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

MicrObiomeS of mArine Copepods in coastal ecosystems (MOSAIC)

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Introduction

Copepods (Arthropoda) are the most important micro-metazoans in terms of abundance, biomass, and diversity in the global ocean[2]. These small crustaceans transfer energy and organic material from phytoplankton primary producers to animals of higher trophic levels, such as planktonic fish and carnivorous invertebrates, thus representing essential food web components (Fig.1). Copepods can also contribute to the microbial loop through the release of nutrients and DOM from fecal pellets, and the decomposition of their moults and carcasses, with an important contribution to biogeochemical cycling and carbon sequestration[4]. There is evidence that associations between copepods and microbes are pervasive (Fig.2), and that the microbiome diversity change with environmental conditions, and among different genera[3,5,6]. The symbiotic bacteria can provide important functions being involved in the processes of host digestion, uptake of nutrients, reproduction, immune response, and other defences[1]. Some bacteria, typically associated with marine copepods, produce chitinases and are able to utilize chitin as a source of carbon and nitrogen. This suggests that some bacteria have adapted to growth in association with the copepods influencing their well-being.
Understanding the diversity of the microbiome and how it interacts with the copepods their lifetime is essential to better understand the functioning of the marine ecosystems also in the light of current global change scenarios.

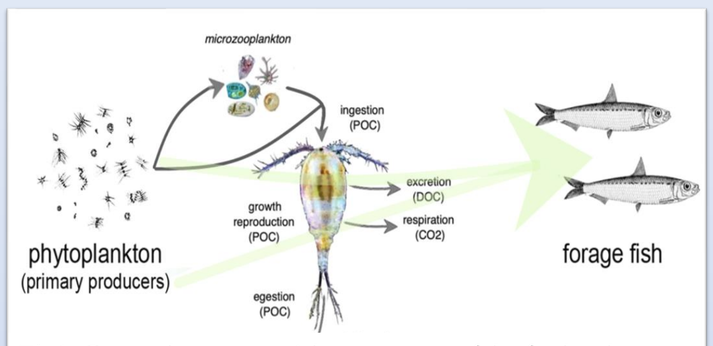

Fig1: Copepods are essential components of the food web

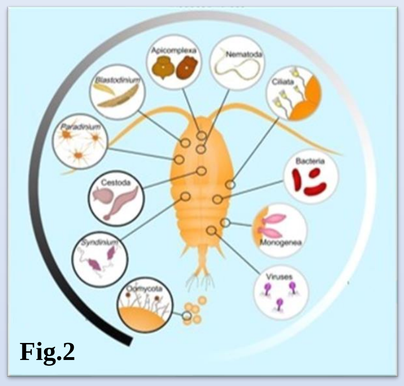

Fig.2

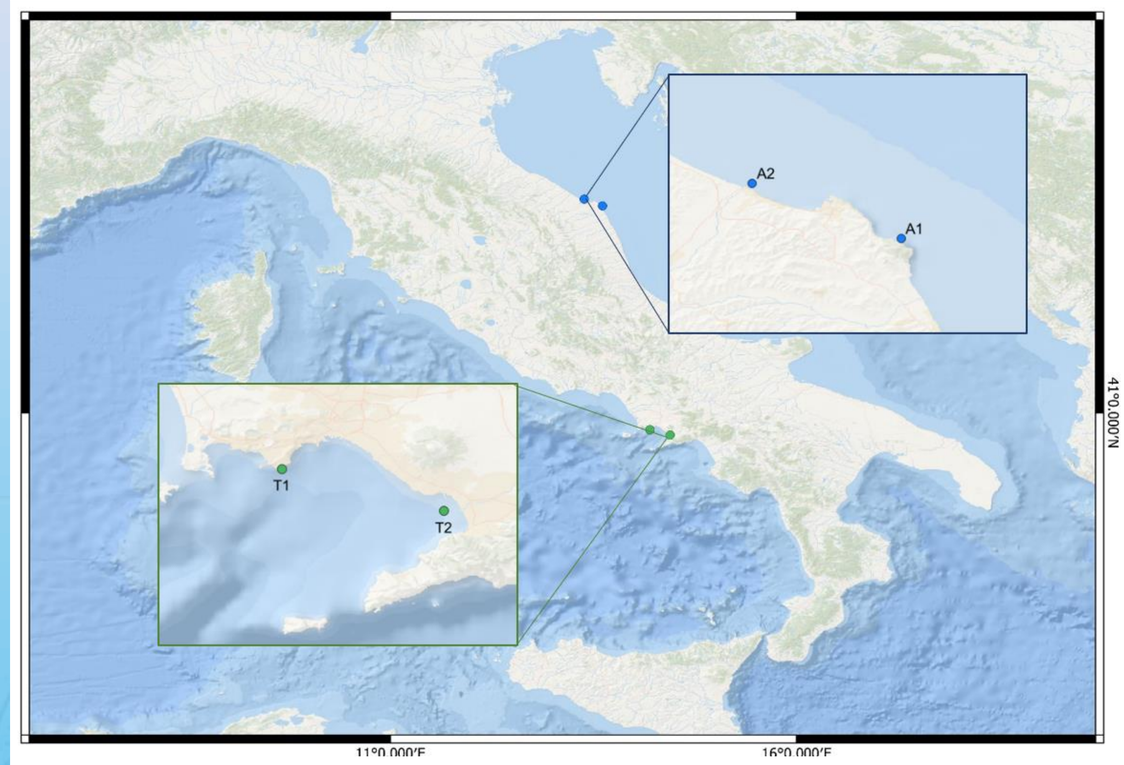

Fig.3: A: Adriatic coast (**A1**: Portonovo bay; **A2**: Esino river); **T**: Tyrrhenian coast (**T1**: LTER- Mare Chiara ; **T2**: Sarno river)

Tasks

1. Exploring the microbiome diversity of different copepod species inhabiting different Mediterranean coastal ecosystems
2. Assessing interactions and functions of the microbial assemblages of different copepods with different trophic strategies
3. Investigating microbiome variability according to the hosts' sex and life stages
4. Assessing the functional roles of microbiomes that can confer abilities to the host to cope with human-driven impacts.

Sample collection and laboratory methods

The study areas identified for the analysis of the copepod microbiomes are located in the coastal Adriatic Sea and the Tyrrhenian Sea (**TASK 1**) (Fig. 3).
In particular, sites with high anthropogenic impact (river mouths) and control sites were compared to identify changes in the microbiome composition and putative functions **(TASK 4).**
The sampling strategy consists of 1-year sampling in relation to copepod seasonal patterns (Fig.4). This will make it possible to identify changes in the microbiome in relation to seasonality. Planktonic copepods will be collected using a WP2 net (200-µm mesh). Sorting of copepods will be carried out under a microscope to identify them at species level. A sub-set of specimens would be stored for SEM analyses to visualize the presence and location of bacteria in the copepod body.

Target species

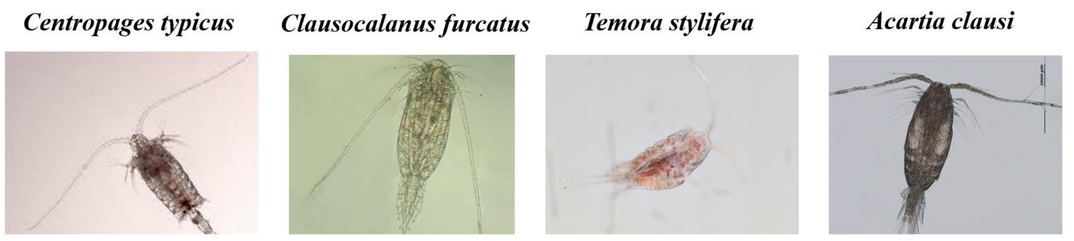

Fig.4

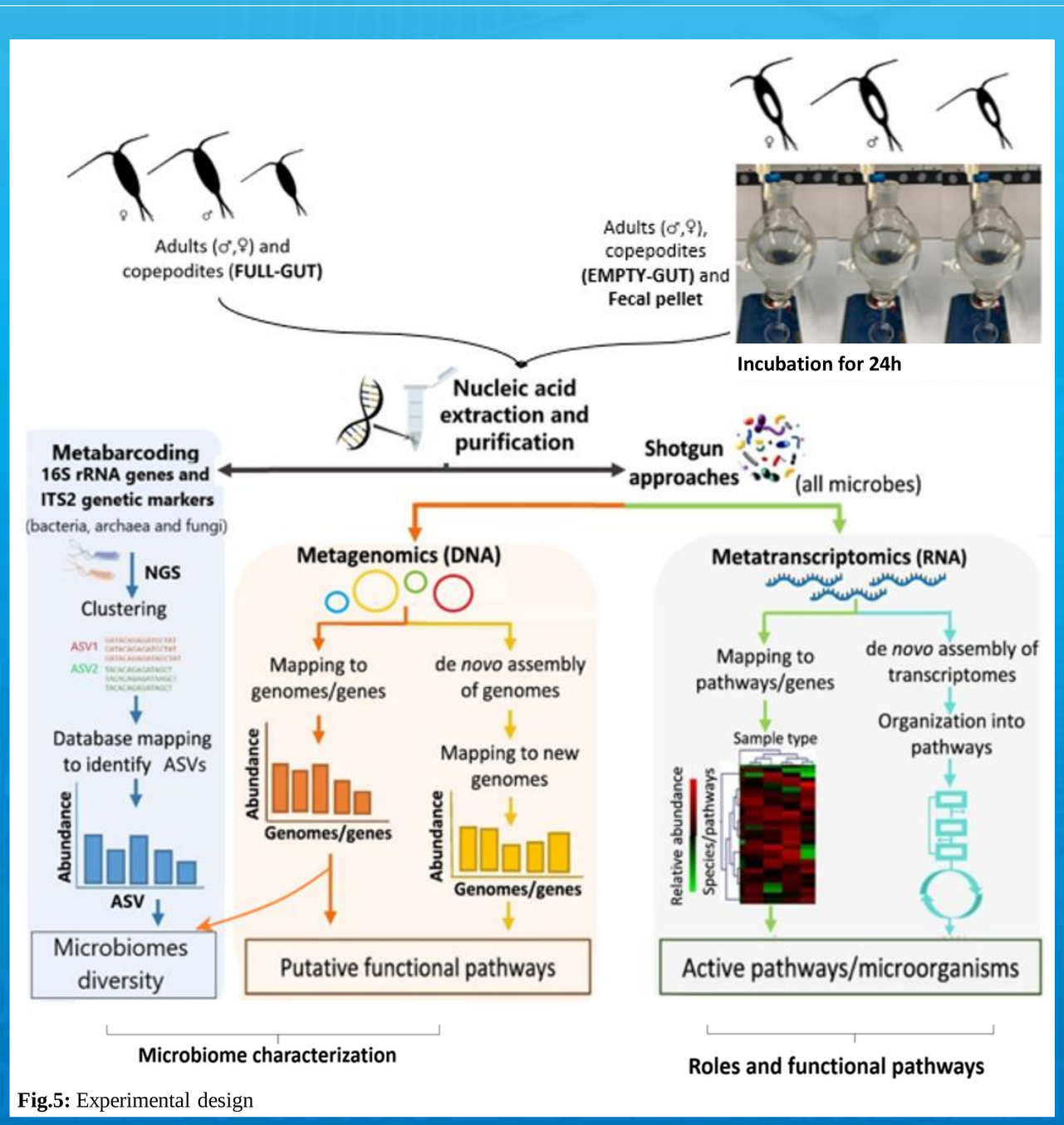

Fig.5: Experimental design

To analyze the diversity of the microbiomes among the different species and the host's sexes, DNA/RNA will be extracted and purified from the specimens using commercial kits. 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 (Illumina Miseq).
To identify the trophic sources of the copepods and their food-related microbiome, the fecal pellets will be collected (Fig.5) and analyzed using different procaryotic and eukaryotic gene markers (**TASK 2).**
The origin of the microbiome will be studied by analyzing the microbiome of the surrounding waters contextually to the copepod microbiome in different life stages (**TASK 3**). To investigate the host-microbiome coevolution, copepods will be reproduced in the laboratory in order to identify the microbiome at different life stages (eggs, nauplii, copepodites, adults). Metagenomic analyses will be also carried out in selected samples to identify Metagenomic Assembled Genomes (MAGs) and putative functions. Metatranscriptomics will be performed to investigate potential metabolic pathways and proteins in order to infer the realized functional potential of the selected microbiomes (Fig.5).

Expected results:

1. **To expand the knowledge of the microbiome diversity associated with different copepod species inhabiting different marine ecosystems**
2. **To identify patterns and functional roles of microbiomes associated with copepods at different life stages and with different trophic strategies**
3. **To explore host-microbiome relationships and their potential changes in relation to different environmental conditions and anthropogenic impacts**

References:
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2. **Benedetti**, et al. "Copepod functional traits and groups show divergent biogeographies in the global ocean". Journal of Biogeography (2022)
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4. **Turner** "Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump." Progress in Oceanography 130 (2015)
5. **Datta**, et al. "Inter-individual variability in copepod microbiomes reveals bacterial networks linked to host physiology." The ISME journal (2018)
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