



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

Microbiomes of marine copepods in coastal ecosystems

Agnese Fumanti

Marine Biology and Ecology Group, DISVA

Tutors: Cinzia Corinaldesi, Iole di Capua

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^{1,5}. So far, information about the copepod microbiome remains limited, despite its key role in the marine food webs^{4,6}. Understanding copepod-microbe associations is essential to unravel ecosystem functioning and this becomes even more critical in the context of ongoing global change and anthropogenic impacts^{2,3}, which may modulate diversity and functions of the holobiont. The main goal of this PhD thesis is to expand our knowledge of the microbiome of the most abundant metazoans on Earth^{1,4}.

GLOBAL COPEPOD MICROBIOME META-ANALYSIS

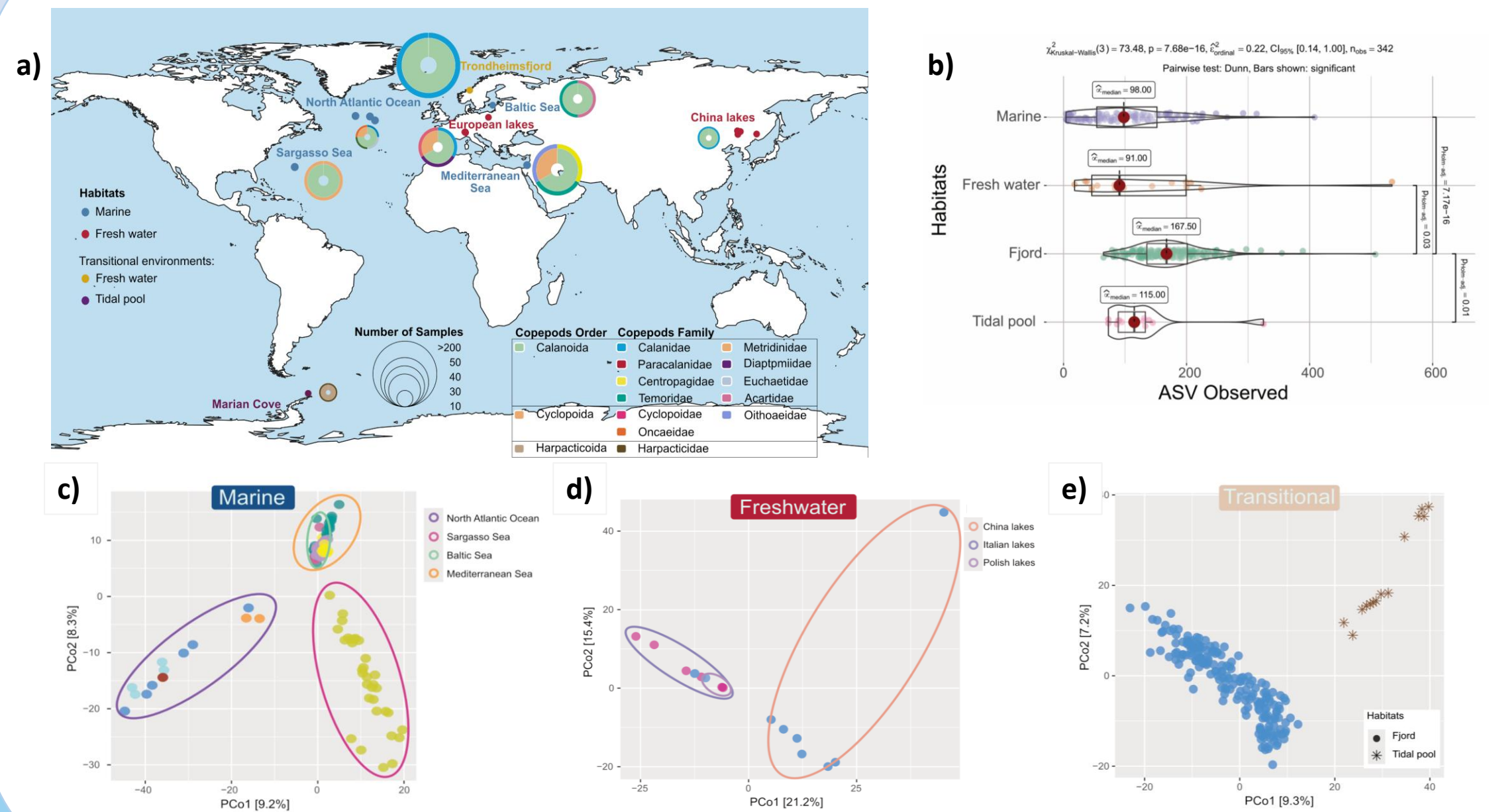


Fig. 1: a) Map showing the sampling areas; b) alpha diversity; c-d-e) beta diversity

MATERIALS AND METHODS

This analysis was based on a systematic review of the published literature on microbiomes associated with copepods (Fig. 1a). We re-elaborated available metabarcoding data to investigate the diversity, origins, and putative co-evolutionary patterns of copepod microbiomes in relation with their hosts across marine, freshwater and transitional ecosystems worldwide.

RESULTS AND DISCUSSION

Herein, thirteen copepod families, and their associated microbiomes were investigated. The meta-analysis revealed differences in microbiome alpha diversity indices (Fig. 1b). Beta-diversity was primarily influenced by habitat and sampling site, with distinct bacterial families characterizing each site (Fig. 1 c-d-e), highlighting the crucial role of the microbiome in the evolutionary success of these globally abundant metazoans.

INTRA- AND INTER- SPECIFIC VARIABILITY OF THE COPEPOD MICROBIOME IN TWO MEDITERRANEAN COASTAL BASINS

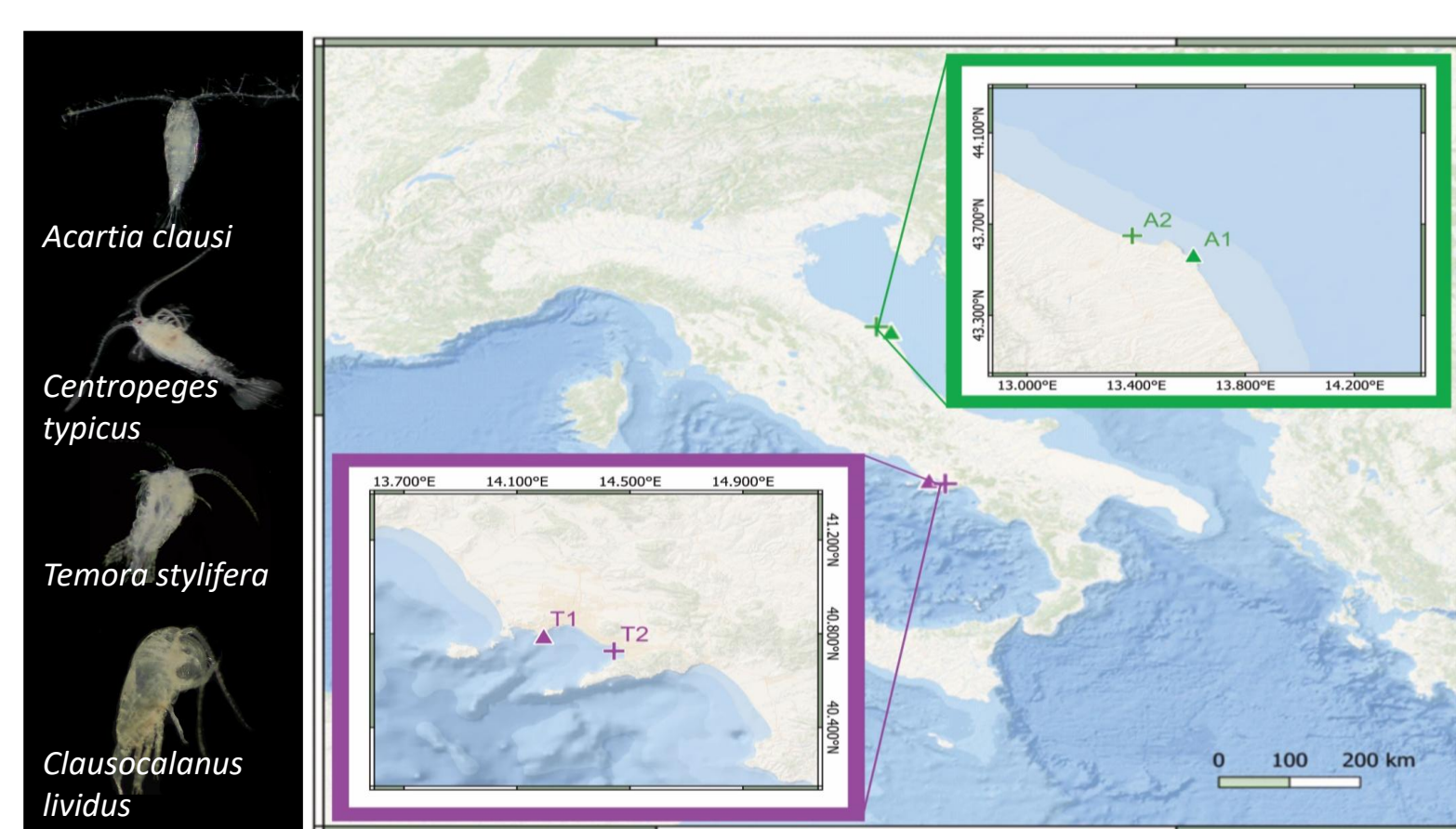


Fig.2: Map showing the sampling areas and target species

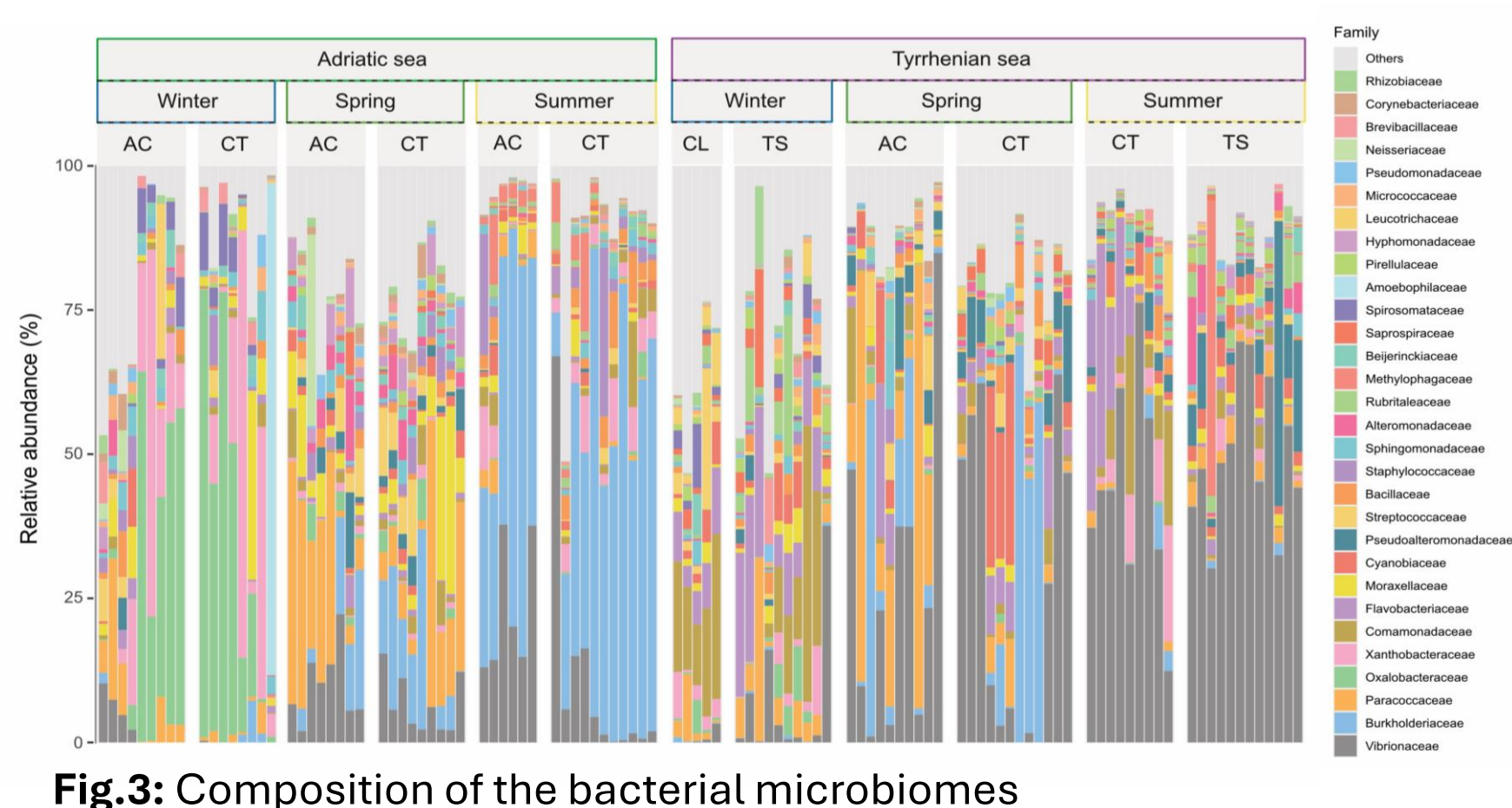


Fig.3: Composition of the bacterial microbiomes

MATERIALS AND METHODS

Planktonic copepods were collected using a WP2 net (200-µm mesh) from two coastal sites in the Adriatic and Tyrrhenian Seas, each with different levels of anthropogenic impact. Four dominant species were identified under a stereomicroscope (Fig. 2). To study their trophic behavior and associated microbiomes, fecal pellets were collected, and DNA was extracted and purified. Trophic behavior and microbiome diversity of copepods were analyzed using metabarcoding of specific gene markers. Bioinformatic analyses were conducted with QIIME2, and statistical analyses were performed in R using specialized packages.

RESULTS AND DISCUSSION

The microbiome composition of the same copepod species was influenced by the areas, sampling sites and seasons (Fig. 3), with temperature and oxygen concentration being key factors explaining the variations (Fig. 4). Specifically, the contribution of certain bacterial families increased with the level of anthropogenic impact at the sampling sites and seasons. Flavobacteriaceae, Rhodobacteraceae, and Vibrionaceae were common across all copepod species. Despite distinct core microbiomes in each copepod species, several bacterial families were shared among different hosts, suggesting their importance in copepod functions.

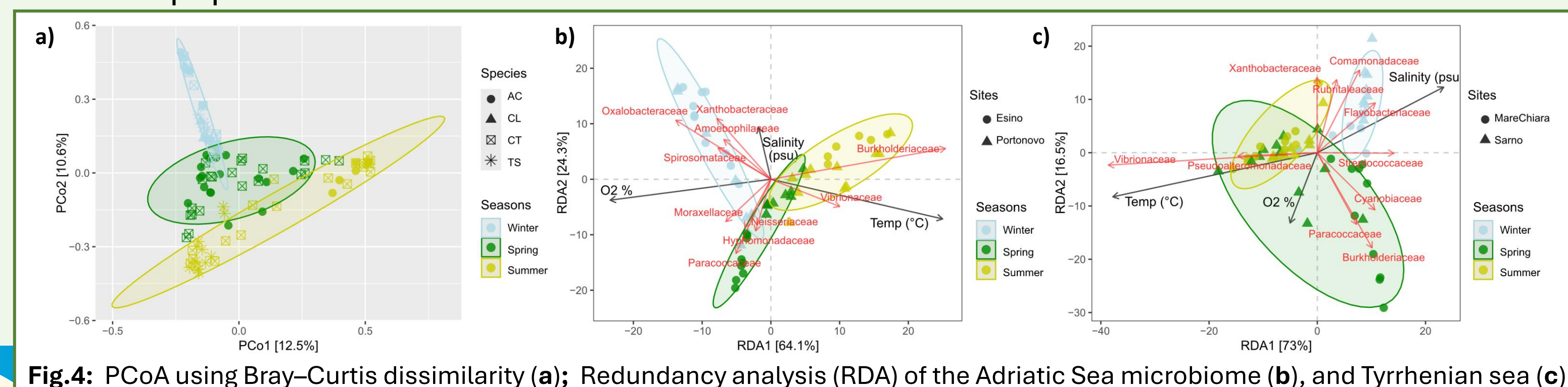


Fig.4: PCoA using Bray-Curtis dissimilarity (a); Redundancy analysis (RDA) of the Adriatic Sea microbiome (b), and Tyrrhenian sea (c)

COPEPOD MICROBIOME CHANGES UNDER DIFFERENT ANTHROPOGENIC IMPACT CONDITIONS

MATERIALS AND METHODS

Sites with high anthropogenic impact (river mouths) and control sites were compared to identify changes in the microbiome composition. Metabarcoding and metagenomic analyses will be carried out to assess putative functions of the holobiont (microbiome and copepods).

RESULTS AND DISCUSSION

The contribution of different bacterial families increased based on the impact level of the sampling sites. Notably, the contribution of bacterial families including Xanthobacteraceae, Oxalobacteraceae, and Comamonadaceae increased based on the pollution level of the areas (Fig. 5).

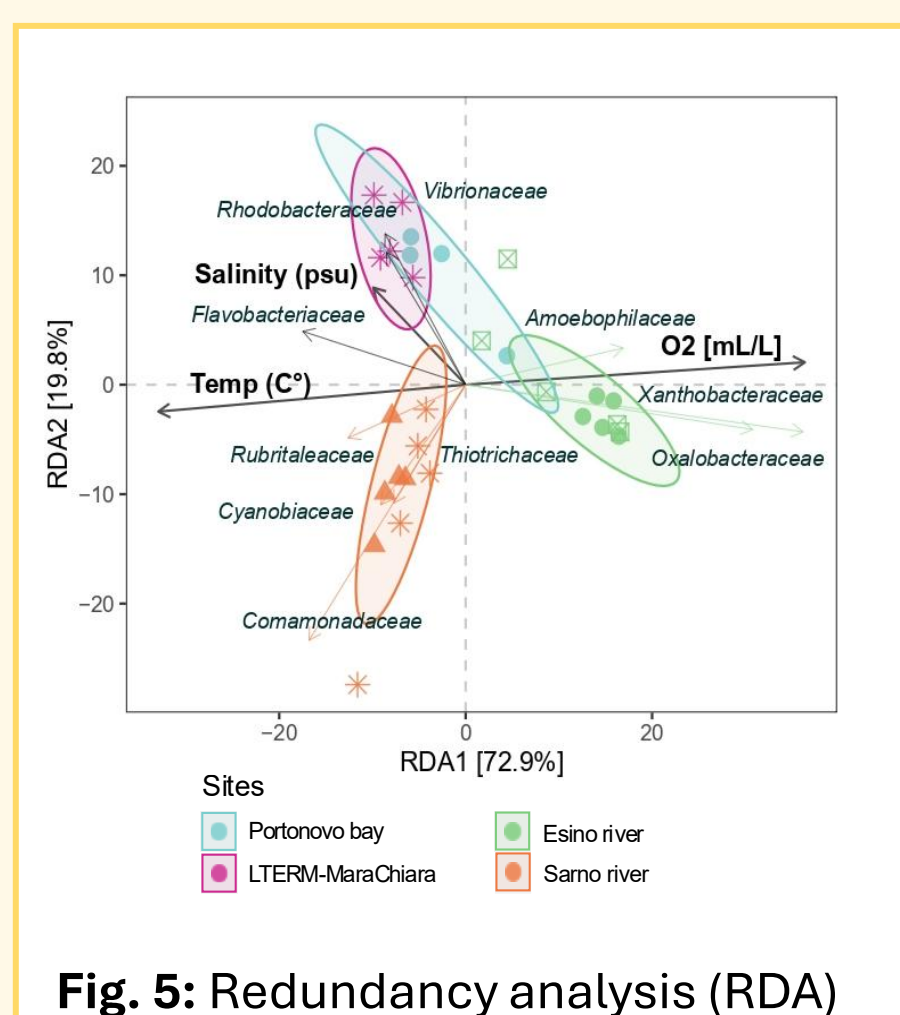


Fig. 5: Redundancy analysis (RDA)

ORIGIN AND CO-EVOLUTIONARY PATTERNS OF COPEPOD MICROBIOME

MATERIALS AND METHODS

The origin of the microbiome was studied by analyzing both the surrounding water microbiome and the copepod microbiome at different life stages. To explore host-microbiome coevolution, copepods will be bred in the laboratory to examine the microbiome across various life stages. Bioinformatic analyses will be conducted with specific R packages.

RESULTS AND DISCUSSION

The number of ASVs shared between copepods and surrounding seawater was limited, suggesting that copepods maintain a specific microbiome independent of the seawater (Fig.6). The rearing experiment is still ongoing and the bioinformatic analysis will be conducted at the end of experiment.

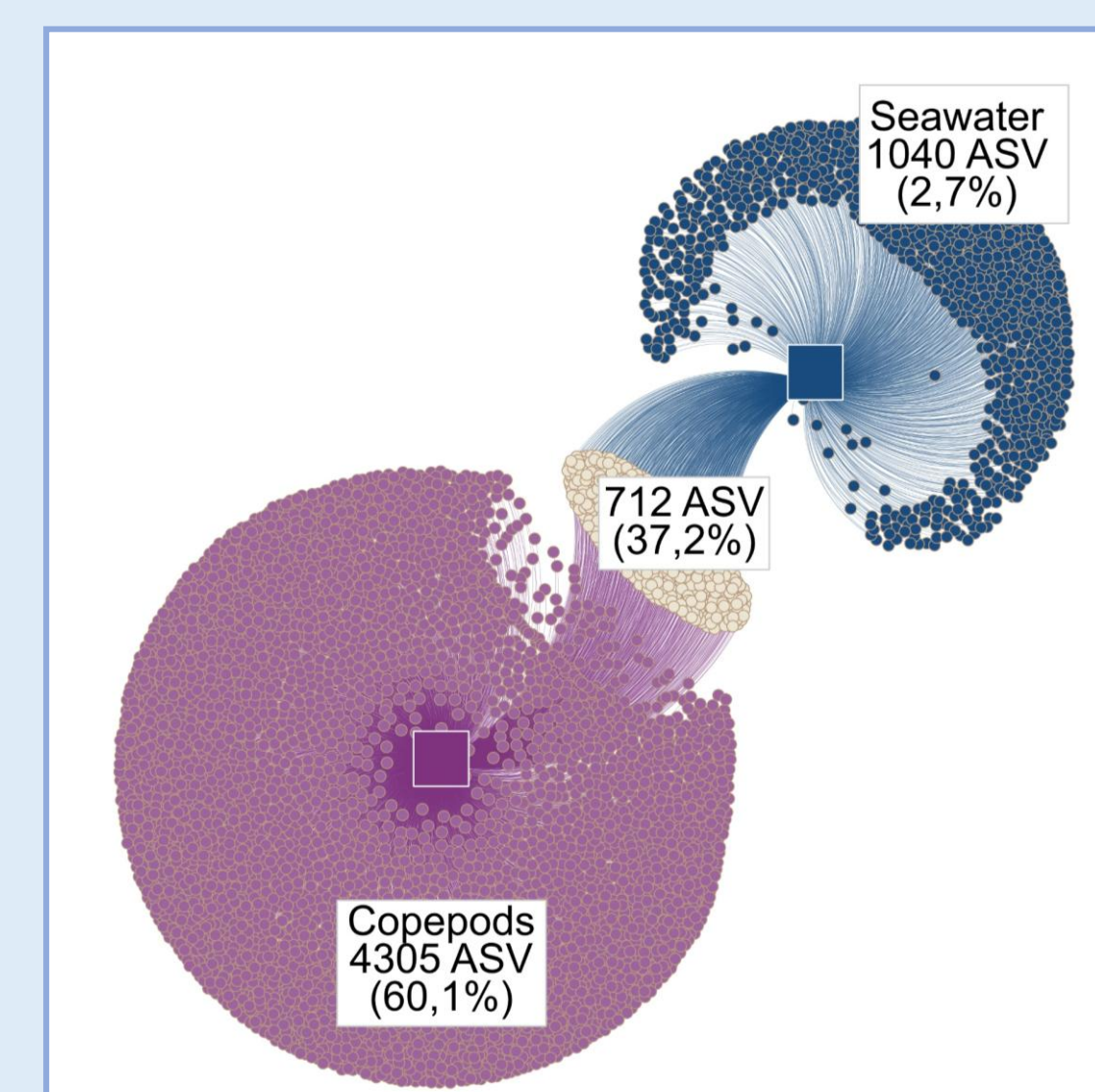


Fig. 6: ASVs shared between copepods and contextual seawater

