# Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVII

## The role of microbiomes in marine systems

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Extreme microbiomes are those microorganisms thriving under the severe conditions that identify extreme environments, where no other living being will have any chance to survive. These environments are characterized by extreme phyisical (high/low temperatures, high pressure) and/or chemical (high/low pH, extremely high salinity) conditions, including high levels of pollution. Although more and more informations regarding the diversity and the potential industrial use of extremophiles are accumulating, their functional role in supporting more complex life forms is still overlooked and needs to be investigated to a greater extent. It is known that marine multicellular organisms live in close relationship with their microbiome. Microbiomes provide the hosts with numerous abilities such as nutrient supply, defence and development, becoming an integral component of the host physiology [1][2]. Moreover, microbiomes show a large variability in composition and structure across hosts, likely due to the capacity of the hosts to select microbes specifically from the environment [3]. There is evidence that organisms own a stable microbiome (core microbiome: set of microbial taxa characteristic of a specific host or environment) which can be useful to better understand the functional feature that are essential for each different species. However, core microbiomes must be investigated in the frame of the significant adaptive and/or maladaptive changes in microbiome composition among individuals in distinct populations under different environmental conditions such as geographic location, presence of pollutants and stressors, including climate change [4]

To increase the understanding of these microbiomes dynamics and evolutionary significance it is necessary to identify core and variable microbial taxa, their adaptative features to environmental conditions and the mechanisms of microbiome establishment and selection. These include horizontally-transmitted microbes (selected from the environment by each host generation) [5] and vertically-transmitted microbes (persisting across life stages and generations, thus coevolving with the host and favoring adaptation) [6].

Despite the scientific community increasing interest on the evolutionary processes and adaptation strategies of marine organisms, information on the specific role of microbiomes to such processes is still limited and further investigations are necessary.

### *Aims*

1) To broaden the knowledge of the roles and the functions of microbiomes in different environmental conditions, including extreme environments and polluted areas. (***Task 1, Task 2***)

2) To investigate the intraindividual, intraspecific diversity of microbiomes associated to invasive species and their role in the adaptation of such species in non native habitats. (***Task 3***)

3) To understand the specific abilities that microbiome could provide to the hosts in order to cope with extreme physical and/or chemical conditions (***Task 2, Task 4***)

### *Task 1*

Bioplastic is rapidly growing as the main alternative to traditional plastics. Although labelled as completely biodiegradable multiple experiments have shown that biodegradation of bioplastic in natural environment is slower than in controlled conditions (such as in composting plants) and it could take years to completely disappear [7] thus creating environmental problems. In order to investigate the biodegradation processes, biofilm formation and evolution and the effects of bioplastic in microbiomes associated to the shallow benthic compartment an experiment was set up. Shore sediment and seawater were collected in Palombina (Ancona, Italy) and equally divided in glass beackers to create microcosms (fig.1). PLA (poly-lactic acid), one of the most produced bioplastic, was added to experimental units in pieces of 1,5 x 1,5 cm. Microcosms were kept at costant temperature of 20°C and with a day/night cycle of 12h in a climatic chamber and sampled every months for a total duration of 5 months (fig.1).

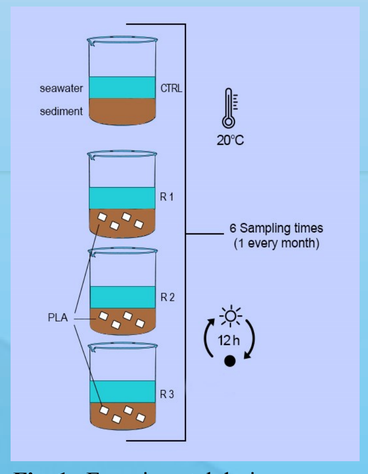


**Fig. 1** Experimental design

### *Methods*

The analyses on sediments included: estimation of prokaryotic abundances (PA),viral abundances (VA), viral production (VP) and EEA (extracellular enzymatic activities), carried out according to [8]. Quantity and quality of organic matter (proteins, carbohydrates, lipids and phytopigments) was estimated spectrophotometrically as in [8]. Bioplastic pieces were weighted before and after the exposure and were analysed through SEM microscopy and FTIR to evaluate the degradation and the evolution of the biofilm associated. DNA was extracted from sediments, water and bioplastic fragment using commercial kits. Metabarcoding analyses for assessing prokaryotic diversity has been carried out by amplifying 16S rRNA genetic marker on high-throughput sequencing platforms.

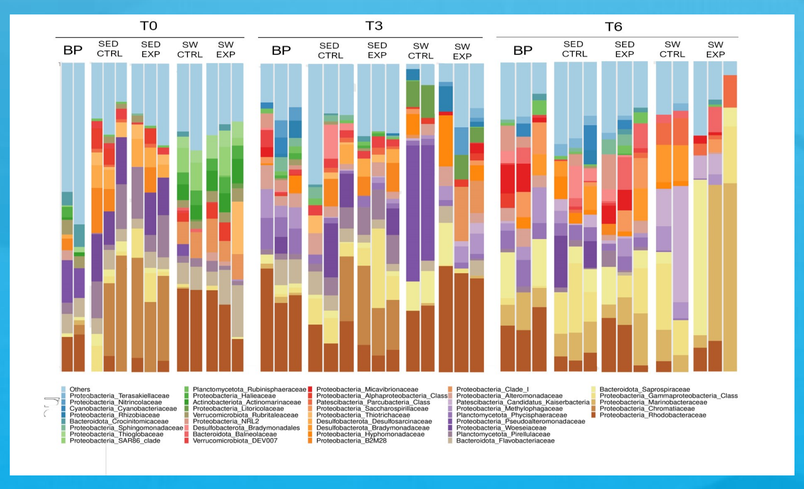


**Fig. 2:** Relative abundance plots of control and PLA-treated microcosms' bacterial ASVs at family level at the beginning of the experiment (T0), after 2 months (T3) and after 5 months (T6). BP: bioplastic; SED: sediment; SW: seawater

### *Task 2*

The Dead Sea reperesents one of the most extreme environment in the world. The basin is characterized by extreme salinity values (approximately 34%), low pH and a unique ionic composition (with Mg+ as main cation) .These features make life almost impossible except for Bacteria, Archea and Fungi. [9] Moreover the Dead Sea is a rapidly changing environment: salinity is expected to increase during the next years mainly due to high evaporation and low regional precipitation [10] and the increase of tourism has already led to the accumulation of litter in some part of the basin. Although some researches have already investigated the microbial diveristy in the basin, it is of fundamental importance to study the Dead Sea area using new and more advanced sequencing techniques and also trying to evaluate the functional role of such extreme microbiomes. To achieve this, sediments, water and salt crystals deposits from the Dead Sea were sampled in 3 different locations (fig. 3) characterized by different levels of pollution in order to investigate the possible effect of the litter in the microbial community. In addition, part of the sediment will be analyzed for the presence of meiofauna and, if present, associated microbiomes will be analyzed to determine the putative advantages that could give to the hosts.

### *Methods*

On sediments collected analyses of biochemical composition of organic matter will be carried out according to [8]. Prokaryotic abundances (PA), viral abundances (VA) and viral production (VP) will be evaluated in sediments and in salt crystal deposits adjusting the protocols given in [8]. In order to evaluate microbiomes diversity DNA extractions, amplification and metabarcoding analyses will be carried out in sediments, water and salt deposits.

### *Task 3*

Marine non indigenous species (NIS) are causing severe ecological and economic impacts worldwide and the Mediterranean Sea is becoming a major hub for the transfer of such invasive species. Moreover, the increase of temperatures due to climate change is accelerating this process with the rate of introductions for the Mediterranean in the 3-year period 2017-2019 of 8 species per year [11]. Among the numerous NIS species (666) in the Mediterranean, ascidians are aggressive competitor for space and resources causing the decrease of native species and thus disrupting the natural ecosystem. Despite the well-known features that help the spread of ascidians (such as high rates of reproduction and production of deterrent substances on the body surface) limited attention has been given to the role of their microbiome in the success of their establishment outside native habitats [12]. Two species of invasive ascidians (present both in the Tyrrhenian Sea and the Adriatic Sea was selected to study its symbiotic microbial community with attention to intraindividual and intraspecific prokaryotic diversity. Additionally, the ascidian associated microbiomes will be investigated also to clarify the positive features that some bacterial taxa may give to the invasive host, contributing to its establishment success.

### *Methods*

Individuals (n=5) of two selected ascidians species will be collected in Thyrrenian Sea and Adriatic Sea along with samples of seawater in the same sampling sites. To analyse the variability of the microbiomes, each ascidian individual will be dissected to separate the inner tunic from which DNA will be extracted and purified as well as from the surrounding waters and sediments using commercial kits. Metabarcoding analyses for assessing prokaryotic taxonomic diversity will be achieved by amplifying 16S rRNA genes on high-throughput sequencing platforms.

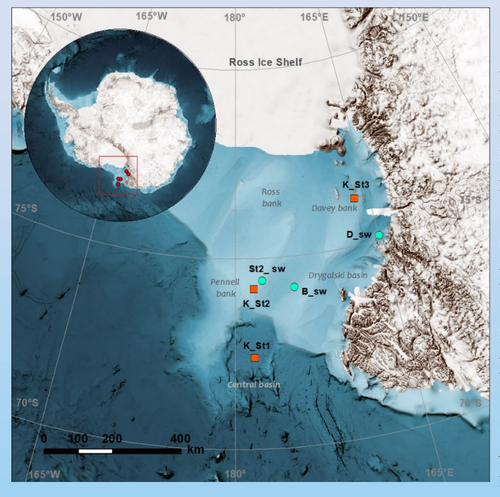


**Fig. 3** Sampling stations of Antarctic krill in January 2022. Two species of antarctic krill were sampled in the Ross Sea Area: *Euphausia superba* and *Euphausia crystallorophias*

### *Task 4*

Antarctic krill are a super-abundant species with a circumpolar distribution and with an important role as keystone species in the Southern Ocean food web. Despite their well-known contribution as a trophic link between Antarctic primary and secondary producers, krill and especially krill-associated microbiota, likely make a substantial contribution to Southern Ocean microbial communities too. In fact, it is known that Antarctic krill support distinct bacterial communities compared to the surrounding seawater, and that each tissue (moult, gut and fecal pellets) represents distinct microhabitats with their own bacterial assemblages [13]. However, the contribution of macro-organisms to marine microbial assemblages is often overlooked and receives limited attention , especially in polar habitats, and additional genomic and metagenomic analyses are necessary to increase the knowledge of krill associated microbiomes and especially their roles in the host physiology . In this context, two krill species (*Euphausia superba* and *Euphausia crystallorophias)* were selected for the study of associated microbiomes in distinct body parts and were sampled, along with seawater, in three different stations located in the Ross Sea (fig. 4), using a low impact plankton net during January 2022.

### *Methods*

To analyze the intra-individual variability of the microbiomes, each krill individual has been dissected to separate different tissues (moult, stomach, gut and photophores). DNA has been extracted and purified from each animal's portion as well as from the surrounding waters using commercial kits. Metabarcoding analyses for assessing prokaryotic and fungal taxonomic diversity have been carried out by amplifying 16S rRNA genes and ITS2 genetic markers on high-throughput sequencing platforms and putative functional layouts of host associated microbiomes have been investigated using FAPROTAX database.

### *Results*

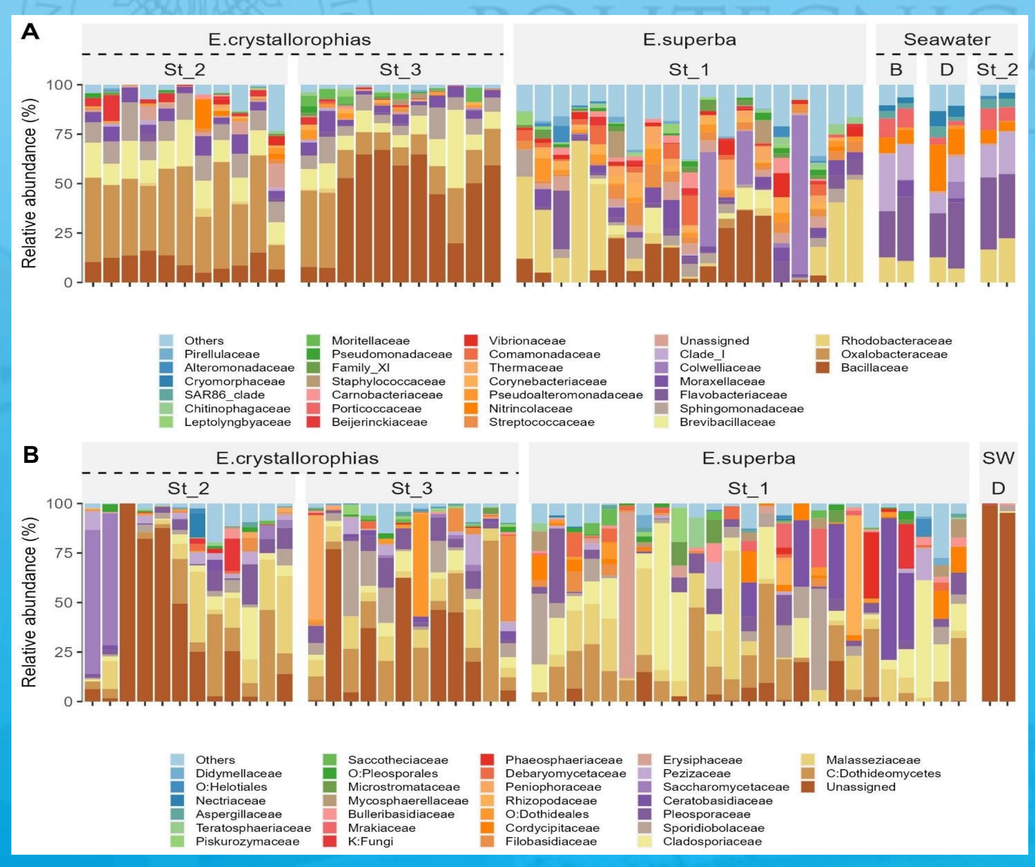


**Fig. 4:** Relative abundance plots of the most frequent bacterial and fungal ASVs at the family level *E. superba*, *E. crystallorophias* and seawater samples. **A**: taxonomy barplots of bacterial communities associated with krill (*E.superba* and *E.crystallorophias* St_2 and St_3) and seawater samples.; **B**: taxonomy barplots of fungal communities associated with krill (*E. superba*, *E. crystallorophias* St_2 and St_3) and seawater samples

**References**

[1] Pollock, F. J., Lamb, J. B., van de Water, J. A., Smith, H. A., Schaffelke, B., Willis, B. L., & Bourne, D. G. (2019). Reduced diversity and stability of coral-associated bacterial communities and suppressed immune function precedes disease onset in corals. *Royal Society open science*, *6*(6), 190355.
[2] Apprill, A. (2020). The role of symbioses in the adaptation and stress responses of marine organisms. *Annual Review of Marine Science*, *12*, 291-314.
[3] Koskella, B., & Bergelson, J. (2020). The study of host–microbiome (co) evolution across levels of selection. *Philosophical Transactions of the Royal Society B*, *375*(1808), 20190604.
[4] Lima, L. F., Weissman, M., Reed, M., Papudeshi, B., Alker, A. T., Morris, M. M., ... & Dinsdale, E. A. (2020). Modeling of the coral microbiome: the influence of temperature and microbial network. *MBio*, *11*(2), e02691-19.
[5] Vijayan, N., Lema, K. A., Nedved, B. T., & Hadfield, M. G. (2019). Microbiomes of the polychaete Hydroides elegans (Polychaeta: Serpulidae) across its life-history stages. *Marine Biology*, *166*(2), 1-13.
[6] Matcher, G. F., Waterworth, S. C., Walmsley, T. A., Matsatsa, T., Parker-Nance, S., Davies-Coleman, M. T., & Dorrington, R. A. (2017). Keeping it in the family: Coevolution of latrunculid sponges and their dominant bacterial symbionts. *Microbiologyopen*, *6*(2), e00417.
[7] Beltrán-Sanahuja, A., Casado-Coy, N., Simó-Cabrera, L., & Sanz-Lázaro, C. (2020). Monitoring polymer degradation under different conditions in the marine environment. *Environmental Pollution*, *259*, 113836.
[8] Danovaro R. (2010) Methods for the study of deep-sea sediments, their functioning and biodiversity. CRC Press, London
[9] Jacob, J. H., Hussein, E. I., Shakhatreh, M. A. K., & Cornelison, C. T. (2017). Microbial community analysis of the hypersaline water of the Dead Sea using high-throughput amplicon sequencing. *MicrobiologyOpen*, *6*(5), e00500.
[10] Georges, O., Fernández, S., Martinez, J. L., & Garcia-Vazquez, E. (2021). DNA metabarcoding illustrates biological pollution threats of Red Sea-Dead Sea water conveyance to Dead Sea biodiversity. *Marine Pollution Bulletin*, *168*, 112451.
[11] Zenetos, A., & Galanidi, M. (2020). Mediterranean non indigenous species at the start of the 2020s: recent changes. *Marine Biodiversity Records*, *13*(1), 1-17.
[12] Goddard-Dwyer, M., López-Legentil, S., & Erwin, P. M. (2020). Microbiome variability across the native and invasive ranges of the ascidian Clavelina oblonga. *Applied and Environmental Microbiology*, *87*(2), e02233-20.
[13] Clarke, L. J., Suter, L., King, R., Bissett, A., & Deagle, B. E. (2019). Antarctic krill are reservoirs for distinct southern ocean microbial communities. *Frontiers in microbiology*, *9*, 3226.