

TITLE: Ecological structure of plankton resting stage assemblages through High Throughput Sequencing metabarcoding approach across the Mediterranean Sea

KEYWORDS: assemblages, data analysis, eDNA, HTS (High Throughput Sequencing) metabarcoding, phytoplankton, resting stages, 18S rDNA, sediments

RESEARCH AREA

FORMAL MODELS, DATA ANALYSIS AND SCIENTIFIC COMPUTING

SSD BIO/07 Ecology

ERC Life Science LS8

GENERAL PRESENTATION OF THE PROJECT AND STATE OF THE ART

Marine phytoplankton are single-cell photosynthetic organisms that live suspended in the euphotic zone of seas and oceans, contributing to about the half of atmosphere's oxygen; they are primary producers and they are at the base of food chain in marine ecosystems. Phytoplankton species can produce resting stages that deposit onto the sea bottom forming seed banks or assemblages. Resting stages are cysts or spores produced by dinoflagellates and diatoms, respectively. Resting stages survive under unfavorable environmental conditions. These stages may remain quiescent for short-term survival, as asexual temporary resting stages [1], or for long-terms perspective (from years to decades) having an ecological role in the resilience and recovery from rapid environmental changes through genetic recombination [2]. These formations have an important role in maintaining the phytoplankton assemblages in the water column [3] and in maintaining the populations in the future environment with the chance of being passively dispersed over long-distances by currents, winds, or through human/animal mediation, extending their distribution and colonizing new areas. The dynamics of resting stages in seawater are not clear: when cysts are found in sediments it is poorly known when and where the encystment occurred. When environmental conditions return favorable, they might germinate determining blooms which can be harmful too (Harmful Algal Blooms), thus severely affecting marine ecosystem. The study of resting phase is hampered by a methodological problem too since cysts are difficult to recognize morphologically. This issue can be solved by using molecular methods. The environmental DNA (eDNA) metabarcoding allows taxa identification directly from environmental samples (e.g. water column, sediments) collected in the study site. The aim of this proposal is improving understanding of abundance, distribution and dynamics of resting stage assemblages in three Mediterranean sub-regions, such as Adriatic, Ionian and Tyrrhenian Sea, through a combination of eDNA metabarcoding and statistical analyses of environmental and human impact factors.

RESEARCH OBJECTIVES

The aim of this project is twofold: a) identifying through eDNA metabarcoding phytoplankton taxa that produce resting stages in three Mediterranean sub-regions (Adriatic, Ionian and Tyrrhenian Sea) and b) inferring the ecological structure of the resting stage assemblages in relation to physical and geomorphological features (i.e. currents, bottom sedimentary type, depth), environmental parameters (i.e. temperature, nutrients, salinity) and human activities [4]. This means that taxa identification by eDNA

metabarcoding will offer some clues for shedding light on the most likely causative factors driving the expected different occurrence of resting stage-forming taxa across the three basins. Moreover, determining patterns of co-occurrence among resting stage-forming will help understand their interactions and underlying ecological factors.

This study aims also at improving our knowledge about the formation process of phytoplankton resting stages in the water column. Through the correlation of biological and environmental data we expect to better understand the reasons why cysts-forming taxa abundance is different across the three Mediterranean basins. This would ultimately translate into increased ability to model and predict future scenarios.

Cyst and spore assemblages are changing in different Mediterranean sediments. Through this study of physical, geomorphological, environmental, and human pressure variables, we would try to understand if resting stage taxa are dependent by these factors and if some taxa preferentially occur under specific conditions. We aim also at understanding how the identified taxa interact and how these interactions change in the sub-regions also in relation to the different environmental and human-driven features.

METHODOLOGY AND EXPECTED RESULTS

The study area includes three sub-regions of Mediterranean Sea: Adriatic, Ionian and Tyrrhenian Sea. A total of 94 surface sediment samples collected from coastal and offshore areas from 2015 to 2017 will be analyzed (Figure 1).

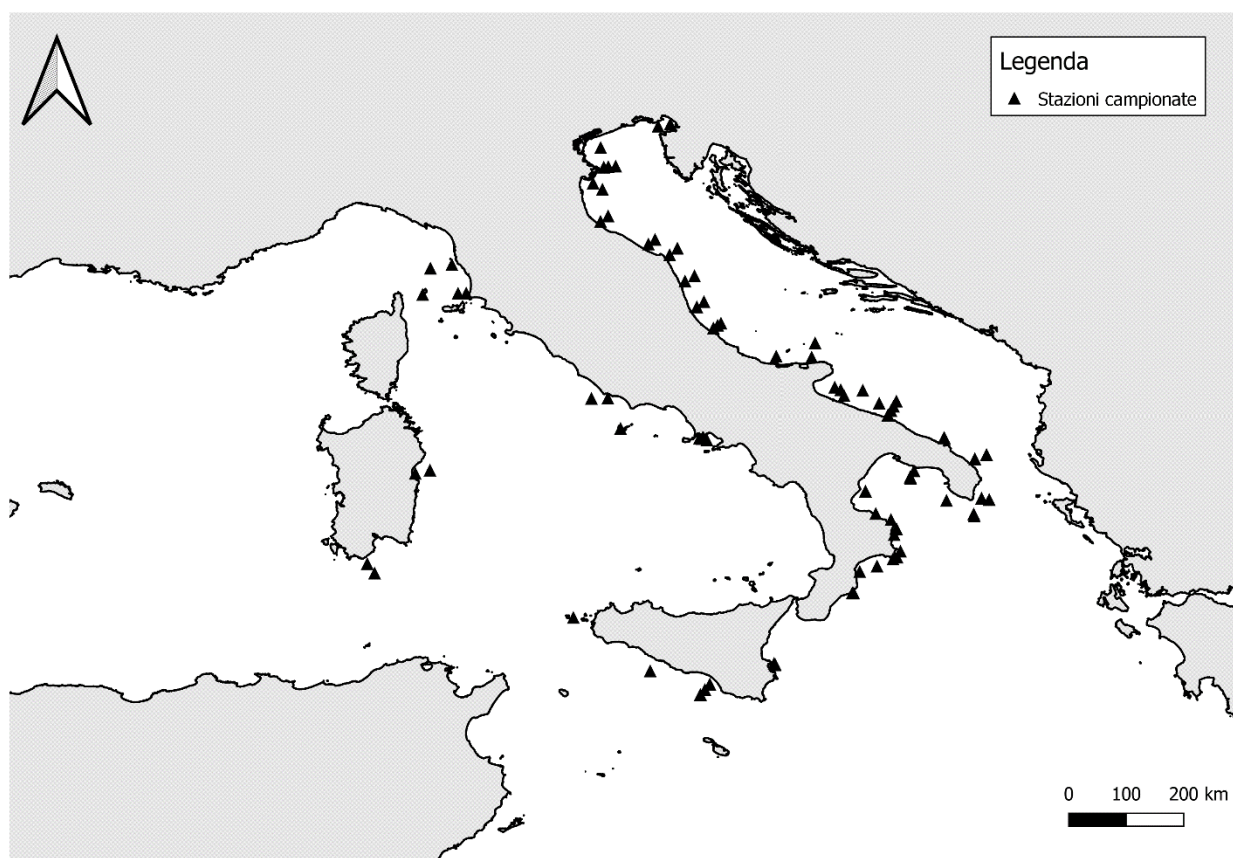


Figure 1 – Graphic representation of the sampled stations.

The eDNA will be extracted from these sediments and it will be analyzed through a metabarcoding approach. After PCR analysis and HTS, millions of reads will be obtained and they will allow us to infer which taxa will

be present in the environmental samples. The massive set of data, which will be generated, will be analyzed with bioinformatic analysis and compared with known sequences in reference database. Results will be correlated with all environmental and human impact data retrieved from existing through appropriate statistical, machine learning and GIS tools. A flowchart of the study is shown in Figure 2.

The eDNA metabarcoding based on PCR amplification gives only qualitative information and it is under continuous development since it seems to amplify some templates with greater efficiency than others [5]. In collaboration with “*Fondazione Edmund Mach*” we would like to extract quantitative information using capture-based approach, a possible solution to overcome the limitations posed by PCR-based approaches [5].

At the end, we expect to have a map of taxa producing resting stages in the Mediterranean Sea with information on their abundance and distribution. Further, we expect to obtain a structure of resting-stage assemblages that can be regarded as a time- and space-integrated response of a subset of phytoplankton species to environmental conditions, including the physical oceanographic dynamics that favor or prevent sedimentation of resting stages. This information will be relevant because the diachronic resting stage assemblages can be regarded as the integral of the biotic responses to seasonal changes and to all the other modifications of the environmental conditions of the water column summarizing a complex time series.

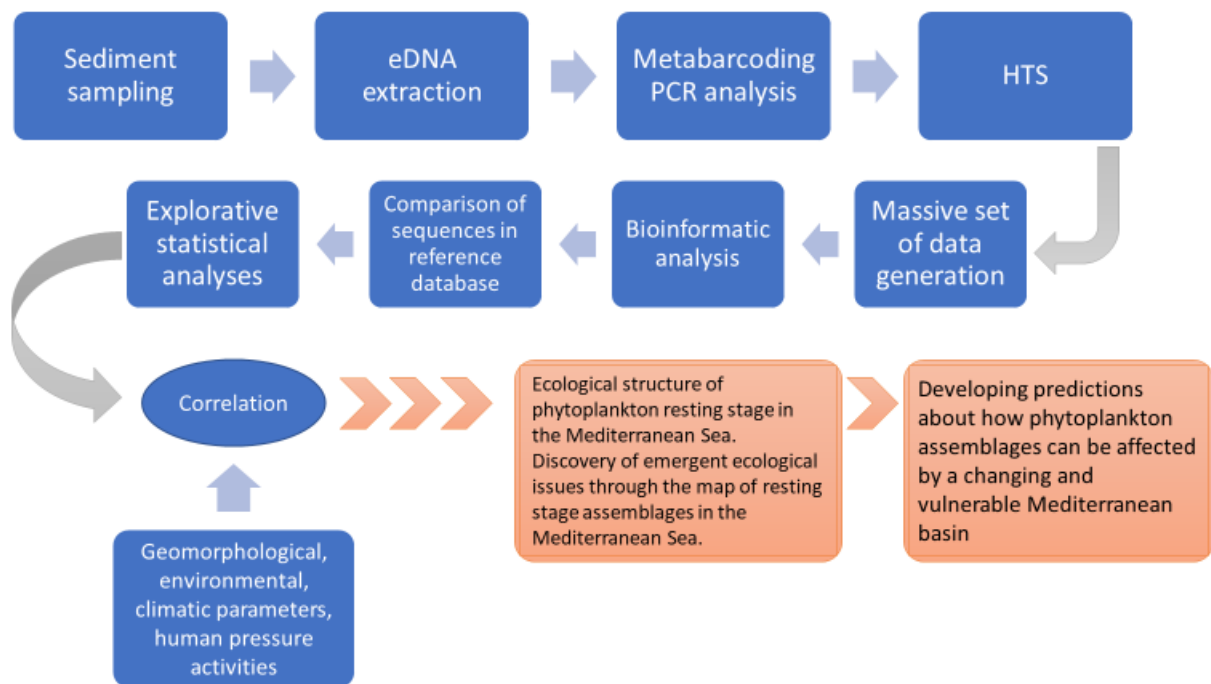


Figure 2 – Flowchart of the activities that will be conducted in this study.

BIBLIOGRAPHY

1. Figueroa R et al. (2005). J Phycol 41: 370–379.
2. Kremp A, et al. (2016). Environ Microbiol 18: 679–691.
3. Montresor M, et al. (2013). Mar Ecol Prog Ser 484: 79–95.
4. Halpern BS et al. (2008). Science 319: 948-952
5. Taylor M. Wilcox et al. (2018). Mol Ecol Resour. 18: 1392-1401.

DESCRIPTION OF THE RESEARCH IN THE THREE-YEAR PERIOD

Figure 3. Gant chart shows the activities that will be carried out over the three years of Ph.D.

Activities	Months	Year 1				Year 2				Year 3			
		10 - 12	1 - 3	4 - 6	7 - 10	10 - 12	1 - 3	4 - 6	7 - 10	10 - 12	1 - 3	4 - 6	7 - 10
Samples preparation													
eDNA extraction													
PCR Metabarcoding													
HTS													
Bioinformatic analysis													
Reference database													
Environmental data collection													
Statistical analyses													
Papers contribution													
Thesis preparation													