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54° Congresso SIBM: Biodiversità e funzionamento attraverso approcci di analisi di DNA ambientale (eDNA)

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TEMPORAL CHANGES IN COMMUNITIES ON PLASTIC PANELS WITHIN A POLLUTED MARINE ENVIRONMENT

CAMBIAMENTI SU SCALA TEMPORALE NELLE COMUNITÀ SU SUBSTRATI DI PLASTICA IN UN AMBIENTE MARINO INQUINATO

Abstract - *This study examined community succession on polystyrene panels deployed for 25 weeks in a harbour impacted by human activities. Microbial and eukaryotic colonisation of plastics was studied using eDNA metabarcoding targeting 16S and 18S rRNA genes. The prokaryotic biofilm community was dominated by Rhodobacteraceae (17%) and Flavobacteriaceae (18%), with only minor changes over time. Concernig eukaryotes, the early stages of colonization were dominated by Alveolata (63%) and Stramenopiles (24%), while the later stages shifted to Chlorophyta (20%) and Opisthokonta (94%). Diversity analyses based on ASVs showed that alpha diversity ranged from 1,875–2,481 for prokaryotes and 159–405 for eukaryotes.. Moreover, potential plastic-degrading bacteria were identified in the prokaryotic community. The ecological dynamics of the eukaryotic community showed the dominance of heterotrophic microrganisms. These findings confirm that plastics may act as substrates and vectors for diverse microbial and eukaryotic assemblages, posing ecological risks to marine ecosystems.*

Keywords: *Biofilm, Diversity, Plastics, Prokaryotic and eukaryotic communities*

Introduction - Plastic waste, primarily originating from land-based sources, is present in various ecosystems, including the most remote and deep oceans (Chiba *et al.,* 2018). The Mediterranean Sea, and particularly the Adriatic Sea, is one of the most heavily impacted areas of the world. Intense human activities such asshipping, aquaculture and tourism, riverine inputs (especially from the Po River), and high population density of the Adriatic region all contribute to high debris densities and the accumulation of plastic waste (Gnip and Velkavrh, 2022). Once in the sea, plastics break down into microplastics that enter the food chain, posing risks to marine and human health. Plastic surfaces can be rapidly colonized by diverse microbial and eukaryotic communities composed of several bacterial taxa, predominantly by the classes Gammaproteobacteria and Alphaproteobacteria. These include the pathogenic *Vibrio* species, as well as various phytoplankton taxa, mainly from the classe Bacillariophyceae and Dinophyceae (Amaral-Zettler *et al.,* 2020). This plastic debris may act as a vector for transporting potential harmful species, pathogens, and non-indigenous species, with consequences such as ecosystem alteration. The present study an environmenta DNA (eDNA) metabarcoding approach to explore the colonization and successional patterns of both microbial and eukaryote communities on expanded polystyrene (EPS) panels deployed in a harbour for 25 weeks.

Materials and methods – Four expanded polystyrene (EPS) panels were placed in the harbour at Pesaro (NW Adriatic Sea) for 25 weeks. Duplicate subsamples were collected at weeks 2, 5, 6, 10, 11, 19, and 25, either being processed immediately for DNA extraction or being frozen at −80 °C for later analysis. At the sampling site, the temperature (°C), salinity, dissolved oxygen (mg/L), and pH were measured with a CTD probe. Nutrient concentrations of total nitrogen (TN) and total phosphorus (TP) were also determined. DNA was extracted in duplicate from each plastic fragment using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) and the DNA concentration was measured with a Qubit fluorometer. Samples were stored at −80 °C until sequencing. To study the plastic surface communities the V3–V4 regions of the 16S rRNA gene and the hypervariable V4 region of the 18S rRNA gene were amplified for prokaryotes and eukaryotes, respectively, using universal primers. The final libraries were sequenced on an Illumina MiSeq platform (2 × 300 bp paired-end). Sequencing reads were processed using the DADA2 pipeline and paired-end reads were merged into amplicon sequence variants (ASVs). Taxonomy was assigned using the SILVA and PR2 database for prokaryotes and eukaryotes, respectively. Moreover, the microbial community was screened for potential plastic-degrading organisms, using the PlasticDB web server (http://www.plasticdb.org/). All statistical analyses were carried out in R (v4.3.1) and PAST (v4.11). Diversity, richness, Shannon, and Simpson indices were calculated for both prokaryotic and eukaryotic ASVs. The eukaryotic ASVs were also classified into three trophic groups: autotrophs/mixotrophs, heterotrophs and parasites. To explore the community composition, non-metric multidimensional scaling (nMDS) with Jaccard distances was applied using the VEGAN R package. Differences between groups were tested by one-way PERMANOVA (999 permutations, Jaccard distance).

Results - The harbour waters showed clear seasonal fluctuation. Temperature peaked at 27.9 °C in week 5, dropping to 10.4 °C by week 25. Nutrient concentrations also varied widely, with total nitrogen ranging from 21.7 to 127.3 µM, and total phosphorus from 0.41 to 4.12 µM. The ASVs community on plastic surfaces analyses showed substantial changes in both prokaryotic and eukaryotic composition over time, highlighting highly dynamic assemblages. About 54 bacterial phyla were detected, with Proteobacteria (39.74 ± 12.76 %), Bacteroidota (28.19 ± 6.17 %), Cyanobacteria (16.13 ± 10.89 %), and Planctomycetota (11.12 ± 4.65 %) dominating the assemblages (Fig. 1A). At the class level, Gamma- and Alphaproteobacteria (13.08 ± 9.02 % and 27.8 ± 6.22 %, respectively), Bacteroidia (24.7 ± 5.8 %), Planctomycetes (9.5 % ± 4.1 %) and Cyanobacteria (15.7 ± 11.71 %) were the main classes (Fig. 1B). At the family level, Rhodobacteraceae (16.97 ± 5.0 %), Flavobacteriaceae (17.99 ± 5.52 %), and Pirellulaceae (5.23 ± 2.85 %) were consistently present, while Xenococcaceae and Phormidiaceae became more abundant in later stages (Fig. 1C).

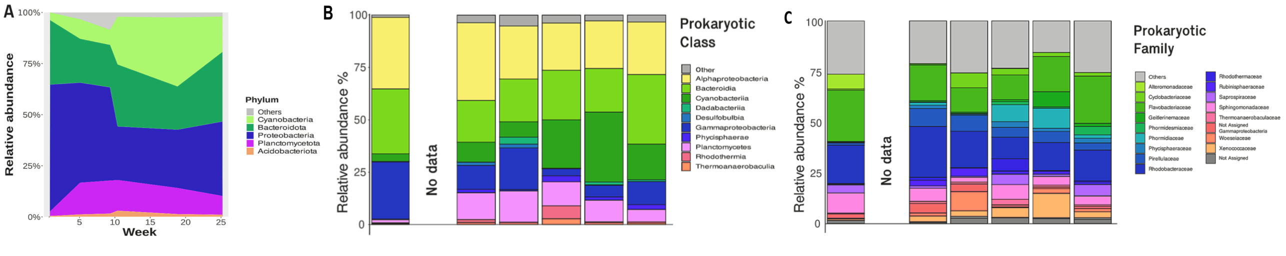

Fig. 1. - (A) Composition of the prokaryotic communities at the phylum level; (B) at class level; (C) at family level, on plastic frames during the 25-week deployment period based on ASV data.
(A) Composizione delle comunità procariotiche a livello di phylum; (B) classe; (C) famiglia su pannelli di plastica durante il periodo di esposizione di 25 settimane base dei dati di ASVs.

A total of nine phyla were found among the eukaryotes with the following dominating the community: Opisthokonta (59.76 ± 12.59 %), Alveolata (18.77 ± 9.17 %), Chlorophyta (10.99 ± 2.26 %), and Stramenopiles (8.99 ± 2.85 %) (Fig. 2A). At the class level, Alveolata (primarily Phyllopharyngea, 62 %) and Stramenopiles (mainly the Bacillariophyceae, 16 %), prevailed during the initial weeks of exposure (Fig. 2B). In the early stages, ciliates of the Acinetidae family dominated the community (63%). Over the following weeks, Annelida and Bryozoa became the most abundant groups (47% and 79%, respectively), together with Maxillopoda and Acinetidae (25% and 26%, respectively). Later, Ostreoida became dominant (39%) and, in the final week, the community shifted back to being dominated by Acinetidae (38%) (Fig. 2C).

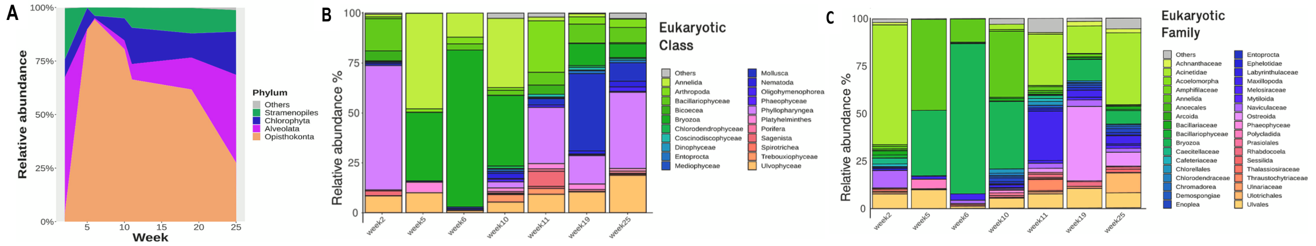

Fig. 2. - (A) Composition of the eukaryotic communities at the phylum level; (B) at class level; (C) at family level, on plastic frames during the 25-week deployment period based on ASV data.
(A) Composizione delle comunità eucariotiche a livello di phylum; (B) classe; (C) famiglia su pannelli di plastica durante il periodo di esposizione di 25 settimane sulla base dei dati di ASVs.

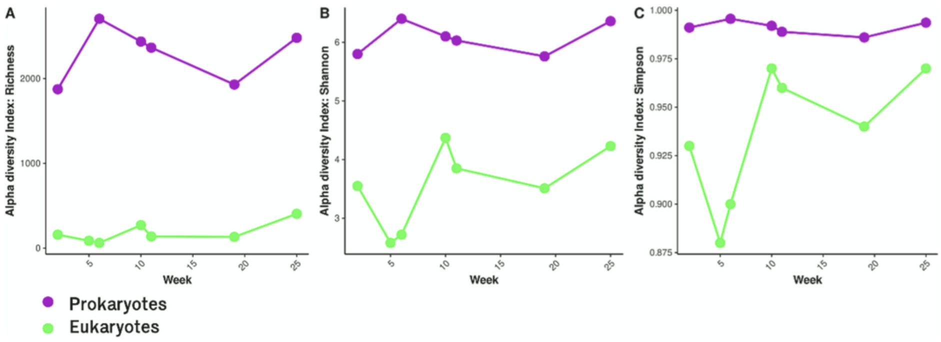

Fig. 3. - Prokaryotic and eukaryotic alpha diversity indices based on ASV data.
Indici di diversità alfa procariotica ed eucariotica basati sui dati ASV.

Alpha diversity was also investigated by richness, Shannon and Simpson indices. For prokaryotes, diversity generally increased in the early stages (weeks 2 and 5), then declined, followed by a secondary rise at week 25. In contrast, eukaryotic diversity initially decreased around weeks 5 and 6, increasing again towards the end of the experiment (Fig. 3). Concerning the community composition and seasonality, nMDS and PERMANOVA analyses showed only a slight seasonal differences in prokaryotic communities ($F = 1.64$, $p = 0.04$), with some overlap between summer and autumn assemblages (Fig. 4A). By contrast, eukaryotic communities clustered clearly by season, with significant differences between summer and autumn assemblages ($F = 3.16$, $p < 0.001$) (Fig. 4B). At the genus level, 58 bacterial groups known for their ability to degrade plastics were detected with highest variability (47 out of 58 genera) at the end of the exposure (data not shown). Finally, the bryozoan *Amathia verticillata* (delle Chiaje, 1822) and the polychaete *Hydroides elegans* (Haswell, 1883) were detected as non-indigenous species (NIS).

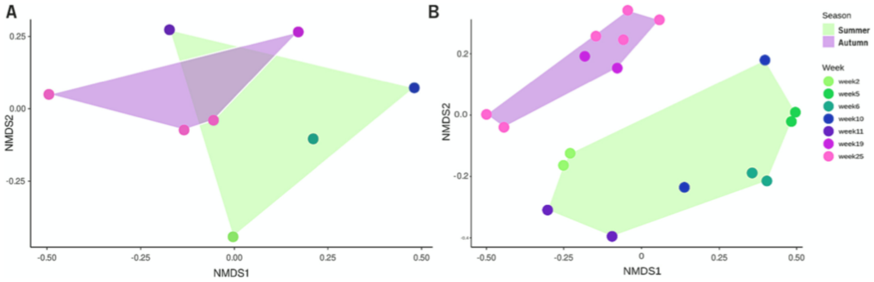

Fig. 4. – (A) Non-metric multidimensional (nMDS) ordination performed on prokaryotic (stress = 0.10); (B) and eukaryotic (stress = 0.14). Convex hulls are shown in green and purple according to the summer and autumn seasons, respectively.
(A) Ordinamento non-metric multidimensional (nMDS) eseguito su procarioti (stress = 0.10); (B) ed eucarioti (stress = 0.14). I convex hulls sono mostrati in verde e viola rispettivamente in base alle stagioni estiva e autunnale.

Although these species are well established in the Mediterranean communities and didn't dominate the plastic-associated eukaryotic assemblages, their potential spread of these species by plastics could pose ecological risks. The functional diversity of eukaryotic community revealed that the plastic were dominated by heterotrophs (68–95%). Autotrophic/mixotrophic organisms were present in lower abundance (5–30%), while parasites were almost absent, never exceeding 8% of the community (data not shown).

Conclusions - This study explored how microbial and eukaryotic communities colonize and develop on plastic debris in the polluted harbour of Pesaro. Using eDNA metabarcoding, we tracked how biofilms formed and changed over time on EPS panels, revealing plastic as a substrate for diverse organisms with different ecological roles. Our results showed that both microbial and eukaryotic communities on plastic debris change following natural successional patterns influenced by local environmental factors and pollution levels. Prokaryotic communities shifted from early pioneer colonizers to more diverse assemblages, while the eukaryotic community, dominated by microeukaryotes in the early stages and by heterotrophic macroinvertebrates at the end of the exposure, reflected a shift towards a complex, multitaxa community. Plastics act as durable surfaces that can transport potentially non-indigenous or harmful species across long distances, thereby threatening biodiversity and marine food webs. Microbial colonizers may contribute to plastic degradation, but they may also introduce ecological risks. Optimizing microbial growth conditions could enhance plastic degradation, reducing marine plastic pollution and protecting ecosystems in the long term. Future research should clarify their metabolic pathways and environmental interactions to support sustainable ecosystem protection.

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