CHANGES IN PHYTOPLANKTON ASSEMBLAGE STRUCTURE DRIVEN BY HUMAN PRESSURES IN THE MEDITERRANEAN SEA THROUGH THE METAGENOMIC 18S RRNA APPROACH

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Abstract
Environmental barcoding based on 18S rRNA revealed a broad genetic diversity of primary producers in a wide range of ecosystems. Environmental drivers can select species and functions of microalgal assemblages. Human activities and climate change are now potent new drivers that significantly alter the functioning of coastal and offshore ecosystems also in the Mediterranean Sea, which is a critical marine ecosystem. Within the framework of OSD (Ocean Sampling Day) campaign 2014 of EU MicroB3 Project, this study elucidated the relationships between composition of phytoplankton assemblages and human impact on Mediterranean Sea, highlighting the potential of a metagenomic 18S rRNA approach in combination with appropriate data analysis techniques.

Keywords: Phytoplankton, Pollution, Mediterranean Sea, Genetics

Introduction. The Mediterranean Sea is a critical marine ecosystem for many reasons, because it is a hot-spot of biodiversity, resource exploitation, maritime traffic and coastal urbanization with high density population pressure. Microalgal diversity composition, in terms of taxonomical hierarchies, and its variations over spatial scales, represents a useful tool to quantify the consequences of the anthropogenic perturbation on marine ecosystem health. The metagenomic analysis of phytoplankton communities based on 18S rRNA sequencing already demonstrated to be a useful tool for genetic diversity knowledge in marine ecosystem.

Materials and methods. All sampling sites were located in the Mediterranean Sea. Samples were collected at different depths within the activity of Ocean Sampling Day (OSD) of EU MicroB3 Project. The metagenomic data set was 18S rDNA workable obtained from LGC (Germany). As for Mediterranean autotrophic plankton, it included 32 samples and 350 OTUs. The whole taxonomical classification data set associated to 18S rDNA sequences was processed to extract the list of unicellular eukaryotic phototrophs (primary producers) exclusively. Data about estimated human impact were obtained from Halpern et al. [1]. Dissimilarities between autotrophic plankton assemblages were computed on the basis of OTUs abundances using the Morisita-Horn index [2].

Results. The structure of autotrophic plankton assemblages varied revealing a biogeographic pattern in the Mediterranean Sea, but, this was not dependent of basin-wide patterns (Mantel test, R=0.016, p=0.408). To reduce the complexity of the available data, only 82 OTUs found in more than 5 samples were kept in the final reduced data set, thus preserving only the most reliable information provided by the full data set. In fact, the relationship between Morisita-Horn dissimilarity and geographical distance was still not significant (Mantel test, R=0.006, p=0.455), and the reduced data set showed a higher correlation to human impact relative to that of the full data set (Mantel test, R=0.266, p=0.008). A Principal Coordinates Analysis (PCoA) was the performed on the same Morisita-Horn dissimilarity matrix (Fig. 1) and the first and second principal axes accounted for 26.8% and 13.6% of the overall variance, respectively. In accordance with the outcome of the Mantel tests, no biogeographical structure emerged from the ordination, while the largest human impact values were associated to samples found in the positive half-plane defined by PCo2. PCo2 scores were highly variable at sites with low to intermediate human impact score (i.e. ≤20), but, they were clearly larger at more disturbed sites, as Messina, Italy (OSD42), Venice, Italy (OSD47), Porto Marghera, Italy (OSD69 and OSD70), Patras (OSD90), Greece. An Indicator Species Analysis [3] was performed on normalized OTU abundances to find out what OTUs based on 18S rRNA metagenomic data set were more frequent and/or abundant at disturbed sites (human impact score >20). It was evident that only ME-Euk-DBT116 and the diatom genus _Skeletonema_ were associated to the more disturbed group showing a significant preference for sites under heavier human impact.
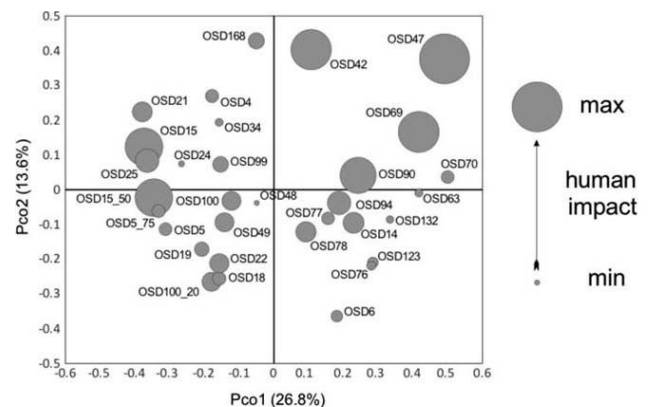

Fig. 1. Principals Coordinates Analysis of the Morisita-Horn dissimilarity matrix based on the reduced data set (82 OTUs, 32 samples). Symbol size is proportional to the human impact score obtained by Halpern et al. [1].

Conclusions. The available data showed significant relationships between autotrophic plankton assemblage structure and human impact. Sites under heavier human impact were significantly associated with assemblages whose recurrent features were low diversity and high dominance. As for the responses of single OTUs, i.e. _Skeletonema_, was significantly more frequent or abundant under heavier human impact, whereas other OTUs showed less clear responses. As for sites where the human impact scores was lower than 20, but still moderate, higher diversity was possibly related to the effects of intermediate disturbance on the autotrophic plankton assemblage. The analysis of metagenomic 18S rRNA, V4 marker, is still to be improved, especially by solving uncertainties in OTU identification and defining an ecologically meaningful level of resolution in that process, but it clearly has a potential, especially if leveraged by appropriate statistical methods.

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