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A PHYTOPLANKTON TIME SERIES IN THE NORTHWESTERN ADRIATIC SEA: STRUCTURE AND DYNAMICS OF THE ASSEMBLAGES IN A COASTAL ECOSYSTEM

STUDIO DELLE SERIE TEMPORALI DEL FITOPLANCTON IN MAR ADRIATICO NORDOCCIDENTALE: STRUTTURA E DINAMICA DEI POPOLAMENTI IN UN ECOSISTEMA COSTIERO

Abstract - *Phytoplankton is responsible for nearly half of the photosynthetic fixation of CO_2 and represents the first level of the food web in oceans. Due to phytoplankton fundamental ecological role, the aim of this study was to assess whether the phytoplankton assemblage structure could vary in time and space and if this variation could be due to such environmental forcing. Surface seawater samples were collected monthly from 2008 to 2019 at two coastal sites in the Northwestern Adriatic Sea together with physico-chemical and biological parameters recorded by a multiparametric probe. Phytoplankton identification and quantification were performed together with chlorophyll a and dissolved inorganic nutrient concentrations determination. Data analysis indicated that* Skeletonema marinoi*,* Thalassionema nitzschioides*,* Dactyliosolen fragilissimus*, and undetermined Dinophyceae, showed a non-random pattern in the binary time series. Moreover, the observed phytoplankton assemblage structure was driven by seasonal and environmental factors.*

Key-words: *Assemblage structure, environmental variables, Northwestern Adriatic Sea, phytoplankton, time series*

Introduction - Phytoplankton is responsible for atmospheric carbon fixation and represents the first level of the food web in marine ecosystems (Treguer *et al.*, 2018). The study of the ecological structure of phytoplankton assemblages, and in particular the variations in time and space is crucial due to their role in primary production (Legendre *et al*., 2015). In this context, time series of phytoplankton data can provide information on spatial and temporal patterns of species populations and assemblages variation due to changes in meteorological and environmental variables, caused by climate change and human disturbances. Since phytoplankton can be considered a good proxy for quantifying environmental changes due to its rapid turnover, also in response to environmental changes, the study of time series assemblage structure may be crucial to move towards water quality targets, as required by both the European Water Framework Directive and Marine Strategy Framework Directive. In this study, phytoplankton assemblage structure was analyzed based on time series data (2008–2019) together with environmental variables in two coastal sites in the northwestern Adriatic Sea to test whether it could be influenced by environmental forcings.

Materials and methods – Sampling was carried out monthly from 2008 to 2019 along two transects in front of Foglia and Metauro river mouths (northwestern Adriatic Sea). In each transect, stations were located at 0.5 and 3 km from the coast. Physico-chemical parameters such as seawater temperature (°C), salinity, density (σт), oxygen concentration (mg L^{-1}) and saturation (%), pH and redox were recorded by a CTD multiparametric probe. Surface seawater samples (0.5 m depth) were collected by Niskin bottles for phytoplankton (stored by adding 0.2% Lugol solution) and nutrient analyses (determined on filtered seawater samples). Phytoplankton identification and quantification were carried out by light microscopy (Zeiss Axiovert 40 CFL), according to the Utermöhl method (Edler and Elbrachter, 2010). Counting was performed at 200 or 400× magnification along transects, after settling a variable volume of seawater (10–25 mL), depending on cell abundance, to count a minimum of 200 cells. Chlorophyll *a* (Chl *a*) and dissolved inorganic nutrients (ammonia-NH_4, nitrite-NO_2, nitrate-NO_3, orthophosphate-PO_4 and orthosilicate-$Si(OH)_4$) determinations were carried out spectrophotometrically (mod. UV-1700, Shimadzu, Japan) on seawater sub-samples. Concerning the statistical analyses, Wald-Wolfowitz runs tests were performed to evaluate deviation from random occurrence in the time series for each phytoplankton taxon. An ordination of phytoplankton assemblage data constrained by environmental variables was obtained by distance-based Redundancy Analysis (dbRDA). Association between phytoplankton taxa was analyzed by means of a species co-occurrence network. All the analyses, except runs tests, were carried out under R 4.1.1 (2021).

Results – Among the 98 identified phytoplankton taxa, four consistently showed a non-random pattern in the binary time series, such as *Skeletonema marinoi* Sarno & Zingone, *Thalassionema nitzschioides* (Grunow) Mereschkowsky, *Dactyliosolen fragilissimus* (Bergon) Hasle and undetermined Dinophyceae (Fig. 1). The latter taxon was excluded by the analyses as it did not occur frequently and did not convey interpretable ecological information.

Fig. 1 - Bar plots show seasonal (blue bars) and annual (green bars) occurrences at the four sampling stations (F3000, F500, M3000 and M500) of *Skeletonema marinoi, Thalassionema nitzschioides,* and *Dactyliosolen fragilissimus*. These taxa are the only ones which deviate from randomness in their time series of occurrence at all sampling stations.
Bar plots delle presenze stagionali (barre blu) e annuale (barre verdi) nelle quattro stazioni di campionamento (F3000, F500, M3000 e M500) di Skeletonema marinoi, Thalassionema nitzschioides, *e* Dactyliosolen fragilissimus*. Questi taxa sono gli unici che si discostano dalla casualità nelle loro serie temporali di presenza in tutte le stazioni di campionamento.*

S. marinoi was more frequent in winter and early spring, whereas *T. nitzschioides* showed an opposite pattern with high frequency in late spring and summer. This was due to a clear and stationary annual cycle. Concerning *D. fragilissimus*, its deviation from randomness depended on the long-term trend of this species time series, as this diatom appeared more frequently since 2016. The phytoplankton assemblages and environmental variable time series were analyzed by distance-based Redundancy Analysis (Fig. 2). Temperature, dissolved oxygen, nitrate, and nitrite concentrations were responsible for the ordination of phytoplankton assemblage data indicating that in the Northwestern Adriatic Sea, phytoplankton assemblage structure was likely driven by seasonal and environmental factors.

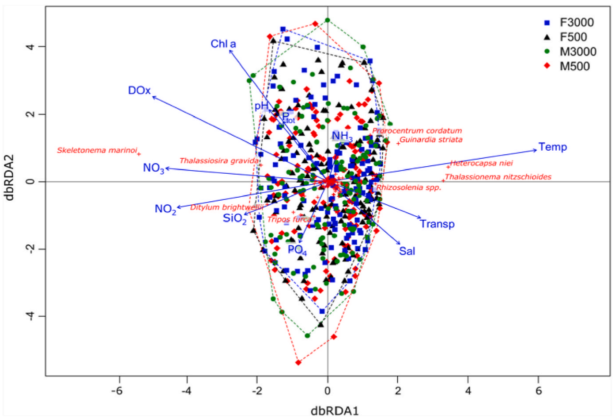

Fig. 2 - Distance-based Redundancy Analysis (RDA) of phytoplankton assemblage structure from data series (2008–2019) in relation to environmental variables. Distance between phytoplankton assemblages is measured based on Jaccard dissimilarity by considering only the lowest, but most reliable level of information, i.e., taxa occurrence. The first two constrained axes account for 56.5% and 8.9% of the total inertia, respectively.

Distance-based Redundancy Analysis (RDA) della struttura dei popolamenti fitoplanctonici da serie temporali (2008-2019) in relazione alle variabili ambientali. La distanza tra i popolamenti fitoplanctonici è misurata in base alla dissimilarità di Jaccard considerando solo il livello di informazioni più basso, ma più affidabile, cioè la presenza di taxa. I primi due assi rappresentano rispettivamente il 56,5% e l'8,9% dell'inerzia totale.

Moreover, a similarity network of the more representative phytoplankton taxa was performed to evaluate the association among them (Fig. 3). The pairwise similarities were shown as black and red solid lines indicating within- and between-clusters similarity, respectively. Only three of the resulting clusters included more than a single taxon (2, 6 and 7, respectively). The smallest and completely separated group from the others clusters was composed by *Ditylum brightwellii* (T.West) Grunow and *Thalassiosira rotula* Meunier. The two clusters, one including *Alexandrium minutum* Halim, *Chaetoceros* spp., *Gymnodinium* spp., *Prorocentrum micans* Ehrenberg, *Prorocentrum cordatum* (Ostenfeld) J.D.Dodge and *S. marinoi* and the other composed by *Cylindrotheca fusiformis* Reimann & Lewin, *Guinardia striata* (Stolterfoth) Hasle, *Heterocapsa niei* (A.R.Loeblich) L.C.Morrill & A.R.Loeblich, *Leptocylindrus danicus* Cleve, *Navicula* spp. (Bory, 1822), *Pseudo-nitzschia* spp. and *T. nitzschioides* were not completely independent showing multiple inter-cluster connections. The remaining 13 taxa did not form clusters.

Conclusions - In conclusion, it was shown that in the northwestern Adriatic Sea, the regular periodic pattern of phytoplankton assemblage structure was driven by seasonal and environmental factors as for the species *Skeletonema marinoi*, *Heterocapsa niei* and *Thalassionema nitzschioides* (Casabianca *et al.*, 2022). The study of long-term monitoring confirmed the efficacy of time series data which showed a regular pattern even in areas subjected to human pressures (i.e., low precipitation induced by climate change, European regulations of nutrient loadings and anthropogenic use) such as the Northwestern Adriatic Sea. Thus, any future change in the pattern of phytoplankton assemblage structure, will be considered as a warning about possible changes in the environmental parameters taken into acount. These potential changes could have an impact on the functioning of the coastal ecosystem and its ability to provide ecosystem services.

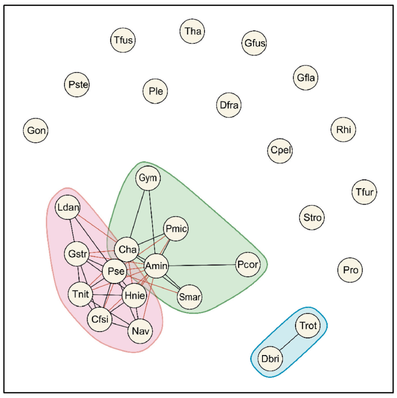

Fig. 3 - Network of phytoplankton taxa association based on Jaccard similarities. Three taxa groups emerged, one of which included only two species. (Cha, *Chaetoceros* spp.; Pse, *Pesudo-nitzschia* spp.; Hnie, *Heterocapsa niei*; Smar, *Skeletonema marinoi*; Nav, *Navicula* spp.; Tnit, *Thalassionema nitzschioides*; Gstr, *Guinardia striata*; Tfus, *Tripos fusus* (Ehrenberg) F.Gómez; Ldan, *Leptocylindrus danicus*; Gym, *Gymnodinium* spp.; Pcor, *Prorocentrum cordatum*; Gfus, *Gyrodinium fusiforme* Kofoid & Swezy; Trot, *T. rotula*; Tfur, *Tripos furca* (Ehrenberg) F.Gómez; Pro, *Prorocentrum* spp.; Stro, *Scrippsiella trochoidea* (F.Stein) A.R.Loeblich III; Cpel, *Cerataulina pelagica* (Cleve) Hendey; Rhi, *Rhizosolenia* spp.; Pste, *Protoperidinium steinii* (Jørgensen) Balech; Gfla, *Guinardia flaccida* (Castracane) H.Peragallo; Ple, *Pleurosigma* spp.; Dfra, *Dactyliosolen fragilissimus*; Dbri, *Dytilum brightwellii*; Gon, *Gonyaulax* spp.; Tha, *Thalassiosira* spp.).
*Network di associazione di taxa di fitoplancton basato su somiglianze di Jaccard. Sono emersi tre gruppi, uno dei quali comprendeva solo due specie. (*Cha, Chaetoceros *spp.*; Pse, Pesudo-nitzschia *spp.*; Hnie, Heterocapsa niei; Smar, Skeletonema marinoi; Nav, Navicula *spp.*; Tnit, Thalassionema nitzschioides; Gstr, Guinardia striata; Tfus, Tripos fusus; Ldan, Leptocylindrus danicus; Gym, Gymnodinium *spp.*; Pcor, Prorocentrum cordatum; Gfus, Gyrodinium fusiforme; Trot, T. rotula; Tfur, Tripos furca; Pro, Prorocentrum *spp.*; Stro, Scrippsiella trochoidea; Cpel, Cerataulina pelagica; Rhi, Rhizosolenia spp.; Pste, Protoperidinium steinii; Gfla, Guinardia flaccida; Ple, Pleurosigma *spp.*; Dfra, Dactyliosolen fragilissimus; Dbri, Dytilum brightwellii; Gon, Gonyaulax spp.; Tha, Thalassiosira *spp.).*

As phytoplankton is considered a good proxy for quantifying environmental changes, the monitoring phytoplankton assemblage structure through the analysis of time series may be therefore crucial to achieve the Good Environmental Status in compliance to environmental directives.

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