Microbial biogeochemistry in the Southern European Seas: the multidisciplinary ADREX survey

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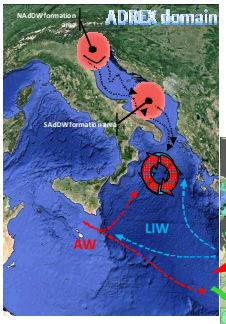
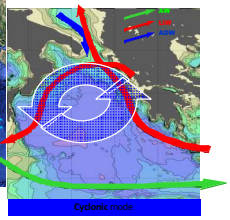
In the frame of PERSEUS EU Fp7 project, part of the multidisciplinary oceanographic campaign ADREX was dedicated to microbial biogeochemistry and its possible connection with climate change in Southern European Seas, according to the European MSFD. Within the Mediterranean, the main physical pressures in the Adriatic-Ionian system are: 1) the dense water formation by both shelf processes in the northern Adriatic and open-ocean convection in the southern basin; 2) the Bimodal Oscillating System mechanism that reverses the Northern Ionian Gyre circulation on decadal scale. In this context, selected stations were investigated in the Ionian and Adriatic Seas (February 2014) by means of seawater samples collection from the surface to a maximum depth of 3698 m. Microbial biogeochemistry was studied by assaying: prokaryotic abundance and size (image analysis and flow cytometry), microbial enzymatic activities, chlorophyll-a and phaeopigments concentrations, viral abundance. The hydrological properties were also monitored.



ADREX considers ***two natural pressures*** (or ***processes***) acting in the Adriatic-Ionian area:

➢**Dense Water Formation (DWF)**
Northern shelf process (in the North Adriatic)
Open sea convection (in the South Adriatic)

➢**North Ionian Gyre reversal (NIGr)**
(on decadal scale)



ADRIATIC SEA

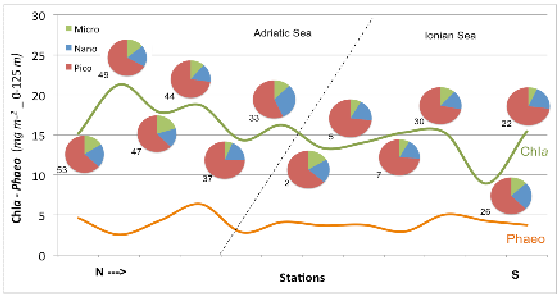
	Station n°	Latitude	Longitude	max depth (m)
South Adriatic	49	42,44	16,60	238
	53	42,59	14,28	258
	44	41,83	17,75	1120
	47	42,28	18,29	794

IONIAN SEA

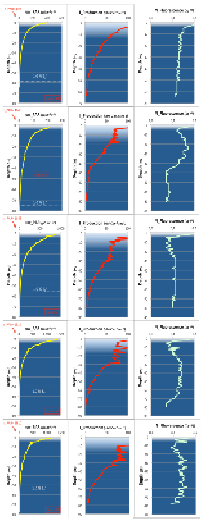
	Station n°	Latitude	Longitude	max depth (m)
N-W Ionian	02	38,50	17,50	1839
	05	38,50	19,00	3115
	33	39,79	18,65	152
	07	38,50	20,00	2377
	37	39,71	19,10	993
South Ionian	22	36,50	18,00	3647
	26	36,50	22,00	2212
	28	37,21	20,26	3300
	30	37,90	18,50	3150

Sampled stations

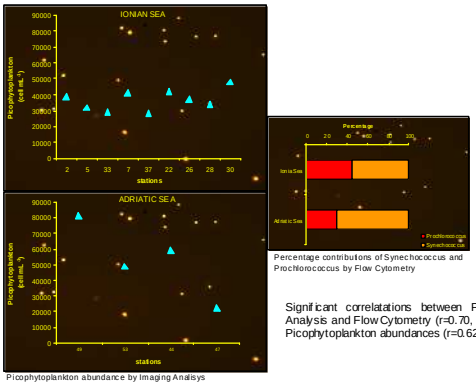
MATERIALS AND METHODS
Prokaryotic abundance by a ZEISS AXIOPLAN 2 IMAGING, according to Porter & Feig, 1980 Lim nol. Oceanogr.
Prokaryotic volume (La Ferla et al., 2014 Hydrobiol.)
Prokaryotic abundance cytom etric counts (Gasol & del Giorgio 2000 Sci. Mar.)
Virus Like Particle (VLP) (Brouss and 2004 Appl. Environ Microbiol.)
Chlorophyll a (Chl-a) (Lazz ara et al., 1990 Nova Thalassia)
Total extracellular enzymatic activity (LAP, AP, B-GLU) (Zaccone et al., 2013 Microb. Ecol.)



Chlorophyll *a* and Phaeopigments concentrations in the layer 0-150 m depth are shown in the graph. The stations are located along the north-south axis and separated between the Adriatic Sea and the Ionian Sea. The low percentage of degraded (Phaeo) compared to chlorophyll *a* (Chla) pigments indicates how the phytoplanktonic community is in the winter stationary growth. Pie charts show the mean contribution of the size fractions (Micro- >10 µm, Nano- <10 - >2.0 µm; Pico- < 2.0 µm) in each station. The smallest size fraction (Pico) is everywhere dominant with average values of 68% and this percentage is constant in both basins (Adriatic and Ionic). The latter condition is also observed for the largest fractions: 12% Micro- and 20% Nano- plankton.



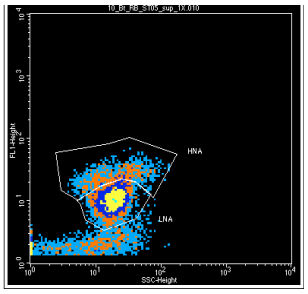
Vertical profile of Under Water (uw) PAR (Photosinthetic Active Radiation), Istantaneous Production and Natural Fluorescence in the Adriatic (stations 49 and 44) and Ionian Seas. On the uwPAR graph, both the intensity of incident PAR to the surface (the value in red at the top left) and depth at which there is 1% of the surface radiation are shown. This level of solar radiation delimits the photic zone (still useful for the process of photosynthesis) from the aphotic one. The values of the Instantaneous Production are around 5.0 nM-C m^{-3} sec^{-1} in the layer 0-30 m depth, then decrease towards the bottom. The Natural Fluorescence (expressed as mg m^{-3} of Chla) shows low values (less than 0.2 mg m^{-3}) but similar to those obtained by the measures of chlorophyll.



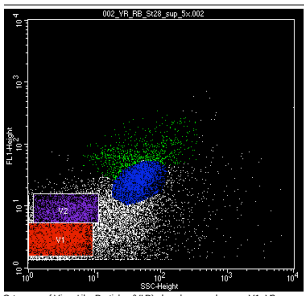
Picophytoplankton abundance by Imaging Analisys

Percentage contributions of Synechococcus and Prochlorococcus by Flow Cytometry

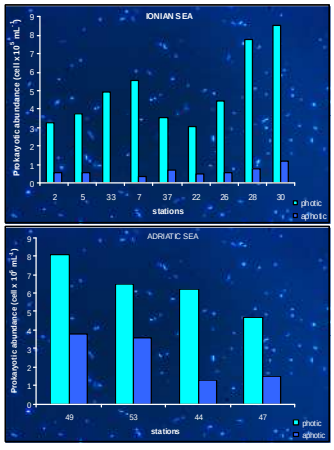
Significant correlations between Prokaryotic abundances by Image Analysis and Flow Cytometry (r=0.70, n=129, P<0.01) as well as between Picophytoplankton abundances (r=0.62, n=61, P<0.01) are determined.



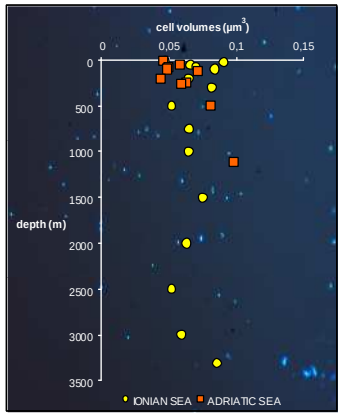
Cytogram of prokaryotic abundance and sub-populations with a different apparent DNA content (HNA High Nucleic Acid and LNA Low Nucleic Acid). Station 5at 5 m depth



Cytogram of Virus Like Particles (VLP) abundance and groups V1, V2. Station 28 at 5 m depth



Integrated and normalized data of prokaryotic abundance

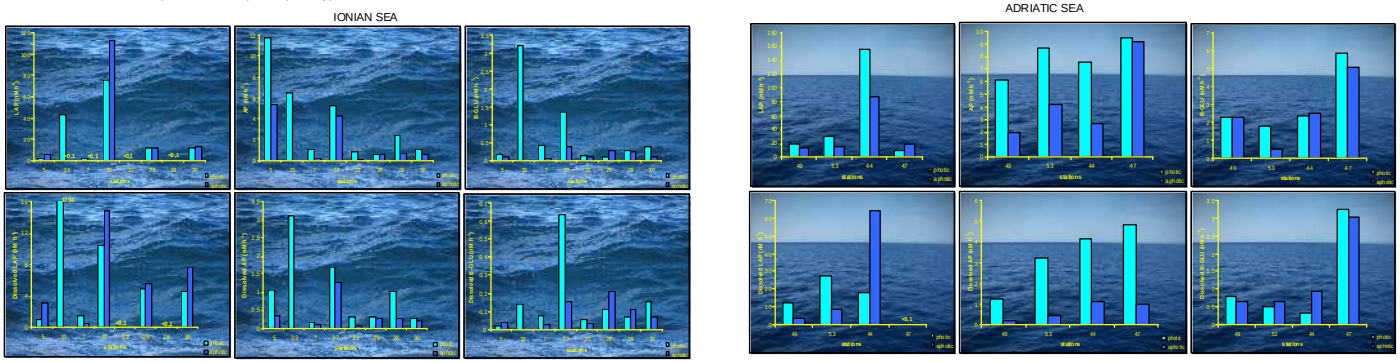


Mean cell volumes along the water column

Differences in the microbiological patterns are found between Western and Eastern stations as well as between photic and aphotic layers of Adriatic and Ionian basins, in relation with anthropogenic and/or natural impacts. Prokaryotic abundances resulted higher in the Adriatic than in the Ionian Sea (mean values: 4.6 ± 3.0 and 2.6 ± 2.7 x 10^5 ml^{-1}, respectively). The ratios between VLP and prokaryotes are higher in the Ionian than in the Adriatic Sea, particularly in the aphotic layers. Higher HNA/LNA ratios also occur with increasing depth. The mean cell volumes of all data, similarly vary in both Ionian and Adriatic Sea (0.068 and 0.062 µm3, respectively). In the Ionian Sea small differences in cell volumes are observed between the photic and aphotic layers: 0.072 and 0.067 $µm^3$, respectively, whereas a more variability is observed in the Adriatic Sea (0.051 and 0.069 $µm^3$, respectively).

IONIAN SEA

ADRIATIC SEA



Integrated and normalized data of the total and dissolved enzymatic activities in the photic and aphotic layers of the different stations. AP = Alkaline phosphatase; B-GLU = beta-Glucosidase; LAP = Leucine aminopeptidase.

As regards the metabolic patterns, differences occur in the spatial distribution of decomposition enzymatic processes on both total and dissolved matter, probably in relation with circulation patterns. The general predominance of proteolytic activity - over the glucosidic and phosphatase ones - suggests a preferential metabolism over the proteinaceous labile matter. The highest rates are detected in the South Adriatic in the photic layer (on total matter) and W-Ionian stations, in the aphotic layer (on dissolved matter). The stations 37 (E-Ionian) and 44 (S-Adriatic) could be involved in deep water formation events. In the Ionian Sea, the high dissolved enzymatic activities on organic carbon (accounting to the 34-70%, 4-97% and 7-58% of the total, for LAP, GLU and AP, respectively) suggest a high efficiency of the microbial carbon pump, involved in the DOC transformation.

The microbial community rapidly responds to environmental changes in terms of both abundance and metabolism. Previous study in the Southern Adriatic Sea highlighted that - when convective phenomenon did not occur - the variability of prokaryotes oxidative metabolism was governed by the seasonal cycle of the organic matter, while at the time of deep water ventilation, it changed the metabolic trend along the water column. The microbial integrated approach appears to be useful to support the knowledge of the evolutionary scenario the Mediterranean sea. The effect of dense shelf water cascading event could have ecological implications over the microbial functioning in the studied areas. A synergic approach - involving the complex circulatory patterns of the study area - is needed to explain the effects anthropogenic and/or natural impacts on the microbial biogeochemistry.

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