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Identifying macroplastic pathobiomes and antibiotic resistance in a subtropical fish farm

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Abstract :

Macroplastics are ubiquitous in aquaculture ecosystems. However, to date the potential role of plastics as a support for bacterial biofilm that can include potential human pathogenic bacteria (PHPB) and antibiotic-resistant bacteria (ARB) has been largely overlooked. In this study, we used a combination of metabarcoding and standard antibiotic susceptibility testing to study the pathobiome and resistome of macroplastics, fish guts and the environment in a marine aquaculture farm in Mauritius. Aquaculture macroplastics were found to be higher in PHPB, dominated by the Vibrionaceae family (0.34 % of the total community), compared with environmental samples. Moreover, isolates from aquaculture plastics showed higher significant multiple antibiotic resistance (MAR) compared to non-plastic samples of seawater, sediment and fish guts. These results suggest that plastics act as a reservoir and fomite of PHPB and ARB in aquaculture, potentially threatening the health of farmed fish and human consumers.

Highlights

► The plastic bacteriome harbors more potential pathogens than environmental communities. ► Aquaculture plastic bacteria show a higher MAR index than non-plastic bacteria. ► Plastic can act as a reservoir for pathogenic and antibiotic-resistant bacteria. ► The fish and macroplastic bacteriome share certain pathogens. ► A nearby river can increase the risk of pathogen introduction in a fish farm.

Keywords : Plastisphere, Aquaculture, Pathogen, Antibiotic, resistance

Introduction

Plastic pollution is a worldwide scourge that particularly affects marine environments, where debris is ubiquitous (Chiba et al. 2018; Galgani et al. 2020). It is estimated that more than one million tons of plastic waste enter oceans just from rivers each year (Lebreton et al. 2017). This debris can persist for centuries in the marine environment. Floating plastics in marine environments have different sizes: large debris (macroplastics) and smaller particles, which are further classified into mesoplastics (< 20 mm), microplastics (< 5 mm) and nanoplastics (< 1 mm) (Barnes et al. 2009; Hanke et al. 2013; Provencher et al. 2017). The Indian Ocean is particularly affected by the accumulation of plastic due to an oceanic gyre that concentrates debris (van der Mheen et al. 2019; Chenillat et al. 2021).

Plastic pervades aquaculture settings. In open water aquaculture, free-floating plastic debris from the ocean can enter the cages, and the aquaculture infrastructure itself is mainly composed of plastic (e.g. buoys, pipes, nylon nets). Over time, this plastic tends to degrade under the action of ultraviolet radiation, waves and wind, releasing small particles into seawater (Song et al. 2017). These particles can subsequently be ingested by marine organisms, including reared animals (reviewed in Barboza et al. 2018; Walkinshaw et al. 2020; Bowley et al. 2021; Chen et al. 2021). A number of consequences of plastic ingestion by aquatic animals have been described: for instance, obstruction, intoxication, or physiological and behavior modifications, all of which can lead to death (Oliveira et al. 2013; Kershaw 2015; Law 2017; Yin et al. 2018; de Sá et al. 2018).

In addition to their direct mechanical action, plastics can also adsorb molecules and serve as potential sources of chemical contaminants of their own composition (Andrady et al. 2009) or from the environment (Mato et al. 2001; Rios et al. 2010). Additionally, the formation and accumulation of microbial biofilms on plastic polymer surfaces, facilitated through various physical and chemical interactions, including van der Waals forces, electrostatic interactions, and hydrophobic interactions, can contribute to the persistence and spread of harmful animal pathogens (Oberbeckmann et al. 2016; Frère et al. 2018; Martínez-Campos et al. 2022) and human pathogens (Zettler et al. 2013; Delacuvellerie et al. 2022; Lear et al. 2022; Liang et al. 2023). For example, the accumulation of pathogenic *Vibrio* species on marine plastic debris has

been described in several studies (Sun et al. 2020, Silva et al. 2019; Laverty et al. 2020; Bhagwat et al. 2021, Pedrotti et al. 2022). Furthermore, biofilms developing on plastics are known to provide a favorable environment for the development of antibiotic resistance (reviewed in Dong et al. 2021) due to slow antibiotic penetration, accelerated horizontal transfer, or antibiotic antagonists (Stewart et al. 2001; Mah 2012; Lebeaux et al. 2012; Uruén et al. 2021). This situation is of particular concern in aquaculture systems, where, driven by economics, antibiotic use is widespread in order to prevent and control bacterial diseases in farmed species. Misuse and overuse of antibiotics in aquaculture settings can lead to the emergence and spread of antibiotic-resistant bacteria (Haya et al. 2001; Boxall et al. 2004), with significant consequences for public health and the environment. Yet the pathogenic risk posed by plastics as fomites of pathogenic bacteria and carriers of antibiotic resistance genes has received little attention to date, and even less in the context of food security.

Assessing the extent of macroplastic contamination by pathogens and associated antibiotic resistance in aquaculture is crucial to identify a potential decline in food production due to bacterial infections as well as to minimize the health risk to consumers. The objective of this study was to characterize the pathobiome of macroplastics used in fish farm infrastructure ("aquaculture plastics") and its antibiotic resistance using metabarcoding and bacterial isolates. More specifically, our first aim was to explore the relative abundance and diversity of macroplastic-associated bacterial communities in aquaculture and to determine whether they differ from those in the surrounding water and sediment, in floating macroplastics, and in aquaculture fish guts. The second aim was to focus specifically on potential pathogens and their antibiotic resistance genes. The third aim was to investigate the influence of the proximity of a river on pathogen diversity and antimicrobial resistance in the marine environment around the farm. Finally, we compared the plastic and fish pathobiomes to determine to what extent these are shared in order to evaluate the role of aquaculture plastics as fomites in fish farms.

Materials and methods

Sample collection

The sampling for this experiment took place in November 2021 in the vicinity of the Mahébourg fish farm in Mauritius, which rears red drum (*Sciaenops ocellatus*) in open water cages. Four sites were sampled: site 1 (20°18'49.4"S, 57°47'03.8"E) and site 2 (20°21'27.1"S, 57°46'54.8"E) were two fish cages in different locations, site 3 (20°17'13.18" S, 57°46'42.49" E) was the estuary of Grand River South East that empties near the farm, and site 4 (20°19'20.36" S, 57°48'57.55" E) was the northern channel of the Mauritian lagoon (Suppl. Fig. 1).

Bacterial communities from water and sediment were collected in triplicate at each sampling site. Sediment was collected in dives at each site, kept in sterile Falcon tubes and stored at 0°C in a portable icebox until arrival at the laboratory, and then stored at -80°C until DNA extraction. Water samples were collected at 1 m below the surface using 500 mL plastic bottles and stored at 4°C in a portable icebox until arrival at the laboratory. Planktonic microbes were collected on 0.2 µm GTTP filters (Whattman®), and the membranes were then placed in sterile cryotubes at -80°C until further analysis. Metadata such as the temperature, salinity and conductivity of the water at the sampling sites were measured and gathered (Suppl. Table 1). Nutrient analyses of the water showed equivalent nitrate and phosphate concentrations in the four sampling sites. Nitrites and ammoniac concentration were under the detection threshold (data not shown).

"Floating plastics" (free-floating macroplastic waste or channel buoys) were collected using sterile forceps to avoid contamination when found on the four sites (Table 1). In addition, at the two farm sites, "aquaculture plastics" (plastic parts of the fish cages such as ties, buoys, nets and pipes) were sampled in five replicates (Suppl. Fig. 2). Each macroplastic was rinsed with MilliQ water to remove non-attached microorganisms. A swab (SK-2S swabs, Isohelix, UK) was then used to collect biofilm from the plastic surface (4 cm^2). Swabs were stored in sterile microtubes at -80°C until DNA extraction.

Fish (n = 8 per site) were collected using nets in the cages (site 1 and 2) by experienced farm staff. Only adult individuals (standard length: 57 ± 18 cm) were collected in order to avoid bacteriome variability due to different ontogenetic stages. Fish were bought to farmers and conserved on ice in coolers for dissection. The content of the last third of the gut (i.e. hindgut)

was homogenized and collected to extract the gut bacteriome (Mouchet et al. 2012; Cheutin et al. 2021). The gut content was stored at -80°C in a 3 mL cryotube until DNA extraction.

Bacterial culture and phenotypic antimicrobial resistance (AMR) testing

Fish gut content, sediment and water samples were diluted at a ratio of 1/10 in sterile seawater. For plastic samples, swabs were immersed in 10 mL of sterile seawater and vortexed for 60 seconds to detach the biofilm from plastic debris. To specifically target PHPB, a 100 µL sample of these solutions was streaked onto Mueller Hinton agar, a non-selective growth medium, and incubated for 24h at 37°C in aerobic conditions, to simulate internal conditions of the human body. All colonies were then collected and inoculated in a tube culture medium (Bio-Rad, France). After 24h of incubation at 37°C with a non-hermetic seal, tubes were kept at room temperature until isolate analyses in the lab (~one week).

All samples were then seeded on five different solid culture media: non-selective (chocolate agar PolyViteX) and selective either for gram negative bacteria (MacConkey agar), gram positive bacteria (Columbia-CNA agar: blood + colistin + nalidixic acid), bacteria resistant to Beta-lactam (chromID® ESBL agar: extended spectrum Beta-lactamase) and bacteria resistant to carbapenems (chromID® CARBA SMART carbapenemase-selective agar). Samples were then incubated at 37°C for 24h in an atmosphere of 5% CO_2 to isolate anaerobic species on blood + CNA agar and chocolate PolyXiteX agar, and in aerobic conditions for the others. Each phenotypically different colony was then isolated and identified with a Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) Mass Spectrometer (Bruker Daltonics, Bremen, Germany) following the manufacturer's instruction for identification acceptance.

Isolated strains were tested against several antibiotics using the Kirby-Bauer disk diffusion method on Mueller Hinton agar. Since all our isolates grew well on Mueller Hinton agar, no NaCl was added to the agar for the antibiotic-resistance tests, even for marine bacteria such as *Vibrionaceae* (Rubin and Tilton 1975; Singleton et al. 1982; Jo et al. 2020). A panel of antibiotics among 36 antibiotic disks (i2a, Pérols, France) were tested for each strain (Suppl. Table 2), according to the European Committee on Guidelines for Antimicrobial Susceptibility Testing (EUCAST) (2021, version 1.0). Measurement of the inhibition zone diameters was performed on a "Sirscan automatic" zone reader (i2a, Pérols, France). The interpretation of the antibiogram results was performed according to EUCAST recommendations (Vong 2021).

The multiple antibiotic resistance (MAR) index is defined as the proportion of resistance to a panel of tested antibiotics. It was calculated for each strain or group of strains, following the method described by Krumperman (1983): the MAR index is equal to a/b, where "a" represents the number of antibiotics to which the strain was resistant, and "b" represents the number of antibiotics to which the strain was exposed.

DNA extraction and sequencing

Total genomic DNA from 200 mg of homogenized fish gut contents, GTTP filters and swabs (for plastic samples) was extracted using the MagAttract PowerSoil® DNA kit according to the manufacturer's instructions (Qiagen, Courtaboeuf, France) with automated processing and the liquid handling system KingFisher FlexTM (ThermoScientific®, Waltam, MA, USA). Nucleic acids were eluted in molecular water (Merck MilliporeTM, Burlington, MA, USA) and quantified on a NanoDrop 8000 TM spectrophotometer (ThermoScientific®, Wilmington, MA, USA). The DNA extracts were stored at -20°C until further analyses and PCR amplification.

The V4-V5 region of the 16S rDNA gene was targeted with the universal primers 515F-Y (5′-GTGYCAGCMGCCGCGGTAA-3′) and 926R (5′-CCGYCAATTYMTTTRAGTTT-3′) (Parada, Needham and Fuhrman 2016) coupled with platform-specific Illumina adaptor sequences on the 5′ ends. Each 50 µL PCR reaction was prepared with 25 µL Taq Polymerase Phusion® High-Fidelity PCR Master Mix with GC Buffer (New England Biolabs®, Inc., Ipswich, MA, USA), 1 µL forward primer (10 µM), 1 µL reverse primer (10 µM), 2 µL template DNA, 1.5 µL DMSO, and 19.5 µL molecular water. PCR amplifications involved the following protocol: an initial 98°C denaturing step for 30 s followed by 35 cycles of amplification (10 s denaturation at 98°C; 1 min at 60°C annealing; 1.5 min extension at 72°C), and a final extension of 10 min at 72°C. Amplification and primer specificity were verified by electrophoresis on a 2.0% agarose gel for confirmation of ~450 bp amplicon size. Extraction of blank samples used as DNA extraction controls and standard mock communities (ZymoBIOMICS Microbial Community DNA Standards II, Zymo Research) was performed to evaluate the quality of our sample-processing pipeline. Sequencing was performed on Illumina Miseq by GeT-Biopuces (INSA, Toulouse, France). Raw reads were deposited in the NCBI database under Bioproject number PRJNA895209.

Sequence processing

Raw sequencing data was analyzed in R (version 4.1.1), using the dada2 pipeline (Callahan et al. 2016). Briefly, sequences were first trimmed and filtered based on read-quality profiles (maxN = 0; maxEE = [2,2]; truncQ = 2; and truncLen = [250,250]). Amplicon sequence variants (ASVs) were inferred using the dada2 algorithm (Divisive Amplicon Denoising Algorithm) after pooling dereplicated reads from all samples. Then forward and reverse reads were merged and chimeric sequences were removed. The taxonomic classification of ASVs was performed with the naive Bayesian RDP classifier implemented in dada2 and using the SILVA reference database nr_V132. The ASV count table, taxonomy and sequences were organized in a phyloseq object using the phyloseq package (v.1.28.0, McMurdie et al. 2013) in R.

We used a combination of two methods to remove contaminants from our dataset. First, the R package decontam (Davis et al. 2018) was used to identify ASV contaminants from the dataset based on the "prevalence method" of the package. However, some known extraction kit contaminants listed by Salter et al. (2014), such as ASVs from the genera *Bradyrhizobium* and *Cupriavidus*, remained in our dataset. They were manually removed from the final dataset. Overall, 293 taxa corresponding to 8% of the total reads were removed.

In order to identify potential human pathogenic bacteria (PHPB) in our pathobiome dataset, all ASV sequences were blasted against a homemade full-length 16S rRNA gene database derived from the enhanced infectious disease database (EID2, Wardeh et al. 2015) containing bacterial species (i.e. cargos) described to have had interactions with the human species. Our database contained 87,405 full-length 16S rRNA from the 878 human bacterial cargos in the EID2 database. Only ASVs matching a 16S rRNA sequence with 100% similarity, 100% coverage and on more than 250 bp were included in our pathobiome dataset. The potential pathogenicity of each ASV was subsequently checked in the literature, and only ASVs matching a pathogen described at least once were kept. Of the 110 PHPB ASVs detected in our study, only 11 were discarded.

Sample read sums were randomly equalized at 11,900 reads per sample using the phyloseq package (McMurdie et al. 2013). Three samples were discarded following this process. After standardization, our final dataset consisted of 1,035,300 sequences belonging to 34,675 ASVs.

Statistics workflow

Taxonomic diversity of each microbial community was measured using richness (number of ASVs) and the Shannon diversity index H. Statistical analyses on alpha diversity comparisons

were carried out using a Kruskal-Wallis test followed by a Dunn post-hoc test (p-value corrected by Bonferroni's method). Beta diversity was assessed using Bray–Curtis distance with the vegan package in R (Oksanen et al. 2022), and statistical analyses were performed using permutational analyses of variance (PERMANOVA). Bray–Curtis dissimilarity was shown in a principal coordinate analysis (PCoA) plot.

ASV biomarkers (i.e. differential abundance of ASV between sample types) were identified by the analysis of bacteriome composition with bias correction (ANCOMBC; p-value corrected by Benjamini–Hochberg method) in the microbiomeMarker package (Cao et al. 2022).

Core bacteriomes were identified by examining species abundance distribution (SAD), patterns of each ASV, and by partitioning the SAD into core and satellite ASVs (Magurran et al. 2003). The index of dispersion for each ASV was calculated as the ratio of the variance to the mean abundance (VMR) multiplied by the occurrence. This index was used to model whether lineages follow a Poisson distribution (i.e. stochastic distribution), falling between the 2.5% and 97.5% confidence interval of the chi-squared ($\chi 2$) distribution. Index values lower or higher than 1 meant that the ASV was under- or overdispersed compared to the Poisson distribution, such that it spread uniformly and could be considered a core ASV. Index values close to 1 meant that the ASV followed a Poisson distribution and corresponded to a satellite ASV.

Results

Composition of the bacterial plastisphere

A total of 34,675 ASVs were retrieved from the 87 samples analyzed in this study. The analysis of these revealed differences in the composition and diversity of bacterial communities in aquaculture plastic, floating plastic, fish guts, and the environment (i.e. water and sediment). Aquaculture plastic (AP) and floating plastic (FP) bacterial communities had significantly lower richness and Shannon diversity values than sediment communities (Dunn post-hoc test, $p < 0.01$), and higher values than communities associated with the fish digestive tract, this was significantly higher in AP (Dunn post-hoc test, $p < 0.05$; Fig. 1A and 1B). The AP and FP communities were as rich and diverse as planktonic bacterial communities in the water. Sample type (i.e. water, sediment, fish guts, AP or FP) explained a higher proportion of the variance (PERMANOVA, $p < 0.001$, $R2 = 0.273$) in bacterial community composition than the sampling

site (PERMANOVA, $p < 0.001$, $R2 = 0.0281$). In particular, AP and FP bacterial composition turned out to be significantly different from the communities in fish guts or the environment (i.e. water and sediment) as shown in PERMANOVA pairwise comparisons ($p < 0.01$; Fig. 1C, Suppl. Table 3). Although it is not clear on the two first axes of the PCoA (Fig 1C), the variability of the plastisphere composition was also higher than the variability found in fish gut or water samples (betadisper, $p < 0.01$; Suppl. Fig. 3). A pairwise comparison between AP and FP bacterial communities also highlighted significant differences in their composition (pairwise PERMANOVA, $p < 0.01$; Suppl. Table 3).

These differences in bacteriome composition were related to high taxonomic rank differential abundance. *Proteobacteria* was the most represented phylum among all samples (38.5% of all reads), and was particularly abundant in plastic samples (48.7% for AP and 44.6% for FP) compared to fish gut, sediment and water samples (18.9%, 19.2%, 35.6%, respectively) (Fig. 1D). At the ASV level, differential abundance analysis using the ANCOM-BC approach highlighted 556 biomarkers from aquaculture plastics and 88 biomarkers from floating plastics. The composition of these markers was similar to the composition of the total communities from plastic samples, with a majority of *Proteobacteria*, *Cyanobacteria* and *Bacteroidota* (49%, 20% and 18% respectively from AP, and 45%, 30% and 16% respectively from FP; Suppl. Fig. 4A).

Plastics harbor an abundant and rich pathobiome

Of the ASV sequences retrieved in our samples compared with the sequences of the infectious disease database, 99 were identified as potential human pathogenic bacteria (PHPB), including 85 different species and 52 different genera (Suppl. Table 4). These PHPB belonged mainly to the families *Listeriaceae* and *Vibrionaceae* (47% and 25% of PHPB reads respectively). In total, PHPB reads represented 4% of all reads in the whole dataset. The percentage of PHPB reads varied according to the sample type. While PHPB reads accounted for less than 1% of reads associated with sediment, water and AP, they represented 2.1% of FP reads and 27% of reads associated with fish digestive tracts (Fig. 2A). We detected 48 ASVs that were part of the core pathobiome of our samples, with 6 species appearing in more than 50% of the samples (*Cutibacterium avidum*, *Photobacterium damselae*, *Staphylococcus haemolyticus*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus* and *Listeria innocua*; Fig. 2B). The potential infections caused by these species are summarized in Suppl. Table 5. Remarkably, in the core pathobiome, metabarcoding analyses also identified *Bacillus anthracis* in 8 samples from sediment and plastic (AP and even more so in FP).

279 The pathobiome associated with FP showed higher taxonomical richness than in AP or
280 environmental samples (Dunn post-hoc test, $p < 0.01$; Fig. 3A). Analyzing PHPB abundance
281 using the Shannon index revealed no significant differences in pathobiome diversity according
282 to sample type. In contrast, PERMANOVA pairwise comparisons showed significant
283 differences in pathobiome community composition according to sample type (Fig. 3C, Suppl.
284 Table 3). In a similar pattern to the whole bacteriome community, AP and FP pathobiome
285 composition differed significantly from fish gut and environmental communities (with an
286 exception between AP and sediment) as shown in PERMANOVA pairwise comparisons ($p <$
287 0.01; Fig. 3C, Suppl. Table 3).

288 The *Vibrionaceae* family represented a high proportion of PHPB in all sample types, accounting
289 for 57% in AP, 36% in FP, 49% in sediment, 60% in water, and 21% in fish gut. Similarly, the
290 *Listeriaceae* family was a ubiquitous PHPB family in all sample types with proportions of 18%
291 in AP, 4.2% in FP, 14% in sediment, 7.1% in water, and 54% in fish gut. *Staphylococcaceae*
292 represented 7.7% of PHPB reads in AP, 4.6% in FP, 6.7% in sediment, 0% in water, and 5.2%
293 in fish gut samples. The *Moraxellaceae* family was more represented in plastic samples, with
294 proportions of 3.8% in AP and 15% in FP, and 2.2% in sediment, 0.44% in water, and 2.5% in
295 fish samples.

296 The ANCOM-BC approach highlighted PHPB biomarkers in all sample types (Fig. 4B).
297 *Moraxella osloensis*, found on FP, was the only species identified as characteristic of plastic
298 substrates.

299 To investigate the potential transfer of PHPB bacteria between plastics and fish in the farm, we
300 analyzed the shared pathobiome (Fig. 4A). We found that 30 bacterial species (30% of PHPB
301 ASVs) were shared between fish and plastics, including the species with the highest occurrence
302 identified in the core pathobiome: *Listeria innocua, Photobacterium damselae, Vibrio*
303 *parahaemolyticus, Vibrio alginolyticus, Staphylococcus haemolyticus, Cutibacterium avidum,*
304 *Moraxella osloensis, Pantoea conspicua, Corynebacterium tuberculostearicum* (Fig. 2B).

305 Investigating the potential effect of sampling site on PHPB richness and the Shannon index, we
306 found significantly higher values for both indices in sampling site 3 (estuary of the Grand River
307 South East) than in aquaculture sampling sites (Dunn post-hoc test, $p < 0.05$; Suppl. Fig. 5A
308 and 5B). PERMANOVA results also showed significant differences in the pathobiome
309 composition of site 3 compared to the two aquaculture sites ($p < 0.01$; Suppl. Fig. 5C, Suppl.
310 Table 6).

311

312 Isolated strains and antibiotic resistance

313 A total of 72 bacterial strains from 27 different species were isolated from all samples, of which
314 48 were gram negative and 24 were gram positive (Suppl. Table 7). The PHPB strains belonged
315 to 19 different species and represented 83% of all isolated strains. Similar to the results from
316 the metabarcoding analyses, isolates were dominated by PHPB from the *Vibrionaceae* family
317 (47% of all isolated strains), and the same dominant species were identified: *Vibrio*
318 *alginolyticus*, *Photobacterium damselae* and, to a lesser extent, *Vibrio parahaemolyticus*. Three
319 PHPB strains from the *Staphylococcaceae* family were also found in isolates (4.2% of all
320 isolated strains), and two PHPB strains of *Moraxellaceae* were isolated (2.8% of all isolated
321 strains). However, no strains from the *Listeriaceae* family were isolated. Isolated strains from
322 FP samples showed greater diversity in bacterial families compared to other sample types, with
323 seven bacterial families isolated (*Aeromonaceae*, *Bacillaceae*, *Enterobacteriaceae*,
324 *Moraxellaceae*, *Nocardiaceae*, *Pseudomonaceae*, *Vibrionaceae*), while the other sample types
325 had only two or three families (Suppl. Fig. 6). The PHPB strains from the *Moraxellaceae* family
326 were found exclusively on plastic samples.

327 As in the metabarcoding analysis, we investigated the isolates shared between fish and
328 macroplastics (Fig. 4). Three species were found in both types of samples: *Vibrio alginolyticus*,
329 *Photobacterium damselae* and *Staphylococcus epidermidis*. In the metabarcoding results, the
330 two *Vibrionaceae* bacteria were also shared between fish and macroplastic samples, and
331 belonged to the core pathobiome, with occurrence of > 40% in all samples.

332 Antibiograms were carried out on all the strains (n = 72), and the MAR index was calculated.
333 We focused on the antibiotic resistance of *Vibrionaceae* strains (n = 34) as they were
334 preponderant and potentially all pathogenic. There was a high level of resistance to certain
335 antibiotics, notably members of the penicillin family. For instance, the tested strains presented
336 88% resistance to ampicillin (n = 33) and 73% to ticarcillin (n = 44; Suppl. Fig 7). However,
337 antibiotics known to be occasionally used in aquaculture (cyclins, quinolones and trimethoprim
338 + sulfonamides) efficiently inhibited the isolates: there was 3% global resistance to the tested
339 quinolones (norfloxacin, ciprofloxacin, ofloxacin and levofloxacin; n = 155) and all tested
340 strains were susceptible to trimethoprim + sulfonamides and to tetracycline (respectively n =
341 69 and n = 65; Suppl. Fig 7).

A MAR index higher than 0.2 is considered a marker for a high risk of antibiotic contamination (Krumperman 1983; Reverter et al. 2020). In our samples, the mean MAR value was 0.098 ± 0.080, and 88% of all isolated strains had MAR values below 0.2, indicating a low level of multiple antibiotic resistance. Nonetheless, bacterial communities on AP had a higher MAR index (mean for all strains: 0.15 ± 0.06; mean for *Vibrionaceae* strains: 0.17 ± 0.06), with significant differences between AP and FP (Fig 5) when considering the MAR index of all isolated strains (Dunn post-hoc test, $p < 0.01$), and significant differences between AP and non-plastic substrates for strains belonging to the *Vibrionaceae* family (Dunn post-hoc test, $p < 0.05$).

Concerning sampling sites, isolates from the estuary area (site 3) had a significantly lower MAR index than other sampling sites. No significant differences were found between the MAR indices of the two aquaculture sites and the lagoon channel (Suppl. Fig. 8).

Discussion

PHPB and antibiotic-resistant bacteria (ARB) represent a significant component of the aquaculture plastisphere

When immerged in aquatic environments, macroplastics act as unique colonization supports on which microbial biofilms quickly develop. Their composition (large carbon polymers) and surface characteristics (hydrophobic) select for specific microorganisms compared to those found in the surrounding environment (Zettler et al. 2013; Dussud et al. 2018; Delacuvellerie et al. 2019). These constitute what has been defined as the "plastisphere" (Zettler et al. 2013). Our results were no exception. The bacterial communities associated with aquaculture and floating macroplastics were different to communities found in the surrounding sediment and water, and in fish gut (Fig. 1C). We found a total of 556 ASV biomarkers from aquaculture macroplastics and 88 biomarkers from floating macroplastics (Suppl. Fig. 4A), mainly belonging to the *Proteobacteria*, *Cyanobacteria* and *Bacteroidia* phyla. Other studies have also found these phyla in high proportions on micro-, meso- or macroplastics (Zettler et al. 2013; Oberbeckmann et al. 2016; Dussud et al. 2018; Martínez-Campos et al. 2022).

The presence of potential pathogens and/or ARB on plastic substrates has been described in a number of studies (Zettler et al. 2013; Zhang et al. 2020; Kesy et al. 2021; Rasool et al. 2021; Lear et al. 2022; Delacuvellerie et al. 2022; Liang et al. 2023), but our study aimed to address

a gap by investigating their contribution to the whole plastisphere. In metabarcoding analyses, we identified 75 PHPB species in aquaculture and floating plastic samples (Suppl. Table 4). The vast majority of the bacterial strains isolated from plastics, mainly dominated by PHPB from the *Vibrionaceae* family, displayed a high level of resistance to antibiotics from the penicillin family. The proportion of PHPB on plastic samples ranged from 0.017% to 7%, with an average of 2.1% on FP and 0.6% on AP (Fig. 2A). Two previous studies have tried to quantify the proportion of pathogenic bacteria in their samples. Using a custom-made 16S rDNA gene database, Hou et al. (2021) looked at potentially pathogenic communities developing on incubated microplastics in a mariculture cage and found that these represented on average 0.81% of the whole bacterial plastisphere. Basili et al. (2020) mainly focused on fecal indicators on macroplastics collected in coastal sites, and found relatively low abundance of these bacterial indicators, ranging from 0% to 5.1% in the sampled plastisphere. These proportions are consistent with our results on plastics (Fig. 2A), although our approach was more conservative than Basili's (based on pathogen taxonomic nomenclature) and Hou's (based not only on PHPB but also on fish, mammal, invertebrate and plant potential bacterial pathogens).

A critical issue is to determine whether macroplastics (AP and FP) provide a favorable matrix for both PHPB and ARB compared to other environmental matrices, acting as potential carriers of infectious diseases and spreading antibiotic resistance from the aquaculture environment to fish stock and, ultimately, to humans. This question has been explored mainly on microplastics; several studies have shown a selective enrichment of PHPB and ARB on microplastics compared to inorganic supports or matrices (Kirstein et al. 2016; Frère et al. 2018; Wu et al. 2019; Junaid et al. 2022; Yu et al. 2022). Zhang et al. (2020) found ARB abundance to be 100 to 5000 times higher in the microplastisphere than in the surrounding environment. In addition, the exchange of antibiotic resistance genes (ARGs) between bacterial cells seems to be enhanced in the microplastisphere, as plasmid transfer frequency can be three orders of magnitude higher than in free-living microbial communities (Arias-Andres et al. 2018). However, other studies have shown no enrichment of PHPB in the microplastisphere compared to control surfaces (glass, wood or cellulose) or particle-attached fraction (Kesy et al. 2019; Hou et al. 2021). Interestingly, we found that aquaculture and floating plastics were enriched in PHPB compared to sediment and water samples, with significant differences between FP and sediment (Fig. 2A). In addition, we found higher MAR indices for bacteria sampled on AP compared to non-plastic samples (water, sediment and fish guts), especially concerning the

406 *Vibrionaceae* family (Fig. 5). Aquaculture macroplastics seem to be enriched in ARB compared
407 to environmental samples. Nevertheless, this must be put into perspective as the MAR indices
408 found in this study (0.15 ± 0.06) were low compared to the average MAR index of aquaculture-
409 related bacteria found in other countries around the Indian Ocean (0.19 in South Africa, 0.35 in
410 Indonesia and 0.36 in India and Sri Lanka; Reverter et al. 2020). This result could be explained
411 by very low antibiotic pressure in this environment.

412 While it is important to note that the molecular detection of PHPB does not prove their
413 pathogenicity, nor their phenotypic resistance to antibiotics, the results of this study suggest
414 that the large amount of plastics typically used in aquaculture infrastructure and the presence
415 of external (non-aquaculture) plastics polluting the farm environment can represent a source of
416 PHPB and ARB in a fish farm. The potential health risks for aquaculture stock and humans
417 caused by these enriched bacteria should not be underestimated, as these plastics may act as
418 dissemination vehicles (fomites) for the spread of infectious diseases and antibiotic
419 resistance.

420 Aquaculture macroplastics are reservoirs of cultivable ARB that are potentially pathogenic for
421 humans and fish

422 Metabarcoding data revealed the high variety of PHPB detected on aquaculture and floating
423 plastics; the families most commonly associated with potentially harmful bacteria were
424 *Vibrionaceae* (for AP 0.34% and for FP 0.78% of the whole community), *Moraxellaceae*
425 (0.02% and 0.31%), *Listeriaceae* (0.11% and 0.09%) and *Staphylococcaceae* (0.05% and
426 0.1%). Except for the family *Moraxellaceae*, the three other PHPB families are commonly
427 found on the marine plastisphere (reviewed in Junaid et al. 2022), with similar relative
428 abundance (Kesy et al. 2019; 2021). Considering the 0.1% threshold generally used to separate
429 the rare bacterial biosphere from more abundant microorganisms (Pedrós-Alió 2012), many of
430 these PHPB families represented a significant component of the plastisphere of our samples
431 (Fig. 2A). However, the detection alone of PHPB on plastics is not enough to classify them as
432 environmental reservoirs. A crucial criterion is whether the plastics can support the survival of
433 these PHPB.

434 Of all the plastic PHPB detected in this study, members of the *Vibrionaceae* family were the
435 most abundant both in metabarcoding and isolate data. More than any other clade, this family
436 has attracted a lot of attention in literature on the plastisphere (reviewed in Junaid et al. 2022),
437 particularly in aquaculture systems (Lu et al. 2019; Sun et al. 2020). This is because

Vibrionaceae are (1) ubiquitous and abundant in marine ecosystems (Haldar 2012; Ina-Salwany et al. 2019), (2) pioneers in the colonization of the plastisphere (Kesy et al. 2021), and (3) capable of causing human illness (Rivas et al. 2013; Mustapha et al. 2013; Schröttner et al. 2020; Letchumanan et al. 2019) or mass mortality of animals reared in aquaculture (Austin et al. 2016; Stentiford et al. 2017; Ina-Salwany et al. 2019; Zhang et al. 2020). In our study, three cultivable species were particularly ubiquitous and abundant on plastics: *Vibrio parahaemolyticus*, *Vibrio alginolyticus* and *Photobacterium damselae* (Fig. 2B). All of these species can be pathogenic for humans, causing mainly foodborne diseases and opportunistic infections (Nelapati et al. 2012; Mustapha et al. 2013; Rivas et al. 2013; Suppl. Table 5). They can also be pathogenic for fish, causing skin lesions and ulcers (Romalde 2002; Lai et al. 2014; Marudhupandi et al. 2017) and losses in aquaculture systems (Ina-Salwany et al. 2019; Zhang et al. 2020). In line with previous studies (Zhang et al. 2020; Moore et al. 2020), we found that *Vibrionaceae* PHPB were particularly resistant to beta-lactam antibiotics of the penicillin family (88% resistant to ampicillin and 94% resistant to ticarcillin) but susceptible to quinolones and trimethoprim + sulfonamides, which are sometimes used in aquaculture. These results indicate that macroplastics, both external to aquaculture infrastructure and those used in aquaculture, are a potential reservoir of ARB that may be pathogenic, and could thus represent a danger to aquaculture stock and human health (Sun et al. 2020; Amaral-Zettler et al. 2020).

A particular cultivable PHPB drew our attention: *Bacillus anthracis*, known to be responsible for the lethal zoonosis anthrax. It was found in the metabarcoding dataset, and its culture proved that it was still viable and physiologically capable of dividing and growing. This bacterium is normally found in soils and is well known for its long persistence in the environment: it can remain in soils for up to 100 years. Its persistence in aquatic environments is not well described, but spores are thought to be able to survive ~20 months in seawater (Sinclair et al. 2008). In humans, there are cutaneous (most common), gastrointestinal or inhalational forms of anthrax, according to the pathway of infection. It particularly affects people working with animals or derived products (Anthrax | CDC 2020; Savransky et al. 2020). No cases in fish or fish farmers have yet been described to our knowledge. Although its identification with MALDI-TOF is qualified by the manufacturer's instructions as "excellent," this result needs to be confirmed, as both the non-pathogenic *Bacillus thuringiensis* and the pathogenic *Bacillus cereus* are very close genetically to *Bacillus anthracis*, and we cannot completely exclude bias resulting from the identification method used (Spencer 2003; Kolstø et al. 2009; Gee et al. 2014; Marston et

al. 2016). The safety rules of the hospital microbiology department where we performed the isolates did not allow antibiograms on *Bacillus anthracis*.

Overall, our results show that macroplastics, both from external sources and those used in aquaculture facilities, may serve as breeding grounds for a great variety of physiologically active PHPB strains and may contribute to the emergence and dissemination of antibiotic resistance.

Potential transfer of plastic-associated PHPB and ARB to reared fish

The role of plastics as fomites or reservoirs of pathogens and antibiotic resistance genes is drawing increasing attention in the scientific community (Goldstein et al. 2014; Viršek et al. 2017; Bowley et al. 2021). The persistence of plastic in aquatic environments and its ability to drift from one place to another make it an effective fomite for potentially harmful microorganisms. Rivers and estuaries are known to be a major source of plastic debris in coastal environments (Lebreton et al. 2017); these plastics can be enriched in pathogens and antibiotic-resistant bacteria from nearby anthropogenic industries and activities (Zhu et al. 2017; Shih et al. 2021). To investigate this, we looked at the influence of the proximity of the estuary of the Grand River South East (3 km and 7.8 km from the two studied aquaculture sites) on the presence of PHPB and ARB in the fish farm. The results showed that samples from the estuary displayed the highest taxonomical richness and Shannon diversity of PHPB compared to the other sampling sites (Suppl. Fig. 4). Moreover, some PHPB species – for instance, *Vibrio parahaemolyticus* and *Photobacterium damselae* – were shared by plastics from the estuary and from the aquaculture sites, but not by plastics from the lagoon channel. Some of the floating plastics sampled in the estuary were drifting, so further analyses to investigate the role these plastics play in coastal environments as fomites, and the risk they represent in the epidemiology of infectious diseases, would be of interest. In contrast, we found that isolated strains from the estuary showed significantly lower MAR indices than other sampling sites, suggesting that the proximity of the river does not represent a risk for the development of ARB in the fish farm. In our study, aquaculture sites, and particularly aquaculture plastics, seem to be the main reservoir of ARB (Suppl. Fig. 8).

The degradation of macroplastics enhances the risks associated with plastic debris because micro- and nanoplastics are more easily ingestible, and potential transfers from these particles to marine biota could occur (Lamb et al. 2018). In an ex situ experiment, Rotjan et al. (2019) proved the persistence of ingested bacteria from microplastic biofilm in coral polyps for several

weeks. However, this issue has been less studied for PHPB and edible marine animals, including reared species, although it is known that these animals ingest plastic (Feng et al. 2019; Priscilla et al. 2019), sometimes in greater quantities than wild animals (Mathalon et al. 2014).

Our metabarcoding analyses identified 30 pathogenic species shared by plastics and fish guts. Of these, two species were cultivable: *Vibrio alginolyticus* and *Photobacterium damselae* (Fig. 4A and 4B). The virulence of these isolates was not tested, so their ability to cause infections is not known. Virulence depends on the presence of various virulence factors, but also on their expression in specific environmental conditions (Wassenaar et al. 2001; Diard et al. 2017). It can be presumed that as these isolates were viable and cultivable, their potential to cause infection in reared fish or in human consumers cannot be excluded.

We also identified a further 28 PHPB species shared between plastic samples and fish guts that were not cultivable. Bacteria can reach a viable but not cultivable (VBNC) state to survive under stressful conditions (such as living on plastic in saltwater). Bacteria in a VBNC state cannot be detected by standard laboratory methods, but they can recover their viability and potential virulence in appropriate culture conditions (Fakruddin et al. 2013; İzgördü et al. 2022). The recovery of virulence in *Vibrionaceae* species after a VBNC state has been shown and merits attention due to its potential impact on food safety and the epidemiology of foodborne diseases (Kahla-Nakbi et al. 2007; Wagley 2023). The observation of 30 PHPB species shared by macroplastics and fish suggests that there may be a potential transfer of bacteria associated with plastics after ingestion by animals (Fig. 4A). As some of these are pathogens for humans and marine animals and responsible for foodborne diseases, this is a potential animal and human health issue. It should be noted that we cannot exclude the possible contamination of fish through the ingestion of bacteria present in the water rather than plastics. However, as we found the abundance of these pathogens in water lower than that on plastics, this route of contamination may be more moderate. Further ex situ controlled experiments would be valuable to confirm this hypothesis and to prove effective transfer (Beloe et al. 2022).

Conclusion

This study, carried out in a tropical aquaculture context, showed that the pathobiome on plastics differed significantly from that in fish gut or the environment (water and sediment). We conducted bacterial analyses using metabarcoding and isolates, and both confirmed that aquaculture plastics and floating plastics were enriched in PHPB and had a higher MAR index compared to environmental samples. These results highlight the risk that plastics could represent as fomites and reservoirs of potential pathogens and antibiotic resistance in aquaculture systems. The findings also showed that a sizable proportion of pathogen species were shared between fish and plastics (both external and internal to aquaculture infrastructure) (30% of the PHPB ASVs), supporting the hypothesis of a potential risk of pathogen transfer from plastics to animals. Further studies would be of interest to test the effectiveness of these transfers in controlled experiments of plastic and bacterial ingestion by fish.

List of abbreviations

ARB: Antibiotic-resistant bacteria; AP: Aquaculture plastic; FP: Floating plastic; PHPB: Potential human pathogenic bacteria; MAR: Multiple antibiotic resistance

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Availability of data and materials

The online version contains supplementary material and is available at xxx

Authors' contributions

TB, JCA and ER obtained the funding for this study. JCA and ER designed the study, collected the samples and revised the manuscript. JN contributed to the design of the study, collected the samples, performed the biometrics and all the analyses, performed the statistics analyses and wrote the manuscript. CRO created the PHPB 16 rRNA gene database. SG supervised some analyses (cultures and antibiograms). SD contributed to the collection of the samples, and performed some cultures and antibiograms. SB welcomed the team to the farm, provided the farm boats and laboratories, and contributed to all the sampling. YB contributed to the collection of the samples and to the biometrics analysis. All authors approved the manuscript before submission.

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Ethics approval and consent to participate

Animal handling was performed respecting ethical animal welfare guidelines; the number of fish sampled was limited to the strictly necessary. Informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable.

Competing of interest

The authors declare no competing interests that could have appeared to influence the work reported in this paper.

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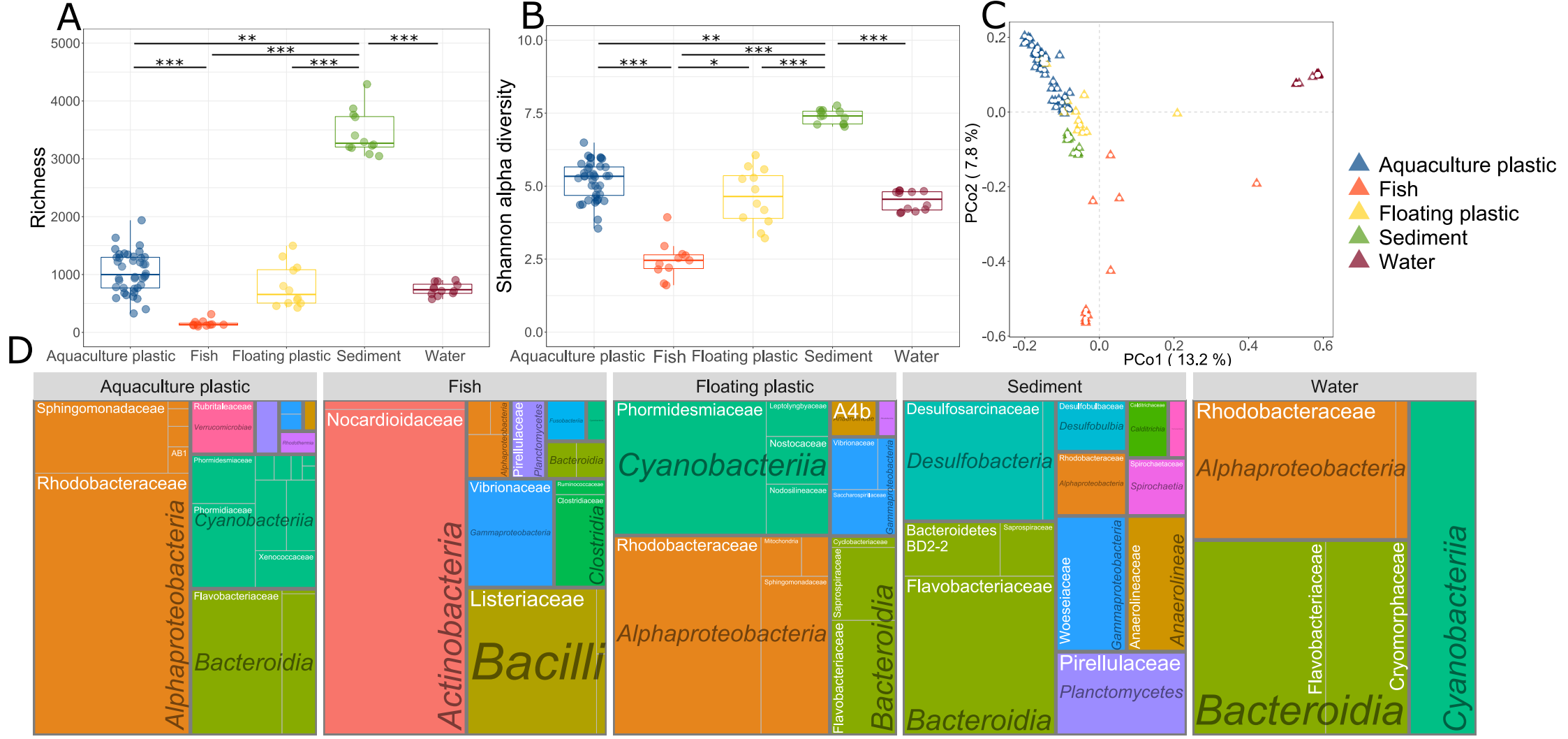


Figure 1: Composition, alpha and beta diversity of the bacterial community. A: taxonomical richness. B: Shannon diversity. A and B: Dunn test between sample origins, ***: p<0.001, **: p<0.01, *: p<0.05. C: Non metric multidimensional scaling (NMDS) ordination of the Bray-Curtis dissimilarities in microbial communities according to the sample type. D: Treemap representing the relative abundance of the most represented phyla (in dark grey) and families (in white) of the microbial community in each sample (> 5%).

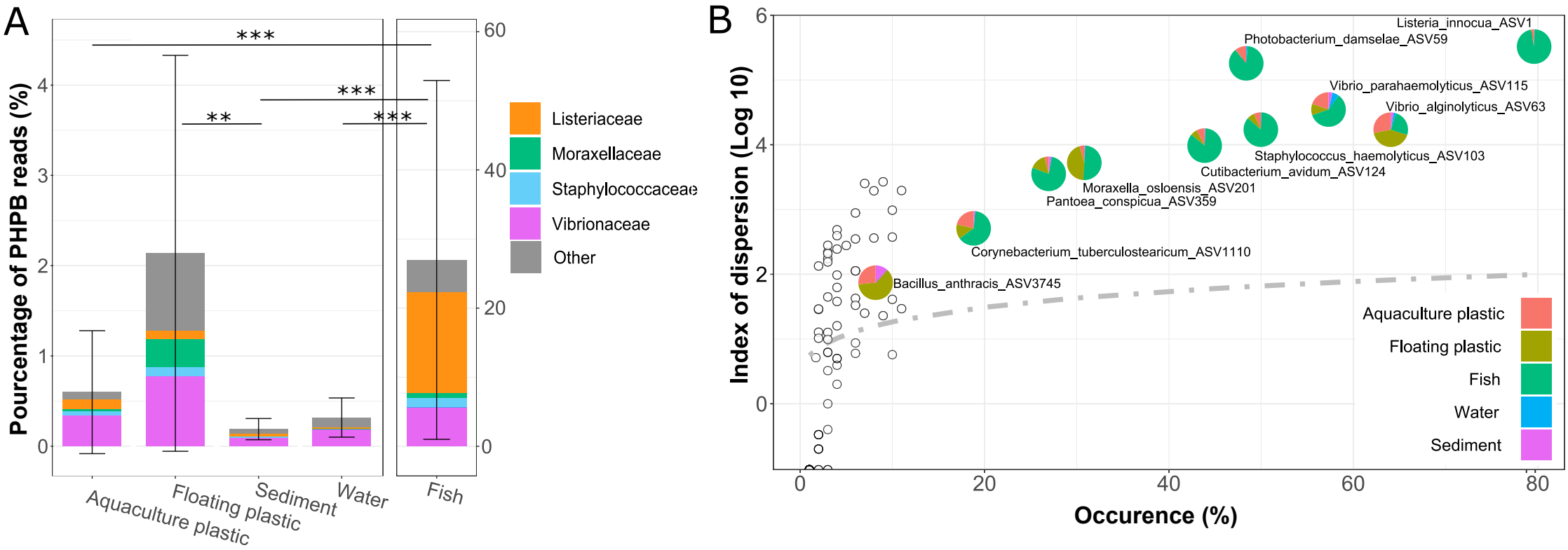


Figure 2: Proportion of pathogenic communities and core pathobiome. A: barplot of the pourcentage of pathogenic reads according to the origin of the sample. Dunn test between sample origins, ***: p<0.001, **: p<0.01. B: occurrence of pathogenic ASVs among samples plotted against its dispersion index. The dotted line depicts the 2.5% confidence limit of the Chi2 distribution: ASVs located above this line are non-randomly distributed among samples, whereas those bellow the line follow a random Poisson distribution. Piecharts display the relative abundance among sample types of the most occurrent ASVs.

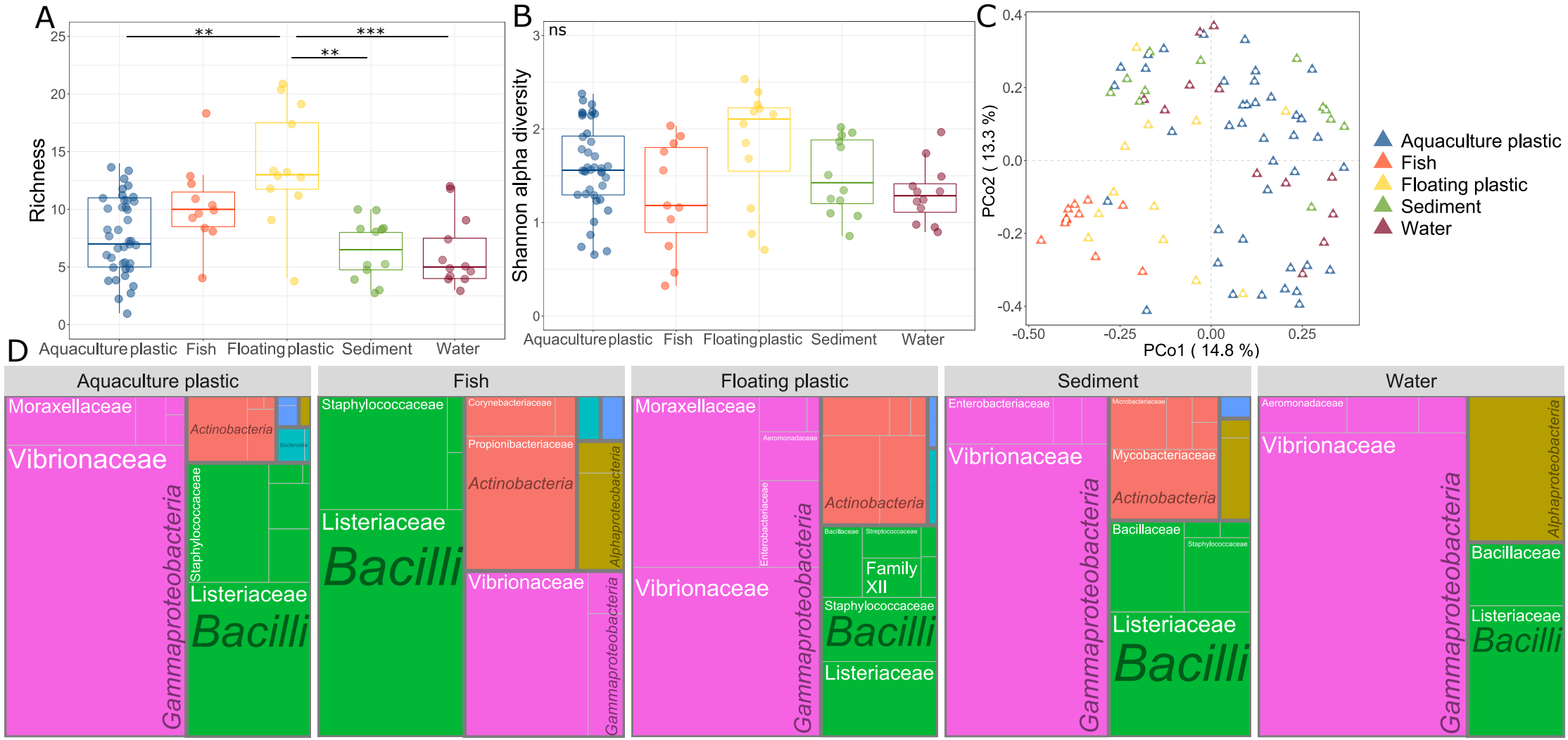


Figure 3: Composition, alpha and beta diversity of the pathobiome. A: taxonomical richness. B: Shannon diversity. A and B: Dunn tests between sample types, ***: p<0.001, **: p<0.01, ns: non significative. C: PCoA plot with Bray-Curtis distances. D: Treemap representing the relative abundance of most abundant phyla (in dark grey) and families (in white) of the pathogenic microbial community in each sample (> 5%).

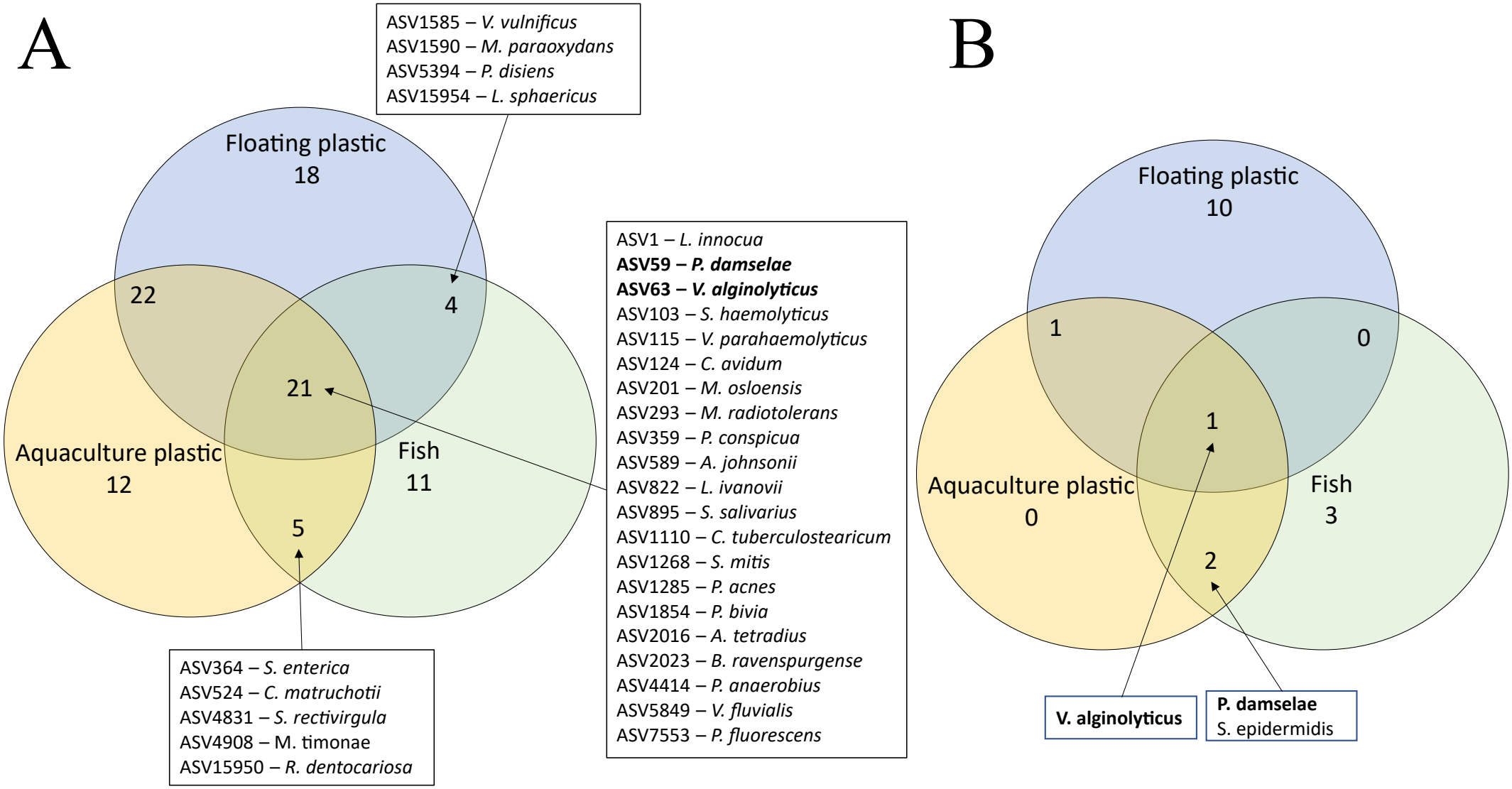


Figure 4: Venn diagram of PHPB detected (A) in metabarcoding analyses or (B) in isolates according to sample type. Species common in (A) and (B) are written in bold.

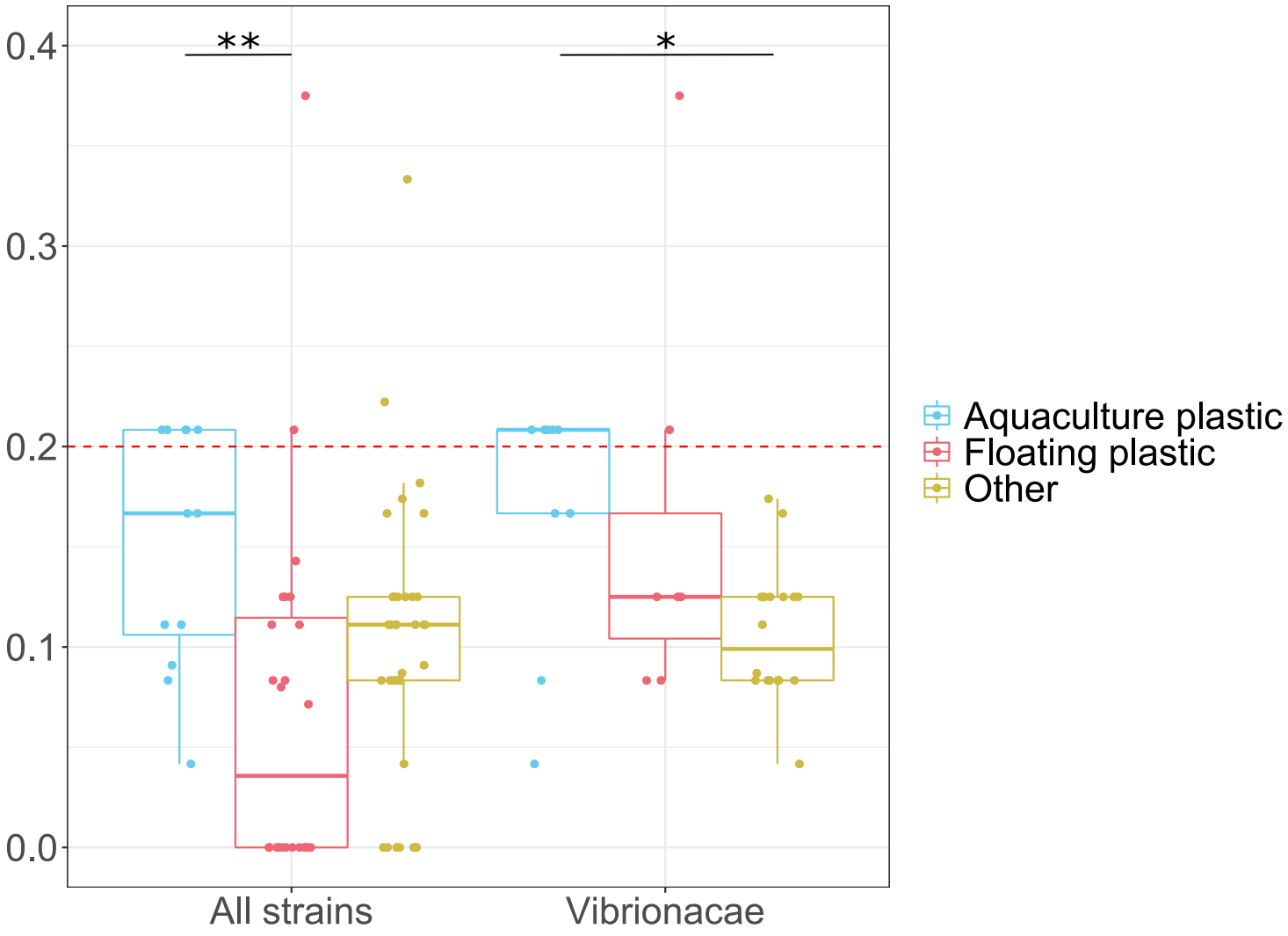
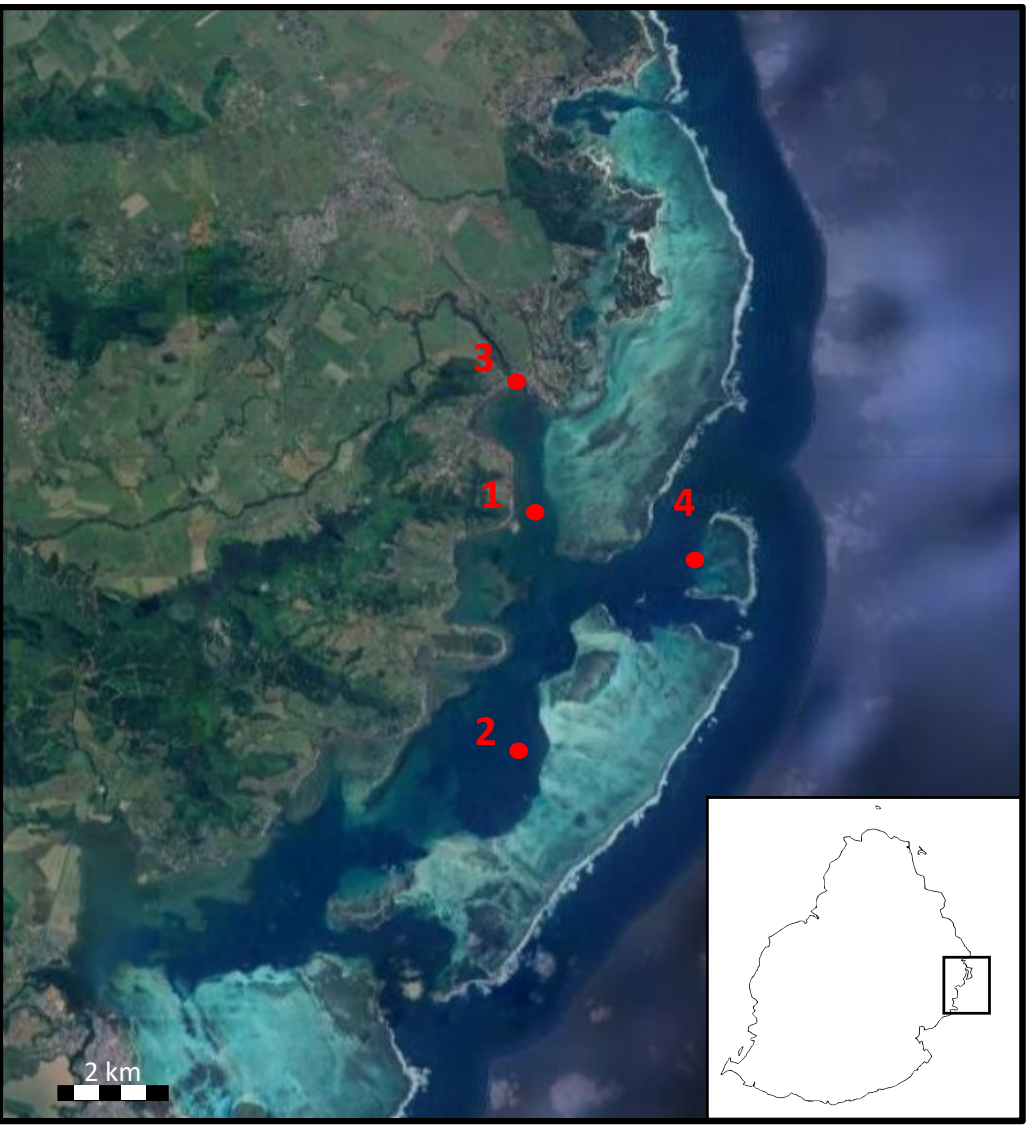
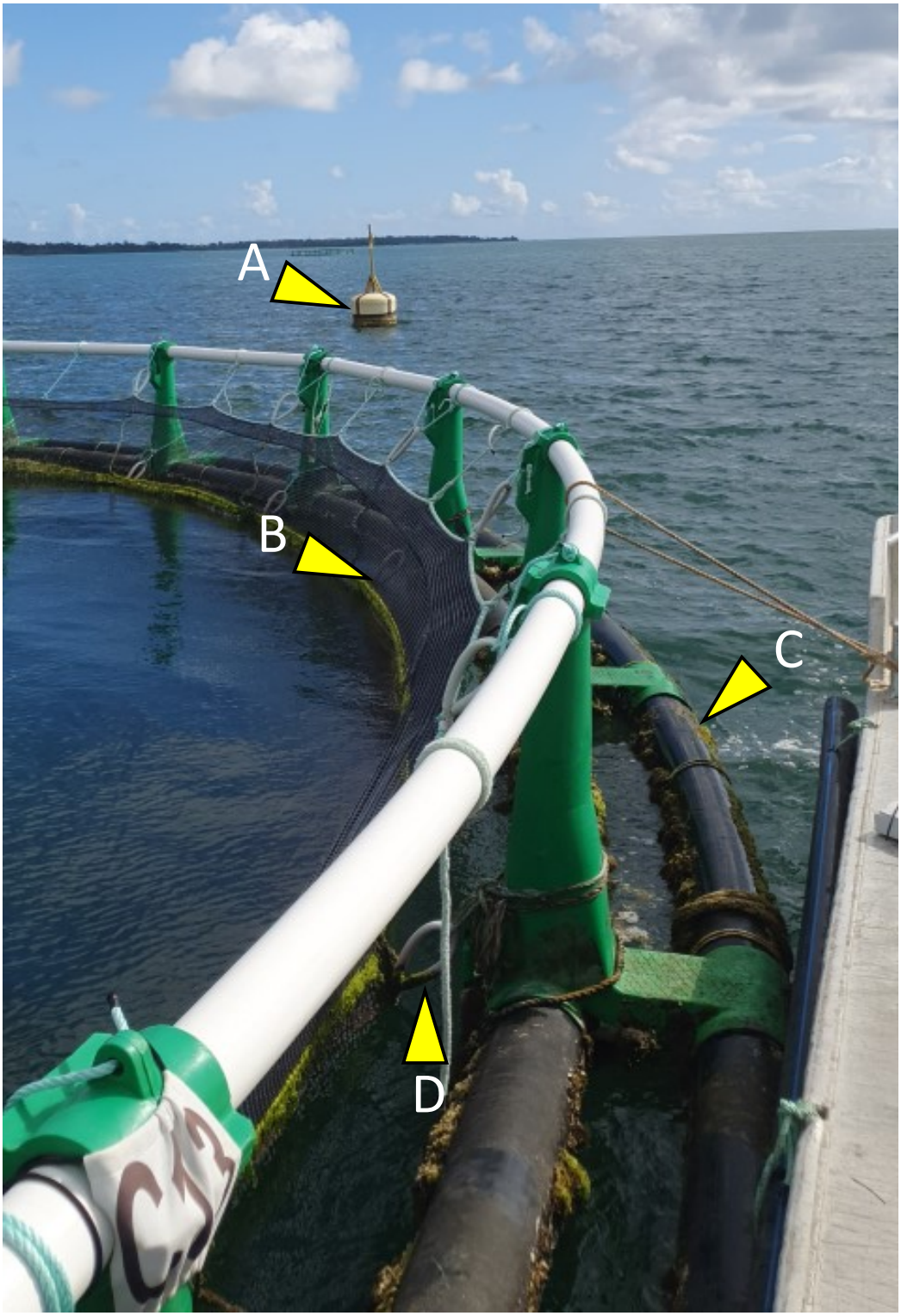


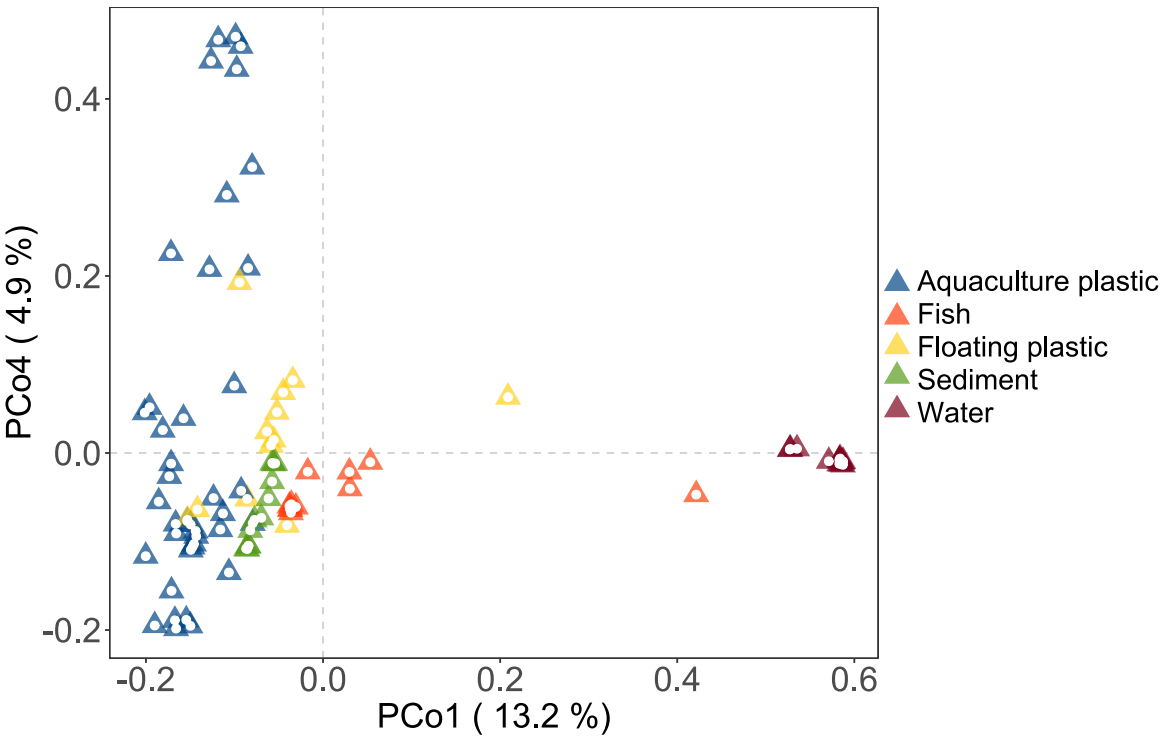
Figure 5: MAR index of all isolated strains and *Vibrionaceae* strains according to the sample type. Boxplot represents the median and quartiles for each sample type. The dotted line depicts the MAR threshold above which resistant bacteria are considered as marker of high antibiotic contamination in the area. Dunn test between sample origins, **: $p<0.01$, *: $p<0.05$.



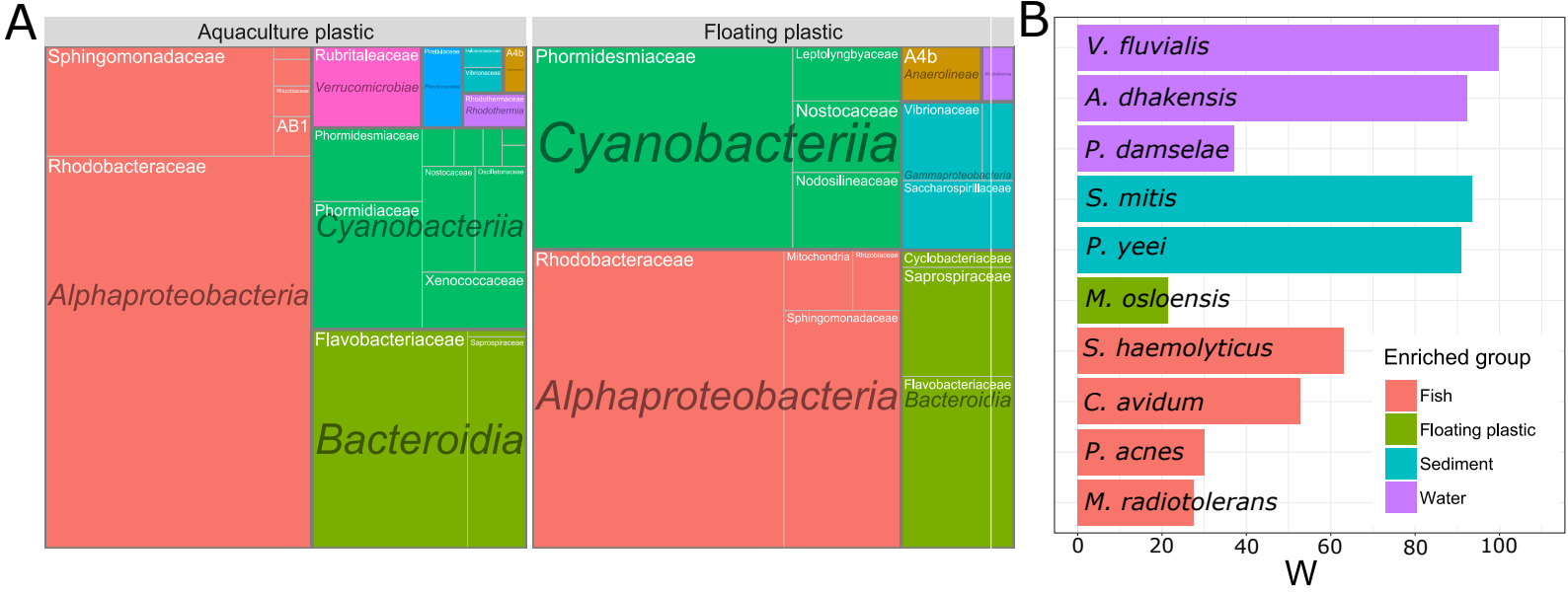
Supplementary figure 1: Geographical representation of the four sampling sites, in Mauritius. Sites 1 and 2 correspond to aquaculture sites, and sites 3 and 4 correspond respectively to the estuary of the South East Grand River and to the lagoon channel.



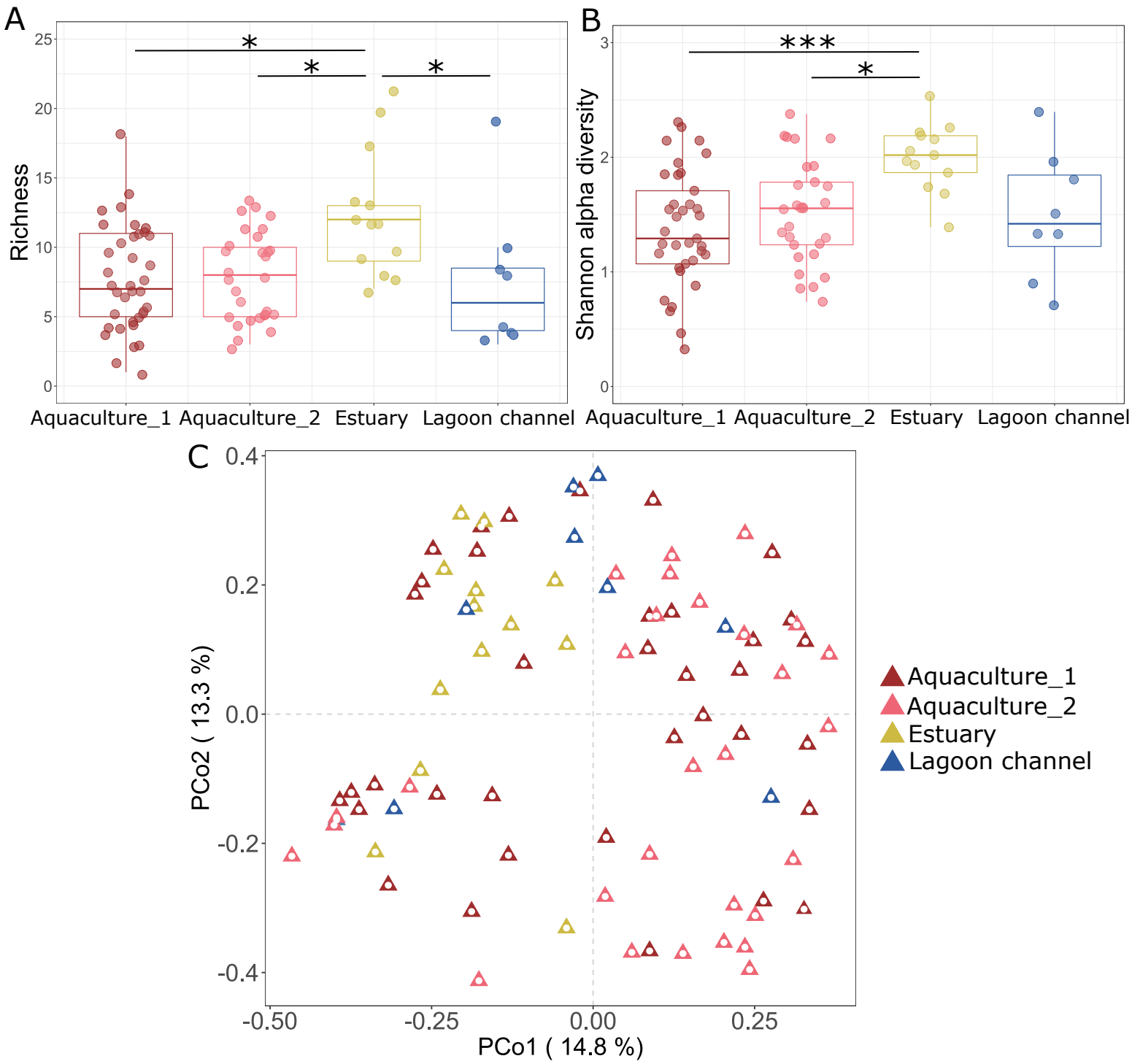
Supplementary figure 2: Photo of the four aquaculture plastics sampled. A: buoy located between cages. B: net of the cage. C: pipe structure of the cage. D: tie linking the net and the pipes.



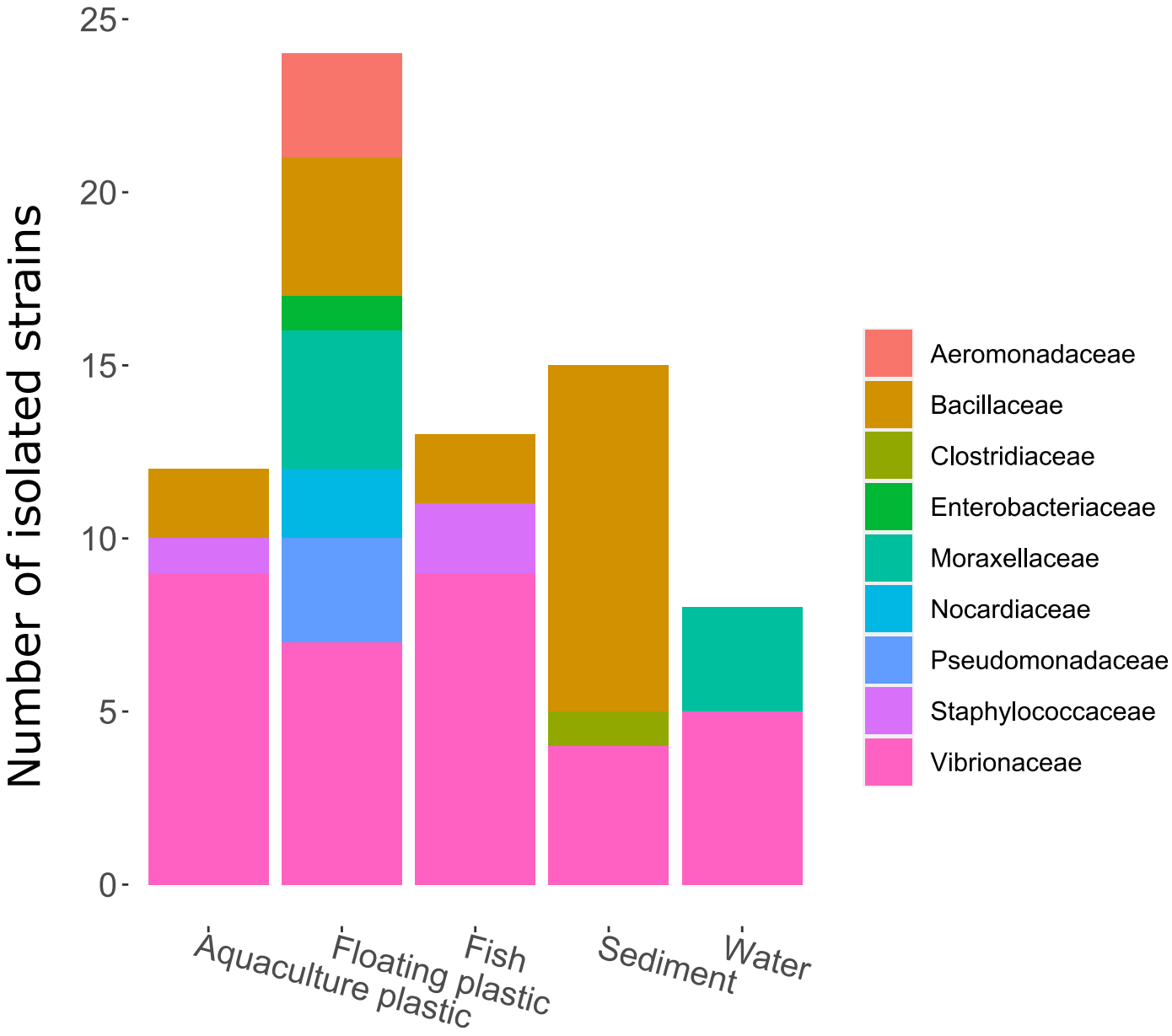
Supplementary figure 3: PCoA plot with Bray-Curtis distances (axes 1 and 4) of the diversity of the bacterial communities according to the sample type.



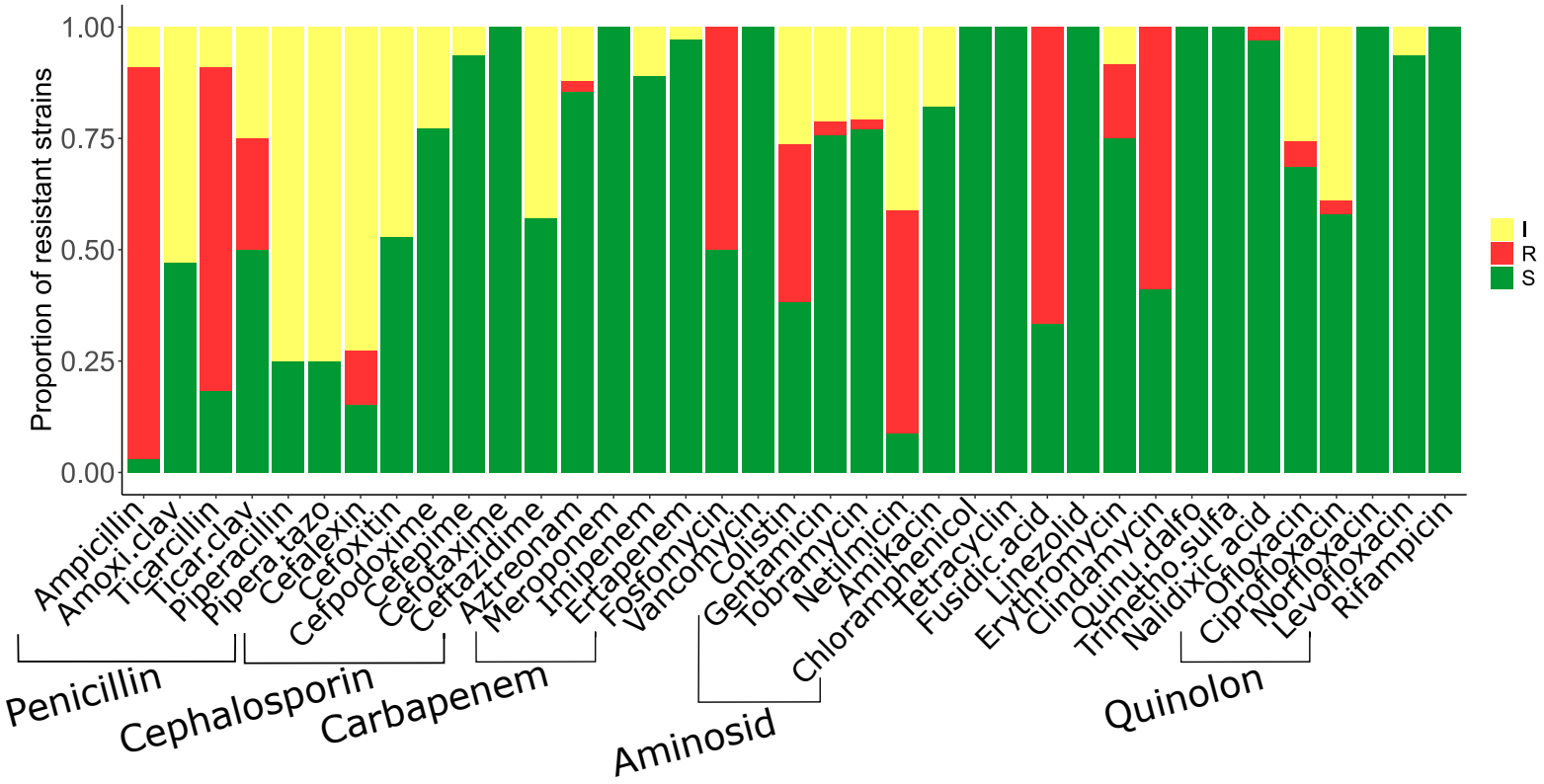
Supplementary figure 4: Biomarkers of the global plastisphere community and of the pathobiome identified by analysis of composition with bias correction (ANCOM-BC). A: treemap representing the relative abundance of the main phyla (>5%, in dark grey) and families (in white) from AP biomarkers (n = 556) and FP biomarkers (n = 88). B: biomarkers of the pathobiome communities according to the sample type, with their standardised effect sizes (W statistic) estimated via the difference on relative abundance between sample types.



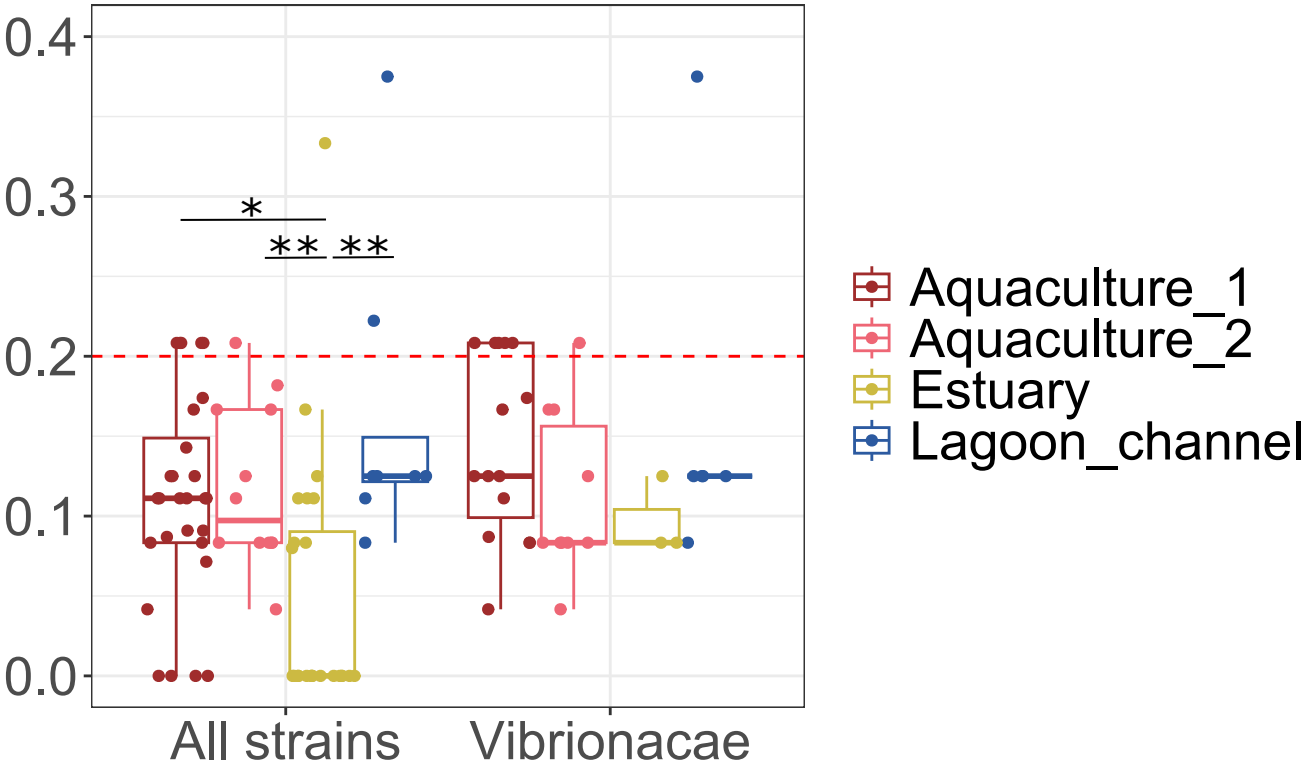
Supplementary figure 5: Alpha and beta diversity of the pathobiome according to the sampling site. A: taxonomical richness. B: Shannon diversity. A and B: Dunn tests between sample origins, ***: p<0.001, *: p<0.05. C: PCoA plot with Bray-Curtis distances.



Supplementary figure 6: Taxonomy at the phylum level of the isolated strains according to the sample type.



Supplementary figure 7: Proportion of resistant strains against each tested antibiotic, according to the EUCAST recommendations. R: resistant, S: sensible, I: intermedirary (sensible at high concentrations). Amoxi.clav: amoxicillin + clavulanic acid, Ticar.clav: ticarcillin + clavulanic acid, Pipera.tazo: piperacillin + tazobactam, quinu.dalfo: quinupristine + dalfopristine.



Supplementary figure 8: MAR index of all isolated strains and Vibrionaceae strains according to the sampling site. Boxplot represents the median and quartiles for each sample site. The dotted line depicts the MAR threshold above which resistant bacteria are considered as marker of high anibiotic contamination in the area. Dunn test between sampling sites, **: $p<0.01$, *: $p<0.05$.

Sampling site	Aquaculture site 1	Aquaculture site 2	Estuary	Lagoonchannel
Longitude	20°18'49.4" S	20°21'27.1" S	20°17'13.18" S	20°19'20.36" S
Latitude	57°47'03.8" E	57°46'54.8" E	57°46'42.49" E	57°48'57.55" E
Sampling date	12/11/2021	12/11/2021	13/11/2021	13/11/2021
Water temperature (°C)	26.53	27.33	26.83	28.07
Water salinity(psu)	30.45	34.9	17.1684	34.3037
Water conductivity (µS/cm)	46782.8	52795.6	27883.8	51954.3

Supplementary table 1: Environmental data of the sampling sites

Supplementary table 2: antibiotics and concentrations (µg) used for each strain according to the EUCAST recommendations

Antibiotic	Acinetobacter sp.	Aeromonas sp.	Bacillus sp.	Clostridium sp. / Exiguobacterium sp. / Lysinibacillus sp. / Rhodococcus sp.	Proteus sp.	Pseudomonas sp.	Staphylococcus sp.	Vibrio sp. / Photobacterium sp.
Ampicillin (10 µg)					X			X
Amoxicillin + clavulanic acid (20-10 µg)					X			X
Ticarcillin (75 µg)	X				X	X		X
Ticarcillin + clavulanic acid (75-10 µg)	X				X	X		X
Piperacillin (100 µg)					X	X		
¨Piperacillin + tazobactam (100-10 µg)					X	X		
Cefalexin (30 µg)					X			X
Cefoxitin (30 µg)					X			X
Cefpodoxime (10 µg)					X			X
Cefepime (30 µg)	X	X			X	X		X
Ceftazidime (30 µg)		X			X	X		
Cefotaxime (30 µg)					X			
Fosfomycin (200 µg)					X	X		
Vancomycin (5 µg)			X					
Aztreonam (30 µg)		X			X	X		X
Meropenem (10 µg)	X		X		X	X		X
Imipenem (10 µg)	X				X	X		X
Ertapenem (10 µg)				X	X			X
Gentamicin (10 µg)	X		X	X	X		X	X
Netilmycin (10 µg)								X
Tobramycin (10 µg)	X				X	X	X	X
Erythromycin (15 µg)			X	X			X	
Clindamycin (2 µg)			X				X	
Quinupristin + dalfopristin (15 µg)							X	
Fusidic acid (10 µg)							X	
Linezolid (10 µg)			X				X	
Amikacin (30 µg)	X				X	X		X
Chloramphenicol (30 µg)					X			X
Tetracyclin (30 µg)	X		X	X			X	X
Colistin (50 µg)								X
Trimethoprim + sulfamids (1,25-23,75 µg)	X	X	X	X	X		X	X
Ofloxacin (5 µg)					X			X
Ciprofloxacin (5 µg)	X	X	X	X	X	X		X
Levofloxacin (5 µg)	X	X			X	X		X
Norfloxacin (10 µg)							X	
Nalidixiq acid (30 µg)								X
Rifampicin (5 µg)							X	

	AP-Fish	AP-FP	AP-Sediment	AP-Water	Fish-FP	Fish-Sediment	Fish-Water	FP-Sediment	FP-Water	Sediment-Water
Global communities	0.127 **(p = 0.01)**	0.0460 **(p = 0.01)**	0.107 **(p = 0.01)**	0.222 **(p = 0.01)**	0.180 **(p = 0.01)**	0.262 **(p = 0.01)**	0.474 **(p = 0.01)**	0.148 **(p = 0.01)**	0.331 **(p = 0.01)**	0.442 **(p = 0.01)**
Pathobiome communities	0.150 **(p = 0.01)**	0.0607 **(p = 0.02)**	0.0450 (p = 0.13)	0.0610 **(p = 0.02)**	0.139 **(p = 0.01)**	0.233 **(p = 0.01)**	0.234 **(p = 0.01)**	0.122 **(p = 0.03)**	0.147 **(p = 0.01)**	0.0956 (p = 0.23)

Supplementary table 3: Summary of results obtained from PERMANOVA pairwise comparisons between beta-diversity estimates, with associated p-values in parenthesis. Bold p-values point out significant differences.

Supplementary table 4: PHPB sequences and taxonomy

	Mean_abundance_inPlasticSamples	Sd_abundance_inPlasticSamples	Sequence	BLAST best match	Kingdom	Phylum	Class	Order	Family	Genus	Species
ASV17246	0.0390243902439024	0	TACGTAGGGCGCGAG	MSLB01000015	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	naeslundii
ASV30006	0	0	TACGTAGGGCGCAAG	ATPE01000577	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Neisseria	gonorrhoeae
ASV2023	0.0115279070256876	0.0114289126226386	TACGTAGGGTACAAG	LPXX01000022	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Brevibacteriaceae	Brevibacterium	ravenspurgense
ASV524	0.0571437210588154	0.0587081442302322	TACGTAGGGTGCGAG	LV535263	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium	matruchotii
ASV1110	0.0442994832622703	0.0396358185040901	TACGTAGGGTGCGAG	LV535283	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium	tuberculostearicum
ASV2839	0	0	TACGTAGGGTGCGAG	X84442	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium	striatum
ASV1590	0.00160513643659711	0	TACGTAGGGCGCAAG	LV535790	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacterium	paraoxydans
ASV16940	0.00638977635782748	0	TACGTAGGGCGCAAG	Y17237	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacterium	schleiferi
ASV15950	0.0833333333333333	0	TACGTAGGGCGCGAG	M59055	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Rothia	dentocariosa
ASV15954	0.0677966101694915	0	TACGTAGGGCGCAAG	KC768807	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Lysinibacillus	sphaericus
ASV540	0.134726346113621	0.220318193667949	TACGTAGGGTGCGAG	X79094	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	chlorophenolicum
ASV6991	0.0120077538859174	0.00109148837243858	TACGTAGGGTCCGAG	KY234243	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	bacteremicum
ASV27673	0	0	TACGTAGGGTGCGAG	Z46664	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	celatum
ASV547	0.430176565008026	0	TACGTAGGGTGCGAG	X79286	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Gordonia	terrae
ASV28754	0	0	TACGTAGGGTGCGAG	KP010754	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Nocardia	beijingensis
ASV124	0.0699241161118568	0.0570011384407591	TACGTAGGGTGCGAG	JVDS01000009	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Cutibacterium	avidum
ASV1285	0.0174901446662843	0.0112092412916308	TACGTAGGGTGCGAG	CP003084	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Propionibacterium	acnes
ASV10630	0	0	TACGTAGGGTGCGAG	FJ785716	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Cutibacterium	granulosum
ASV4831	0.0625	0	TACGTAGGGTGCGAG	LT547818	Bacteria	Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Saccharopolyspora	rectivirgula
ASV1854	0.110421286031042	0.100970458333511	TACGGAAGGTTCGGG	KF280289	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	bivia
ASV2813	0	0	TACGGAAGGTCCGGG	LG086091	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	copri
ASV5394	0.0390243902439024	0	TACGGAAGGTCCAGG	LV536048	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	disiens
ASV17602	0.186046511627907	0	TACGGAAGGTCCGGG	AB547677	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	corporis
ASV33137	0.0833333333333333	0	TACGGAGGATTCAAG	LT558807	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	onderdonkii
ASV13114	0.0263264343018944	0.00614958368321072	TACGGAGGGTGCAAG	KJ806354	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	Chryseobacterium	indologenes
ASV16803	0	0	TACGGAGGGTGCAAG	KU358930	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	Chryseobacterium	indologenes
ASV23894	0.0508474576271186	0	TACGGAGGGTGCAAG	KR297230	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	Chryseobacterium	indologenes
ASV27450	0.0625	0	TACGTAGGTGGCAAG	NBTM01000001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Aerococcus	viridans
ASV2780	0	0	TACGTAGGTGGCAAG	EU723827	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	megaterium
ASV3745	0.0683400316021617	0.0414052864216921	TACGTAGGTGGCAAG	MF062970	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	anthracis
ASV6520	0.0620474943629978	0.0371678944845661	TACGTAGGTGGCAAG	JF784023	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Staphylococcus	epidermidis
ASV17869	0.00319488817891374	0	TACGTAGGTGGCAAG	FJ607352	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Pseudomonas	monteilii
ASV895	0.0936735260802562	0.0501589092881768	TACGTAGGTGGCAAG	JVBO01000005	Bacteria	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Streptococcus	salivarius
ASV6179	0	0	TACGTAGGTGGCAAG	AB647330	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium	butyricum
ASV2016	0.0407181571815718	0.018300460055099	TACGTAAGGTCCGAG	LV534711	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XI	Anaerococcus	tetradius
ASV3558	0.0335788746142974	0.0255863544788092	TACGTATGGAGCGAG	LRPW01000090	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XI	Finegoldia	magna
ASV26945	0.00746268656716418	0	TACGTAGGTGGCAAG	Y13364	Bacteria	Firmicutes	Bacilli	Bacillales	Family XI	Gemella	sanguinis
ASV3373	0.0848250081476919	0.126509417723832	TACGTAGGTGGCAAG	KU922148	Bacteria	Firmicutes	Bacilli	Bacillales	Family XII	Staphylococcus	epidermidis
ASV18610	0.0277777777777778	0	TACGTAGGTGGCAAG	KT275954	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Pediococcus	acidilactici
ASV30100	0.0425531914893617	0	TACGTAGGTGGCAAG	MRUN01000012	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	delbrueckii
ASV1	0.1712583831779	0.191884510114332	TACGTAGGTGGCAAG	X98527	Bacteria	Firmicutes	Bacilli	Bacillales	Listeriaceae	Listeria	innocua
ASV822	0.00358381891499693	0.00183120805084868	TACGTAGGTGGCAAG	FR687253	Bacteria	Firmicutes	Bacilli	Bacillales	Listeriaceae	Listeria	ivanovii
ASV4414	0.0512067226830009	0.0525353451447666	TACGTAGGGGGCTAG	LSQZ01000058	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Peptostreptococcus	anaerobius
ASV7543	0.0318339852238157	0.0316054855441813	TACGTAGGTGGCAAG	KF471508	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	Proteus	mirabilis
ASV103	0.105676012259031	0.074894087309083	TACGTAGGTGGCAAG	NDWY01000001	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	haemolyticus
ASV2327	0.17930086410055	0.179137420999734	TACGTAGGTGGCAAG	KT720156	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	pettenkoferi
ASV7317	0.093558282208589	0.06857008067762	TACGTAGGTGGCAAG	MUXI01000016	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	saprophyticus
ASV16554	0.0578666547767671	0.0736188506371449	TACGTAGGTGGCAAG	KU740175	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	cohnii
ASV1268	0.0429307986844112	0.0423330254436766	TACGTAGGTCCCGAG	NCVL01000053	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	mitis
ASV2956	0	0	TACGTAGGTCCCGAG	NCVK01000046	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	mitis
ASV5890	0.113230519480519	0.162664038796611	TACGTAGGTCCCGAG	KT725398	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Pantoea	agglomerans
ASV7829	0.0475193534251911	0.0500766673916112	TACGTAGGTCCCGAG	LAWM01000014	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	gordonii
ASV12654	0.0195121951219512	0	TACGTAGGTCCCGAG	LXOU01000013	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	salivarius
ASV13421	0.0149224956766198	0.0100231780709363	TACGTAGGTCCCGAG	MCQT01000004	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	agalactiae
ASV18416	0.0265478424015009	0.016848510546096	TACGTAGGTGGCAAG	KF933808	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Veillonella	parvula
ASV23784	0.0113636363636364	0	TACGTAGGTGGCAAG	LT223661	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Dialister	invisus
ASV2549	0.0702990349069125	0.0942167523620176	TACGGAGGGTGCAAG	LZFM01000016	Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Aeromonas	dhakensis
ASV293	0.0180618225373098	0.0101286574224738	TACGAAGGGGGCTAG	LV535779	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	radiotolerans
ASV1872	0	0	TACGAAGGGGGCTAG	LQAN01000054	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	radiotolerans
ASV2695	0	0	TACGTAGGGTGCGAG	LV535921	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Oligella	urethralis
ASV4908	0.0649760052315942	0.058545693579623	TACGTAGGGTGCAAG	LK391531	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Massilia	timonae
ASV11305	0	0	TACGTAGGGTGCAAG	AJ871463	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Massilia	timonae
ASV20137	0.00975609756097561	0	TACGTAGGGTGCAAG	Y14907	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Achromobacter	denitrificans
ASV8602	0.0201286518359689	0.012925404040987	TACGAAGGGGGCTAG	LT600611	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	vesicularis
ASV15284	0	0	TACGTAGGGTGCAAG	KR780470	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Chromobacteriaceae	Chromobacterium	violaceum
ASV20135	0	0	TACGTAGGGTGCGAG	KJ725364	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Chromobacteriaceae	Vibrio	cholerae
ASV359	0.0630395303362356	0.0771893451640729	TACGGAGGGTGCAAG	MF083088	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Pantoea	conspicua
ASV364	0.023474607234198	0.00245433482691714	TACGGAGGGTGCAAG	NBRN01000022	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Salmonella	enterica
ASV371	0	0	TACGGAGGGTGCAAG	X96965	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Shigella	boydii
ASV8168	0.0627898005198619	0.0496781265193134	TACGGAGGGTGCAAG	LVUN01000018	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	aerogenes
ASV1342	0.0945715446053878	0.109909300955516	TACGGAGGGTGCAAG	KY606629	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Pseudomonas	otitidis
ASV201	0.142132973963753	0.151295222101217	TACAGAGGGTGCGAG	Y15855	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxella	osloensis
ASV589	0.0535400465846262	0.0500302887970606	TACAGAGGGTGCGAG	X89775	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	johnsonii
ASV2174	0.0508474576271186	0	TACAGAGGGTGCAAG	X81665	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	lwoffii
ASV2372	0.0652535735869974	0.0451400662076874	TACAGAGGGTGCGAG	GU593616	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	junii
ASV3552	0.065187967918957	0.0744073427822617	TACAGAGGGTCGAG	HQ739095	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Staphylococcus	pasteuri
ASV5999	0.0464269772780411	0.0137884734905322	TACAGAGGGTGCGAG	LV534584	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	haemolyticus
ASV9331	0	0	TACAGAGGGTGCGAG	Z93438	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	junii
ASV13890	0.0416666666666667	0	TACAGAGGGTGCGAG	FPFL01000003	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Chlamydia	abortus
ASV23891	0.0245398773006135	0	TACAGAGGGTGCAAG	LN774706	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	ursingii
ASV6233	0	0	TACAGAGGGTGCAAG	KT695838	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	fluorescens
ASV6474	0.0674157303370786	0	TACGAAGGGTGCAAG	AJ312161	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	stutzeri
ASV7553	0.0610047846889952	0.0253746452578905	TACAGAGGGTGCAAG	KU883600	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	fluorescens
ASV22588	0.0277777777777778	0	TACGAAGGGTGCAAG	LQ684665	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	oryzihabitans
ASV23150	0.0377601122281973	0.00677843769666786	TACAGAGGGTGCAAG	MRVJ01000048	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	mosselii
ASV4015	0.0297055559388648	0.0458993816243672	TACGGAGGGGGCTAC	LK391535	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	yeei
ASV23889	0	0	TACGGAGGGTGCGAG	X81621	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Shewanellaceae	Shewanella	algae
ASV1020	0	0	TACGGAGGGAGCTAG	JF459973	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	aromaticivorans
ASV59	0.170794786525767	0.163819878834397	TACGGAGGGTGCGAG	X78106	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Photobacterium	damselae
ASV63	0.274733213936411	0.218365549936639	TACGGAGGGTGCGAG	X74691	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	alginolyticus
ASV115	0.153649571490619	0.1335242111907664	TACGGAGGGTGCGAG	X74720	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	parahaemolyticus
ASV1585	0.00993720575774992	0.00267745016014306	TACGGAGGGTGCGAG	HM996971	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	vulnificus
ASV4057	0.0830670926517572	0	TACGGAGGGTGCGAG	HQ616143	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	furnissii
ASV5849	0.0266245416865503	0.0241192321509624	TACGGAGGGTGCGAG	X76335	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	fluvialis
ASV11337	0	0	TACGGAGGGTGCGAC	CP009765	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	parahaemolyticus
ASV13210	0	0	TACGGAGGGTGCGAC	LFWL01000043	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	alginolyticus
ASV23454	0	0	TACGGAGGGTGCGAC	MVKQ01000003	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	vulnificus
ASV7838	0.0425531914893617	0	TACGAAGGGTGCAAG	EU434550	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	maltophilia
ASV23034	0	0	TACGAAGGGTGCAAG	LLXV01000060	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	maltophilia

Bacteria	Hosts (human and/or fish)	Human saprophyte	Infections in humans
Moraxella osloensis	Human	yes	Endocarditis, meningitis, osteomyelitis, septic arthritis, vaginitis, and bacteremia (Shah, Ruth, et Coffin 2000)
Cutibacterium avidum	Human	yes	Breast infections, skin abscesses, infective endocarditis, and device-related infections (Corvec 2018)
Photobacterium damselae	Human and fish	no	Wound infections, necrotizing fasciitis, sepsis, urinary tract infections, food intoxications (Rivas, Lemos, et Osorio 2013, Schröttner et al. 2020)
Staphylococcus haemolyticus	Human	yes	Meningitis, endocarditis, prosthetic joint infections, bacteremia, septicemia, peritonitis, and otitis (Eltwisy et al. 2022)
Vibrio parahaemolyticus	Human and fish	no	Foodborne disease: gastroenteritis, diarrhea, stomach pains, nausea, fever (Letchumanan et al. 2019)
Vibrio alginolyticus	Human and fish	no	Foodborne disease (gastroenteritis, diarrhea, stomach pains, nausea, fever), conjunctivitis, tissue necrosis, peritonitis (Mustapha, Mustapha, et Nozha 2013)
Listeria innocua	Human	no	Bacteriemia, meningitis (Perrin, Bemer, et Delamare 2003, Favaro et al. 2014)

Supplementary table 5: Summary of infections that can be caused by some potential pathogens.

	Aquaculture 1 – Aquaculture 2	Aquaculture 1 – Estuary	Aquaculture 1 – Lagoon channel	Aquaculture 2 – Estuary	Aquaculture 2 – Lagoon channel	Estuary – Lagoon channel
Global communities	0.0282 **(p = 0.042)**	0.0514 **(p = 0.006)**	0.0461 **(p = 0.048)**	0.0655 **(p = 0.006)**	0.542 **(p = 0.03)**	0.0883 (p = 0.198)
Pathobiome communities	0.0373 **(p = 0.018)**	0.0576 **(p = 0.006)**	0.0267 (p = 1)	0.0116 **(p = 0.006)**	0.0661 **(p = 0.030)**	0,0955 (p = 0.108)

Supplementary table 6: Summary of results obtained from PERMANOVA pairwise comparisons between beta-diversity estimates, with associated p-values in parenthesis. Bold p-values point out significant differences.

Supplementary table 7: MALDI-TOF identification of isolates according to their sampling site and type

Organism	Family	Site	Source
Acinetobacter tandoii	Moraxellaceae	3	Water
Aeromonas hydrophila	Aeromonadaceae	3	Floating plastic
Acinetobacter venetianus	Moraxellaceae	3	Floating plastic
Pseudomonas mendocina	Pseudomonadaceae	3	Floating plastic
Aeromonas veronii	Aeromonadaceae	3	Floating plastic
Aeromonas jandaei	Aeromonadaceae	3	Floating plastic
Bacillus cereus	Bacillaceae	1	Sediment
Bacillus pumilus	Bacillaceae	1	Sediment
Photobacterium damselae	Vibrionaceae	1	Sediment
Proteus hauseri	Enterobacteriaceae	3	Floating plastic
Photobacterium damselae	Vibrionaceae	1	Fish
Bacillus subtilis	Bacillaceae	1	Fish
Vibrio alginolyticus	Vibrionaceae	1	Fish
Vibrio navarrensis	Vibrionaceae	1	Water
Vibrio alginolyticus	Vibrionaceae	1	Water
Rhodococcus pyridinivorans	Nocardiaceae	1	Floating plastic
Pseudomonas resinovorans	Pseudomonadaceae	1	Floating plastic
Pseudomonas mendocina	Pseudomonadaceae	1	Floating plastic
Vibrio alginolyticus	Vibrionaceae	1	Floating plastic
Bacillus anthracis	Bacillaceae	1	Floating plastic
Vibrio alginolyticus	Vibrionaceae	3	Sediment
Lysinibacillus pakistanensis	Bacillaceae	3	Sediment
Clostridium sartagoforme	Clostridiaceae	3	Sediment
Vibrio alginolyticus	Vibrionaceae	1	Floating plastic
Vibrio parahaemolyticus	Vibrionaceae	1	Floating plastic
Photobacterium damselae	Vibrionaceae	1	Fish
Acinetobacter tandoii	Moraxellaceae	3	Water
Vibrio alginolyticus	Vibrionaceae	1	Aquaculture plastic
Vibrio alginolyticus	Vibrionaceae	1	Aquaculture plastic
Vibrio alginolyticus	Vibrionaceae	1	Aquaculture plastic
Vibrio alginolyticus	Vibrionaceae	1	Aquaculture plastic
Lysinibacillus fusiformis	Bacillaceae	3	Sediment
Bacillus pumilus	Bacillaceae	3	Sediment
Bacillus amylequofaciens	Bacillaceae	3	Sediment
Vibrio alginolyticus	Vibrionaceae	1	Fish
Photobacterium damselae	Vibrionaceae	1	Fish
Lysinibacillus fusiformis	Bacillaceae	1	Fish
Bacillus pumilus	Bacillaceae	1	Aquaculture plastic
Acinetobacter junii	Moraxellaceae	3	Floating plastic
Bacillus pumilus	Bacillaceae	3	Floating plastic
Acinetobacter junii	Moraxellaceae	3	Floating plastic
Vibrio alginolyticus	Vibrionaceae	4	Floating plastic
Vibrio harveyi	Vibrionaceae	4	Floating plastic
Bacillus amylequofaciens	Bacillaceae	3	Sediment
Bacillus pumilus	Bacillaceae	4	Sediment
Vibrio alginolyticus	Vibrionaceae	4	Sediment
Bacillus pumilus	Bacillaceae	4	Sediment
Vibrio alginolyticus	Vibrionaceae	4	Water
Vibrio alginolyticus	Vibrionaceae	4	Floating plastic
Rhodococcus hoagii	Nocardiaceae	3	Floating plastic
Exiguobacterium sp	Bacillaceae	3	Floating plastic
Photobacterium damselae	Vibrionaceae	2	Aquaculture plastic
Vibrio alginolyticus	Vibrionaceae	2	Water
Vibrio alginolyticus	Vibrionaceae	2	Aquaculture plastic

Staphylococcus warneri	Staphylococcaceae	1	Fish
Staphylococcus epidermidis	Staphylococcaceae	1	Aquaculture plastic
Staphylococcus epidermidis	Staphylococcaceae	2	Fish
Photobacterium damselae	Vibrionaceae	2	Fish
Vibrio alginolyticus	Vibrionaceae	2	Fish
Vibrio alginolyticus	Vibrionaceae	2	Fish
Vibrio alginolyticus	Vibrionaceae	2	Fish
Vibrio alginolyticus	Vibrionaceae	2	Aquaculture plastic
Photobacterium damselae	Vibrionaceae	2	Aquaculture plastic
Vibrio alginolyticus	Vibrionaceae	2	Aquaculture plastic
Bacillus pumilus	Bacillaceae	2	Sediment
Acinetobacter tandoii	Moraxellaceae	3	Water
Acinetobacter venetianus	Moraxellaceae	3	Floating plastic
Vibrio alginolyticus	Vibrionaceae	4	Sediment
Vibrio alginolyticus	Vibrionaceae	3	Water
Bacillus pumilus	Bacillaceae	1	Aquaculture plastic
Bacillus pumilus	Bacillaceae	1	Floating plastic
Vibrio alginolyticus	Vibrionaceae	3	Floating plastic