PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

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

Name of PhD student: Agnese Fumanti

Title of PhD research: Microbiomes of marine Copepods in coastal ecosystems

Name of PhD supervisor: Cinzia Corinaldesi (UNIVPM)**,** Iole Di Capua (SZN)

Research lab name: Marine Biology and Ecology Group, DISVA

Cycle:
[ ] XXXVI
[ ] 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:
[X] Actea Mobile Laboratory
[X] Advanced Instrumentation lab
[ ] Aquarium
[ ] MassSpec lab
[ ] MaSBiC
[ ] Simulation/informatics lab
[ ] Other. Please, indicate: ....

Part 1. Scientific case of the PhD Research

-ABSTRACT:

Copepods 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. 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, and that the microbiome diversity change with environmental conditions, and among different genera[3,5,6]. The symbiotic bacteria can provide important functions involved in 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. In the present study, we investigated the microbiome of planktonic copepods through a molecular approach based on metabarcoding (amplicon sequencing of the 16S rRNA gene) and metagenomics (DNA shotgun sequencing). We characterized the microbiome of four dominant coastal epipelagic species in two areas of the Mediterranean Sea investigating, origin, and functional role. Our first

results show that copepod-associated microbial communities in the winter season are host-specific rather than determined by sex. Furthermore, although most bacterial families are shared between hosts, each species displays a specific core microbiome.

- SCIENTIFIC AIMS

1. Exploring the microbiome diversity of different copepod species inhabiting different Mediterranean coastal ecosystems
2. Assessing the microbiome in copepods with different trophic strategies to identify their trophic sources and the potential transfer patterns from the environment.
3. Investigating copepod microbiome variability and origin 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.

- WORKPLAN AND RESEARCH ACTIVITIES

WP 1. Objective

Exploring the microbiome diversity of different copepod species inhabiting different Mediterranean coastal ecosystems

Methods

The study areas identified for the analysis of the copepod microbiomes are located in the coastal Adriatic Sea and the Tyrrhenian Sea (Fig. 1). The sampling strategy consists of 1-year sampling in relation to copepod seasonal patterns. 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). To analyze the diversity of the microbiomes among the different species inhabiting different Mediterranean coastal ecosystems, DNA was extracted and purified from the specimens using commercial kits. Metabarcoding analyses for assessing prokaryotic and fungal taxonomic diversity were carried out by amplifying 16S rRNA genes on high-throughput sequencing platforms (Illumina MiSeq).

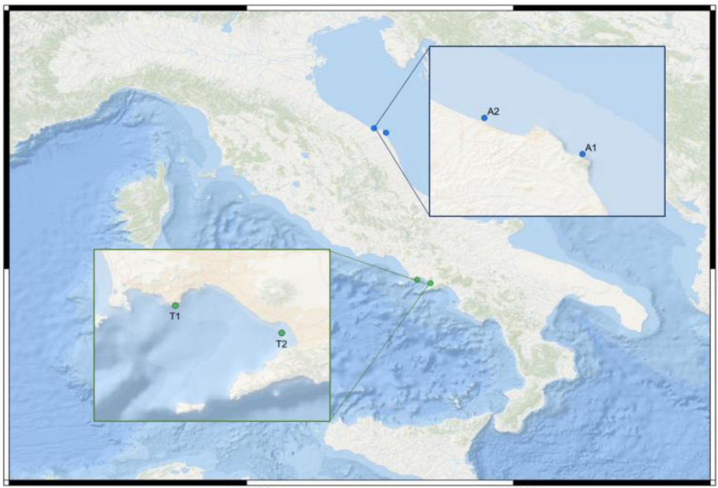
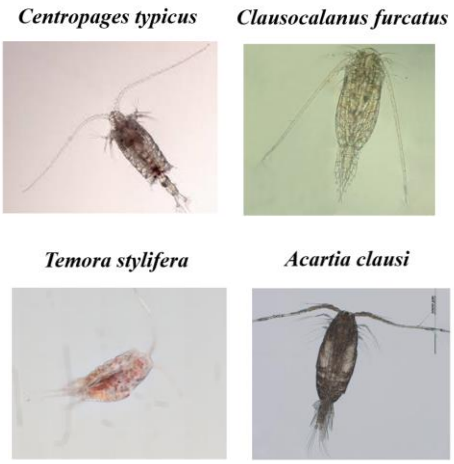


Fig.1: A: Adriatic coast (A1: Portonovo bay; A2: Esino river); T: Tyrrhenian coast (T1: LTER- Mare Chiara ; T2: Sarno river)



Centropages typicus
Clausocalanus furcatus
Temora stylifera
Acartia clausi

Fig.2: Target species

Obtained Results

16S rDNA sequencing and bioinformatic analyses on 96 samples have been performed and the microbiome composition of different copepod species inhabiting different marine ecosystems is showed in Figure 3.

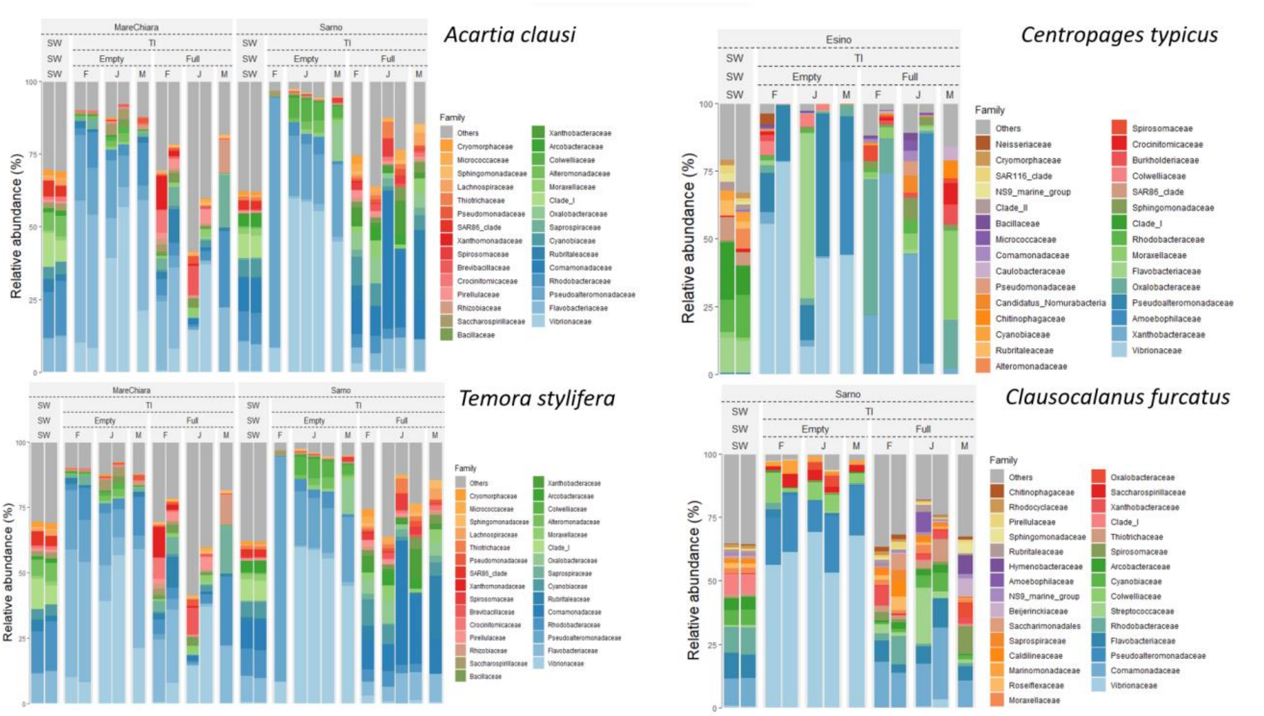


FIG 3. Taxa barplot for each copepod species.

The taxonomic composition of copepod microbiomes showed significant differences between the species. particular, Vibrionaceae, Rhodobacteraceae, and Flavobacteriaceae were the families associated with all copepod species. Each copepod revealed a specific *core* microbiome shown in Figure 4.

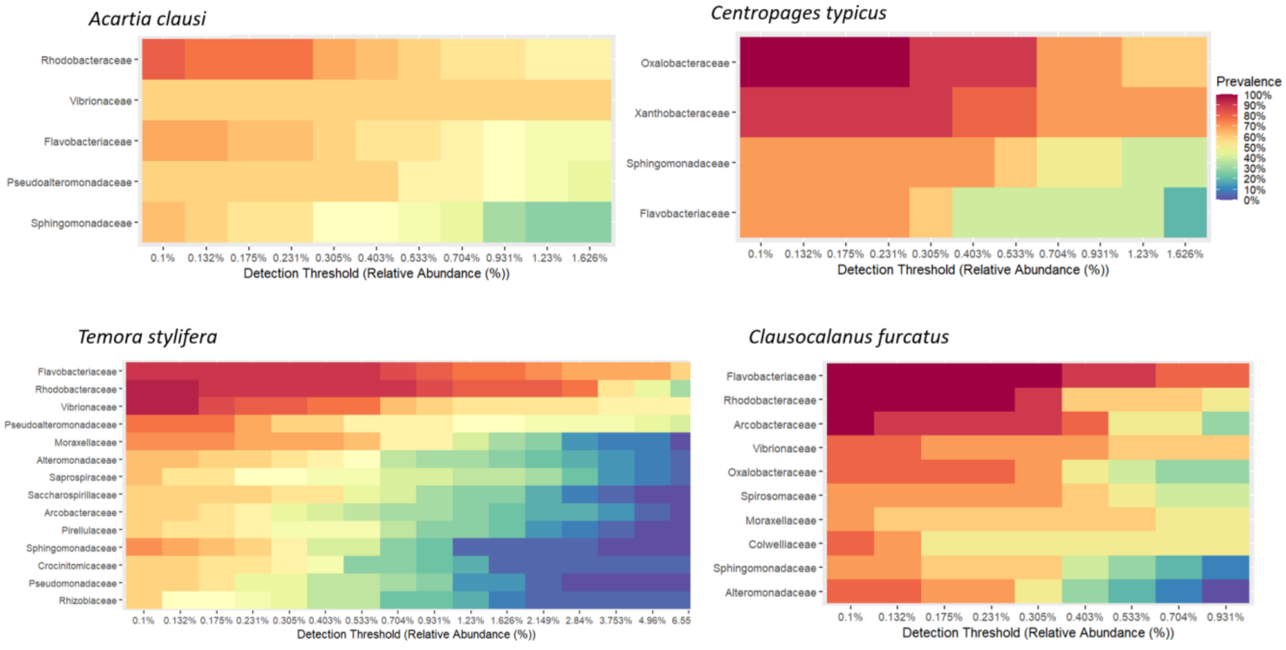


FIG 4. Core microbiome for each copepod species

Vibronaceae and Rhodobacteriaceae were present in core of *Temora stylifera*, *Acartia Clausi* and *Clausocalanus furcatus*, while Xantobacteriacea was exclusive of *Centropages typicus*. Psudomonadaceae and Moraxellaceae were mostly observed in *Temora stylifera* while *Clausocalanus furcatus* was mainly associated with Oxalobacteriaceae, Spirosomaceae, Colwelliaceae.

WP 2. Objective

Assessing the microbiome in copepods with different trophic strategies to identify their trophic sources and the potential transfer patterns from the environment

Methods

To analyze the diversity of the microbiomes among planktonic copepods with potentially different trophic strategies, after sampling (see WP1 for details) through a WP2 net (200-µm mesh), individuals were sorted under a stereomicroscope to identify the species and the presence of food in the intestine. In addition, the fecal pellets of the selected individuals were collected and procaryotic and eukaryotic gene markers (16S and 18S rDNA) were used to investigate the trophic sources. Results obtained will be compared to those obtained by the analysis of the copepod and seawater microbiomes.

Expected Results

The identification of the microbiome composition in copepods with different trophic strategies, both in the fecal pellets, in their body and in the surrounding environment, will allow us to identify the role of the diet and environment in the transmission patterns of the copepod microbiome. The analyses are in progress.

WP 3. Objective

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

Methods

Changes of the microbiomes depending on their sex will be studied selecting for each species male and female. The potential vertical transmission of the microbiome will be studied by analyzing the microbiome of the copepods in different life stages (eggs, nauplii, copepodites, adults). To investigate the host-microbiome phylosymbiosis/coevolution, copepods will be reared in the laboratory to identify the microbiome at different life stages.

Obtained Results

The analyses of the microbiome composition in male and female individuals suggests that no significant differences are present depending on the sex of the copepods (Fig 4). However, these analyses have to be finalised and dataset elaborated.

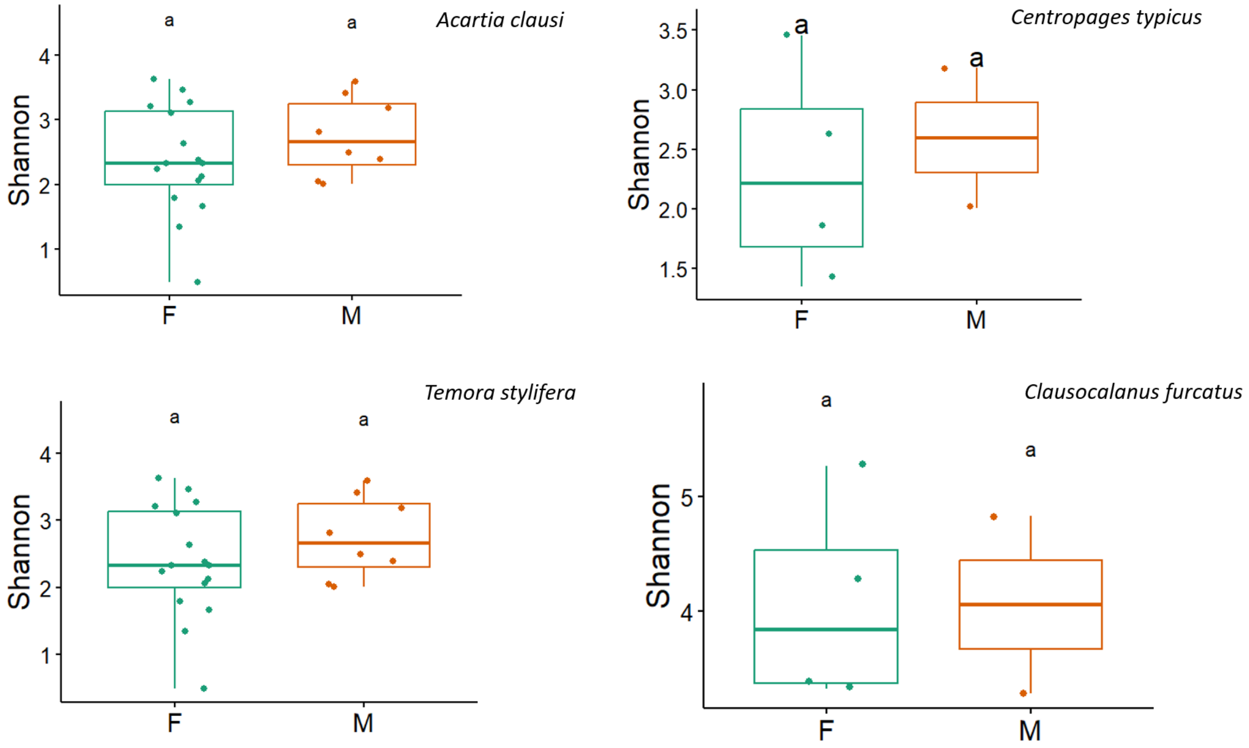


FIG 4. Alpha diversity based on Shannon index and statistical analysis (ANOVA) between Female (F) and Male (M) for each copepod species.

WP 4. Objective

Assessing the functional roles of microbiomes that can confer abilities to the host to cope with human-driven impacts

Methods

Sites with high anthropogenic impact (river mouths) and control sites (Fig.1) will be compared to identify changes in the microbiome composition and putative functions. Metagenomic analyses will be also carried out in selected samples to identify Metagenomic Assembled Genomes (MAGs) and putative functions. Proteomics analysis will be performed to investigate bacterial protein production that can confer abilities to the host to cope with human impacts.

Expected Results

To explore host-microbiome relationships and their potential changes in relation to different environmental conditions and anthropogenic impacts.

- REFERENCES

1. **Bass**, et al. "Parasites, pathogens, and other symbionts of copepods." Trends in Parasitology 37.10 (2021): 875-889
2. **Benedetti**, et al. “Copepod functional traits and groups show divergent biogeographies in the global ocean”. Journal of Biogeography (2022)
3. **McGinty**, et al. "Anthropogenic climate change impacts on copepod trait biogeography." Global Change Biology 27.7 (2021): 1431-1442

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)
6. **Moisander**, et al.. "Stable associations masked by temporal variability in the marine copepod microbiome." PloS one(2015)

Part 2. PhD student information on the overall year activity (courses/seminars/schools, mobility periods, participation to conferences)

Seminars:

1. William Martin, Institute of Molecular Evolution -Heinrich-Heine-Universität Düsseldorf, Germany May 5 th. "Hydrothermal vents, the origin of the first cells, and their physiology (lifestyle)"
2. Kyle J. Lauersen, Biological & Environmental Science and Engineering Div. - KAUST, Saudi Arabia June 23rd. "A Green Vision for Future Algal Biotechnology Innovations and Applications"
3. Michael Behrenfeld, Department of Botany and Plant Pathology -Oregon State University. OR, USA July 7 th.
4. Antonios Mazaris, Dept Ecology, School of Biology -Aristotle University of Thessaloniki, Greece October 6 th
5. Prof. P. Lenz, University of Hawai'i at Manoa, October 12th. "Physiological Adaptations in Zooplankton in the Sub-Arctic".
6. Susana Coelho, Max Planck Institute for Biology Tübingen, Germany October 13th. "Brown algal development, reproduction and evolution".

Courses:

1. TECHNOLOGY TRANSFER AND INNOVATION A.A. 2022/2023 (Iacobucci)
2. DESIGN OF RESEARCH: EUROPEAN PROJECTS A.A. 2022/2023 (Paone)
3. ANALISI DELLE REGRESSIONI LINEARI A.A. 2022/2023 (Beolchini)
4. CONFOCALE A.A. 2022/2023 (Frontini)
5. MICROBIAL-MEDIATED PROCESSES IN ACQUATYC ECOSYSTEM: FROM BASIN TO APPLIED RESERCH TOWARD SOLVING ENVIRONMENT PROBLEMS A.A.2022/2023 (Dell'Anno)

Courses SZN:

1. PRESENTING WITH ZEN (Marco Signore)
2. COMMUNICATING SCIENCE: WHY, TO WHOM AND HOW (Marco Signore)
3. STATISTICS PRISCILLA LICANDRO (Marco Uttieri)
4. NEW PERSPECTIVES IN MARINE BIOTECHNOLOGY (Donatella De Pascale)
5. MARINE BIODIVERSITY MARINA MONTRESOR (Gabriele Procaccini)

List of periods spent abroad

1. Oceanographic campaign: Aegean Sea (3 weeks)

Part 3. PhD student information on publications

List of publications on international journals

J1. Systematic review and meta-analysis: Copepod microbiomes – *in preparation*

J2. Polar microbiomes – *in preparation*

J3. Microbial assemblages associated with two bioluminescent mesopelagic fishes (*Argyropelecus hemigymnus and Maurolicus muelleri*) – *in preparation*

[Date: 12/10/23]

Student signature

Supervisor signature