, X., Wang, S., Gao, F., Li, J., Zheng, L., Sun, C., et al., 2019. Marine microplastic-associated bacterial community succession in response to geography, exposure time, and plastic type in China's coastal seawaters. Mar. Pollut. Bull. 145, 278–286.
Yang, K., Chen, Q.-L., Chen, M.-L., Li, H.-Z., Liao, H., Pu, Q., et al., 2020. Temporal dynamics of antibiotic resistome in the plastisphere during microbial colonization. Environ.Sci. Technol. 54, 11322–11332.
Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the “plastisphere”: microbial communities on plastic marine debris. Environ.Sci.Technol. 47, 7137–7146.
Zhang, S.-J., Zeng, Y.-H., Zhu, J.-M., Cai, Z.-H., Zhou, J., 2022. The structure and assembly mechanisms of plastisphere microbial community in natural marine environment. J. Hazard. Mater. 421, 126780.