

RIUNIONE SCIENTIFICA NAZIONALE  
GRUPPO DI ALGOLOGIA  
Società Botanica Italiana

Orto Botanico – Catania  
16/17 Novembre 2018

A cura di  
Giuseppina Alongi, Francesca Toscano, Rosalinda Turnaturi

Comitato organizzatore:  
Giuseppina Alongi

# Programma



## VENERDÌ 16 NOVEMBRE

- 9.30 **Registrazione**  
 10.00 **Saluti istituzionali**  
 10.15 **Ricordo del prof. Giaccone**  
 F. Cinelli  
 10.30 **Il Codice: questo sconosciuto (?). Manuale d'uso**  
 G. Furnari  
 11.15 *Coffee break*

### **MICROALGHE 1 – Moderatore: Roberta Congestri**

- 11.45 **Beyond peptidase activity of cyanobacterial phytochelatin synthases: the case of *Nostoc* sp., *Gleiterinema* sp., *Gloeobacter violaceus***  
 E. Bellini, L. Rugnini, A. Saba, R. Congestri, L. Sanità di Toppi, L. Bruno  
 12.00 **Cultivation of *Haematococcus pluvialis* for astaxanthin extraction through a milking process**  
 C. García Díez, C. Samorì, M. Semeraro, R. Pistocchi, L. Pezsolesi  
 12.15 **Proteins, pigments content and ultrastructure of *Arthrospira platensis* in blu and yellow light and in increasing white intensities**  
 M. Pelagatti, G. Mori, R. Ballini, P. Di Falco, C. Tani, A. Papini, L. Lazzara  
 12.30 **Microalgae monitoring in freshwater reservoirs intended for human consumption: application of innovative fluorimetric tools**  
 M. Simonazzi, F. Guerrini, L. Pezsolesi, S. Vanucci, R. Pistocchi  
 12.45 **Interdecadal variability in phytoplankton community structure in the coastal area of the LTER-Senigallia transect (northern Adriatic Sea) over a 40 years period (1977-2016)**  
 C. Totti, T. Romagnoli, S. Accoroni, A. Coluccelli, S. Giuliotti, M. Pellegrini, A. Campanelli, F. Grilli, M. Marini  
 13.00 *Pausa pranzo*

### **MACROALGHE 1 – Moderatore: Giusi Alongi**

- 15.00 **Promoting biodiversity enhancement by Restoration Of *Cystoseira* Populations – ROC Pop Life project**  
 M. Chiantore, V. Asnaghi, G. De La Fuente, S. Kaleb, S. Ciriaco, G. Fanciulli, P. Scarpellini, L. Kastelic, A. Falace  
 15.15 **Long-term changes of Fucales in the Gulf of Naples**  
 D. Grech, M.C. Buia

- 15.30 **Il declino delle comunità a *Cystoseira* (Fucales, Ochrophyta) lungo le coste della Sicilia orientale: osservazioni sul grazing dei pesci erbivori**  
G. Marletta, D. Serio
- 15.45 **Seasonal variability of epiphytic micro and macroalgal communities on *Cystoseira barbata* and *C. compressa* in the Conero Riviera**  
F. Moroni, T. Romagnoli, F. Rindi, S. Accoroni, C. Totti
- 16.00 **The role of phytobenthic biomass in the natural capital assessment of a marine protected areas**  
F. Toscano, G. Alongi, E. Conti, R. Turnaturi, C. Mulder

## **MICROALGHE 2 – Moderatore: Cecilia Totti**

- 16.15 **Phosphatase activities of the microbial community during the bloom of *Ostreopsis cf. ovata* in the northern Adriatic Sea: the role of temperature and organic phosphorus sources**  
S. Accoroni, M. Pasella, T. Romagnoli, E. Razza, C. Totti, N.T.W. Ellwood
- 16.30 **Environmental metabarcoding and local reference barcoding reveal remarkably high diversity in the planktonic diatom family Chaetocerotaceae**  
W.H.C.F. Kooistra, C.C. Gaonkar, A. Zingone, R. Piredda, D. Sarno, M. Montresor
- 16.45 **First detection of *Prorocentrum cf. shikokuense* bloom in the Mediterranean Sea**  
L. Roselli, M.R. Vadrucchi, F. Fanelli, N. Ungaro, C. Caroppo
- 17.00 *Coffee break*

## **MACROALGHE 2 – Moderatore: Maria Cristina Buia**

- 17.30 **Shipping does not seem an effective vector for the introduction of non-indigenous seaweeds in Italian ports**  
A. Petrocelli, E. Cecere, L. Bolognini, M. Marini, F. Rubino, A. Falace
- 17.45 **Le alghe di alcuni tratti sorgentizi del fiume Liri (Italia centro-meridionale)**  
N. Abdelahad, O. De Castro, M. Iberite
- 18.00 **Macrophyte diversity in a coastal lagoon inside the Marine Protected Area of Porto Cesareo (Italy)**  
A. Bottalico, A. Lisco, N. Ungaro, N. Dipierro
- 18.15 **Coralline algae of calcareous biological concretions of the northern Adriatic Sea (Tegnue and Trezze)**  
A. Caragnano, S. Kaleb, F. Rindi, A. Falace
- 18.30 **Amenità ficologiche**  
G. Furnari

18.45 *Assemblea dei soci*

20.30 *Cena sociale*

## SABATO 17 NOVEMBRE

### **MICROALGHE 3 – Moderatore: Wiebe Kooistra**

- 9.00 **Maintenance of a photosynthetic capacity by *Trentepohlia umbrina* in stressful subaerial environments**  
N. T.W. Ellwood, L. Bruno and G. Caneva
- 9.15 **Phytochelatin Synthase involvement in Cr(VI) tolerance in *Scenedesmus acutus* (Chlorophyceae)**  
M. Ferrari, M. Marieschi, R. Ruotolo, V. Reverberi, R. Cozza, A. Torelli
- 9.30 **Preliminary study of the effect of iron on the growth and morphology of extremophile green alga *Coccomyxa melkonianii* SCCA 048**  
V. Malavasi, S. Soru, A. Concas, S. Montinaro, M. Afzal, M. Pisu, G. Cao
- 9.45 **A different expression of two H<sup>+</sup>/SO<sub>4</sub><sup>2-</sup> transporters characterizes two strains of *Scenedesmus acutus* with different chromium sensitivity**  
M. Marieschi, M. Ferrari, A. Locatelli, C. Zanni, R. Cozza, A. Torelli
- 10.00 **Diatom biorefineries: mycosporine-like amino acids (MAAs), lipids and biosilica from *Phaeodactylum tricornutum*, *Staurosirella pinnata* and *Thalassiosira weissflogii***  
S. Savio, S. Farrotti, S. Antonaroli, R. Lauceri, R. Congestri
- 10.15 **If Dante had known Phytoplankton: A comparison between literature and science through the didactics of the metaphors**  
M.R. Vadrucci, F. Vitale, M.T. Duggento, C. Alberani, A. Calò, G. Giancane, B.B. Rizzelli, S. Schipa, R. Visconti, F. Del Vecchio, B. Paiano, F. Imperiale, D. Brunetti, L. Pancosta
- 10.30 **How biodiversity and structure help to improve biofilm control in cooling industrial systems**  
L. Di Gregorio, S. Rossetti, R. Congestri, F. Di Pippo
- 10.45 *Coffee break*

### **MACROALGHE 3 – Moderatore: Annalisa Falace**

- 11.15 **The diversity of Halymeniales (Rhodophyta) from Madagascar and Guadeloupe explored by DNA tools**  
A. Manghisi, M. Saitta, M. Morabito, L. Le Gall

- 11.30 **Risultati preliminari di uno studio molecolare e citologico su *Polysiphonia caretia* e "*Neosiphonia sertularioides*" (Rhodomelaceae, Rhodophyta)**  
K. Sciuto, I. Moro, G. Furnari, D. Serio
- 11.45 **The DNA barcode-assisted floristic list of red algae from Tunisia**  
M. Morabito, A. Manghisi, R. Miladi, S. Armeli Minicante, L. Le Gall, S. Abdelkafi, G.W. Saunders and G. Genovese
- 12.00 ***Asparagopsis*: a rich source of allelochemical compounds**  
D. Spagnuolo, A. Manghisi, M. Morabito, G. Genovese
- 12.15 **Total phenolic content in brown algae from the Sicilian coasts**  
A. M. Mannino, E. Oddo
- 12.30 **Chiusura lavori**

# Riassunti



## **Il Codice: questo sconosciuto (?). Manuale d'uso.**

G. Furnari

Dipartimento di Scienze Biologiche, Geologiche e Ambientali dell'Università di Catania, Via A.  
Longo 19, 95125 Catania - Italia

Dopo una breve introduzione sulle origini e su come si sia evoluto il *Codice Internazionale di Nomenclatura delle alghe, funghi e piante* (ICN), vengono presentate le basi che governano la nomenclatura e le principali regole che consentono di riconoscere come validamente pubblicato il nome di un *taxon*: effettiva pubblicazione del nome, forma corretta, presenza di una descrizione o diagnosi, ecc. Vengono quindi illustrate le regole che consentono di pubblicare nuove combinazioni valide e la differenza tra il concetto di validità e quello di legittimità dei nomi. Vengono infine presentate le principali norme che regolano la corretta ortografia dei nomi dei generi e delle specie.

## Beyond peptidase activity of cyanobacterial phytochelatin synthases: the case of *Nostoc* sp., *Gleiterinema* sp., *Gloeobacter violaceus*

E. Bellini<sup>1,2</sup>, L. Rugnini<sup>1</sup>, A. Saba<sup>3</sup>, R. Congestri<sup>1</sup>, L. Sanità di Toppi<sup>2\*</sup>, L. Bruno<sup>1\*</sup>

<sup>1</sup>University of Rome 'Tor Vergata', Dept. Biology, Via Cracovia 1, 00133 Roma, Italy

<sup>2</sup>University of Pisa, Dept. Biology, via Luca Ghini 13, 56126, Pisa, Italy

<sup>3</sup>University of Pisa, Dept. of Surgical, Medical and Molecular Pathology and Critical Care Medicine, via Paolo Savi 10, 56126, Pisa, Italy

\*co-senior authors and corresponding

The enzyme phytochelatin synthase (PCS) is a  $\gamma$ -glutamylcysteine dipeptidyl (trans)peptidase (EC 2.3.2.15), belonging to the clan CA of the papain-like cysteine proteases. The PCS catalyzes the prompt formation of some peculiar thiol-peptide compounds, the so-called "phytochelatins", starting from the reduced form of glutathione (GSH) *via* a transpeptidation reaction. Phytochelatins (PCs) are thiol-peptide compounds whose general structure is  $(\gamma\text{-glutamate-cysteine})_n\text{-glycine}$ , with  $n$  usually ranging from 2 to 5 (Grill et al., 1985). Due to the thiol group of the cysteine residues, PCs can bind cadmium (Cd) and other thiophilic heavy metals and prevent them from circulating in the cytosol, thus dramatically reducing their toxicity. It is now well known that land plants as well as some marine and freshwater algae (e.g. members of *Chlorophyta*, *Chrysophyceae*, *Phaeophyceae*, *Rhodophyta*), several fungi, lichens and even animal species do actually produce PCs in response to heavy metal stress, in particular Cd. Indeed, PCS is of particular interest from an evolutionary prospect, due to its constitutive expression and its widespread presence in nature. Recently, some PCS-like enzymes, sharing significant sequence homologies with land plant PCSs, were identified in cyanobacteria and in some gamma- and beta- proteobacteria. Previous evidences suggested that predicted product of PCS gene of *Nostoc* sp. (*alr0975*) contains the conserved N-terminal domain, but not the variable C-terminal domain found in eukaryotic PCSs (Tsuji et al., 2004; Vivares et al., 2005). Proteins encoded by the cyanobacterial genes seem to be primitive forms of PCS and to represent an early stage in the evolution of the enzyme in photoautotrophic organisms, since up to now only peptidase and almost absent transpeptidase activities have been reported for cyanobacteria (Tsuji et al., 2005). However, studies on functional characterization of prokaryotic PCS are scant, despite their importance as fundamental landmarks in evolution. Thus, the aim of our study is to investigate possible functional and molecular differences between phototrophic eukaryotic and prokaryotic PCS, by studying the enzymes in three cyanobacterial strains (*Nostoc* sp. PCC 7120, *Geitlerinema* sp. PCC 7407 and *Gloeobacter violaceus* PCC 7421). Preliminary HPLC-mass-spectrophotometry results evidenced a noteworthy PC synthesis in all the strains upon Cd-exposure, thus demonstrating that cyanobacterial PCS-like enzymes do possess transpeptidase activity, likewise eukaryotic PCSs. This evidence highlights a remarkable evolutionary conservation of PCS functionality between cyanobacteria, algae and land plants.

### References

- Grill E., Winnacker E.-L., and Zenk M.H. 1985. Phytochelatins: the principal heavy-metal complexing peptides of higher plants. *Science*, 230, 574 – 576.
- Tsuji N., Nishikori S., Iwabe O., Shiraki K., Miyasaka H., Takagi M., Hirata K., and Miyamoto K. 2004. Characterization of phytochelatin synthase-like protein encoded by *alr0975* from a prokaryote, *Nostoc* sp. PCC 7120. *Biochem Biophys Res Commun*, 315,751–755.
- Vivares D., Arnoux P., and Pignol D. 2005. A papain-like enzyme at work: Native and acyl-enzyme intermediate structures in phytochelatin synthesis. *PNAS*, 102, 18848–18853.
- Tsuji N., Nishikori S., Iwabe O., Matsumoto S., Shiraki K., Miyasaka H., Takagi M., Miyamoto K., and Hirata K. 2005. Comparative analysis of the two-step reaction catalyzed by prokaryotic and eukaryotic phytochelatin synthase by an ion-pair liquid chromatography assay. *Planta*, 222, 181-191.

## Cultivation of *Haematococcus pluvialis* for astaxanthin extraction through a milking process

C. García Díez<sup>1</sup>, C. Samorì<sup>2</sup>, M. Semeraro<sup>1</sup>, R. Pistocchi<sup>1</sup>, L. Pezolesi<sup>1</sup>

<sup>1</sup> Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Bologna

<sup>2</sup> Dipartimento di Chimica "Giacomo Ciamician", Università di Bologna

Carotenoids have received increasing attention due to their high antioxidant activity and their suitability as components of various types of products (e.g. cancer prevention agents, inhibiting agents for heart attack and coronary artery disease). Astaxanthin is one of the most effective carotenoids with 10 times stronger antioxidant properties than those of any other carotenoid. Due to the high application potential in the nutraceutical, pharmaceutical, cosmetics, food and feed industries, astaxanthin has a high commercial importance. The green microalga *Haematococcus pluvialis* is the richest source for the production of astaxanthin, which is a secondary carotenoid not essential for the growth and metabolism of the alga. The extraction of astaxanthin from red cyst *Haematococcus* cells is usually performed by using organic solvents, such as ethanol, acetone and hexane. However, these petroleum-derived solvents are potentially toxic and unacceptable for pharmaceutical and food industries, thus green downstream processes have been proposed. Recently, it has been reported that milking of microalgae can strikingly reduce the time and cost associated with the algal biorefinery process. The idea is to reuse the biomass for the continuous production of astaxanthin, while maintaining microalgal vitality, just like milking cows. *Haematococcus pluvialis* was optimally grown using three slightly different culture media. Afterwards the culture was stressed to induce the transformation of green vegetative cells to red cysts under unfavorable environmental conditions, such as strong light intensity and nitrogen source exhaustion. Milking using various unconventional solvent systems was performed, then the photosynthetic efficiency of the algal cells was measured. Solvents performances were evaluated in terms of astaxanthin extraction, toxicity towards algae and general safety. Among the tested solvents, 2-methyltetrahydrofuran and dimethyl carbonate provided the best extraction performance but did not preserve the algal cells vitality, even after 5 min. Contrarily, cyclohexane allowed to maintain healthy cells even after 30 minutes, but low extraction efficiencies were observed. Vegetable oil and isoamyl acetate showed the best milking potential in terms of algal vitality and extraction efficiency. The milking process using vegetable oil was then applied for a longer time (24 and 48 h), resulting in a better extraction performance as well as in a good physiological status of the algal cells. The alternative milking process using bio-solvents, such as vegetable oils, proposed for astaxanthin extraction hints towards a continuous accumulation of target compounds in microalgae while keeping the cells alive.

## Proteins, pigments content and ultrastructure of *Arthrospira platensis* in blu and yellow light and in increasing white intensities

M. Pelagatti, G. Mori, R. Ballini, P. Di Falco, C. Tani, A. Papini\*, L. Lazzara

Dipartimento di Biologia, Via Micheli 3, Università di Firenze; \*alpapini@unifi.it

*Arthrospira platensis* (Nordstedt) Gomont is a multicellular filamentous cyanobacteria usually known as Spirulina. Owing to its high protein content, that gave to it popularity in the health food industry and to its possible mass cultivation, we wanted to investigate possible differences in growth and biochemical content, particularly the differences in proteins and pigment concentrations of *A. platensis* cultivated under different light intensity and spectral composition (blue, yellow and white). To check if there were effects induced by the light condition at ultrastructural level, we investigated essential morfological characters of *A. platensis* ultrastructure by transmission electron microscopy. Before cultivation under different light conditions, *A. platensis* cells were grown under white light at low PAR ( $20 \mu\text{E m}^{-2} \text{s}^{-1}$ ) in semi-continuously refreshed Zarrouk medium. Then *A. platensis* was exposed to different light colors and intensities: blue ( $\lambda$  max 460 nm) and yellow ( $\lambda$  max 590 nm) with PAR 160  $\mu\text{E}$  and white with both PAR 160 and 550  $\mu\text{E}$ . In each phase of the 8 day experiment we measured: spectral *in vivo* absorption, pigments (chlorophyll, phycocyanine, carotenoids), proteins, dry weight and at the end of the experiment in blue and yellow light, samples were observed at the transmission electron microscope. The results show that the ratios of Proteins, Chlorophyll and Phycocyanine/dry weight as well as Phycocyanine/Chl and Phycocyanine/Protein are higher at 20  $\mu\text{E}$  compared to all other cases. In addition to the general decrease in pigment and protein content at medium and high PAR, there are differences between the treatment with yellow light and blue light. Summarising, there is a stronger and quicker response in yellow light compared to blue light, after eight days treatment, in certain cases in opposite direction between blue and yellow light. In particular there is an increase in the Protein/dry weight ratio and Chl/dry weight in yellow light whereas the two ratios decrease in blue light. As far as Phycocyanine/dry weight and Phycocyanine/Chl ratios are concerned, they decrease in both treatments, but in a stronger way in yellow light. T8 Phycocyanine/dry weight and Phycocyanine/Chl ratios are 33 and 25 % respectively when compared to T0 ratios in yellow light, while the T8 ratios under blue light are 76 and 87% of the initial value. Samples under the transmission electron microscope show some differences. The sample of yellow light experiment has a more viscous cytoplasm, that could be linked to the higher protein content. Many gas vesicles are visible close to the plasma membrane. In blue light carboxysomes are more numerous, while gas vesicles are reduced in quantity and some lipid droplets appeared. A possible explanation may be related to the perception of the cells of blue light as a wave length typical of higher depth and lower intensity. The lipid droplets may represent a beginning of a phase of reserve storage. The capability of *Arthrospira* to change its protein, pigment and gas vesicles content may be related to its capability of adaptation to different water depth and may be considered as one relevant factor for speciation, since ecological differences among ecotypes may be considered as key factor for the existence of species delimitations in prokaryotes (Kopac et al. 2014; Dvorak et al. 2015).

### References

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- Kopac S, Wang Z, Wiedenbeck J, Sherry J, Wu M, Cohan FM (2014) Genomic heterogeneity and ecological speciation within one subspecies of *Bacillus subtilis*. *Appl Environ Microbiol* 80:4842-4853.

## Microalgae monitoring in freshwater reservoirs intended for human consumption: application of innovative fluorimetric tools

M. Simonazzi<sup>1</sup>, F. Guerrini<sup>2</sup>, L. Pezolesi<sup>2</sup>, S. Vanucci<sup>3</sup>, R. Pistocchi<sup>2</sup>

<sup>1</sup> Centro Interdipartimentale di Ricerca per le Scienze Ambientali, Università di Bologna, via Sant'Alberto 163, 48123 Ravenna (mara.simonazzi2@unibo.it)

<sup>2</sup> Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Bologna, via Sant'Alberto 163, 48123 Ravenna

<sup>3</sup> Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche e Ambientali, Università degli Studi di Messina, Via Stagno d'Alcontres, 98166 S. Agata Messina

During the past decades a higher concern about the occurrence of potential harmful algae in freshwater bodies has arisen due to the increase of toxic species reported worldwide. Among these organisms, cyanobacteria count several species with toxigenic potential being able to produce a wide range of cyanotoxins, whose presence in drinking water has been associated with different human health-risks. Monitoring the presence of both cyanobacteria and microalgae is required to determine the quality of water intended for human consumption. The qualitative and quantitative analysis of phytoplankton via microscopic observation is time-consuming with late results that could prevent a prompt response by quality control agencies. Several tools have been developed to obtain algae composition estimation in a water sample based on specific class characteristics (e.g. flow cytometry, pigments analysis via HPLC), but they usually have high costs or require advanced expertise. A German company, bbe Moldaenke GmbH, has developed a user-friendly fluorimeter that could discriminate four major algae classes (Chlorophyta, Cyanophyta, Diatoms and Cryptophyta) and recognize the cyanobacteria genus *Planktothrix* which includes some toxic species. The principle of the method is based on the specific algal fluorescence spectrum which represents a fingerprint of an algal class; in fact the chlorophyll *a* spectrum of each algal group is influenced by the presence of different accessory pigments. In this study we compared the chlorophyll *a* content of algal cultures and natural freshwater samples using either portable and on-site bbe fluorimeters and traditional spectrophotometric determination. Preliminary results of an intercalibration performed among five distinct laboratories to compare the two methods, showed similar values of total chlorophyll *a* concentrations in algal cultures. Identification at algal class level performed in natural samples with fluorimeters and compared with microscopic observations was in most of the cases accurate. Our data also confirm that the fluorimeter was able to discriminate the genus *Planktothrix* from other cyanobacteria (*Microcystis aeruginosa*) in culture. Data obtained in this preliminary work demonstrate that bbe fluorimeters could be a useful tool for a rapid algae screening in natural freshwater samples to underline the presence of cyanobacteria by quality control agencies.

## Interdecadal variability in phytoplankton community structure in the coastal area of the LTER-Senigallia transect (northern Adriatic Sea) over a 40 years period (1977-2016)

C. Totti<sup>1</sup>, T. Romagnoli<sup>1</sup>, S. Accoroni<sup>1</sup>, A. Coluccelli<sup>1</sup>, S. Giulietti<sup>1</sup>, M. Pellegrini<sup>1</sup>, A. Campanelli<sup>2</sup>, F. Grilli<sup>2</sup>, M. Marini<sup>2</sup>

<sup>1</sup>Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona (Italy)

<sup>2</sup>Istituto per le Risorse biologiche e le Biotecnologie Marine (IRBIM) CNR, Largo Fiera della Pesca, 60125 Ancona (Italy)

The northern Adriatic (NA) is characterized by shallow waters, weak bathymetric gradient and a high riverine input mainly due to discharge of the Po River. The Senigallia-Susak transect is located in the lower part of the NA, where the Western Adriatic Current become sharper, clearly separating nutrient rich coastal waters from oligotrophic offshore ones. This transect represents a LTER (Long-Term Ecosystem Research) site, where physical parameters, nutrient concentration and phytoplankton abundance and biomass along a trophic gradient are recorded since 1988. In this study the interannual variability of phytoplankton in the coastal site of the transect was investigated, in order to document patterns related to environmental and climatic drivers.

Comparing meteorological, physical, chemical and biological data of the periods 1988-2002 and 2007-2016 periods, we showed that (i) the trend of T anomalies along the entire study period showed significant positive tendencies in spring and summer, (ii) in the last decade the atmospheric pressure negative anomalies were more frequent, (iii) in the last decade the occurrence of heavy rainfall increased, (iv) inorganic nutrient concentrations significantly increased in the second period, (v) phytoplankton abundances significantly increased in the second period. These results indicate that the tendency to oligotrophication due to the drop of the Po River outflow in the years 2002-2007 was reversed in the period 2007-2016. The P-limited conditions typical of the NA seem to be attenuated in the study area, and the observed P levels were not explained by the P concentrations in the Po River waters, suggesting the possible influence of other local P sources most probably related to the anomalous and intense meteorological events occurred in the 2007-2016 period. In the 1988-2002 phytoplankton annual cycle showed the maximum peak in January due to *Skeletonema marinoi* winter bloom. Diatom blooms also occurred in spring and autumn with variable intensity, depending on rainfall regimes. In periods between diatoms blooms, phytoplankton communities were dominated by heterogeneous communities of small phytoflagellates, while dinoflagellates increased only in spring-summer, with abundance peaks 1-2 orders of magnitude lower than those of diatoms. Coccolithophorids were a minor but persistent component of winter communities. In this period, during summer the N Adriatic experienced the appearance of large mucilage aggregates. In the last decade, phytoplankton abundance was significantly higher than in 1988-2002. The timing of the seasonal cycle of major groups changed. *Skeletonema marinoi* winter bloom shifted onward and other diatom blooms occurred irregularly throughout the year reflecting the irregularity of meteorological events. Dinoflagellate abundances decreased, but the occasional proliferation of large sized species (*Noctiluca scintillans*) caused sporadic biomass peaks. Coccolithophores showed a remarkable decline in the last decade particularly in winter months: some species that were among the most relevant indicator species in the 1988-2002 period, such as *Emiliania huxleyi* in winter, and *Syracosphaera pulchra* in spring, lost such role in the last decade. The causes of such decline are not clear and deserve to be in-deep investigated.

## Promoting biodiversity enhancement by Restoration Of *Cystoseira* Populations – ROC Pop Life project

M. Chiantore<sup>1</sup>, V. Asnaghi<sup>1</sup>, G. De La Fuente<sup>1</sup>, S. Kaleb<sup>2</sup>, S. Ciriaco<sup>3</sup>, G. Fanciulli<sup>4</sup>,  
P. Scarpellini<sup>5</sup>, L. Kastelic<sup>6</sup>, A. Falace<sup>2</sup>

<sup>1</sup> DiSTAV - University of Genoa (Italy), <sup>2</sup> DSV – University of Trieste (Italy), <sup>3</sup> WWF Oasi Miramare MPA (Italy),

<sup>4</sup> Portofino MPA (Italy), <sup>5</sup> Cinque Terre MPA (Italy), <sup>6</sup> Landscape Park Strunjan (Slovenia).

The genus *Cystoseira* C. Agardh is ecologically relevant as an ecosystem engineer, and plays a key functional role in controlling spatial habitat heterogeneity, productivity, and nutrient cycling in temperate rocky reefs. Currently, some *Cystoseira* populations (depending on species and location) are declining/lost throughout the Mediterranean, largely due to multiple human impacts such as urbanization, overfishing and climate change, emphasizing the urgency to develop an active intervention to restore this endangered habitat. ROC Pop Life project aims at triggering the recovery of *Cystoseira* populations in two Natura 2000 sites: the Marine Protected Areas (MPAs) of Miramare (Northern Adriatic Sea) and Cinque Terre (Eastern Ligurian Sea), where this taxon was present, as proven by museum and scientific literature records. Disappearance causes have been presently removed and the protection is guaranteed by the MPAs. The project will develop non-destructive and eco-compatible protocols, which include an innovative outplanting approach that consists in the production of germlings in the laboratory to be introduced in the areas to be restored, starting from small portions of macroalgae fertile apices. This approach, particularly convenient in terms of time, costs and ecological impact, will cause no harm to donor populations in Landscape Park Strunjan, Slovenia (for N Adriatic) and Portofino MPA (for Ligurian Sea), which is essential given the critical conservation status of the species. The proposed process is appropriate to large-scale application and replication in other Mediterranean areas is desirable in a project capitalization perspective. Preliminary results about the restoration experience during summer 2018 will be presented.

## Long-term changes of Fucales in the Gulf of Naples

D. Grech, M.C. Buia

Stazione Zoologica Anton Dohrn, Napoli

Coastal areas are in continuous transformation to sustain the increasing residential, commercial, and tourist functions. However, the proliferation of artificial infrastructures (e.i. marinas and breakwaters) and maritime activities may contribute to destroy and fragment marine natural habitats, alter connectivity among populations and of consequence the natural seascape (Airoldi et al., 2007; Coleman et al., 2008; ). In particular, the upper subtidal rocky fringe, where the coastal transformations are more conspicuous, represents the natural habitat of dominant engineering algae, such as *Fucales*, that play a relevant structural and functional role in marine ecosystems but are experiencing a huge decline in many areas of the Mediterranean Sea (Thibaut et al., 2015). Despite the long tradition of phycological studies in the Gulf of Naples (Italy) (Buia et al., 2013), a gap of knowledge on the dynamics and current status of *Cystoseira* and *Sargassum* spp. in this area has been highlighted for the last 60 years. A complete re-monitoring of the fucoids distribution along the neapolitan coast has been planned and the main threats affecting their distribution (e.i. coastal transformation, urbanization, sewage outfall, fishing gears and maritime traffic) have been taking into account in order to provide an overview of long-term changes and pressures on the distribution and abundance of *Cystoseira* spp. and *Sargassum* spp. along different sectors of the Gulf of Naples, in the bathymetric range between 0 and 50 m depth (Grech, 2017). Results from the comparison of past and present occurrence of Fucales in the gulf showed that in more than 84% of the historical sites the species previously recorded have not been found anymore, highlighting a decline of *Cystoseira* and *Sargassum* spp. mainly in the most anthropized area. Information on their distribution was compiled to produce a local-scale distribution map. Seven of the eighteen species recorded in the first half of the 20th century were no longer recorded; the remaining species persist in few localities but with very low abundances. For these last species the local decline could end up with their extinction if no action will be pursued for their conservation.

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## Il declino delle comunità a *Cystoseira* (Fucales, Ochrophyta) lungo le coste della Sicilia orientale: osservazioni sul *grazing* dei pesci erbivori

G. Marletta, D. Serio

Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Catania  
Via A. Longo, 19 – 95125 Catania

Le cistoseire sono considerate specie costruttrici di habitat e hanno una notevole importanza nelle acque costiere del Mediterraneo, tuttavia nonostante l'inestimabile valore, si sta assistendo ad un loro graduale declino. Recenti indagini hanno suggerito che i pesci erbivori possono avere un'influenza significativa sulla struttura di tali comunità algali (Gianni *et al.*, 2017). In questo studio è stato preso in esame l'aspetto riguardante il pascolamento dei pesci erbivori sulle comunità a *Cystoseira*, mirato anche a verificare l'ipotesi di espansione lungo le coste orientali della Sicilia delle specie aliene appartenenti alla famiglia dei Siganidi, *Siganus rivulatus* (Forsskål e Niebuhr, 1775) e *S. luridus* (Rüppell, 1829) che, come dimostrato da diversi studi, in Mediterraneo prediligono la dieta ad alghe brune (Lundberg e Golani, 1995). Per realizzare quest'indagine sono state scelte due località, Santa Maria La Scala e Santa Tecla, ricadenti entrambe nel Comune di Acireale (Sicilia orientale), in cui in passato è stata documentata la presenza di popolamenti ben strutturati a *Cystoseira* (Furnari e Scammacca, 1970; 1973; 1975; 1979; Cormaci *et al.*, 1979; Pizzuto, 1999; Catra *et al.*, 2007). Per valutare lo stato di tali comunità e per effettuare le osservazioni delle comunità ittiche, è stato svolto un monitoraggio, tramite la tecnica del *visual census* e la tecnica delle riprese subacquee, nel corso di nove mesi (dal giugno 2017 al febbraio 2018) nelle fasce batimetriche 0-10 m e 20-25 m. Il monitoraggio ha mostrato la presenza di talli sparsi di *Cystoseira montagnei* J. Agardh nella fascia batimetrica 0-10m e *C. zosteroides* (Turner) C. Agardh nella fascia batimetrica 20-25m. Su alcuni talli di *C. montagnei*, sono stati osservati evidenti segni di *grazing* da parte di pesci erbivori. Inoltre, le osservazioni hanno evidenziato la presenza, a partire da Dicembre, di giovani talli di *C. zosteroides* che hanno continuato ad accrescersi fino al mese di Febbraio in entrambi i siti, mentre i talli di *C. montagnei*, osservati più sporadicamente durante l'intero periodo di rilevamento, hanno emesso raramente nuove ramificazioni. Lo studio delle comunità ittiche non ha messo in evidenza la presenza dei Siganidi, ma ha mostrato un numero consistente di esemplari di *Sparisoma cretense* (Linnaeus, 1758), specie autoctona in forte espansione in Mediterraneo. In generale, è stato riscontrato un numero maggiore di erbivori nella stagione estiva rispetto a quella invernale e nella fascia batimetrica 0-10 m rispetto alla fascia batimetrica 20-25 m. Ciò potrebbe essere correlato con la ridotta capacità di accrescimento e riproduzione di *C. montagnei* maggiormente interessata dal fenomeno del *grazing* rispetto a *C. zosteroides*. Le osservazioni condotte dimostrano che lo stato di conservazione di tali cistoseireti risulta essere estremamente scarso e dunque è necessario effettuare ulteriori monitoraggi affinché i fattori di impatto vengano limitati e sia possibile la naturale ripresa dei popolamenti a *Cystoseira*.

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## Seasonal variability of epiphytic micro and macroalgal communities on *Cystoseira barbata* and *C. compressa* in the Conero Riviera

F. Moroni, T. Romagnoli, F. Rindi, S. Accoroni, C. Totti

Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona

Rocky coastal substrata are colonized by sessile organisms such as invertebrates and macroalgae, which include encrusting forms, algal turfs and large canopy-forming seaweeds, such as *Cystoseira* spp. (Fucales, Phaeophyceae). The tridimensionality provided by canopy-forming organisms supports highly diverse communities of great ecological value. In particular, *Cystoseira* canopies are referred to as "habitat formers" that produce spatial heterogeneity and host a high number of algal and animal species. Our study was conducted at the rocky shore of Passetto, in the Conero Riviera (northern Adriatic Sea), where two species of *Cystoseira* have been reported since the 1990's (*Cystoseira barbata* (Stackhouse) C. Agardh and *Cystoseira compressa* (Esper) Gerloff & Nizamuddin). *C. compressa* was found in a sheltered site where the presence of breakwater structures reduces hydrodynamism, while *C. barbata* was collected from an exposed site. Our aim was to examine micro- and macroalgal epiphytic communities associated with *C. barbata* and *C. compressa* over one year with a seasonal frequency. Samples were collected in February, May, July and November 2017. Surface temperature and salinity measurements, and water samples for nutrient analysis were collected before sampling to avoid disturbance. For each species, three thalli were collected in each season. For each thallus two apical branches were examined for analysis of the microalgal epiphytes and one basal branch for analysis of the macroalgal epiphytes (overall, nine samples per sampling date for each of the two species). Microalgal epiphytes were identified and counted using the Utermöhl method (1958) and their abundance was expressed as cells/cm<sup>2</sup>. Macroalgae were observed under a stereomicroscope and a light microscope, and their coverage was estimated (cm<sup>2</sup>/cm<sup>2</sup> of *Cystoseira* thallus surface). For *C. compressa*, the highest microepiphytic abundance was observed in spring, while summer and autumn showed the lowest abundance. Diatoms represented the dominant group, contributing for the ≥90% of the total abundance, followed by dinoflagellates and cyanobacteria. The highest macroepiphytic coverage was found in spring, with a decreasing trend until autumn, when the lowest abundance was observed. Rhodophyta were the dominant macroalgal group in terms of coverage, both in spring and summer, while in autumn there was a dominance of Phaeophyceae. For *C. barbata*, the highest microepiphytic abundance was observed in summer and the lowest value was recorded in autumn. As for *C. compressa*, diatoms represented the dominant microalgal group (>88% of total abundance), followed by cyanobacteria and dinoflagellates. The highest macroepiphytic coverage was observed in spring, followed by summer and autumn, and was lowest in winter. As for *C. compressa*, red algae were the dominant macroalgal epiphytes, with a contribution higher than 91% in all seasons. Despite some statistically significant correlations between environmental parameters and biological data (Spearman's correlation coefficient), distance-based Linear Model analysis did not detect temperature, salinity and nutrient concentrations as predicting factors for epiphyte distribution. Only silica concentrations were a significant predictor of microalgal community structure on *C. barbata*, explaining 91% of variability. To date, very few studies on epiphyte distribution were carried out on the Conero Riviera, making comparisons difficult. However, our results show that the highest microalgal abundance values found in this study were comparable with those seen for epilithic microalgae in the same area by Totti et al. (2007), while the lowest were one order of magnitude higher than those observed in that study. In general, a marked seasonal variability in epiphytic communities was noted, as expected based on previous studies. However, the environmental parameters measured in this study do not appear to be strong drivers in shaping the structure of the epiphytic communities. For this reason, we suppose that other parameters related to the geomorphology and exposure conditions of the two sites may influence the epiphytic communities associated with these two macroalgae. The results of this study represent a background of information that will be useful for future investigations aimed at clarifying the factors that affect the structure of micro- and macroalgal communities living on *Cystoseira* spp. along the Conero Riviera, and the effects that these communities have on their basiphytes.

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# The role of phytobenthonic biomass in the Natural Capital assessment of a Marine Protected Area.

F. Toscano<sup>1</sup>, G. Alongi<sup>1</sup>, E. Conti<sup>2</sup>, R. Turnaturi<sup>1</sup>, C. Mulder<sup>2</sup>

<sup>1</sup>Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Catania -Sezione di Biologia Vegetale, Via A. Longo, 19 – 95125 Catania

<sup>2</sup>Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Catania -Sezione di Biologia Animale, Via Androne, 81 – 95125 Catania

Marine and coastal ecosystems are subjected to continue anthropogenic pressure generating a threat for the biodiversity and long-term sustainability of marine environment. The overexploitation in order to generate goods and services for human well-being have to be controlled to maintain a good status for any marine ecosystem. In this perspective, the presence of Marine Protected Area can be considered as crucial, because if used properly it becomes an important tool to achieve a trade-off between nature conservation and human activities from a sustainability point of view. Sometimes, an integrate approach of evaluation, taking into account both human preferences and biophysical needs, can be difficult to obtain. Regarding this, Environmental Accounting Emery-based provides a measure of Natural Capital (biophysical value of stocked biomass) in terms of natural resources consumption required to support generation of goods and fruition of services. This is due to the ability of Emery Accounting methods integrating commonly unquantified processes such as those provided by the environment (for example solar radiation, rain, wind) into market evaluations as introduced by Odum (1996). His model shape as a helpful support for local manager and policy makers to develop strategies, which aim to the sustainable use of natural resources. The evaluation protocol involves the analysis of bionomic map of MPA to identify the main benthonic biocenosis or habitats. To calculate energy and matter flows (providing as such natural capital), benthic components have been chosen because they seem to be more stable than pelagic ones. In fact, they control water column biomass converting it into macrofauna with lower respiration rate, enhancing in such a way regulatory effects on the total system metabolism (Paoli et al., 2016). Moreover, they are able to respond to change much more smoothly than other components. The evaluation of MPAs natural capital starts with the estimation of phytobenthic biomass. It represents a good starting point for the environmental assessment because is the basis of the ecological pyramid of Elton on which the whole food-web structure depends, in a more or less direct way. Throughout *ad hoc* data of benthic communities can be achieved in terms of biomass expressed per square meter. Then, biomasses of different taxa are turned into grams of carbon (gC) using appropriate conversion factors. The amount of autotrophic biomass of each biocenosis, here expressed in gC m<sup>-2</sup>, can be obtained through the sum of the biomass of the primary producers. Then, the biomass of heterotrophic groups for the different biocenosis is transformed into the primary biomass required for its formation according to Elton's theory. Finally, the total primary biomass, supporting stocks formation in each biocenosis, is calculated as the sum of autotrophic biomass and primary biomass associated to heterotrophic groups at higher trophic levels. The biomass data, collected for each biocenosis, are the basis to provide a biophysical accounting, through the calculation of primary productivity supporting the trophic web of MPAs, and environmental flows by means of emery analysis.

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## Phosphatase activities of the microbial community during the bloom of *Ostreopsis cf. ovata* in the northern Adriatic Sea: the role of temperature and organic phosphorus sources

S. Accoroni<sup>1</sup>, M. Pasella<sup>1</sup>, T. Romagnoli<sup>1</sup>, E. Razza<sup>1</sup>, C. Totti<sup>1</sup>, N.T.W. Ellwood<sup>2</sup>

<sup>1</sup>Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy

<sup>2</sup>Dipartimento di Scienze, Università Roma Tre, Viale G. Marconi 446, 00146 Roma

During the last few decades, recurrent and intense blooms of the toxic benthic dinoflagellate *Ostreopsis cf. ovata* have been frequently reported during summer along several sections of the Mediterranean coast. Field studies about *Ostreopsis* blooms highlighted the important role of hydrodynamics, water temperature and inorganic nutrients, among the other environmental parameters. However, all factors (and the interactions among them) that drive the bloom development throughout its phases are not totally understood yet. For example, the potential utilization of organic phosphorus (DOP) and its role on the *Ostreopsis* bloom are still unclear.

The potential utilization of dissolved organic phosphorus (DOP) by the toxic dinoflagellate *Ostreopsis cf. ovata* within its microbial-mat community was investigated during a bloom along the Conero Riviera (N Adriatic Sea). Measurements of phosphomonoesterase (PMEase) and phosphodiesterase (PDEase) activities of the epiphytic mats (including cells and exopolymeric substances) and a range of chemico-physical parameters were performed from late summer to early autumn at Passetto station. Analyses of ambient nutrient fractions revealed very high N:P values, very low filterable reactive P (FRP) concentrations and DOP concentrations that were on average 85% of the total dissolved P. A rapid increase in PMEase and PDEase activities in the microbial community was recorded coinciding with the onset of the *Ostreopsis* bloom. Chromogenic staining of samples showed that activity was closely associated with the *Ostreopsis* cells, located both extracellularly (cell surface and within the EPS) and intracellularly (ventral cytoplasm). The increase in both phosphatases indicates that *Ostreopsis*-mat community can utilize a wide range of DOP types confirmed through tests in laboratory. *Ostreopsis cf. ovata* can utilize both phosphomonoester (D-Fructose 1,6-disphosphate,  $\beta$ -Glycerophosphate,  $\alpha$ -D-Glucose 1-phosphate, Guanosine 5'-monophosphate and Phytic acid) and phosphodiester (DNA and RNA) sources to grow. The experiments also demonstrated that PMEase and PDEase were strongly influenced by water temperature, with maximum values recorded at 30-35 °C. Based on the present findings, the *Ostreopsis* bloom seems to be maintained thanks to some adaptations that allow it to thrive in P-limited environments where organic P is the main source of P, until water temperature is enough high to allow PMEase and PDEase activity.

## Environmental metabarcoding and local reference barcoding reveal remarkably high diversity in the planktonic diatom family *Chaetocerotaceae*

W. H. C. F. Kooistra, C. C. Gaonkar, A. Zingone, R. Piredda, D. Sarno, M. Montresor,

Stazione Zoologica Anton Dohrn, Villa Comunale, Napoli, Italia.

Assessment of morphological and molecular diversity in the planktonic diatom family *Chaetocerotaceae* (using isolation and cultivation) uncovered ca. 80 species, 44 of which were observed in the Gulf of Naples (GoN, Mediterranean Sea). Subsequently, we assessed the diversity in this family by means of high throughput sequencing (HTS) metabarcoding of 48 protist samples collected at the LTER MareChiara (GoN) between 2011 and 2013. As metabarcode we used the V4 hypervariable region in the 18S rDNA. The 13.6M obtained eukaryotic contigs grouped into 615,142 haplotypes, of which 18,625 were assigned to *Chaetocerotaceae*. Following elimination of haplotypes with <3 contigs, the retained 650 haplotypes were aligned with the ca 80 *Chaetocerotaceae* reference barcodes to build an ML tree. This tree resolved 66 terminal taxa assigned to *Chaetoceros* species and ten to *Bacteriastrum*. Of these 76 taxa: 39 were assigned to species known from the GoN, seven to a species not known from the GoN, and the remaining 30 lacked a reference barcode. These species have probably been observed in the regular cell counts at the LTER, but are categorized as *Chaetoceros* spp as these are difficult to distinguish in LM. Thus, HTS-meta-barcoding works well in detecting – and discriminating among the members of - this common and abundant family. Moreover, HTS metabarcoding results show that even in the taxonomically well-explored GoN a considerable percentage of the *chaetocerotaceae* species is still to be described.

## First detection of *Prorocentrum cf. shikokuense* bloom in the Mediterranean Sea

L. Roselli<sup>a</sup>, M. R. Vadrucchi<sup>a</sup>, F. Fanelli<sup>a</sup>, N. Ungaro<sup>a</sup>, C. Caroppo<sup>b</sup>

<sup>a</sup> Regional Agency for the Environmental Prevention and Protection (ARPA Puglia), Corso Trieste 27, Bari

<sup>b</sup> CNR-IRSA National Research Council-Water Research Institute, Unit of Taranto Via Roma, 3 - 74121 9 Taranto, Italy

Increasing intensity and spreading of toxic and non-toxic blooms, as well as global transport of non-indigenous marine species, have expanded worldwide during the last decades (Hallegraeff, 1993; McGeoch et al. 2010; Pyšek et al. 2012; Simberloff et al. 2013). HABs and NIS introduction will undoubtedly constitute a growing threat to human activities related to the sea, including fishery, aquaculture, recreational activities and tourism representing a serious ecological and economical menace (Zingone et al., 2017; Occhipinti-Ambrogi and Savini, 2003). A bloom of non-indigenous species (NIS) *Prorocentrum cf. shikokuense* was detected for the first time in the Mediterranean Sea at the Brindisi port (Southern Adriatic Sea) on September 2016, during a plankton survey aimed to detect and monitor non-indigenous species in the ports (Marine Strategy Framework Directive MSFD, 2008/56/EC). Morphological detailed observations by light and electron scanning microscopy and cell density were reported. In the Brindisi port this dinoflagellate reached the exceptionally high concentration  $10^5$  cell/L and represented from 30 to 50 % of the total phytoplankton population. *Prorocentrum shikokuense* Hada (1975) is globally scarcely studied and its toxicity is unknown. However, is usually monitored in the East China Sea and Japanese and Korean waters since it causes huge red tide (Su-Myat and Koike, 2013). Although the bloom of *Prorocentrum cf. shikokuense* from Brindisi port did not seem to cause any toxic event (e.g. fish kills), the presence of this species, suggests the need for improving risk assessment to manage and prevent the occurrence of harmful blooms, especially those caused by non-indigenous species, in such a sensitive area.

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## Shipping does not seem an effective vector for the introduction of non-indigenous seaweeds in Italian ports

A. Petrocelli<sup>1</sup>, E. Cecere<sup>1</sup>, L. Bolognini<sup>2</sup>, M. Marini<sup>2</sup>, F. Rubino<sup>1</sup>, A. Falace<sup>3</sup>

<sup>1</sup>Water Research Institute (IRSA), secondary location of Taranto, CNR, via Roma 3, 74123 Taranto, Italy

<sup>2</sup>Institute for Biological Resources and Marine Biotechnologies (IRBIM), secondary location of Ancona, Largo Fiera della Pesca 1, 60125 Ancona, Italy

<sup>3</sup>University of Trieste, Department of Life Sciences, via Giorgieri 10, 34127 Trieste, Italy

Hull fouling is the most ancient vector for the introduction of non-indigenous species (NIS) throughout the world, and, like ship ballast waters for microalgae, it is considered the most important mean of transportation for macroalgae in oceanic zones (Hewitt et al. 2007). For this reason, port areas are considered as particularly exposed to the risk of new introductions. In order to assess the presence of NIS in these areas, Port Biological Baseline Surveys (PBBS) were devised, that are periodic scientific campaigns of samplings and observations *in situ* aiming to follow changes in biodiversity (Awad et al. 2014). However in despite of their importance, very few PBBS were performed into the Italian ports (Petrocelli et al. 2018). The recent BALMAS Project, carried out in 2014-2015 in 12 Adriatic ports, including the four Italian ports of Ancona, Bari, Trieste, and Venezia, allowed a first evaluation of the current situation (Petrocelli et al. 2018). Into each port, four different typologies of sampling sites were chosen to assess the possible influence of the different activities here performed on the introduction of NIS. To this end, notwithstanding the low number of species, statistical analysis was carried out to evaluate significant differences. nMDS analysis on presence/absence data confirmed that the Italian ports have peculiar features. Indeed, the non-indigenous seaweed species (NISS) recorded in each of them were different, except for *Aglaothamnion feldmanniae* Halos (Rhodophyta, Ceramiales) present in all the three ports where NISS were detected. In this connection, numerically Venezia ranked the first with four NISS (i.e. *Agardhiella subulata* (C. Agardh) Kraft et M.J. Wynne, *A. feldmanniae*, *Polysiphonia morrowii* Harvey, and *Undaria pinnatifida* (Harvey) Suringar), followed by Bari with three species (i.e. *A. feldmanniae*, *Antithamnion nipponicum* Yamada et Inagaki, and *Asparagopsis armata* Harvey), and Ancona with two NISS (i.e. *A. feldmanniae*, and *Grateloupia turuturu* Yamada). Trieste was the only Italian port where no NISS was found (Petrocelli et al. 2018). In terms of different sampling zones, according to SIMPER test, the most important activity resulted the industrial shipment, followed by passenger terminal, cargo, and anchorage outside the port (Petrocelli et al. 2018). The last updated list of NISS along the Italian coast counts 52 species (Petrocelli and Cecere, 2018), net of four *Antithamnionella* spp. (Rhodophyta, Ceramiales), which are considered questionable (Zenetos et al. 2017) and *Ascophyllum nodosum* (Linnaeus) Le Jolis (Ochrophyta, Fucales), which disappeared from the Mar Piccolo of Taranto (A. Petrocelli pers. observ.). Most of these species were reported from transitional waters (e.g. Venice Lagoon, Mar Piccolo of Taranto, Faro Lake) and from some well studied coastal zones (e.g. Tuscan Archipelago, Sicilian Isles) (Petrocelli and Cecere 2018). Apart from Bari area, where few floristic information are available, Ancona, Trieste and, above all, Venice areas are rather well known from a phytobenthic point of view and count a higher number of NISS (Petrocelli and Cecere 2018). Therefore, in these port areas, the low NISS number recorded would suggest that shipping is unimportant for their introduction, and, it would confirm that in Italian waters the importation of shellfish is the most relevant vector of introduction for seaweeds (Cecere et al. 2016).

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## Le alghe di alcuni tratti sorgentizi del fiume Liri (Italia centro-meridionale)

N. Abdelahad<sup>1</sup>, O. De Castro<sup>2</sup>, M. Iberite<sup>1</sup>

<sup>1</sup> Dipartimento di Biologia ambientale, Sapienza Università di Roma, P. le Aldo Moro 5, 00185 Roma

<sup>2</sup> Dipartimento di Biologia; Università di Napoli Federico II, Via Foria 223 - Orto Botanico, 80139 Napoli

La ricerca è stata condotta, a partire dal 2010, in alcune tratti sorgentizi del fiume Liri e dei suoi affluenti. Nelle diverse stazioni (Cappadocia, Zompo Lo Schioppo, Carpello, Rio, Fonteoco e Canalara), sono state studiate alghe, muschi, epatiche e piante vascolari. In questa sede si riferisce prevalentemente delle alghe rinvenute durante le escursioni. Le analisi chimico-fisiche effettuate in queste stazioni hanno evidenziato che le acque sono oligotrofiche e di tipo bicarbonato-alcantino-terroso. Le specie algali (18) rinvenute appartengono a Cianobatteri, Crisoficee, Xantoficee e Cloroficee. Nell'ambito di ciascuno di questi gruppi algali sono state rinvenute specie che presentano interesse per motivi diversi. Tra i Cianobatteri osservati, due specie, *Phormidium simplicissimum* (Gomont) Anagnostidis et Komárek e *Blennothrix brebissonii* (Kützing ex Gomont) Anagnostidis et Komárek, citate anche in letteratura per essere caratteristiche di acque incontaminate (Komárek & Anagnostidis, 2005), sono potenzialmente buone indicatrici di qualità delle acque. Di particolare rilievo è il rinvenimento, in due delle sorgenti studiate (Fonteoco e Carpello) di *Tetrasporopsis fuscescens* (A. Braun ex Kützing) Lemmermann, Crisoficea che risulta finora non segnalata in Italia (Starmach, 1985; Entwistle & Andersen (1990); Guiry, 2018). Tra le alghe verdi riscontrate appare particolarmente interessante il ritrovamento, nella sorgente di Zompo Lo Schioppo, di una specie di *Ulvaes* d'acqua dolce, piccola e molto ramificata, che, da una preliminare caratterizzazione molecolare tramite DNA barcoding risulta essere maggiormente affine al genere *Blidingia* Kylin (vedi Bliding, 1963; Cormaci et al. 2014). Tale caratterizzazione molecolare è stata eseguita su due esemplari su cui sono stati amplificati e sequenziati lo spaziatore interno trascritto dei geni ribosomali del DNA nucleare (ITS2; Chen et al., 2010). Le sequenze ottenute sono state successivamente analizzate attraverso BLASTn (Basic Local Alignment Search Tool – nucleotides; Camacho et al., 2009) per ottenere una comparazione di identità con gli altri taxa presenti nella banca dati usando un *cut-off* di identità  $\geq$  del 95% (*query cover*  $\geq$  85%) ed un *e-value*  $< 1e^{-4}$ . Per definire la corretta attribuzione tassonomica del ceppo afferente a *Blidingia* di Zompo Lo Schioppo, ulteriori analisi di caratterizzazione molecolare dovranno essere eseguite con un maggior numero di marcatori molecolari di DNA barcoding come definito dal CBOL (Consortium for the Barcode of Life) e da Chakraborty et al. (2014) per le comunità algali.

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## Macrophyte diversity in a coastal lagoon inside the Marine Protected Area of Porto Cesareo (Italy)

A. Bottalico<sup>1</sup>, A. Lisco<sup>1</sup>, N. Ungaro<sup>2</sup>, N. Dipierro<sup>1</sup>

<sup>1</sup>Dipartimento di Biologia, Università degli Studi di Bari "A. Moro", Via E. Orabona 4, 70125, Bari

<sup>2</sup>Agenzia Regionale per la Prevenzione e Protezione Ambientale (ARPA Puglia), Corso Trieste 27, 70126 Bari

Coastal lagoons are highly dynamic and productive ecosystems providing shoreline protection and water quality improvement. They are protected by national and international agreements due to their value as natural resources (Christia *et al.*, 2018). Being able to respond to environmental changes, macrophyte assemblages are among the Biological Quality Elements (BQEs) proposed by the European Water Framework Directive (WFD, 2000/60/EEC) to assess the ecological status of transitional waters. Thus, knowledge of macrophyte diversity in such priority habitats is a fundamental pre-requisite for their conservation and sustainable management.

The Bay of Porto Cesareo is an inlet partially delimited by a narrow peninsula called "La Strea", a tongue of land in front of the municipality of Porto Cesareo (Gulf of Taranto, Ionian Sea). The south-eastern part of the bay, characterized by shallow waters with a muddy sandy bottom, is included in the C zone (area under partial protection) of the Porto Cesareo MPA. It has also been classified as a "transitional water body", due to freshwater inputs deriving from some underground springs, and its annual monitoring for the BQE "macrophyte" is performed by the Department of Biology, University of Bari, on behalf of the Apulian Environmental Agency (ARPA Puglia). The lagoon is subjected to many human impacts such as tourism activities, due to the seasonal increase in population density, boat anchoring and illegal local fishing. Inventory of the submerged macroflora in this lagoon was completed during the monitoring program carried out from 2011 to 2018. A total of 67 macrophytes were identified. The vegetation was dominated by: (i) attached and unattached forms of both *Rytidhalea tinctoria* (Rhodophyta) and *Anadyomene stellata* (Chlorophyta), (ii) the protected species *Cystoseira barbata* (Ochrophyta), (iii) meadows of *Cymodocea nodosa* (Spermatophyta), especially in the central part of the bay. The chorological spectrum shows a high occurrence of Circumtropical elements, in agreement with previous records of benthic organisms with tropical affinity (Parenzan, 1983; Pardi *et al.*, 1988). Throughout the study period the ecological status of this transitional water body was evaluated as "High", according to MaQI classification (Sfriso *et al.*, 2009), due to the stable large percentage of sensitive species. The increasing water turbidity, on the other hand, is a factor that in the long term might disturb macrophyte assemblages, especially *C. nodosa* and *C. barbata* populations. Some regression of algal communities was highlighted in the past, both within and near the MPA (Cecere *et al.*, 2005), corroborating the importance of protection measures and continuous monitoring activities.

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## Coralline algae of calcareous biological concretions of the northern Adriatic Sea (Tegnue and Trezze)

A. Caragnano<sup>1</sup>, S. Kaleb<sup>2</sup>, F. Rindi<sup>1</sup>, A. Falace<sup>2</sup>

<sup>1</sup> Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy

<sup>2</sup> Dipartimento di Scienze della Vita, Università di Trieste, Via L. Giorgieri 10, 34127 Trieste, Italy

The northern Adriatic Sea is a shallow basin with hydrological features remarkably different from the rest of the Mediterranean. Its bottom is mostly soft, but in the area between the Gulf of Trieste and the delta of the River Po numerous biogenic outcrops are scattered within it, at depths ranging between -5 and -25 m. These outcrops, locally known as Tegnue or Trezze, consist of concretions derived from the building action of calcareous organisms on hard substrata of diverse geological origins. In recent years these habitats have received great attention; however, due to logistical sampling constraints related to their offshore location, their benthic communities are still imperfectly known. Based on observations made in the last two decades and collections made in summer 2017, we studied the diversity and distribution of coralline algae living on the Trezze and Tegnue. *Lithophyllum incrustans* was the most common coralline species and was a major contributor to bioconstruction of some outcrops, but its abundance varied considerably among outcrops; its identity was confirmed using molecular data (*psbA* sequences). A species of *Lithophyllum* in need of taxonomic assessment was the main coralline in some outcrops in the easternmost part of the area; molecular data (*psbA* and *cox2,3* sequences) show that this species is closely related to *Lithophyllum stictiforme*, but distinct from it at species level. Additional species recorded include *Hydrolithon boreale*, *H. farinosum*, *Lithophyllum corallinae*, *L. cystoseirae*, *L. pustulatum*, *L. racemus*, *Lithothamnion corallioides*, *L. minervae*, *L. sonderi*, *Mesophyllum macroblastum*, *M. philippii*, *Neogoniolithon brassica-florida*, *Pneophyllum confervicola*, *P. fragile*, *Phymatolithon calcareum* and *P. lenormandii*. Overall the results suggest that the communities of these outcrops differ substantially from the coralligenous communities of other parts of the Mediterranean and that different outcrops are characterized by different coralline species.

## Maintenance of a photosynthetic capacity by *Trentepohlia umbrina* in stressful subaerial environments

N.T.W. Ellwood<sup>1+2</sup>, L. Bruno<sup>2</sup> and G. Caneva<sup>1</sup>

<sup>1</sup> Department of Science, University of Rome 'Roma Tre', Viale G. Marconi 446, 00146 Roma, Italy

<sup>2</sup> Department of Biology University of Rome 'Tor Vergata', Via Cracovia 1, 00133 Rome, Italy

Subaerial green algae are exposed to extreme hydration stress, yet cells need to remain fully hydrated and ultrastructurally intact in order to function physiologically. To compound the problems of dehydration, there is also an increased possibility of photochemical damage from high levels of irradiance. Algae can retain their chlorophyll viability during desiccation, but under high light these pigments can continue to absorb damaging radiation even though the chemical reactions of photosynthesis are stalled. Cells need effective protective mechanisms to allow harmless dissipation of this excess excitation energy. Species of the genus *Trentepohlia* are known to produce significant quantities of the photo-protective compounds and suppress photosynthesis during periods of desiccation, with rates of photosynthesis associated with diurnal changes in relative humidity (*T. odorata*) and full, rapid recovery of photosynthesis upon rewetting cells following extended periods of desiccation of 40 days (*T. umbrina*). Studies of the desiccation tolerance of *Trentepohlia* have been conducted *ex situ* and restricted to samples taken from single environments, yet, ecological ranges of some species, e.g. *T. umbrina*, can be quite wide and so *in situ* investigations of such species would be highly informative regarding stress tolerance. The study site, the Grassi Hospital, Ostia, is very particular as it has multiple sites with extensive growths of *T. umbrina* on substrates with multiple aspects and a wide range of light intensity and associated environmental conditions. Four sites were chosen that ranged from highly exposed to highly shaded. For each site, the environmental conditions were characterised and biofilm samples for chlorophyll a and b and total carotenoids were taken. Photosynthetic parameters were measured *in situ* with a mini-PAM. Diurnal environmental conditions that would normally inhibit photosynthesis occurred at each site. Pigment analysis of the biofilms showed a high variability (chl a and b, CV 70%; carotenoids, CV 40%) with higher concentrations in biofilms associated with the higher light exposure. The ratio of total carotenoids and total chlorophyll was lowest at the shaded site (3:1) compared the sites receiving direct light (mean 12.5:1). Estimations of biomass showed that the lowest biomass occurred at the exposed site and the highest in the shaded site. A 3 to 6-fold increase in the yields of photosynthesis following re-hydration of biofilms was identified at all sites. The results showed that *T. umbrina* could physiologically adapt to diverse solar irradiation exposure and dehydration. All biofilms suffered diurnal desiccation yet maintained chlorophyll viability allowing *T. umbrina* to be photosynthetically active during the briefest window when conditions are met for photosynthesis. Adjusting pigment content to adapt environmental stress allows *T. umbrina* to maximise its rate of photosynthesis before inhibitory levels of light and relative humidity are reached throughout the day. The diverse biomass levels at each site suggest that growth is restricted, but the survival capacity of *T. umbrina* allows for slow colonisation of highly hostile substrates.

## Phytochelatin Synthase involvement in Cr(VI) tolerance in *Scenedesmus acutus* (Chlorophyceae)

M. Ferrari<sup>1,2</sup>, M. Marieschi<sup>2</sup>, R. Ruotolo<sup>2</sup>, V. Reverberi<sup>2</sup>, R. Cozza<sup>1</sup>, A. Torelli<sup>2</sup>

<sup>1</sup>University of Calabria, Department of Biology, Ecology and Earth Science, Via P. Bucci, 87036, Arcavacata di Rende, Cosenza, Italy

<sup>2</sup>University of Parma, Department of Life Sciences, University of Parma, Parco Area delle Scienze 11/A, 43124 Parma, Italy

Metal contamination represents a serious concern for the environment and mostly affects aquatic ecosystems. Chromium (Cr) is one of several heavy metals causing serious environmental contamination in soil, sediments, and groundwater where it is present as Cr(VI) or as the less mobile Cr(III) form. Heavy metals resistance in microalgae can be mediated by several mechanisms: exclusion through binding to the cell wall or low plasma membrane permeability; active extrusion; biotransformation; compartmentalization of HM into vacuoles and other intracellular organelles; complexation with chelating agents, such as non-proteinaceous compounds (as malate, citrate, ascorbate and polyphosphates) or metal-binding proteins, such as metallothioneins and phytochelatins. PCs are cysteine rich metal-binding peptides with the general structure  $(\gamma\text{-Glu-Cys})_n\text{-Gly.}$ , synthesized in plants by the enzyme Phytochelatin Synthase (PCS) which uses glutathione (GSH) as substrate. Several physiological studies in plants, indicated the role of PCs in the homeostasis and detoxification of toxic metals including Cr, However, synthesis of PCs has received little attention in algal cells and no data regarding Cr-induced PC synthesis in microalgae exist. We recently reported the first evidence of a PCS gene from the microalga *Scenedesmus acutus*. Using degenerate primers, we amplified a partial cDNA fragment of nearly 1900 bp (*SaPCS*) corresponding to a single nearly 5000 bp sequence of genomic DNA. The deduced protein shows a high identity homology (52%-75%) with other PCS of algae and plants corresponding to a putative protein of near 73kDa. Western blot analysis conducted with a polyclonal antibody raised vs PCS of *Arabidopsis thaliana* reveals an immunoreaction signal on two proteins of molecular weight of about 73 and 37 kDa, suggesting that the shorter form comes from a putative alternative splicing. Both the two forms show higher levels in the Cr-tolerant strain and increase after S-starvation; whereas in the wild type only the 37 kDa protein become more abundant after pre-culture in sulfur deprived medium. Albeit chromium induced an increase in *SaPCS* transcripts, western blot does not evidence difference in the protein levels after metal exposure. A differential PCs production after cadmium, but not after Cr(VI), exposure were observed in the two *S. acutus* strains (Torricelli *et al.*, 2004; Gorbi *et al.*, 2006). Nevertheless, the higher level of PCS found in the Cr-tolerant, suggests a more promptness of this strain in response to metal stresses. Moreover the *SaPCS* protein forms increase observed after S-starvation indicates an involvement of PCS in the transient Cr(VI) tolerance increase induced by this nutritional stress (Gorbi *et al.*, 2007). These observations indicate that in *S. acutus* PCS is not only involved in Cr detoxification, but can also play a role in the balance the intracellular sulphur. The PC production in the experimental conditions leading to increased protein levels should be investigate to better clarify the role of the enzyme in chromium detoxification or in the cell homeostasis during sulphur shortage.

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## Preliminary study of the effect of iron on the growth and morphology of extremophile green alga *Coccomyxa melkonianii* SCCA 048

V. Malavasi<sup>1</sup>, S. Soru<sup>1</sup>, A. Concas<sup>2</sup>, S. Montinaro<sup>1</sup>, M. Afzal<sup>1</sup>, M. Pisu<sup>2</sup>, G. Cao<sup>1,3</sup>

<sup>1</sup>Interdepartmental Center of Environmental Science and Engineering (CINSA), University of Cagliari, Via San Giorgio 12, 09124, Cagliari (CA), Italy;

<sup>2</sup>Center for Advanced Studies, Research and Development in Sardinia (CRS4), Loc. Piscina Manna, Building 1, Pula (CA), Italy;

<sup>3</sup>Department of Mechanical, Chemical and Materials Engineering, Piazza d'Armi, 09123, Cagliari (CA), Italy

A heavy-metal-resistant green alga *Coccomyxa melkonianii* SCCA 048 was investigated to evaluate its ability to grow in culture media with different concentrations of Iron Sulphate ( $\text{FeSO}_4$ ). The microalga was sampled in the Rio Irvi river (Sardinia, Italy) which is severely polluted by heavy metals including Fe, Mn, Zn, Cd, Co, Ni, and Pb (Malavasi et al., 2016). Previous studies demonstrated that this strain grew well in the pH range 4.0–8.0, and the optimal value for its growth was 6.8 (Soru et al., 2018). In the current research, elemental concentration of iron-enriched medium and microalgal bioaccumulation ability were investigated. For this purpose, two independent techniques were used: inductively coupled plasma optical emission spectroscopy (ICP-OES) was used to analyze the iron concentration of the solutions, and inverted light microscope was used to examine the morphology of the cells. This strain was cultivated in standard liquid BBM medium, to which the following  $\text{Fe}^{2+}$  concentrations (mM) were added: control 0.089, 0.25, 0.30, 0.4, 0.8, 0.9, 1.2, 1.9. The biosorption experiments were performed in Erlenmeyer flasks containing 150 mL of solution continually shaken at 100 rpm. All cultures were illuminated with 80–100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  grown at 25 °C. The obtained experimental data were then elaborated to evaluate the growth rate and iron dependent kinetics. Preliminary results showed 0.089 mM  $\text{Fe}^{2+}$  to be the optimal concentration for the microalga growth. Under such conditions, the growth rate was equal to about 0.16  $\text{day}^{-1}$ . Higher concentration of iron sulphate led to a decrease of the *C. melkonianii* growth rate, demonstrating that, over a certain concentration, iron acts as an inhibiting substrate. In this experiment, iron dependent kinetics were well interpreted by Haldane-type or substrate-inhibition kinetics. Under very high iron sulphate concentration, microalgae growth rate decreased, but continued to grow, demonstrating that *C. melkonianii* can tolerate very harsh environmental conditions, such as the conditions of mine drainage. It should be noted that such high salt concentration well simulates metal-contaminated waters since very low water pH are attained. In addition, during the investigation, a significant phenotypic plasticity of this strain was observed by optical light microscopy. The algal biomass with permanently bound microelements have many industrial applications (feed, natural fertilizers, etc.). It is therefore essential not only to study the influence of iron, but also the influence of other heavy metals, on the laboratory growth of this organism.

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The financial support of the COMISAR project (POR FESR 2014/2020 - ASSE PRIORITARIO I "RICERCA SCIENTIFICA, SVILUPPO TECNOLOGICO E INNOVAZIONE" Regione Autonoma della Sardegna, Italy) is gratefully acknowledged.

## A different expression of two H<sup>+</sup>/SO<sub>4</sub><sup>2-</sup> transporters characterizes two strains of *Scenedesmus acutus* with different chromium sensitivity

M. Marieschi<sup>1</sup>, M. Ferrari<sup>1,2</sup>, A. Locatelli<sup>1</sup>, C. Zanni<sup>1</sup>, R. Cozza<sup>2</sup>, A. Torelli<sup>1</sup>

<sup>1</sup>University of Parma, Department of Life Sciences, University of Parma, Parco Area delle Scienze 11/A, 43124 Parma, Italy ([anna.torelli@unipr.it](mailto:anna.torelli@unipr.it))

<sup>2</sup>University of Calabria, Department of Biology, Ecology and Earth Science, Via P. Bucci, 87036, Arcavacata di Rende, Cosenza, Italy

In the freshwater green alga *Scenedesmus acutus*, sulfur starvation induces a transient increase of Cr(VI) tolerance, both in the wild type and in a Cr-tolerant strain, associated to an increased capacity of sulfur uptake and assimilation leading to an increase in cysteine synthesis during the recovery in standard medium (Gorbi *et al.*, 2007). The tolerance to this metal seems strictly connected to sulfur metabolism (Pereira *et al.*, 2008; Schiavon *et al.*, 2008), since end products of sulfur assimilation pathway (cysteine, GSH and phytochelatins) can be directly involved in metal binding and/or detoxification of its noxious effects. Moreover Cr(VI) enter the cells exploiting cell sulfate transporters. It is well known that sulfur starvation induces a huge variation in gene expression in *Chlamydomonas reinhardtii* (Zhang *et al.*, 2004) as well as in other algae and in land plants (Bochenek *et al.*, 2013). Among the genes overexpressed there are plasma membrane high affinity sulfate transporters and many enzymes of the sulfate assimilation pathway. Primers designed on the sequence of *C. reinhardtii* and used both on genomic DNA and cDNA from 24h S-starved algae allowed the partial cloning of two sulfate transporters, *SULTR1* and *SULTR2*, codifying for H<sup>+</sup>/SO<sub>4</sub><sup>2-</sup> co-transporters. These transporters belong to the class of SLC26 transporters characterized by 12-14 transmembrane spans and a cytoplasmic STAS domain with regulative functions. Albeit very similar, the aminoacidic sequences shows some differences in the pocket and in the regulative STAS domain in the 3' end of protein. To evaluate if these differences were related to a different sulfate affinity and a different inducibility we analyzed gene transcription in different experimental conditions. The analysis has been conducted by means of RT-PCR on algae pre-cultured for 3 days in standard (+S) or S deprived medium (-S) and subsequently transferred into standard medium or in standard medium supplemented with Cr(VI) supplied as potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>). The two transporters show a different inducibility. *SULTR2* results constitutively expressed and weakly enhanced by sulfur starvation in both strains whereas *SULTR1* appear to be strongly induced by sulfur starvation in both strains and expressed in only the Cr-tolerant strain at the end of pre-culture in standard medium. An increase of *SULTR1* expression was observed in both strains after 48h recovery in S-sufficient condition, when likely nutrient consumption by growing cell populations start to cause sulfur starvation. The induction of *SULTR1* by S-starvation and its silencing after standard medium renewal allow to hypothesize a role of this transporter in the transient increase of Cr(VI) tolerance observed after S-starvation. The differential expression in the Cr-tolerant strain moreover seems directly linked to the enhanced sulfate uptake/assimilation pathway shown by this strain and putatively involved in its Cr(VI) tolerance.

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## Diatom biorefineries: mycosporine-like amino acids (MAAs), lipids and biosilica from *Phaeodactylum tricornutum*, *Staurosirella pinnata* and *Thalassiosira weissflogii*

S. Savio<sup>1</sup>, S. Farrotti<sup>1</sup>, S. Antonaroli<sup>2</sup>, R. Lauceri<sup>3</sup>, R. Congestri<sup>1</sup>

<sup>1</sup>University of Rome 'Tor Vergata', Dept. of Biology, Via Cracovia 1, 00133, Rome, Italy

<sup>2</sup>University of Rome 'Tor Vergata', Dept. of Chemical Sciences and Technologies,  
Via della Ricerca Scientifica s.n.c,

<sup>3</sup>National Research Council, Institute of Ecosystem Study, Largo Tonolli 50,  
28922 Verbania Pallanza (VB), Italy

Diatoms are key microalgal component in marine and freshwater habitats. They successfully thrive in a wide range of environmental conditions exhibiting complex mechanisms to perceive and adapt to external changes. This is also reflected in the biosynthesis of a variety of organic compounds that constitute a potential source of added-value chemicals and biofuels. In addition, diatoms produce silica cell walls with species-specific nanopore patterns which showed photonics and nanotechnology applications. Thus diatom-based biorefinery, involving multiple extractions of diatom products from the same biomass, is prospect to novel development in biomedical and materials sciences while reducing pipeline costs. In this work, a native strain, VRUC 290 strain, of the colonial, araphid diatom *Staurosirella pinnata* (Ehrenberg) D.M. Williams & Round, isolated from biofilms of a Mediterranean coastal lagoon, and two commercial strains, SAG 1090-1b and 122.79, of the marine planktonic species *Phaeodactylum tricornutum* Bohlin and *Thalassiosira weissflogii* (Grunow) G.Fryxell & Hasle respectively, were mass cultivated, batch cultures, in indoor photobioreactor (30 L). *P. tricornutum* reached the stationary phase at day 10 while *S. pinnata* and *T. weissflogii* at day 14 and 12, respectively. *P. tricornutum* also showed the highest biomass production, with  $0.29 \pm 0.017$  g DW L<sup>-1</sup>, while  $0.22 \pm 0.0013$  and  $0.263 \pm 0.005$  g DW L<sup>-1</sup> were recorded for *S. pinnata* and *T. weissflogii*. No stress/optimized condition was applied in order to preserve biosilica pore integrity and homogeneity. The biomass of each culture, at the stationary phase, was dewatered, by settling and centrifuging, and mycosporine-like amino acids (MAAs), cellular lipids and frustule biosilica were sequentially obtained by means of different compatible protocols. Data on MAAs extracts showed that all strains are capable to produce MAAs. The highest MAA diversity and quantities were found in the *S. pinnata* extract, while in *T. weissflogii* and *P. tricornutum* only 2 MAAs were recognized. The potential biomedical application of the MAAs extracts were assessed by flow-cytometry analysis on human melanoma cell line. Lipid analyses showed that *S. pinnata* had the highest lipid content 11.85% w/w and the presence in the three strains of long chain polyunsaturated fatty acids as omega-3 C20:5 (Eicosapentaenoic acid, EPA) and omega-6 C20:4 (Eicosatetraenoic acid) prospecting employment in food ingredients and the nutraceutical field. Frustule analysis, by SEM microscopy, revealed the absence of ultrastructural anomalies in all strains. *Staurosirella pinnata* frustules showed structural properties suitable for random lasing effect and dye trapping applications, the latter also tested preliminarily using phycobiliproteins obtained from cyanobacterial biomass.

*We acknowledge Giuseppe Torzillo and Graziella Chini Zittelli, National Research Council-Institute of Ecosystem Study, for providing cyanobacterial biomass and the ES1408 COST Action EUALGAE for networking and knowledge transfer.*

## **If Dante had known Phytoplankton: A comparison between literature and science through the didactics of the metaphors**

M.R. Vadrucci<sup>1</sup>, F.Vitale<sup>1</sup>, M. T., Duggento<sup>2</sup>, C. Alberani<sup>2</sup>, A. Calò<sup>2</sup>, G. Giancane<sup>2</sup>, B. B. Rizzelli<sup>2</sup>, S. Schipa<sup>2</sup>, R. Visconti<sup>2</sup>, F. Del Vecchio<sup>3</sup>, B. Paiano<sup>3</sup>, F. Imperiale<sup>3</sup>, D. Brunetti<sup>3</sup>, L. Pancosta<sup>3</sup>

<sup>1</sup> ARPA Puglia – Agenzia Regionale per la Protezione Ambientale della Regione Puglia –  
Dipartimento di Lecce, via Miglietta 2, Italy.

<sup>2</sup> Liceo Classico Musicale «G. Palmieri», viale degli studenti, Lecce, Italy.

<sup>3</sup> Liceo Classico, Scientifico, Linguistico, delle Scienze Applicate e delle Scienze Umane “Virgilio-Redi”  
via G.Galilei, 4, Lecce; via Marinelli, 8 Squinzano (Le)-Italy

Here we present a learning project addressed to Classical High School students, developed under the "Alternanza Scuola - Lavoro" project (Italian Law n. 107/2015), between two High Schools "G. Palmieri" and Virgilio-Redi of Lecce and the Environmental Protection Agency of Puglia (ARPA), Department of Lecce. In particular, this project aimed to analyze allegorically the "phytoplankton communities" with topics of Italian literature. The learning project is developed in three study sheets that foresee the analysis and interpretation of characters or salient moments of the three Cantiche of Dante Alighieri's Divine Comedy with the purpose to create links with the microscopic aquatic organisms included in the phytoplankton communities. Such organisms are further known to the scientific community, for their important role as producers of energy and oxygen for their unbelievable variety of forms, colors and functions. Two study sheets were developed, the first, entitled "If Dante had known Phytoplankton: part I. The Inferno: the Harmful Algae Blooms" want to describe allegorically the "HABs' world"; the second entitled "If Dante had known Phytoplankton: part II: The Purgatory: how phytoplankton are and what they do", focused on association of shape and function of phytoplankton with features of selected characters in Purgatory. The results of this project concerned the design, by the students, of a narrative process that reconstructed the nodal points of Divine Comedy. The students, starting from the text in its rhetorical complexity (from the cosmological references, to the imagination of Dante expressed in 'figures'), have constructed a map of events and characters of the first two Cantiche of Divine Comedy in order to linked them to comparable events and characteristics of phytoplankton communities in the aquatic environment. The products of this training process were a hypertext with dissemination purposes, the preparation of manuscript for a scientific journal and a short essay.

## How biodiversity and structure help to improve biofilm control in cooling industrial systems

L. Di Gregorio<sup>1,2</sup>, S. Rossetti<sup>1</sup>, R. Congestri<sup>2</sup>, F. Di Pippo<sup>1</sup>

<sup>1</sup>CNR-IRSA, National Research Council, Water Research Institute, Rome, Italy.

<sup>2</sup>University of Rome Tor Vergata, Department of Biology, Rome, Italy

Matrix-enclosed, attached microbial communities, known as biofilms, colonize available surfaces of a wide range of industrial plants, where they may cause serious equipment damages. Particularly relevant are problems occurring in systems where natural aquatic bodies are used as water sources for industrial purposes, including cooling water systems. The colonizing microbes enter in these systems either from the source water or from the atmosphere. Here, the presence of nutrients and organic matter, concentrated by evaporation encourages biofilm formation on suitable surfaces. Such attached microbial communities can interfere with operational requirements of the systems, promoting micro-biofouling, particularly in those that are open to the atmosphere and hence exposed to sun light, where phototrophic biofilms can produce high amounts of biomass, whose removal requires high costs for regular cleaning to avoid performance loss and equipment plant damages. Conventionally, management strategies to limit biofouling do not take into account either compositional or structural features of biofilms. Indeed, biofouling is monitored and diagnosed indirectly by determining the number of free-living bacteria in bulk water samples. Moreover, biofilm control, mainly based on continuous addition of biocides directly to the process flow, may not be fully effective in biofilm removal due to the protection provided by the matrix to biofilm cells. A thorough insight in the microbial diversity and structure of biofilms occurring in cooling systems should be useful to encourage the development and the application of sensitive and effective biofouling control strategies. Even though many aspects of the ecology of biofilms in natural habitats have been disclosed, only scant information are available on composition and structure of biofilm communities growing in cooling tower systems.

This study aimed to fill the gaps in the knowledge of biofilms occurring in cooling plants, by focusing on the effect of source communities, types of cooling systems and seasonal variations of environmental conditions on the biodiversity and structure of biofilms sampled from different full-scale cooling systems. To this end, Next Generation Sequencing (NGS) and Catalyzed Reporter Deposition Fluorescence *In Situ* Hybridization (CARD-FISH) as well as microscopy (transmitted light, epifluorescence, confocal and electron microscopy) techniques were used.

Biofilm communities growing in the screened plants were substantially different from the corresponding planktonic communities, sharing only a few taxa. Alphaproteobacteria dominated the biofilm communities along with Beta- and Gammaproteobacteria. The phototrophic components were mainly Cyanobacteria, diatoms and green algae, with species composition similar to stream and river biofilms. Biofilm diversity and structure changed, reflecting only partially source water variations and appeared to be mostly related to type of plant and seasonal environmental changes. The tower operating conditions, e.g. pH, water hardness, biocide presence, most likely select, from the source communities, microorganisms able to survive under these conditions. Those of them capable to adhere to the available surfaces in the cooling tower, (e.g. members of the bacterial families Sphingomonadaceae and Comamonadaceae) may initiate the biofilm formation. Subsequently, seasonal variations in irradiance and water temperature could shape communities, accounting for the marked seasonal variations in diversity observed in the assemblages, with the increase of cyanobacterial and microalgal richness from winter to autumn, concurrently with reduction in bacterial diversity. Differences in structure and spatial organization were also observed among biofilm communities, with low stratification of microorganisms occurring in winter samples and the highest spatial heterogeneity and structural layering found in summer. In particular, the effect of irradiance and water temperature may drive firstly the composition of the phototrophic fractions, dominated by diatoms in winter, green algae in summer and cyanobacteria in the period at intermediate temperatures, and then, indirectly, the diversity of non-photosynthetic bacteria, the latter being mainly affected by the interactions between microorganisms.

Deciphering changes in the composition and structure of biofilms is crucial to defining specific control actions, beyond the application of one-shot solutions which may efficiently counter the dynamic evolution of biofouling in cooling towers. A single control strategy indeed cannot be used all over the year and in all cooling tower types due to the biofilm variability retrieved.

## The diversity of Halymeniales (Rhodophyta) from Madagascar and Guadeloupe explored by DNA tools

A. Manghisi<sup>1</sup>, M. Saitta<sup>1</sup>, M. Morabito<sup>1</sup>, L. Le Gall<sup>2</sup>

<sup>1</sup> Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali - Botanica, Università di Messina, Italia

<sup>2</sup> Institut de Systématique, Évolution, Biodiversité, ISYEB – UMR 7205 – CNRS, MNHN, UPMC, EPHE, Muséum National d’Histoire Naturelle, Sorbonne Universités, Paris, France

In the present study, we report on a survey of the diversity of Halymeniales (Rhodophyta) collected during two expeditions along the coasts of southern Madagascar and Guadeloupe. The aim of these marine expeditions was to overcome the lack of knowledge on the algae and more generally on the diversity of benthic organisms by means of a modern systematic approach. Among the collected material, specimens morphologically identified as halymeniacean taxa have been sequenced for the DNA barcode gene (COI-5P). Subsequently, those recognized as putative new taxa were thoroughly observed to document their vegetative and reproductive anatomical structures. Phylogenetic analyses inferred from additional genetic markers (*rbcL*, LSU) were also conducted to assess the phylogenetic relationships of the Malagasy and Guadeloupian samples with their worldwide relatives. The first published result was a report of two novel species of the genus *Yonagunia* from Madagascar (Manghisi et al. 2015), moreover additional new halymeniacean taxa were uncovered and will be formally described.

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## Risultati preliminari di uno studio molecolare e citologico su *Polysiphonia caretta* e “*Neosiphonia sertularioides*” (Rhodomelaceae, Rhodophyta)

Sciuto K.<sup>1</sup>, Moro I.<sup>1</sup>, Furnari G.<sup>2</sup>, Serio D.<sup>2</sup>

<sup>1</sup>Dipartimento di Biologia, Università di Padova, Via U. Bassi, 58/B - 35131 Padova

<sup>2</sup>Dipartimento di Scienze Biologiche, Geologiche e Ambientali dell'Università di Catania,  
 Via A. Longo, 19 - 95125 Catania

È noto che la tassonomia del “genere *Polysiphonia*” è molto complessa e i caratteri diacritici proposti per la suddivisione sono stati più volte rivisti. Inoltre, studi recenti condotti da Díaz-Tapia *et al.* (2017) hanno ulteriormente dimostrato l'eterogeneità di tale gruppo e una nuova risistemazione della tribù delle Polysiphoniae è stata proposta. Due generi sono stati riconosciuti: *Vertebrata* (che include le specie tipo dei generi *Ctenosiphonia*, *Enellittosiphonia*, *Boergeseniella* e *Brongnartella*) e *Melanothamnus* (che include le specie tipo dei generi *Fernandosiphonia* e *Neosiphonia*). Nell'ambito di tale problematica, al fine di chiarire la posizione generica di due specie morfologicamente molto simili, *Polysiphonia caretta* e “*Neosiphonia sertularioides*”, e confermare o meno la loro conspecificità, si è avviato uno studio su esemplari di *P. caretta* (tetrasporofiti e gametofiti femminili) raccolti a Lampedusa sul carapace di *Caretta caretta* e di “*N. sertularioides*” (tetrasporofiti, gametofiti femminili e maschili) raccolti lungo il litorale catanese. *P. caretta* e “*N. sertularioides*” mostrano, infatti, le medesime caratteristiche morfologiche, anatomiche e riproduttive: assi eretti e prostrati, corticazione assente, ramificazione pseudodicotoma, rami che non si originano all'ascella dei tricoblasti, rizoidi separati dalla cellula pericentrale (non in aperta connessione), 4 cellule pericentrali, ramo carpogoniale di tre cellule (osservato in *P. caretta* per la prima volta), cistocarpi globulari, cellule dell'ostiolo simili alle sottostanti, tetrasporangi disposti a spirale. Per quanto riguarda l'origine dei rami spermatangiali non è possibile fare una comparazione adeguata. Infatti, in “*N. sertularioides*” essi rimpiazzano uno dei rami del tricoblasto, come da noi osservato e in accordo con i dati di letteratura, mentre in *P. caretta* tale carattere non è stato evidenziato da Rojas-González *et al.*, (1994), gli unici autori che hanno segnalato il ritrovamento dei gametofiti maschili. Al fine di chiarire la posizione tassonomica di *P. caretta* e “*N. sertularioides*” sono state quindi condotte delle analisi molecolari, utilizzando un approccio multigenico (*rbcL*, *cox1* e geni ribosomali). Tali indagini e i recenti risultati riportati da Díaz-Tapia *et al.* (2017) hanno suggerito di effettuare delle ulteriori osservazioni al microscopio su sezioni semifine di *P. caretta* e “*N. sertularioides*”, al fine di chiarire l'aspetto di determinati caratteri citologici considerati diagnostici per discriminare i generi recentemente descritti nella tribù delle Polysiphoniae. Verranno qui discussi i risultati preliminari ottenuti finora e la posizione sistematica delle due specie indagate.

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## The DNA barcode-assisted floristic list of red algae from Tunisia

M. Morabito<sup>1</sup>, A. Manghisi<sup>1</sup>, R. Miladi<sup>1,2</sup>, S. Armeli Minicante<sup>3</sup>, L. Le Gall<sup>4</sup>, S. Abdelkafi<sup>2</sup>, G.W. Saunders<sup>5</sup> and G. Genovese<sup>1</sup>

<sup>1</sup> Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali - Botanica, Università di Messina, Italia;

<sup>2</sup> Unité de Biotechnologie des Algues, Département de Génie Biologique, Ecole Nationale d'Ingénieurs de Sfax, Université de Sfax, Tunisia;

<sup>3</sup> ISMAR-CNR, Venezia, Italia;

<sup>4</sup> Institut de Systématique, Évolution, Biodiversité, ISYEB – UMR 7205 – CNRS, MNHN, UPMC, EPHE, Muséum National d'Histoire Naturelle, Sorbonne Universités, Paris, France;

<sup>5</sup> Centre for Environmental and Molecular Algal Research, Department of Biology, University of New Brunswick, Fredericton, Canada.

In the present study, we produced a DNA barcode-assisted floristic list of florideophycean algae collected along the Tunisian coast. This allowed us to reveal cryptic species, allochthonous introductions and to identify problematic taxa. Tunisia holds a key position in the Mediterranean Sea as it constitutes a transitional area between the eastern and western basins benefitting from rich habitat diversity. The Strait of Sicily, the waters between Tunisia and Sicily, is the crossroad from south to north and from east to west, and is crucial in the analysis of the distribution of marine organisms in the Mediterranean Sea (Coll et al., 2010). The most recent inventory of marine macrophytes dates back to 1987 with an update in 1995, but these were limited to morphological observations, which can be misleading for many seaweed species (Ben Maiz et al., 1987, Ben Maiz, 1995). To the best of our knowledge this is the first study dealing with macroalgal inventory in Tunisia using a DNA approach. As opposed to morphological identifications, DNA barcodes are more useful for uniting biological specimens into genetic groups as a first step to assigning them to species and genera (Saunders, 2005). This methodology is particularly useful in organisms with simple morphologies, a high degree of phenotypic plasticity or convergence, and heteromorphic life histories, as is so common in marine macroalgae (e.g. Manghisi et al., 2015, Filloramo and Saunders, 2016). To ensure an effective monitoring of biodiversity change over time we propose the use of DNA barcode-assisted floristic lists, which are more convenient and accurate than lists devised from traditional approaches, because associated barcode sequences are easily trackable and each specimen is unequivocally linked to a permanent genetic label regardless of any subsequent taxonomic or nomenclature variation. DNA barcoding surveys contribute new records to DNA libraries of life such as the BOLD catalogue, increasing our biodiversity knowledge, which is linked to geographical information and is also freely available to the scientific community (Le Gall et al., 2017). Furthermore, in the context of environmental management, the added value of DNA barcode-assisted identifications is that they give objective and verifiable data, an essential strength if we consider that floristic lists are extensively used by ecologists and environmental agencies as the basis for monitoring studies.

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## ***Asparagopsis*: a rich source of allelochemical compounds**

D. Spagnuolo, A. Manghisi, M. Morabito, G. Genovese

Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali –  
Botanica, Università di Messina, Italia

Macroalgae produce a wide variety of allelopathic metabolites with a broad spectrum of effects, including immunostimulant, cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities, related to abiotic or biotic stress factors, in order to attract or discourage other organisms (Amsler 2008; Marino et al. 2016). Among red algae, species of *Asparagopsis* (Bonnemaisoniales, Rhodophyta), as well as other species of the family Bonnemaisoniaceae, are well known as sources of halogenated compounds with strong antimicrobial activity (Genovese et al. 2008). This genus produces a plethora of compounds containing bromine and iodine, which are active against several pathogens including the protozoan *Leishmania* spp. (Kinetoplastida, Euglenozoa), the mycetes *Aspergillus* (Ascomycota) and *Cryptococcus* (Basidiomycota), responsible for both humans and animals diseases, and bacteriosis relevant in aquaculture (Genovese et al. 2012; Genovese et al. 2013a, 2013b; Vitale et al. 2015; Rizzo et al. 2017).

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## Total phenolic content in brown algae from the Sicilian coast

A.M. Mannino, E. Oddo

Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche, Università di Palermo,  
Via Archirafi 38, I-90123 Palermo, Italy

Phlorotannins are polyphenolic secondary metabolites found in almost all brown algae that function as defense against grazers, pathogens and epiphytes but are also involved in photoprotection mechanisms. These compounds, produced in the Golgi apparatus, are accumulated in cytoplasm, within vesicles called physodes, or bound to the cell wall. The concentration of phlorotannins differs within and between species, shows geographical variations but may be also affected by abiotic or biotic factors. We present here an overview of the studies carried out on total phenolic content in brown algae collected along the north-western coast of Sicily. The aims of these studies were in particular a) to analyse total phenolic content in four Mediterranean brown algae (*Cystoseira amentacea*, *Cystoseira compressa*, *Dictyopteris polypodioides* and *Padina pavonica*), b) to follow the seasonal changes in total phenolic content in *D. polypodioides* and *C. amentacea* and c) to test the effects of temperature on total phenolic content of *C. amentacea*. Results showed significant differences in total phenolic content between leathery and sheet-like algae and also within each morphological group. Among the four species, the sheet-like alga *D. polypodioides* showed the highest concentration of phenolic compounds. Differences in the seasonal pattern of total phenolic content and in the period of maximum production were observed between *D. polypodioides* and *C. amentacea*. In *D. polypodioides* the peak was observed during winter and autumn whereas for *C. amentacea* the peak was observed during spring and summer. Moreover, *C. amentacea* responded significantly to the exposition to an increase of temperature, suggesting that increasing global temperatures predicted in the coming century might have effects on the chemical defences and then on the trophic interactions of these algae. The results of these studies seem to confirm that total phenolic content in brown algae is a response to a combination of several factors. However, due to the complexity of total phenolic content responses in brown algae and the multiple roles of phlorotannins, for a better understanding of this process, it is still necessary to identify which types of phlorotannins are responsible for the different activities in order to clarify *who does what*. Therefore, studies are currently in progress in order to characterize phlorotannins in some species belonging to the Fucales and Dictyotales and to test their biological activities.

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