PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

Report Form for PhD student annual evaluation (XXXVI and XXXVII cycles)

Name of PhD student: Simoncini Nicola

Title of PhD research: The role of microbiomes in marine ecosystems

Name of PhD supervisor: Cinzia Corinaldesi, **Co-supervisor**: Antonio dell'Anno
Research lab name: Laboratory of microbial and molecular ecology

Cycle:
[ ] XXXVI
[x] XXXVII

PhD Curriculum::
[x] Marine biology and ecology
[ ] Biomolecular Sciences
[ ] Civil and environmental protection

DISVA instrumentation labs/infrastructure eventually involved in the project:
[ ] Actea Mobile Laboratory
[ ] Advanced Instrumentation lab
[ ] Aquarium
[ ] MassSpec lab
[ ] MaSBiC
[ ] Simulation/informatics lab
[ ] Other. Please, indicate: .....

ABSTRACT (1000 characters, including spaces):

Multicellular organisms live in close association with microbial assemblages, which provide their hosts with strategic functionalities including nutrient supply, defense mechanisms, and metabolic pathways, thus representing an integral component of the host and influencing the whole ecosystem's health. Microbiomes could have a role also in the host-microbe adaptation to extreme environmental conditions and global changes. Although microbiomes may shift over time and within species and habitats, some specific bacterial taxa, potentially important for the health of the organism, can be shared representing a stable core (i.e., any set of microbial taxa characteristic of a specific host or environment). Identifying such a core and the factors shaping microbiomes is of fundamental importance for understanding the role of microbial associations in host adaptability to different environmental conditions (i.e. geographic location, presence of pollutants, and other stressors).

Part 1. Scientific case of the PhD Research (2 to 3 pages, including figures)

- SCIENTIFIC AIMS

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

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

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

- WORKPLAN AND RESEARCH ACTIVITIES

WP1.

Bioplastic is rapidly growing as the main alternative to traditional plastics. Although labelled as completely biodegradable, their biodegradation in natural environment might be slower than in controlled conditions (such as in composting plants) and it could take years to completely disappear [1] thus creating environmental problems as dangerous as those of traditional plastics. To investigate the responses of microbiomes to bioplastic contamination, the biodegradation processes of this polymer, the microbial biofilm formation, and successions a 5-month time-course experiment was performed.

Methods

Shore sediment and seawater were collected in Palombina (Ancona, Italy) and equally divided in glass beakers to create microcosms. PLA (poly-lactic acid), one of the most produced bioplastics was added to the experimental units in pieces (1.5 x 1.5 cm). Microcosms were kept aerated and at constant temperature of 20°C with a day/night cycle of 12h in a climatic chamber and sampled every month for a total duration of 5 months (figure 1). 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 [2]. Chlorophyll-*a* and phaeopigment analyses were carried out according to [3] and the concentrations were reported as $\mu g\ g^{-1}$. The total phytopigments were defined as the sum of chlorophyll-*a* and phaeopigments. Protein, carbohydrate, and lipid contents were determined spectrophotometrically, and their concentrations were expressed as bovine serum albumin, glucose and tripalmitin equivalents. The content of biopolymeric carbon (BPC) was assessed converting into carbon equivalents proteins, carbohydrates and lipids concentrations using the conversion factors of 0.49, 0.40 and 0.75 $\mu g\ C\ mg^{-1}$, respectively, and their sum was defined as the biopolymeric organic carbon. Seawater from each microcosm was analyzed for the estimation of PA, VA and VP. Bioplastic pieces were weighted before and after the experiment to assess the total lost weight and were subsequently analyzed through SEM microscopy and FTIR analysis to evaluate the degradation and the evolution of the biofilm associated to the plastic overtime. DNA was extracted from sediments, seawater, and bioplastic fragments using QIAGEN commercial kits. Metabarcoding analyses for assessing prokaryotic taxonomic diversity will be carried out by amplifying 16S rRNA genetic markers on high-throughput sequencing platforms.

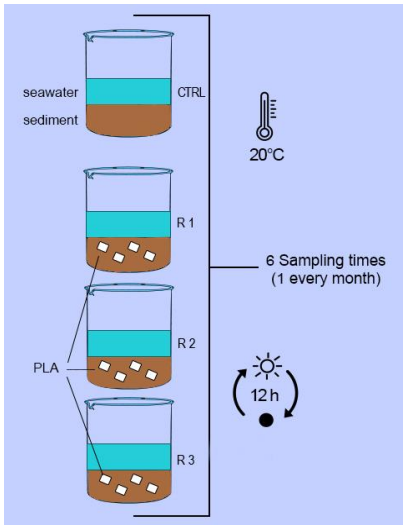

Figure 1: Experimental design. A climatic chamber was used to maintain temperature at 20°C and to have a regular day/ night cycle of 12 hours

Results

Prokaryotic abundances and prokaryotic biomasses are showed in figure 2. Prokaryotic abundances in control sediments varied from $7.56 \pm 0.49 \times 10^7$ cell g^{-1} at the beginning of the experiment (t0) to $1.93 \pm 0.06 \times 10^8$ cell g^{-1} at the end of the experiment (T6, 150 days). The exposed sediments showed a number of prokaryotes of $7.07 \pm 1.07 \times 10^7$ at the t0 and of $2.21 \pm 0.23 \times 10^8$ cell g^{-1} at the end of the experiment showing the highest value after 90 days of exposure with $2.23 \pm 0.16 \times 10^8$ cell g^{-1} (figure 2A). Prokaryotic biomasses increased over time both in the control and in the exposed sediments, with control sediments showing the lowest biomass at t0 (0.96 ± 0.05 µgC g^{-1}) as well as sediments exposed to PLA (0.86 ± 0.16 µgC g^{-1}). The highest value of biomass found in the control sediments (2.89 ± 0.28 µgC g^{-1}) was recorded after 150 days whereas in the exposed sediments, the highest biomass value (2.88 ± 0.43 µgC g^{-1}) was observed after 90 days of exposure.

The composition of the organic matter (expressed as percentage) in the control and exposed sediments are shows in figure 3. In the control sediments (figure **3A**) the percentage of proteins ranged from 57 % (t0) to 37 % (t4) whereas in the exposed sediments (figure **3B**) from 55 % at t2 to 37.5 % at t4. Carbohydrates, instead, showed the highest percentage (38 %) at the end of the experiment (t6) in control sediments and at the t5 in exposed sediments with a percentage of 38.5 %. For what it concerns the total lipids, the lowest percentage was at the beginning of the experiment (t0) in control sediments (16 %) and at t5 in exposed sediments. with a percentage of 7.2 % while the highest percentages were found at t5 in control sediments and at t4 in exposed sediments. The BPC concentrations were estimated for all the sampling times both in control and exposed sediments. In control sediments the amount of BPC varied from 0.15 ± 0.04 mgC g^{-1} at t0 to 0.29 ± 0.10 mgC g^{-1} at t5. In sediments exposed to PLA the biopolymeric carbon content ranged from 0.16 ± 0.01 mgC g^{-1} (t1, after 15 days of exposure) to 0.43 ± 0.30 mgC g^{-1} at t4.

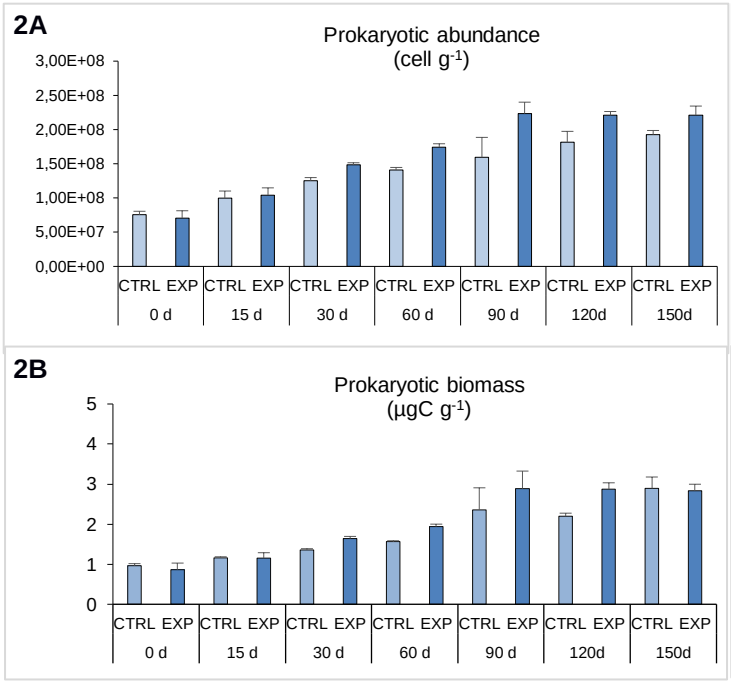

Figure 2 (A, B). 2A: Prokaryotic abundances expressed as cell g^{-1} estimated in both control sediments (light blue) and sediment exposed to PLA (dark blue) for every sampling time (expressed as days from the start of the experiment).
2B: Prokaryotic biomasses expressed as µgC g^{-1} calculated for both control and exposed sediments in every sampling time (expressed as days from the start of the experiment).

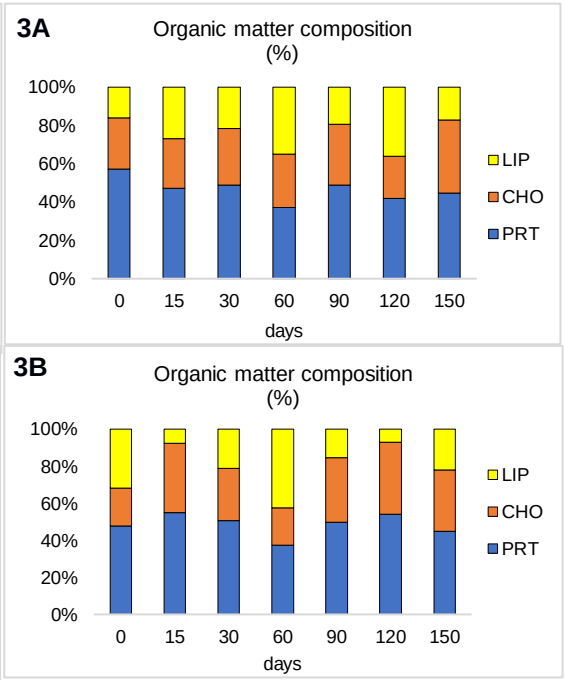

Figure 3 (A, B). Organic matter composition expressed as percentage of proteins, carbohydrates and lipids**.**
3A: Composition of organic matter in control sediments
3B: Composition of organic matter in sediments exposed to PLA

WP2

The Dead Sea represents one of the most extreme environments 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, Archaea and Fungi [4]. 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 [5], and the increase of tourism has already led to the accumulation of litter in some parts of the basin. Although some research have already investigated the microbial diversity 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 collected in 3 different locations 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

The biochemical composition of sedimentary organic matter will be carried out spectrophotometrically according to [2], to investigate the content of proteins, carbohydrates, and lipids. Prokaryotic abundances (PA), viral abundances (VA) and viral production (VP) will be determined in sediments and salt crystal deposits adjusting the protocols reported in [2]. To evaluate microbiome diversity, DNA extractions will be carried out using QIAGEN commercial kits and amplification and metabarcoding analyses will be carried out in sediments, water and salt deposits.

WP3

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 in the Mediterranean with a rate of introduction in the period 2017-2019 of 8 species per year [6]. Among the numerous NIS species (666) in the Mediterranean, ascidians are aggressive competitors 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 [7]. Two species of invasive ascidians (*Styela plicata* and *Ciona robusta*) and one species of native ascidian (*Pyura dura*) present both in the Tyrrhenian Sea and the Adriatic Sea were selected for the study of their symbiotic microbiome focusing on the intraindividual and intraspecific prokaryotic diversity. Additionally, the ascidian-associated microbiomes will be investigated also to clarify the positive features that some bacterial taxa may provide to the invasive host, contributing to its establishment success.

Methods

Individuals (n=5) of selected ascidians species (native: *Pyura dura*, aliens: *Styela plicata* and *Ciona robusta*) were collected in the Adriatic Sea, near the Marina Dorica marina, and in the Tyrrhenian Sea, near the Miseno lake in August 2022. The individuals were immediately preserved in RNA Later to prevent nucleic acids deterioration. Additional individuals (n=2) of each species were then collected and fixed in 4% formalin for the preservation of their inner structures for subsequent SEM analyses. Along with ascidians individuals 5 L of contextual seawater was collected for each sampling site. To analyze the variability of the microbiomes, each ascidian individual will be dissected to separate the inner tunic and the branchial sac from which DNA will be extracted and purified. DNA extractions will be carried out also in the surrounding waters and using QIAGEN commercial kits. Metabarcoding analyses for assessing prokaryotic taxonomic diversity will be achieved by amplifying 16S rRNA genes on high-throughput sequencing platforms.

WP4

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 (cuticle, gut and fecal pellets) represents distinct “microhabitats” with their own bacterial assemblages [8]. However, the contribution of macro-organisms to marine microbial assemblages is often overlooked and receives limited attention, especially in polar habitats, and additional 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 (cuticles, gut, stomach and photophores) and were sampled, along with seawater, in three different stations located in the Ross Sea, using a low impact plankton net during January 2022.

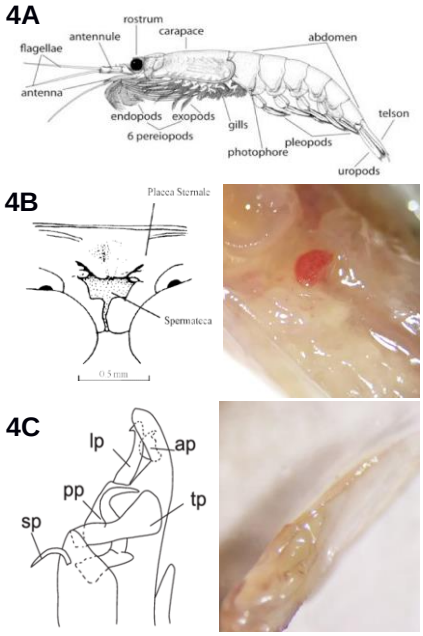

Figure 4 (A,B,C): 4A: *Euphausia superba* bauplan highlighting the main characteristics of krill species. **4B**: Telicum (spermatheca, female organ) of female individual of *Euphausia superba* **4C**: Petasma (male organ) of male individual

Methods

Individuals of *Euphausia superba* and *Euphausia crystallorophias* (n=32 for each species) collected in the Ross Sea were selected considering sub-set of larger individuals with a similar size to facilitate the dissection processes. Then, every individual of each species was observed under a stereomicroscope to discriminate the sex of individual, weighted, and measured with a caliper. Subsequently every individual (n=10) of each species was dissected under a stereomicroscope with sterile forceps and scalpels to collect the cuticle, stomach, gut and photophores (for *Euphuasia superba*) and to collect cuticle and stomach (for *Euphuasia crystallorophias*). DNA from each part of every individual selected was immediately extracted using QIAGEN Blood and Tissue kit and then quantified using Nanodrop spectrophotometer. Metabarcoding analyses for assessing prokaryotic and fungal taxonomic diversity will be carried out by amplifying 16S rRNA genes and ITS2 genetic

markers on high-throughput sequencing platforms. Functional layouts of host associated microbiomes will be provided by shotgun metagenomics of the total DNA on the NexSeq Illumina palatform .

Results

DNA concentrations in *Euphausia superba* cuticles ranged from 1 ng/uL to 23.16 ng/uL (mean 9.3 ± 6.3 ng/uL) whereas the concentrations found in *Euphausia crystallorophias* cuticles were generally lower, ranging from 2.6 ng/uL to 11.66 ng/uL (mean 6.55 ± 6.3 ng/uL). Concentrations of DNA extracted from stomach of *Euphuasia superba* were generally higher than cuticles of the same species (mean 18.68 ± 41.2 ng/uL) as well as the DNA extracted from gut (mean 16.82 ± 34.4 ng/uL) whereas lower DNA concentrations were recorded in photophores (mean 3.49 ± 2.4 ng/uL). DNA concentrations in stomach of *Euphausia crystallorophias* were the lowest with a mean of 0.29 ± 0.2 ng/uL.

- REFERENCES

[1] 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.

[2] Danovaro R. (2010) Methods for the study of deep-sea sediments, their functioning and biodiversity. CRC Press, London

[3] C.Lorenzen, J.Jeffrey (1980) Determination of chlorophyll in seawater. Technical Paper in Marine Science (UNESCO), 35 (1980), pp. 1-20

[4] 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.

[5] 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.

[6] Zenetos, A., & Galanidi, M. (2020). Mediterranean non indigenous species at the start of the 2020s: recent changes. *Marine Biodiversity Records*, 13(1), 1-17.

[7] 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.

[8] 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.

Part 2. PhD student information on the overall year activity (courses/seminars/schools, mobility periods, participation to conferences)

List of attended courses/seminars/schools

1. Technology transfer and innovation A.A. 2021/2022

2. Design of research A.A. 2021/2022

List of periods spent abroad

1.

2.

List of conferences/workshops attended and of contributions eventually presented

1.

2.

Part 3. PhD student information on publications

If not yet published, please indicate the publication status (submitted, accepted, in preparation...)

List of publications on international journals

J1. Corinaldesi, C., Bianchelli, S., Rastelli, E., Varrella, S., Canensi, S., Gambi, C., ... & Dell'Anno, A. (2022). The paradox of an unpolluted coastal site facing a chronically contaminated industrial area. *Frontiers in Marine Science*, *8*, 813887.

J2. Baldrighi, E., Correa, M.L. , Donnarumma, L., Gambi, M.C., Ferrigno, F., Simoncini, N., Appolloni, L., … & Semprucci, F. (2022). First insight into meiofauna inhabiting the shallow CO_2-seeps around Castello Aragonese (Ischia, Italy) IN PREPARATION !

List of publications on conference proceedings

C1. ...

C2. ...

List of other publications (books, book chapters, patents)

B1. ...

B2. ...

13-10-2022

Student signature

Supervisor signature