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

Effect of ocean acidification on changes in the microbiota of coastal habitats: focus on macroalgae, water column and the sea urchin *Arbacia lixula*

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Background

Echinoderms play a crucial ecological role in marine benthic ecosystems by shaping plant and animal community dynamics and functioning as primary consumers of algal biomass and detrital material. Among them, the sea urchin Arbacia lixula, widespread throughout the Mediterranean Sea, is characterized as a herbivore or opportunistic omnivore, predominantly feeding on algae and seagrasses (Liu et al., 2024). The term "microbiota" refers to the assemblage of microorganisms residing within a specific biological or environmental niche. In sea urchins, these microbial communities are integral to the modulation of ecological interactions and host metabolic functions (Rodríguez-Barreras et al., 2021). Anthropogenic stressors such as ocean acidification—frequently co-occurring with ocean warming—can exert additive, synergistic, or antagonistic effects on microbial community composition and functionality (Brothers et al., 2018). Investigating microbiome diversity across seawater pH gradients and its interaction with host organisms is essential for elucidating ecosystem-level responses to global climate change. This study contributes to an ongoing multidisciplinary research project focused on assessing the effects of ocean acidification on the ecological, physiological, and behavioral dynamics of organisms inhabiting acidified marine coastal environments.

Fig.1 ***Arbacia lixula*** in sand-rocky area

Fig. 2. **Castello Aragonese of Ischia**. To the right of the bridge, the southern part

Tasks

- Assess the taxonomic composition of microbiomes across varying seawater pH conditions.
- Investigate microbiome diversity among different matrices (seawater, primary producers, herbivores) .
- Evaluate functional shifts in microbial communities under varying seawater pH regimes.

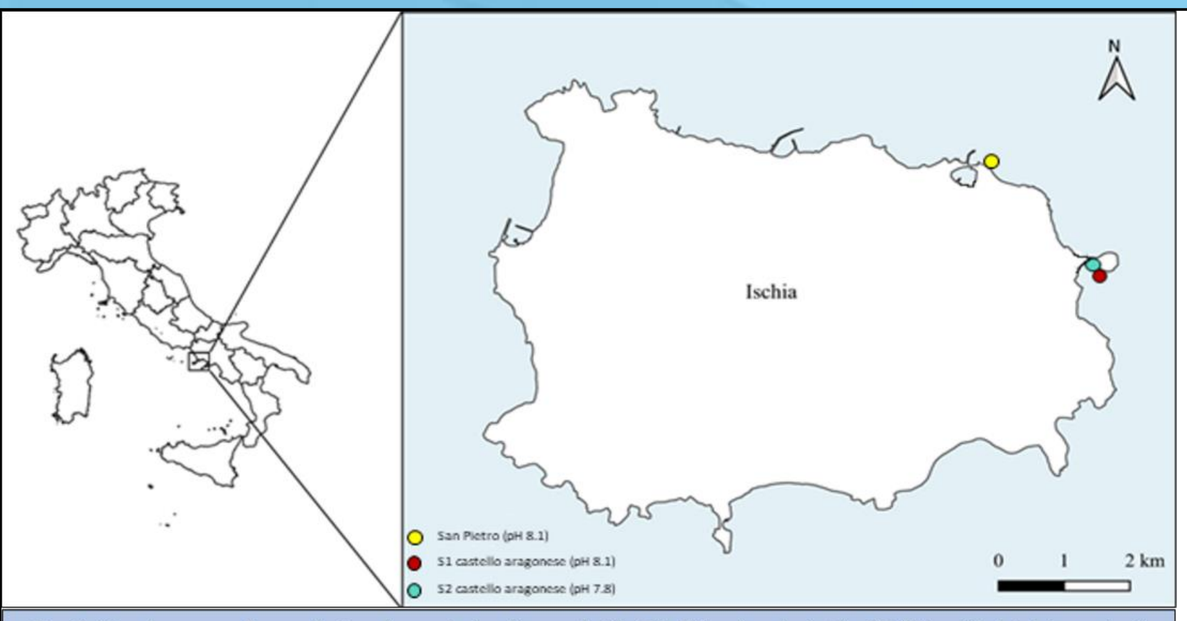

Fig.3 Study **area.San pietro beach** (yellow dot); **S1 Site** (red dot); **S2 Site** (light blue dot)

Study Area

Sampling was conducted around Ischia Island (Campania, Italy).
Three sites were chosen as control and acified locations:

- **San Pietro beach**.Sand-rocky area in the north-east part of the island. The mean pH is 8.1, control site.
- **S1 site**.Sand-rocky area sited in the southern part of the Castello Aragonese. The mean pH is 8.1, control site.
- **S2 site**.Sand-rocky area sited in the southern part of the Castello Aragonese. The mean pH is 7.8, acified site.

Methodologies

Sample Collection and Processing

A total of 103 samples were collected from four matrices: A. lixula gut, macroalgae, interstitial water, and water column. Samples were collected by professional SCUBA divers, with water matrices filtered through 0.22 µm sterile filters. All samples were preserved in RNAlater™ to prevent nucleic acid degradation.

DNA Extraction and Quantification

Genomic DNA was extracted using three commercial kits, and quality/quantity assessed via DeNovix DS-7 and Qubit 4 spectrophotometer and fluorimeter

Metabarcoding and Sequencing

Bacterial and archaeal diversity will be analyzed through 16S rRNA gene metabarcoding (V3–V4 region), using universal primers 341F and 805R (Klindworth et al., 2013; Apprill et al., 2015) and sequenced using a high-throughput Next Generation Sequencing (NGS) platform.

Bioinformatics

Data will be processed using QIIME 2 for quality control, taxonomic assignment, and diversity analysis.

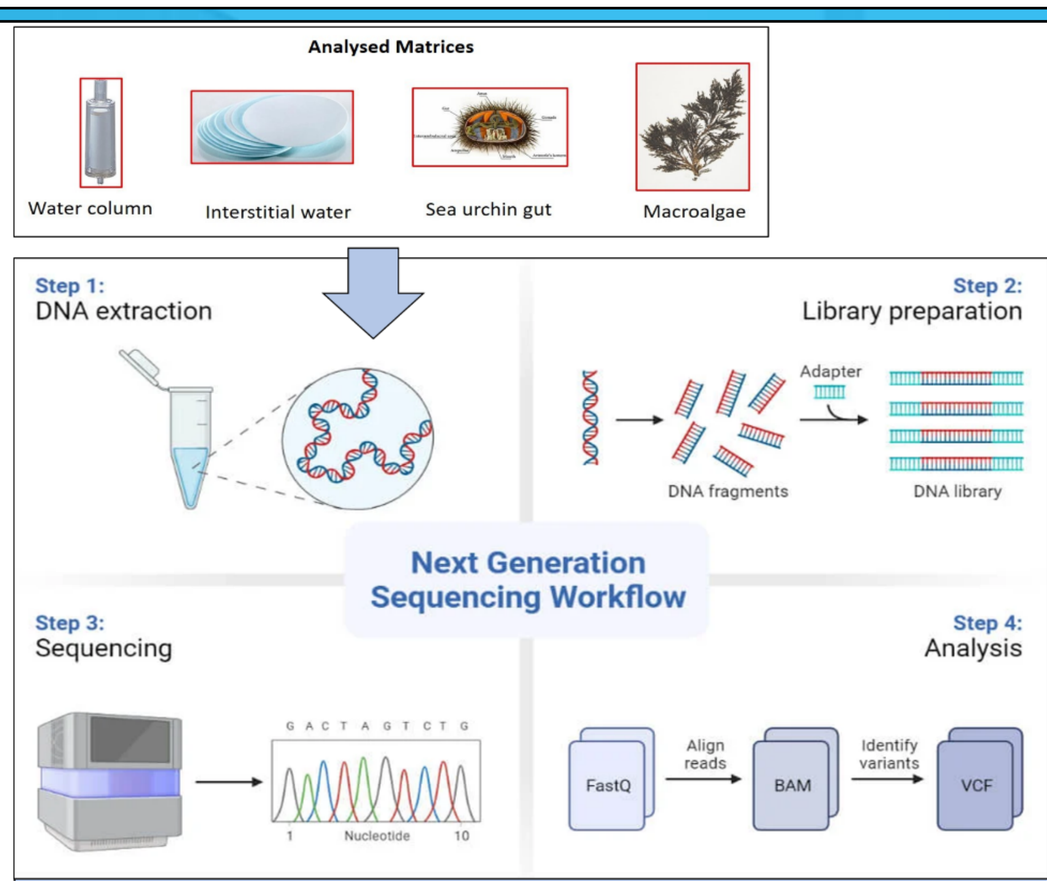

Fig.4 **Next generation sequencing** workflow for metabarcoding analyses

Expected Outcomes

- Evaluate microbiome taxonomical diversity among different pH scenarios.
- Characterize microbiome diversity across distinct sample matrices within various coastal habitats
- Investigate alterations in microbial functions in response to environmental pH variation

Citations

- Apprill, A., McNally, S., Parsons, R., & Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquatic microbial ecology*, *75*(2), 129-137.
- Brothers, C. J., Van Der Pol, W. J., Morrow, C. D., Hakim, J. A., Koo, H., & McClintock, J. B. (2018). Ocean warming alters predicted microbiome functionality in a common sea urchin. Proceedings of the Royal Society B, 285(1881), 20180340.
- Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic acids research*, *41*(1), e1-e1
- Liu, Z., Guo, Y., Qin, C., Mu, X., & Zhang, J. (2024). High-Throughput Sequencing Analysis Revealed a Preference for Animal-Based Food in Purple Sea Urchins. Biology, 13(8), 623.
- Rodríguez-Barreras, R., Tosado-Rodríguez, E. L., & Godoy-Vitorino, F. (2021). Trophic niches reflect compositional differences in microbiota among Caribbean sea urchins. PeerJ, 9, e12084.