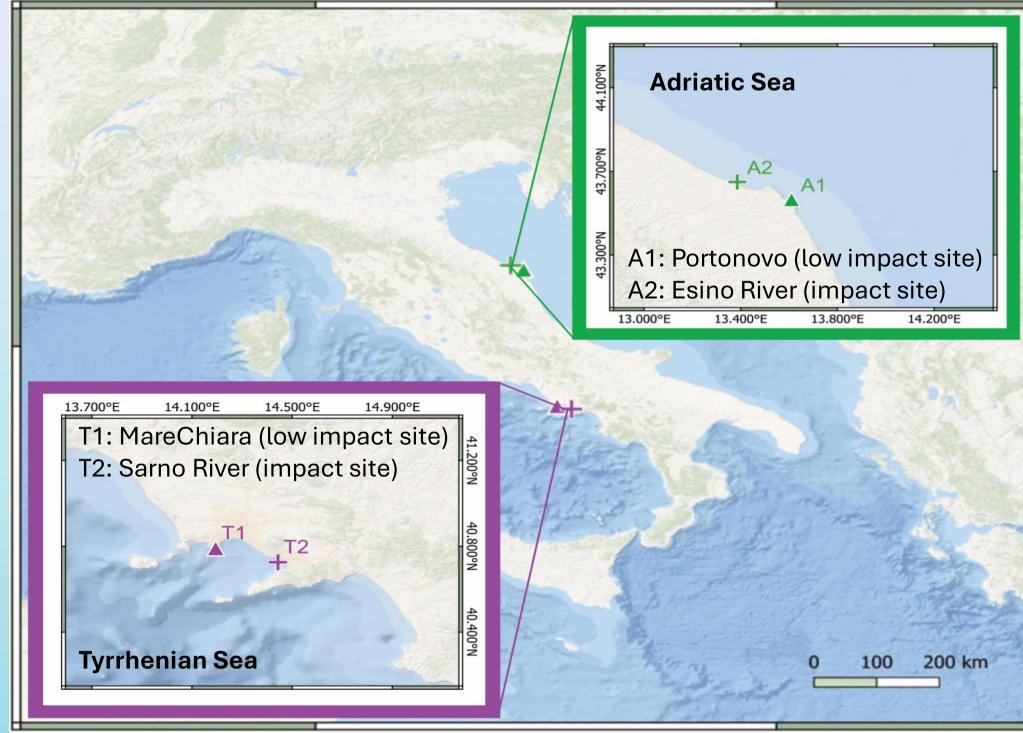
# Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente, Ciclo XXXVIII Microbiomes of marine copepods in coastal ecosystems Agnese Fumanti

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### INTRODUCTION

The associations between marine invertebrates and microbes are widespread throughout the oceans. Microbiota can play crucial roles in the digestion, nutrient uptake, reproduction, immune response, and defense mechanisms of almost all marine animals, thus influencing their health and well-being<sup>1,5</sup>. So far, information about the copepod microbiome is limited although copepods represent key components in the marine food webs<sup>4,6</sup>. Knowledge of the copepod-microbe associations is essential to understanding ecosystem functioning and this is even more important in the context of ongoing global change and anthropogenic impacts<sup>2,3</sup>, which could modulate diversity and functions of the holobiont.



TASK 🍌

1.

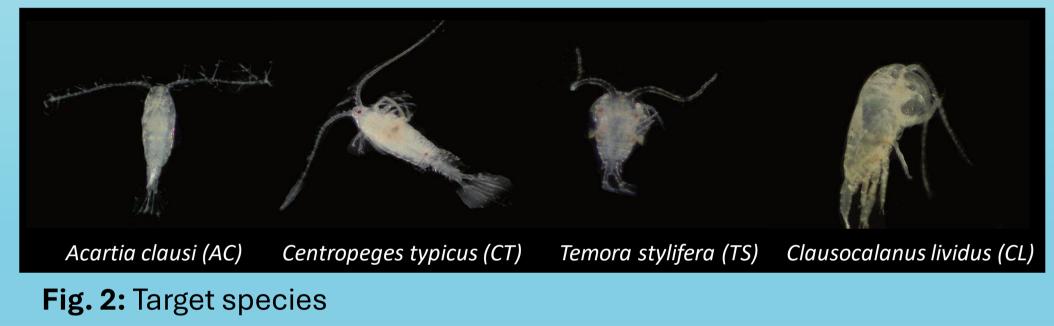
Exploring the microbiome diversity of different calanoid copepod species Assessing its potential changes under different anthropogenic impact conditions

2.

#### **MATERIALS AND METHODS**

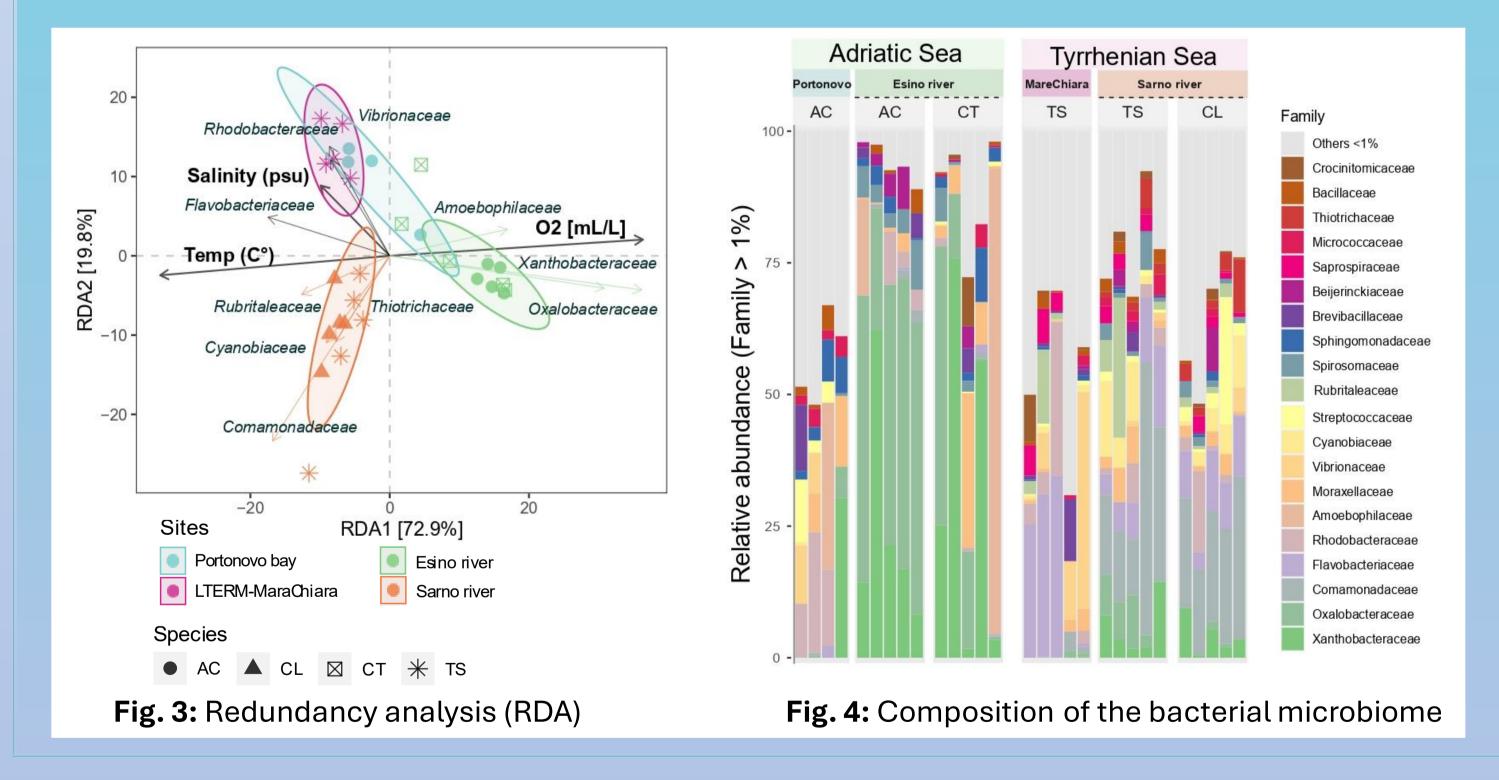
Planktonic copepods were collected using a WP2 net (200-µm mesh) in the coastal Adriatic Sea and the Tyrrhenian Sea in two sites each, with different human impact levels (Fig. 1). Dominant copepod species were selected and identified under the stereomicroscope (Fig. 2). DNA was extracted and purified from the specimens to analyze the diversity of the microbiomes. Metabarcoding analyses for assessing microbiome diversity were carried out by amplifying 16S rRNA genes on high-throughput sequencing platforms (Illumina MiSeq). Bioinformatics was run on QIIME2 and statistical analyses were performed using R with specific packages.

Fig. 1 Map showing the sampling areas



#### **RESULTS AND DISCUSSION**

In the winter season, the composition of the microbiome associated with the same species was influenced by the sampling sites. Temperature and  $O_2$  concentration largely explain the variation in microbiome composition (Fig. 3). In particular, the contribution of different bacterial families increased based on the impact level of the sampling sites 4). Flavobacteriaceae, Rhodobacteraceae, (Fig. and Vibrionaceae were the families associated with all copepod species. Although copepod species exhibited a distinct core microbiome, several bacterial families were shared among different hosts leading to hypothesize their key role in the copepod's functions. Notably, the contribution of bacterial families including Xanthobacteraceae, Oxalobacteraceae, and Comamonadaceae increased based on the pollution level of the areas.



#### **MATERIALS AND METHODS**

The origin of the microbiome was studied by analyzing the microbiome of the surrounding waters contextually to the copepod microbiome in different life stages (TASK 3). To investigate the hostmicrobiome coevolution, copepods will be reproduced in the laboratory to identify the microbiome at different life stages (eggs, nauplii, copepodites, adults). To identify the trophic sources of the copepods and their food-related microbiome, the fecal pellets were collected and will be analyzed using different procaryotic and eukaryotic gene markers (TASK 4).

#### **PRELIMINARY RESULTS**

3.
Investigating microbiome variability and origin according to the hosts' sex and life stages

2

### Assessing interactions between microbiome and copepods with different trophic strategies

Copepod microbiomes were characterized by low inter-individual variability within the same sites and no significant differences were present

#### between females, males, and copepodites, in the winter season. Besides, the ASVs shared between copepod and contextual seawater were low

#### in number and abundance.





TASK