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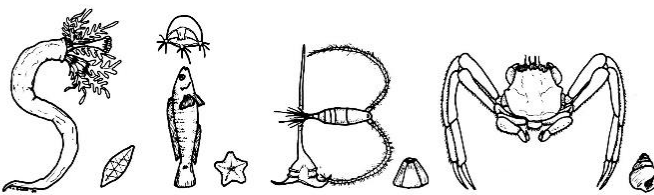
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PHYTOPLANKTON COMPOSITION AND LONG-TERM VARIATIONS IN TWO COASTAL AND OFFSHORE MARINE SITES (NORTHERN ADRIATIC SEA)

COMPOSIZIONE E VARIAZIONI A LUNGO TERMINE DEL FITOPLANCTON IN UNA STAZIONE COSTIERA E UNA AL LARGO (ADRIATICO SETTENTRIONALE)

Abstract - Two long term (1988-2019) data sets collected at a coastal and offshore stations located in the LTER Senigallia-Susak Transect were compared. The multivariate statistical analysis highlighted the role of the oceanographic conditions in discriminating the study areas. Environmental parameters at the coastal station were more variable and with a marked seasonality due to direct riverine inputs. Different mean annual cycles of physico-chemical parameters and of phytoplankton abundances were found, together with a different community composition. Higher mean abundances values and lower diversity were found at the coastal station, although the two stations were more similar in summer, due to the spreading southeastwards of riverine waters in stratified conditions. Graph network analysis highlighted that species not relevant following the IndVal were able to influence the overall community in both sites. The offshore community showed higher number of strong interactions among species than the coastal one.

Key-words: phytoplankton, LTER, graph-network analysis, diversity, ecosystem comparison.

Introduction - Phytoplankton community dynamics and patterns can highlight changes in the marine ecosystems, as they are directly related to environmental conditions. Therefore, given the high temporal coverage, Long-Term Ecological Research (LTER) marine areas are of crucial importance to detect changes and trends of biogeochemical descriptors in relation to meteorological drivers (Cerino *et al.*, 2019; Totti *et al.*, 2019; Neri *et al.*, 2022). In the Northern Adriatic Sea (NAS), which is one of the most productive areas of the Mediterranean Sea, four LTER marine areas are present. Among these, the Senigallia-Susak Transect (SST) is located in the lower part of the NAS. Since 1988 to 2019, data of phytoplankton (abundances and biomasses) and abiotic parameters were collected in the SST during oceanographic cruises and at a fixed-point observatory, with a ca. monthly frequency. To study the multidimensionality of phytoplankton diversity and changes, a combination of factors should be addressed, combining abundances, biomass, community diversity indices but also functional diversity, which takes into account the role of the organisms in the ecosystems (Lyashevskaya and Farnsworth, 2012; Francé *et al.*, 2021). In this regard, graph-network analysis based on interactions, regardless the type, can give insights into the properties and dynamics of communities (Delmas *et al.*, 2019). In this study we compared environmental conditions and phytoplankton community structure between two stations (one coastal and one offshore), combining the use of different methods, in order to (i) highlight the main forcings affecting the phytoplankton community dynamics, (ii) compare the phytoplankton group abundances, diversity and community structure and (iii) highlight the phytoplankton intra- and interannual variability at the two stations.

Materials and methods - The sampling stations were located along the SST, one coastal (SG01, bottom depth 12 m) and one offshore (SG05, bottom depth 55 m), located at 1.2 and 15 nautical miles from the western NAS coast, respectively. At both stations, environmental parameters (temperature, salinity, dissolved inorganic nitrogen, orthophosphate, silicate) and phytoplankton community composition, abundances and biomass (not presented in this study) were collected from 1988 to 2019 on board of several oceanographic vessels. Although several depths were sampled, only the surface data (0.5 m) of both stations were considered. Principal Component Analysis (PCA) and Hierarchical Clustering on Principal Components (HCPC) were performed on environmental parameters and on the main phytoplankton group abundances. Data were scaled to allow comparability. In the offshore station, the interannual trends of physico-chemical parameters and phytoplankton group abundances were investigated through linear regression and analysis of variance (ANOVA), due to a sampling interruption between 2003 and 2012 (on ranked transformed data), respectively. Phytoplankton groups abundances were analysed through Non-Metric Multidimensional Scaling (NMDS), performed on the different seasons. To test for significant differences among the groups, a PERMANOVA was performed and the potential significant dispersion was checked. Shannon diversity index, Pielou's evenness and rarefied richness were used to compare the biodiversity of the two stations and two-samples Wilcoxon test to check statistically significant differences. The phytoplankton community was tested through the IndVal analysis, to identify phytoplankton key species on a seasonal basis. Furthermore, the graph-network analysis was used to investigate the interactions within the phytoplankton community at each station. Pearson correlations were calculated on log transformed species abundance data. Only significant ($p \leq 0.05$) and positive correlations were taken into account. The closeness centrality measure was considered for the analysis. All the data analysis were performed using the R (<https://CRAN.R-project.org/package=vegan>, <https://CRAN.R-project.org/package=FactoMineR>, <https://igraph.org/r/>) and STATISTICA 12 softwares.

Results - At both stations, the mean annual cycle of physico-chemical parameters showed a seasonal behaviour, although with different periods of minima and maxima. Salinity showed a different behaviour, as the minimum was found in December and June in the coastal and offshore stations, respectively. At the coastal station, the nutrients showed the maxima in spring and summer and the minima in December. Instead, offshore the maximum of DIN (Dissolved Inorganic Nitrogen), PO_4 and $Si(OH)_4$ were found in November, June and January, respectively. A peak of DIN was observed in summer. These peaks and maxima of DIN and PO_4 at the offshore stations are related to the spreading of riverine waters in stratified conditions. The mean annual cycle of phytoplankton differed between the two stations (Fig. 1). At the coastal station, the maxima were observed in winter, followed by other peaks in spring and autumn, while the minimum in summer. Offshore, diatoms, dinoflagellates and phytoflagellates showed the annual maximum in June, in concomitance with high values of DIN and PO_4 , while the coccolithophores in April. Important interannual trends (increases in temperature, DIN, PO_4 and decreases of coccolithophores) were also found at both stations. The PCA performed on physico-chemical parameters highlighted that the discrimination between coastal and offshore stations was primarily driven by DIN, followed by Salinity, $Si(OH)_4$ and DIN/PO_4 and that the coastal station was more variable than the offshore one. This was also confirmed by the HCPC, as SG01 clustered in three

groups, while SG05 only in one. A pattern similar to that of physical and chemical parameters was also observed with the PCA and HCPC performed on the main phytoplankton group abundances. The NMDS analysis found a higher difference, in terms of phytoplankton group abundances, in winter and autumn, although the PERMANOVA showed a significant difference in all the seasons ($p < 0.001$). Significant dispersion values were found in winter and autumn ($p < 0.001$), as a high variability of coastal samples can be observed (as highlighted also by HCPC analysis). Considering the mean abundances of the main phytoplankton group for each season, higher values were found at the coastal station than at the offshore, with the exception of coccolithophores that in winter showed significantly higher values at offshore than at the coastal station. The diversity was higher offshore than at the coastal station. All the three indices were found to be significantly higher in SG05 than in SG01 ($p < 0.001$), while no difference was observed in summer. In spring, Evenness and Rarefied Richness were significantly higher offshore than in the coastal station ($p < 0.01$ and $p < 0.05$, respectively). The IndVal analysis revealed some similarities in terms of significant seasonal species (e.g. *Skeletonema marinoi* Sarno & Zingone in the winter communities of both stations) and differences (e.g. *Guinardia striata* (Stolterf.) Hasle in the offshore autumn community, not present as an indicator in the coastal station). The Graph-Network analysis showed that the taxa that are not considered as indicator following the IndVal, were the ones characterized by high values of closeness in both sites. The offshore networks showed a higher percentage of strong interactions, than the coastal ones.

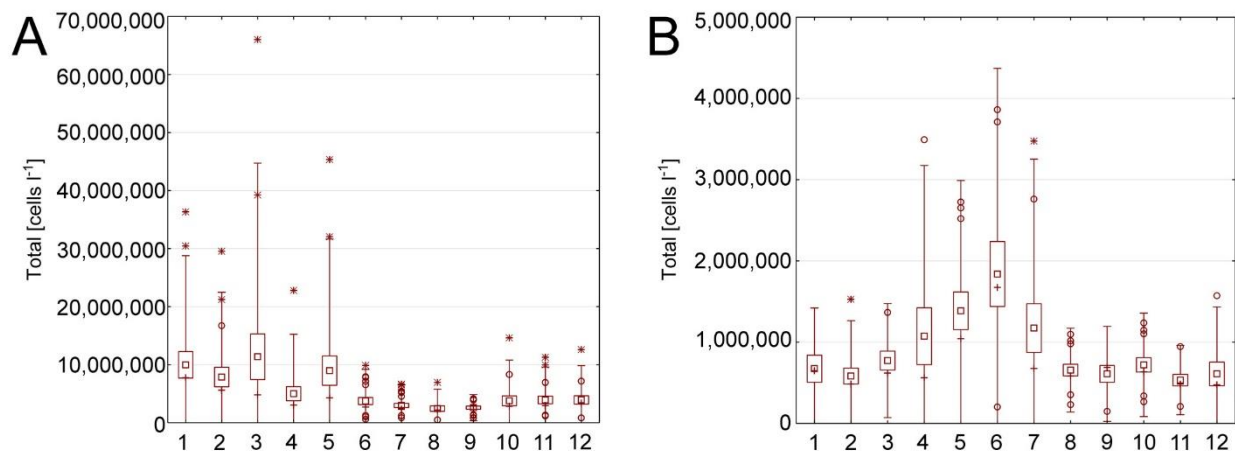


Fig. 1 - Mean annual cycle, on a monthly basis, of total phytoplankton abundances at the coastal (A) and offshore (B) stations.

Ciclo annuale medio su base mensile delle abbondanze del fitoplancton totale alla stazione costiera (A) e al largo (B).

Conclusions - In this study we showed the role of the riverine inputs and of the spreading of fresher and nutrient-rich waters during stratified conditions in discriminating the two stations of the transect and in affecting the phytoplankton community. The statistical analysis highlighted that the offshore station was more stable than the coastal one, as revealed by the HCPC, and that the two stations were more similar in summer, as showed by the NMDS and the diversity indices. Indeed, the coastal area was affected by the riverine waters from the Northern Adriatic, which are conveyed by the Western Adriatic Current (WAC), reducing salinity and increasing nitrate and silicate concentrations (Artegiani *et al.*, 1997), those factors discriminating the coastal

sampling points in the PCA. On the contrary, the offshore station, located beyond the WAC, was affected by riverine inputs only in stratified conditions (Neri *et al.*, 2022) and was more stable throughout the year. As the effect of the riverine input rivers was more evident along the coast than offshore, higher phytoplankton abundances were found always in the coastal site (with the exception of winter coccolithophores, that were higher in SG05), while diversity values were higher in SG05, as already found in other oligotrophic areas (Varkitzi *et al.*, 2020; Francé *et al.*, 2021). The effect of the different environmental conditions and oceanographic circulation along the SS transect was reflected in a different mean annual cycle of nutrients and phytoplankton and in differences in the community composition, as highlighted by the IndVal. Graph network analysis highlighted that taxa not indicator of a certain season (following the IndVal) were the ones with high values of closeness, and thus able to influence the overall community (in both sites) as they were more homogeneously distributed throughout the year. On the contrary, the indicator taxa were more or less strictly related to the specific environmental conditions of a certain period. In the offshore network, where stronger relationships were observed due to the lower seasonal behaviour and higher stability, the lowest percentage of strong correlations was found in summer, underlying again the role of water stratification in affecting phytoplankton community. Important interannual trends have been highlighted at both stations, underlying the importance of long term observational studies.

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