PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

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

Name of PhD student: Anna Salvatori

Title of PhD research: Assessing sponge-microbiome acclimatization to changing environmental conditions through reciprocal transplantation

Name of PhD supervisor: Antonio Dell'Anno (DISVA), Laura Nunez Pons (SZN)
Research lab name: Microbial and Molecular Ecology Lab

Cycle:
[ ] XXXVII
[x] XXXVIII

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):

Marine sponges are ecologically important components of the benthic communities that provide habitats for various species and mediate nutrient recycling and benthopelagic coupling. They are mostly filter-feeders heterotrophs, with some exceptions (i.e. mixotrophic, phototrophic). Despite their simple anatomical characteristics, the sponges have been deeply studied as models of holobiont systems (complex consortia comprising the host, its associated microbiome, and their interactions). The intimate microbial partnerships enlarge their metabolic arsenal allowing them to exploit different pools of nutrients and to obtain UV-protection and chemical defence. It has been hypothesized that sponges may be "winners" in future global change scenarios compared with other benthic invertebrates. The purpose of this PhD research is to reveal the phenotypic strategies of acclimatization in Porifera in relation with different environmental scenarios related to climate change and anthropogenic impacts, investigating microbial dynamics, trophic patterns, and metabolic profiles.

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

- BACKGROUND

The dispersive capacity of marine sponges has been attributed to 1) a well-developed aquiferous system that affords efficient filter feeding on living particulate organic matter POM (phytoplankton and bacteria), detritus, and dissolved organic matter DOM; 2) the intimate association with microorganisms that enlarge the metabolic

arsenal, supplementing host heterotrophic diet with photosynthates or DOM re-cycling products and supplying allelochemicals as UV-protectants and chemical defences (Freeman et al., 2021).
Porifera host a wide variety of microorganisms comprising 52 phyla and candidate phyla across Bacteria, Archaea, and Eukarya domains and taxa including Gammaproteobacteria, Alphaproteobacteria, Chloroflexi, Thaumarchaeota and Cyanobacteria. According to their microbial densities, sponges can be divided into two general groups: HMA (high microbial abundance, with 10^8–10^{10} cells per gram of sponge), and LMA (low microbial abundance, with 10^5–10^6 cells per gram of sponge) (Thomas et al., 2016).
Marine sponges reveal a continuum of trophic strategies ranging from photosynthetic, mixotrophic, and heterotrophic feeding, which largely depend on the metabolic exchanges with their symbiotic microbes. Photosynthetic hosts often contain cyanobacterial cells or Symbiodiniaceae dinoflagellates that may supplement heterotrophic feeding with inputs of fixed carbon, while the presence of certain prokaryotic taxa allows the exploitation of different heterotrophic carbon (POM, detritus, or DOM) pools (Freeman and Thacker, 2011; Rix et al., 2020).
Plasticity in symbiotic microbial communities, and/or in metabolic functionalities and nutrient utilization may allow sponges to rapidly adapt to changing environmental conditions (Kiran et al., 2018). Human activities are modifying the marine environment and some of the major stressors threatening the ocean are climate change (ocean warming and acidification), and the deterioration of water quality. It has been hypothesized that sponges may be "winners" in future global change scenarios compared with other benthic invertebrates. This is because fast microbial generation rates, community reorganization, and vertical transmission of symbionts can contribute to microbiome-mediated acclimatization and even adaptation (Bell et al., 2018; Glasl et al., 2018). Nevertheless, the metabolic interactions between the microbiome and the host remain largely unknown.

- SCIENTIFIC AIMS

The scope of the present research is to acquire a holistic comprehension of the acclimatization potential of the sponge holobionts in future forecasted environmental scenarios. In particular, the main research objectives are:
1. to investigate microbiome diversity associated with different sponge species reciprocally transplanted in diverse environmental conditions (in terms of acidification, pollution, and light vs dark conditions);
2. to disclose the trophic interactions between microbial symbionts and hosts under different perturbating scenarios;
3. to correlate the microbial dynamics with trophic patterns and metabolic profiles of sponge holobionts exposed to different environmental conditions

- WORKPLAN AND RESEARCH ACTIVITIES

WP 1. Objective. Development of transplantation methods

Methods. Three sponge species commonly inhabiting Mediterranean hard bottom substrates have been selected on the basis of the feasibility for transplants and their trophic strategy:

1. *Petrosia ficiformis* – HMA sponge with spicules and spawning reproduction. The trophic strategy is mixotrophic to heterotrophic.

2. *Chondrosia reniformis* – HMA sponge with lack of spicules and spawning reproduction. The trophic strategy is largely heterotrophic.

3. *Chondrilla nucula* – HMA sponge with spicules and spawning reproduction. The nutritional strategy is mostly photosynthetic (it establishes trophic associations with cyanobacteria).

Three study sites and respective control areas for reciprocal transplantation have been selected (paired experimental settings):

1. Site Grotta Punta Vico, at the Island of Ischia (Naples) for acclimatization to light vs dark.
2. Site Grotta Del Mago, at the Island of Ischia (Naples) for acclimatization to low vs normal pH.

3. Site Bagnoli Bay (Naples) for acclimatization to polluted vs non-polluted conditions

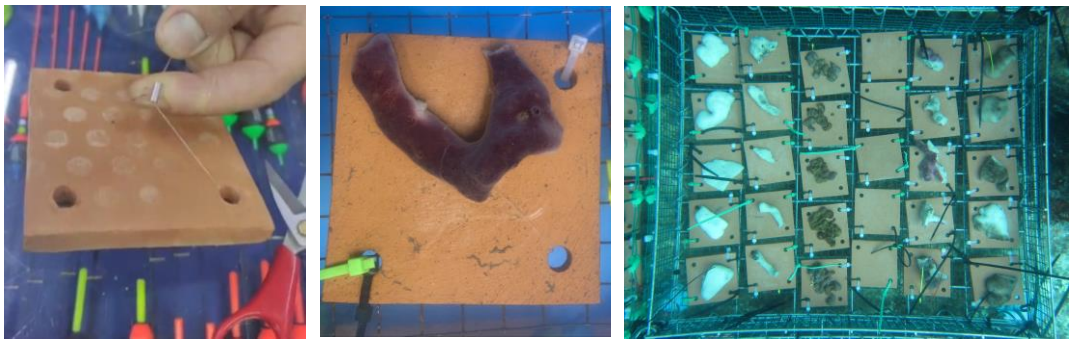
Before field experiments, preliminary studies have been conducted in aquaria to select fixation tools and substrates for the sponge attachment. In particular, different attachment tools (cable ties, fishing line, and free attachment) and different substrates (porphyry and clay tile) with "smooth" or "rough" surfaces have been tested.

Cross and reciprocal transplants of the three sponge species in the first two study areas were carried out in June 2023. Some of these transplants were recovered in September 2023 (after ca. 2.5 months), whereas others will be collected in November 2023 (i.e., after 5 months). Donor specimens (~30 cm^3 n = 5) of the three target species were collected by scuba diving, at each of the target sites and then divided into 7 clonal replicates (~8–10 cm^3). One clonal replicate has been processed immediately while the remaining 6 clones have been used for reciprocal and cross transplants.

Expected/Obtained Results.

By comparing in aquaria clonal grafts and intact individuals (not cut) we found that the clonal grafts heal rapidly (about 1 – 2 days) showing no differences with intact individuals after a few days. Moreover, we observed that the fishing line is the right compromise between the free attachment and the cable ties to secure the sponge to the substrate. We also found that raw clay tiles, not subjected to industrial processing are good substrates for sponges and that a "rough" surface guarantees a quicker attachment.

In the field, we collected the transplants in September 2023 in two study areas: Grotta del Mago and Grotta Punta Vico with a sufficient number of replicates to evaluate the acclimatization after 2.5 months. An additional collection of the transplants is planned for November 2023, but not for all the sponge species (because of mortality). The experiment in the third study area (Bagnoli Bay) will be instead performed in April 2024.



WP1. A) Fishing line as a tool to attach the sponge clones to the tiles; B) Example of a clone of *Petrosia ficiformis*; C) Example of panels disposition in the field.

WP 2. **Objective.** Characterization of the microbiome associated with different sponge species subjected to reciprocal transplantation.

Methods. DNA from frozen sponge samples and 0.22 µm polycarbonate membranes (seawater microbes) have been extracted with DNeasy PowerSoil Kit (QIAGEN) and the DNeasy PowerWater Kit, respectively. The extracts will be submitted for MISeq amplicon sequencing targeting the hypervariable V4 of 16S rRNA for Bacteria/Archaea. Bioinformatics analyses will be run on QIIME2, and statistics on R.

Expected/Obtained Results. We expect to broaden knowledge on the microbiome diversity associated with these marine sponge species exposed to different environmental conditions. Particularly, we expect a shift in the microbiome composition which can favour the sponge acclimatization.

WP 3. Objective. To disclose the trophic interactions between microbial symbionts and hosts and to correlate the microbial dynamics with trophic patterns and metabolic profiles of sponge holobionts exposed to different environmental conditions.

Methods. SIA (stable isotopic analyses) will be applied to determine the trophic interactions of host and symbiont cells. Sponge samples from -20ºC will be thawed and submitted to cellular separations by centrifugation and gravitational density separation using Ca2+ and Mg2+ free artificial seawater, to separate larger host sponge cells from smaller prokaryotic cells. Cellular fractions will be dried at 60ºC and submitted for 13C and 15N isotopic analyses, along with the 0.7 µm GF/F filters from seawater samples (serving as baseline DOM/POM resources). Statistical and graphical analyses will be done in R.

Concerning the metabolic profile, frozen sponge samples will be lyophilized and extracted, along with Ix/Ex fluxes and sponge-derived POM (PeriSIP), with methanol-based protocols for a) untargeted and b) targeted metabolomics; and c) quantification of specific metabolites (e.g., pigments, UV-absorbing products, other secondary metabolites,) applying chromatographic techniques (e.g., GC-MS, GC/LC-IMRS).

The functional profile of the microorganisms associated with the sponges will be assessed through metatranscriptomic or metagenomic analysis by the application of physical enrichment methods before sequencing or rRNA depletions in case of metatranscriptomics.

Expected/Obtained Results. To shed light on the implications of microbiomes in metabolism and nutrition of the sponges and to identify phenotypic traits of holobiont acclimatization under diverse natural environmental scenarios.

- REFERENCES

Bell JJ, Rovellini A, Davy SK, Taylor MW, Fulton EA, Dunn MR, et al. (2018). Climate change alterations to ecosystem dominance: how might sponge-dominated reefs function? Ecology 99, 1920–1931. doi: 10.1002/ecy. 2446

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Freeman, C. J., and Thacker, R. W. (2011). Complex interactions between marine sponges and their symbiotic microbial communities. Limnol. Oceanogr. 56, 1577–1586. doi: 10.4319/lo.2011.56.5.1577

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Kiran GS, Sekar S, Ramasamy P, Thinesh T, Hassan S, Lipton AN, Ninawe AS, Selvin J. Marine sponge microbial association: Towards disclosing unique symbiotic interactions. Mar Environ Res. 2018 Sep;140:169-179. doi: 10.1016/j.marenvres.2018.04.017

Rix, L., Ribes, M., Coma, R., Jahn, M. T., de Goeij, J. M., van Oevelen, D., et al. (2020). Heterotrophy in the earliest gut: a single-cell view of heterotrophic carbon and nitrogen assimilation in sponge-microbe symbioses. ISME J. 14, 2554–2567. doi: 10.1038/s41396-020-0706-3

Thomas, T., Moitinho-Silva, L., Lurgi, M., Björk, J. R., Easson, C., Astudillo- García, C., et al. (2016). Diversity, structure and convergent evolution of the global sponge microbiome. Nat. Commun. 7, 1–12. doi: 10.1038/ncomms 11870

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. Course: Design of Research (European Projects) – Prof. Nicola Paone

2. Course: Technology Transfer and Innovation – Prof. Donato Iacobucci

3. Course: Microbial-mediated processes in aquatic ecosystems: from basic to applied research toward solving environmental problems – Prof. Antonio Dell'Anno

4. Course SZN: Presenting with Zen - Marco Signore

5. Course SZN: Communicating science: why, to whom and how - Marco Signore

6. Course SZN: Statistics – Priscilla Licandro and Marco Uttieri

7. Course SZN: New perspectives in marine biotechnology - Donatella De Pascale

8. Seminar: "Evolution of epithelial organization from sponges to human" Prof. André Le Bivic of the Institut de Biologie du Développement de Marseille (IBDM).

9. Seminar: "A Green Vision for Future Algal Biotechnology Innovations and Applications" Prof. Kyle J. Lauersen of the Biological and Environmental Sciences and Engineering Division (BESE) King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia.

10. Seminar: "Thoughts on plankton biodiversity, succession, and mechanisms of coexistence" Prof. Michael J Behrenfeld (Department of Botany and Plant Pathology, Oregon State University).

11. Seminar: "Can algae support our transition to a sustainable bioeconomy?" Prof. Mathieu Pernice (Deputy Director, Climate Change Cluster, University of Technology Sydney).

List of periods spent abroad

1. Oceanographic campaign in the Aegean Sea (three weeks)

List of conferences/workshops attended and of contributions eventually presented

None.

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. Investigating the Antarctic krill bacterial micro- and mycobiome (in preparation)

J2. Polar microbiome (in preparation)

J3. Marine microbial successions on prototypes of offshore oil and gas platforms under mineral accretion technology (in preparation)

List of publications on conference proceedings

None.

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

None.

[13/10/23]

Student signature

Supervisor signature