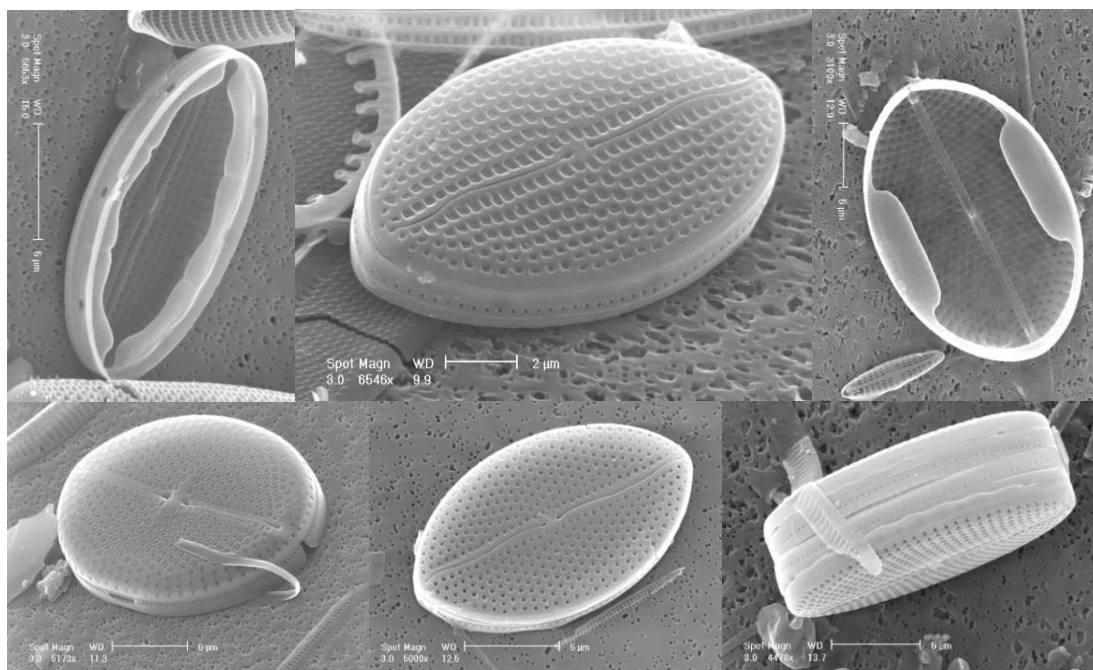




# RIUNIONE SCIENTIFICA ANNUALE DEL GRUPPO DI ALGOLOGIA DELLA SOCIETA' BOTANICA ITALIANA (SBI)

Amendolara, 11-12 novembre 2022



Stazione Zoologica Anton Dohrn - Sede Calabria



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**Società Botanica Italiana**  
**Gruppo di Algologia**  
**Riunione Scientifica Annuale**  
Amendolara, 11-12 novembre 2022  
Stazione Zoologica Anton Dohrn – Sede Calabria

**Comitato organizzatore:**  
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Si ringraziano:  
il Comune di Amendolara per aver patrocinato l'evento,  
Deltek per aver finanziato il kit congressuale.



## Programma

### Venerdì, 11 novembre 2022

- 8.30 Inizio registrazione dei partecipanti
- 9.15 Saluti di benvenuto e introduzione ai lavori
- 10.00 Relazione ad invito:  
**Prof. Christopher S. Lobban**, University of Guam  
What if we assume everything is NOT everywhere?

#### Sessione Microalghe I - Moderatore: Rossella Pistocchi

- 10.30 **M. De Stefano**, C. Auciello, M. Iovinella, C. Ciniglia, C. Pennesi  
From Antarctica to Tropics, a long term study on biodiversity and ecological role of benthic diatom communities
- 10.45 **R. Trentin**, E. Moschin, A. Duarte Lopes, S. Schiaparelli, L. Custódio, I. Moro  
Molecular, morphological and chemical diversity of two new species of Antarctic diatoms, *Craspedostauros ineffabilis* sp. nov. and *Craspedostauros zucchellii* sp. nov.
- 11.00 **R. Bentivogli**, L. Pezzolesi, N. Caputo, F. Costantini, J. Fondo, R. Pistocchi  
Morphological vs. innovative DNA metabarcoding approach for the phytoplankton monitoring in an Adriatic coastal lagoon (cascade project)

#### 11.15 - 11.45 Coffee break

#### Sessione Macroalghe - Moderatore: Fabio Rindi

- 11.45 **G. Denti**, A. Petrocelli, E. Cecere, G. Fanelli, L. Papa, F. Rubino  
Further evidence of the presence of *Caulerpa cylindracea* (Bryopsidales, Chlorophyta) and *Asparagopsis* sp. (Bonnemaisoniales, Rhodophyta) in the Taranto seas (Northern Ionian Sea, Mediterranean Sea), two non-indigenous species (NIS) of macroalgae
- 12.00 **D. Lenzo**, A. Bartual, B. Úbeda, E. Zubía, B. Cuevas, M.A. Colangelo, A. Pasteris, L. Pezzolesi  
Allelopathic effects in the invasive macroalga *Rugulopterix okamurae* (Phaeophyceae):



potential role of the newly discovered compound Dilkamural

- 12.15 **D. Oliva, A. Piro, M. Carbone, E. Mollo, F. Scarcelli, V. Osso, D.M. Nisticò, S. Mazzuca**  
Study of the effects of metabolites of invasive *Caulerpa* spp. on *Posidonia oceanica* seedling growth: unexpected effects of the vehicle (DMSO)
- 12.30 **A. Tursi, F. Mastrototaro, G. Chimienti, A. Bottalico**  
Algal assemblages' composition of two semi-submerged marine caves at Tremiti Islands Marine Protected Area (Italy, Adriatic Sea)
- 12.45 **S. Kaleb, E. Descourvières, V. Bandelj, F. Gianni, M. Orlando-Bonaca, A. Falace**  
New insights into the reproductive strategies of the endangered *Fucus virsoides* useful for implementing restoration measures

### 13.00– 15.00 Pausa pranzo

#### **Sessione Microalghe II - Moderatore: Stefano Accoroni**

- 15.00 **M. Marieschi, G. Molinari, S. Savi, R. Bolpagni, A. Torelli**  
Search of freshwater algal species with adaptive mechanisms to Cr (VI) stress
- 15.15 **G. Molinari, M. Marieschi, R. Bolpagni, A. Torelli**  
Interaction between OAS-TL and desulfhydrase activity in Cr (VI) tolerance in the freshwater green alga *Scenedesmus acutus*
- 15.30 **L. Mollo, F. Drigo, A. Norici**  
Effetti di composti fenolici presenti in acque di vegetazione sulle microalghe
- 15.45 **C. Auciello, C. Pennesi, C. Ciniglia, M. Iovinella, L. Rabaoui, M. De Stefano**  
Benthic diatom communities on seagrass and corals in Arabian Gulf: floristic analysis, population dynamics and estimate of contribute to the primary production of the basin
- 16.00 **M. Blasio, G.V. de Klerk, P. Marotta, A. Cutignano, G. Trancone, G. Dupont, A. Sardo, P. De Luca, S. Papirio, S Balzano**  
Potential of *Nannochloropsis oceanica* for bioremediation
- 16.15 **S. D'Arpa, M.R. Vadrucchi, P.C. Ciciriello, E. Stanca, A. Pastorelli, N. Ungaro**  
A photo guide of harmful algae along coastal areas of Apulian Region



## 16:30 – 17.00 Coffee break

### Sessione Microalghe III - Moderatore: Marina Montresor

17.00 **V. Natali, T. Cibic**

Valutazione dell'effetto inibitorio di materiali legnosi modificati o trattati con impregnanti di nuova generazione sullo sviluppo della comunità microfitobentonica nella laguna di Grado (nord Adriatico)

17.15 **F. Neri, M. Ubaldi, T. Romagnoli, S. Accoroni, C. Totti**

Phytoplankton indicators for GES assessment: a case study in the N Adriatic Sea

17.30 **A. Pelusi, L. Ambrosino, M. Miralto, M.L. Chiusano, A. Rogato, M.I. Ferrante, M. Montresor**

Espressione genica durante la formazione di spore nella diatomea marina *Chaetoceros socialis*

17.45 **L. Roselli, S. Bevilacqua, A. Terlizzi**

Trait-based approach for rapid and effective characterization of phytoplankton patterns

18.00 **G. Furnari**

Amenità ficologiche e non solo

18.15 **ASSEMBLEA DEI SOCI**

## 20.30 Cena Sociale

### Sabato, 12 novembre 2022

### Sessione Microalghe IV - Moderatore: Laura Pezzolesi

9.30 **K. Sciuto, A.A. Sfriso, M.A. Wolf, A. Sfriso, E. Moschin, C. Munari, M. Mistri, I. Moro**  
Dati preliminari su una microalga verde isolata dal Lido di Spina (Comacchio, Ferrara)

9.45 **M. Ubaldi, S. Bacchiocchi, M. Siracusa, F. Neri, T. Romagnoli, C. Totti, S. Accoroni**  
Effect of salinity on growth and microcystin production by the cyanobacterium



*Planktothrix rubescens*

- 10.00 **L. Barra, S. Balzano, M. Moros, A. Smerilli, E. Cavalletti, I. Percopo, P. Chiaiese, A. Sardo**  
Potential of a green algal strain collected from the Sarno River mouth for heavy metal bioremediation
- 10.15 **M. Simonazzi, L. Pezzolesi, T.K. Shishido, D.P. Fewer, R. Pistocchi**  
Investigation of potentially toxic metabolites in a newly isolated freshwater cyanobacterium of the family Pseudanabaenaceae through a genomic approach
- 10.30 **M. Iovinella, C. Ciniglia, S.J. Davis**  
Organelle phylogenomics unveil the diverging evolution of 6 lineages in *Galdieria sulphuraria* (Cyanidiophyceae)

**10.45 - 11.15 Coffee break**

**Sessione: Microalghe V - Moderatore Chiara Pennesi**

- 11.15 **M. Mutalipassi, V. Mazzella, N. Ruocco, V. Zupo**  
*Halomicronema metazoicum*, la storia di un cianobatterio, del suo ambiente e dei suoi vicini
- 11.30 **I. Orefice, V. Di Dato, G. Romano**  
Induzione di stadi di resistenza nella diatomea marina *Thalassiosira rotula*
- 11.45 **G. Zoffoli, C. Samorì, M. Simonazzi, R. Pistocchi, L. Pezzolesi**  
Production of the biopolymer polyhydroxybutyrate (PHB) from the Chlorophyceae *Desmodesmus communis*
- 12.00 Arrivederci!**



## Sessione Microalghe I

Moderatore: Rossella Pistocchi



## From Antarctica to Tropics, a long term study on biodiversity and ecological role of benthic diatom communities

**M. De Stefano, C. Auciello, M. Iovinella, C. Ciniglia, C. Pennesi**

In Polar and tropical marine waters, microalgal communities associated to macrophytes, seagrasses, and invertebrates can be often more productive than phytoplanktonic ones (Colijn & De Jonge, 1984; Sundbäck & Jönsson, 1988). These benthic microalgal assemblages, known as “microphytobenthos” are mainly represented by diatoms (Bacillariophyta) (Mac Intyre et al., 1996). and specifically by a well-circumscribed group of “pennate” diatoms genera that can be considered adapted to this peculiar epibenthic life habitus. However, despite to their crucial ecological role in the trophic chain, the biodiversity of epibenthic diatom communities worldwide is poorly studied and greatly underestimated. Floristic studies at species or intraspecific level are rare since most of these species exhibit mean dimensions lower than 20 micrometers with taxonomic features often below the solving power of the light microscope. Therefore, a floristic and ecological analysis of these communities needs scanning electron microscopy (SEM) as common approach. In the framework of an international project aimed to decrypt and document the hidden biodiversity of epibenthic diatom assemblages worldwide, we will report the results of comparative analysis, in term of specie richness, community structure, and ecological role, within Antarctic and Tropical diatom communities associated to macrophytes and different invertebrates including gastropods, crustaceans bryozoans and bivalves. Our results demonstrate that either in Polar or Tropical regions, significant benthic diatom communities can be hosted by macrophytes whereas those on invertebrates are generally poor in term of species and relative abundances. Specifically, cell densities of seagrasses diatom communities resulted more than one hundred times higher than those of invertebrates with mean values approximately of 6000-7000 cell/mm<sup>2</sup> (up to 13000 cell/mm<sup>2</sup>) in the former vs 100-300 cell/mm<sup>2</sup> in the latter. Noteworthy is that the specie composition of seagrasses diatom communities in both biogeographic regions was characterized by the almost full dominance of adnate genus *Cocconeis* (represented by four species) exhibiting cell densities mean values more than one hundred times higher than those of all the other diatom genera present in the communities. *Cocconeis* predominance in epibenthic diatom assemblages was been also confirmed by previous similar studies on Mediterranean and indo-pacific regions thus confirming the crucial ecological role of such genera in all epibenthic diatom communities worldwide.

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- Sundbäck, K., Jönsson, B. (1988). Microphytobenthic productivity and biomass in sublittoral sediments of a stratified bay, south-eastern Kattegat. *J. Exp. Mar. Biol. Ecol.* 122: 63-81.

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## Molecular, Morphological and Chemical Diversity of two new species of Antarctic Diatoms, *Craspedostauros ineffabilis* sp. nov. and *Craspedostauros zucchellii* sp. nov.

**R. Trentin, E. Moschin, A. Duarte Lopes, S. Schiaparelli, L. Custódio, I. Moro**

The current study focuses on the biological diversity of two strains of Antarctic diatoms (strains IMA082A and IMA088A) collected and isolated from the Ross Sea (Antarctica) during the XXXIV Italian Antarctic Expedition. Both species presented the typical morphological characters of the genus *Craspedostauros*: cribrate areolae, two 'fore-and-aft' chloroplasts and a narrow 'stauros'. This classification is congruent with the molecular phylogeny based on the concatenated 18S rDNA-*rbcl*-*psbC* alignment, which showed that these algae formed a monophyletic lineage including six taxonomically accepted species of *Craspedostauros*. Since the study of the evolution of this genus and of others raphe-bearing diatoms with a 'stauros' is particularly challenging and their phylogeny is still debated, we tested alternative tree topologies to evaluate the relationships among these taxa. Metabolic fingerprinting approach was implemented for the assessment of the chemical diversity of IMA082A and IMA088A. In conclusion, combining: (1) traditional morphological features used in diatoms identification, (2) phylogenetic analyses of the small subunit rDNA (18S rDNA), *rbcl* and *psbC* genes, and (3) metabolic fingerprint, we described the strains IMA082A and IMA088A as *Craspedostauros ineffabilis* sp. nov. and *Craspedostauros zucchellii* sp. nov. as new species, respectively.

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Isabella Moro ([isabella.moro@unipd.it](mailto:isabella.moro@unipd.it)), Department of Biology, University of Padova, 35131 Padova, Italia.



## Morphological vs. innovative DNA metabarcoding approach for the phytoplankton monitoring in an Adriatic coastal lagoon (cascade project)

**R. Bentivogli, L. Pezzolesi, N. Caputo, F. Costantini, J. Fondo, R. Pistocchi**

The INTERREG project CASCADE (CoAstal and marine water integrated monitoring systems for ecosystems protection And management) aims to assess and to evaluate the quality and the vulnerability of coastal ecosystems, with the final purpose to enhance the knowledge, restore endangered species and support an integrated management of coastal areas. Microalgae have a crucial role in water management since they represent the mainstay of coastal ecosystems. Given that algal blooms have become a widespread phenomenon across the world (Wurtsbaugh et al., 2019) and established that traditional monitoring methods based on the experience of the surveyor are not appropriate for a large-scale measurement of phytoplankton, new methodologies are needed to overcome the issue and face the diversified factors driving algal blooms (Wood et al., 2013). As part of the CASCADE project, during the last year a monthly monitoring campaign has been carried out in a coastal lagoon situated in the northern Adriatic Sea (Sacca di Goro, FE) to assess the planktonic communities and evaluate the ecological status of the area. The analysis of the phytoplankton communities relied on qualitative and quantitative evaluation of the species present using a morphological analysis (microscope), however, an innovative technique, mainly the environmental DNA metabarcoding using Oxford Nanopore MinIon sequencing, has been tested during seasonal samplings and compared with the microscope analysis to better define phytoplankton dynamics in a coastal lagoon characterized by peculiar hydrological trends. This innovating technique has enabled to detect new multiple, cryptic and picoplanktonic taxa that were not identified using microscope and, in addition, it was found to be effective in the identification of harmful algal bloom species and non-indigenous species in this shallow water lagoon. DNA metabarcoding has been largely applied in the last decade as an instrument to fulfil ecosystem biotic information (Brown et al., 2022). Although it represents a powerful tool for valuable insights, it still needs to be improved, specifically in the implementation of the barcoding libraries, thus it cannot yet be treated as a complete substitute for the traditional monitoring methods.

### Letteratura citata

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## Sessione Macroalghe

Moderatore: Fabio Rindi



## Further evidence of the presence of *Caulerpa cylindracea* (Bryopsidales, Chlorophyta) and *Asparagopsis* sp. (Bonnemaisoniales, Rhodophyta) in the Taranto seas (Northern Ionian Sea, Mediterranean Sea), two non-indigenous species (NIS) of macroalgae

**G. Denti, A. Petrocelli, E. Cecere, G. Fanelli, L. Papa, F. Rubino**

The introduction of non-indigenous species (NIS), organisms arriving from beyond their natural (past or present) geographical region and outside of their natural dispersal potential, represents one of the most important threats for biodiversity. This arrival in many cases leads to detrimental community-level modifications and, consequently, to alterations of ecosystem functioning together with socio-economic dimension that is unavoidably affected (Tsirintanis et al., 2022). 1.288 trillion \$ are reported as the minimum value of the global economic loss related to biological invasion assessed in the past few decades (1970-2017). The Mediterranean Sea is considered a hotspot for biological invasions counting more than 1000 registered non-indigenous species; of these, seaweeds are approximately 200 to date. Taranto seas, Mar Grande and Mar Piccolo, host 43 NIS of which 15 are macroalgal species; their presence is related to the pathways of introduction which typify the site (Cecere et al., 2016). Here we further report the accidental observation of two NIS considered among the worst in terms of negative impact on biodiversity and ecosystem functioning: *Caulerpa cylindracea* Sonder and *Asparagopsis* sp. *Caulerpa cylindracea* (as *Caulerpa racemosa* (Forsskål) J. Agardh var. *cylindracea* (Sonder) Verlaque, Huisman et Boudouresque) was firstly recorded along the coasts of North Africa in the 80's; the presence of this seaweed in the Gulf of Taranto dates back to 1996. The thalli consist in photosynthetic sub-cylindrical/claviform ramules attached to stolons. After a rapid colonization of the Taranto marine area, due to its impressive invasive ability (stolon elongation approx. 20mm d<sup>-1</sup>), a regression of coverage was observed almost to its disappearance. In May 2022, only one erect axe was again observed in the Mar Piccolo of Taranto, at 1 meter of depth. In July 2022, a scattered but dense coverage of *C. cylindracea* was observed colonizing rocks in the intertidal zone, few centimetres of depth in the Gulf of Taranto; at the same site, in September it seemed to be disappeared. In June and July 2022, thalli of *Asparagopsis* sp. were observed on rocky bottom in the Mar Grande of Taranto, at 1 meter of depth. The genus *Asparagopsis* comprehends two species *A. armata* and *A. taxiformis* featuring a triphasic heteromorphic cycle. The gametophytic stage is represented by pink thalli attached to the substratum with pyramidal structure, and feathery branches; *A. armata* differs for the presence of harpoon-like branchlets. The tetrasporophyte stage appears as filamentous turfs and was previously considered as a separated species, i.e. *Falkenbergia hillebrandii* (Bornet) Falkenberg. Moreover, while *A. armata* is considered genetically uniform, for *A. taxiformis* molecular analysis showed the existence of 4 different evolutionary lines, two of them present in the Mediterranean Sea (Zanotta et al., 2014). In 1992, the presence of *A. armata* on the bottom of Cheradi Islands in front of the city of Taranto was reported. The allochthonous lineage of *A. taxiformis* was probably observed in 2015 in the Mar Piccolo of Taranto (Bottalico et al., 2015). The next step includes the use of molecular analysis on macroalgal samples to correctly identify the species.

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## Allelopathic effects in the invasive macroalga *Rugulopteryx okamurae* (Phaeophyceae): potential role of the newly discovered compound Dilkamural

**D. Lenzo, A. Bartual, B. Úbeda, E. Zubía, B. Cuevas, M. A. Colangelo, A. Pasteris, L. Pezolesi**

Climate change is altering the distribution of organisms across the globe and is likely to change co-occurrence patterns and interspecific interactions of native, and invasive species, whose negative impact on marine ecosystems could affect the biodiversity and economy of coastal areas (Salvaterra et al., 2013). In aquatic environments, macroalgae provide habitat for fauna but are also able to produce different allelopathic compounds with high structural variability, that play an important role in species' successions (Pezolesi et al., 2021). Whether invasive algae could produce allelopathic and toxic compounds, and how they could potentially change the population dynamics of native species remained unknown. In the last decade, the brown macroalga *Rugulopteryx okamurae* has shown an intensive proliferation in the southwestern coasts of Europe (Strait of Gibraltar). Currently, there is no clue regarding the reasons of such huge invasive potential, although the involvement of chemical defenses has recently been suggested. Chemical study on *R. okamurae* from the Strait of Gibraltar led to the isolation of different secondary metabolites, among which the compound Dilkamural stands out because of its high concentration (Casal-Porras et al., 2021). In this context, this work was aimed to investigate the effects of Dilkamural on unicellular phototrophs to understand its potential role as allelochemical. In particular, this ecotoxicological study was carried testing two different species of microalgae (the diatoms *Phaeodactylum tricorutum* and *Cyclotella cryptica*) and a cyanobacterium (*Synechococcus elongatus*, PCC 7002) with different concentrations of Dilkamural. The effects on cells were evaluated for each species after 0, 3, 24, 48, 72 hours, adding "SYTOX green" to mark dead cells and analysing them through image flow cytometry (IFC) (Amnis ImageStream X MkII, Luminex Corporation). Results showed an acute effect of Dilkamural, after only three hours, on all the analysed species; particularly, effects on the viability and cell morphology of *P. tricorutum*, as well as cell integrity and reproduction of *S. elongatus* and *C. cryptica* were reported. The observed effects highlighted as the production of Dilkamural by this invasive species could affect algal population dynamics, reporting allelopathic effects on target organisms, thus potentially reducing the biodiversity of the coastal ecosystems where this invasive algal species has been introduced and extensively proliferates.

### Letteratura citata

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## Study of the effects of metabolites of invasive *Caulerpa* spp. on *Posidonia oceanica* seedling growth: unexpected effects of the vehicle (DMSO)

**D. Oliva, A. Piro, M. Carbone, E. Mollo, F. Scarcelli, V. Osso, D.M. Nisticò, S. Mazzuca**

The Mediterranean hosts the largest number of allochthonous macrophytes (Zenetos et al., 2012) that can replace indigenous species, causing a decrease in the biodiversity of native species and altering the ecosystem. The species of greatest concern are mainly *Caulerpa taxifolia* and *Caulerpa cylindracea*, both infesting tropical algae whose role towards the biodiversity of Mediterranean macrophytes has not yet been clarified (Marbà et al., 2005). The algae of the genus *Caulerpa* also have the ability to compete with seagrasses (Williams SL 2007). It has been hypothesized that, at the basis of this competition, there may be an allelopathic interaction between species (Defranoux et al., 2021). Among the secondary metabolites produced by algae, the alkaloid *caulerpin* and the sesquiterpene *caulerpenyne* have been considered to play a role in the regression of endemic communities (Piazzi et al., 2001); their mechanism of action, however, is currently unknown.

The aim of the present research is to clarify the effects of molecules purified from *C. cylindracea* on growth, phenology, and molecular responses of *Posidonia oceanica* cuttings acclimated under controlled conditions in a mesocosm, and thus identify the metabolic pathways that are induced by the chemical interaction between the species. Here we will present the *i*) optimized conditions for the cultivation of *P. oceanica* cuttings in the aquarium, *ii*) the results of the extraction, purification and characterization of the main secondary metabolites in *Caulerpa* spp. and *iii*) the results of a pilot experiment on *P. oceanica* treated with caulerpin and caulerpenyne dissolved in DMSO (dimethyl sulfoxide). An unexpected result will also be presented which concerns the effect of DMSO on the studied parameters. This data will be discussed in relation to the recent publication which has shown that, among the seagrass, *P. oceanica* is the largest producer of DMSO together with its precursor, dimethylsulfoniopropionate (DMSP) (Richir et al., 2021).

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## Algal assemblages' composition of two semi-submerged marine caves at Tremiti Islands Marine Protected Area (Italy, Adriatic Sea)

**A. Tursi, F. Mastrototaro, G. Chimienti, A. Bottalico**

Semi-submerged marine caves are peculiar coastal habitats widely spread all over the Mediterranean Sea (Gerovasileiou & Bianchi, 2021). These environments enrich the coastal landscape complexity, being extremely important for marine biodiversity. In fact, they represent valuable hotspots of biodiversity, being colonized by peculiar species generally found at higher depths, well adapted to semi-submerged marine caves characterized by low light intensity and scarce hydrodynamism (Ouerghi et al., 2019). The benthic communities in these caves include a rich and diverse macroalgal flora which plays a key role in the stability of these habitats. Nevertheless, only a few studies have been carried out about the algal assemblages of such environments (Alongi et al., 2012; Bottalico et al., 2016). In this context, Tremiti Islands Marine Protected Area (MPA) hosts several semi-submerged marine caves, whose algal composition is scarcely known (Pignatti et al., 1967; Rizzi Longo, 1972). This study is focused on the monitoring of two semi-submerged marine caves in the MPA, namely Violo and Rondinelle caves. A qualitative sampling was carried out by SCUBA diving in two different monitoring periods (spring and autumn 2021). A total of 42 species were identified in the two semi-submerged marine caves. At Violo cave, Rhodophyta showed to be the most abundant taxa with 24 species, followed by Chlorophyta and Ochrophyta-Phaeophyceae, which were represented by 7 and 6 species, respectively. A total of 20 algal species was observed at Rondinelle Cave, with 13 Rhodophyta, 5 Chlorophyta and 2 Ochrophyta-Phaeophyceae. In both caves, Rhodophyta were particularly abundant in the inner portions of the caves, where the light intensity was scarce, while Chlorophyta and Ochrophyta-Phaeophyceae tended to be more abundant nearby the entrance and the end of the caves, which had collapsed vaults, allowing the presence of sunlight. Cave walls were dominated by encrusting algae belonging to the genus *Peyssonelia*, confirming what observed more than 50 years earlier (Pignatti et al., 1967). This study contributed to implementing the knowledge about the algal assemblages of two semi-submerged marine caves, particularly important for the tourism industry of Tremiti Archipelago. Our results showed the presence of several algal species of great ecological and conservation values, highlighting the importance of monitoring and conservation of these coastal habitats. Future perspectives will focus on the algal communities of other semi-submerged marine caves of the MPA, in order to identify any similarities and differences, possibly also related to different environmental conditions and/or to anthropic actions.

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## New insights into the reproductive strategies of the endangered *Fucus virsoides* useful for implementing restoration measures

**S. Kaleb, E. Descourvières, V. Bandelj, F. Gianni, M. Orlando-Bonaca, A. Falace**

Canopy-forming algae of the order Fucales and Laminariales (Phaeophyceae) are among the most valuable marine species in temperate waters. They provide a number of important ecosystem services, including carbon sequestration, shelter and nursery (Fabbrizzi et al., 2020). They also play a central role in understory growth by influencing light, desiccation and water movement. *Fucus virsoides* J. Agardh (Fucales, Phaeophyta), endemic to the Adriatic Sea (Linardić, 1949; Giaccone & Pignatti, 1967; Munda, 1972), is a glacial relict that has survived in the Adriatic Sea thanks to the specificities of this basin (Munda, 1972, Orlando-Bonaca et al., 2013). In the past, its range extended from the Venice Lagoon to the Boka Kotorska (Mačić, 2006). In recent decades, it has experienced dramatic declines or local extinctions (e.g. Battelli, 2016; Mačić, 2006; Orlando-Bonaca et al., 2013). Due to its endangered status and ecological role, it has been included in the "List of Threatened or Endangered Species" of the Barcelona Convention (amended Annex II of the Protocol on Specially Protected Areas and Biological Diversity in the Mediterranean, UNEP; 2019).

The lack of adult plants producing recruits hinders the natural recovery of degraded areas. Therefore, active restoration is recommended to recover populations within a reasonable time frame. In this study, for the first time, an ex situ pilot restoration of *F. virsoides* was carried out by outplanting cultivated recruits in the Gulf of Trieste. The following aspects were addressed: the efficiency of seedling culture in the laboratory; the effectiveness of an intermediate step in the field in suspended structures; and the subsequent anchoring on rocky substrate. Fertile apical fronds were collected from the donor site and the development of the recruits on clay tiles was followed under controlled conditions. Two different enriched culture media were tested. The recruits were further cultivated on lantern nets at the recipient site until they reached a "refuge size".

The effectiveness of restoration was measured by quantifying juvenile cover, growth and survival at different time points. This study enlarges the biological knowledge of the early life and juvenile stages of *F. virsoides* and draws some practical methodological considerations for implementing restoration measures. It provides new information to optimise best practises in laboratory culture and addresses the first steps of recovery and introduction of juvenile thalli into the natural environment.

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## Sessione Microalghe II

Moderatore: Stefano Accoroni



## Search of freshwater algal specie with adaptive mechanisms to Cr (VI) stress

M. Marieschi, G. Molinari, S. Savi, R. Bolpagni, A. Torelli

Sulfate uptake/assimilation has a pivotal role in enhancing cell defense and on the onset of tolerance mechanisms toward abiotic stresses, in particular to heavy metals, chromium included. This phenomenon, known as SIR/SED (Sulfur Induced Resistance/Sulfur Enhanced Defense) (Rausch and Watcher 2005), relies on a high capability to synthesize cysteine (cys) and reduced glutathione (GSH). These molecules are directly involved in chelating various metal ions, Cr(VI) included, and in the reduction of metal induced oxidative stress. In previous studies we observed that differences in sulfate pathway are involved in different Cr(VI) sensitivity in two strains of *Scenedesmus acutus* (Gorbi et al 2007, Marieschi et al. 2015, Sardella et al 2019, Ferrari et al 2022). To verify if an enhanced cys production could represent a mechanism of environmental adaptation, we tried to isolate microalgae in habitats subject to abiotic stress to be addressed to laboratory research. Algal samples were collected in a chromigen spring at Mount Prinzera, an ophiolitic mountain nearby Parma. The spring water shows Cr(VI) levels above low limits, with an average of 12 Cr(VI) µg/l depending on water flow. The eco-toxicological assay for algal growth (OECD 201/2006) indicate that the water has an inhibitory effect on algal growth. This sampling area thus proved to be an optimal source for the isolation of organisms which could have evolved tolerance mechanisms to Cr(VI). We isolated nearly 60 colonies, among them some of green algae, diatoms and cyanobacteria. Isolation of pure colonies and species identification is still in progress. Some of the tested species shows a Cr(VI) tolerance to concentration 10fold higher that of the spring in which they were isolated, thus constituting a good experimental material for future researches.

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## Interaction between OAS-TL and desulphydrase activity in Cr(VI) tolerance in the freshwater green alga *Scenedesmus acutus*

**G. Molinari, M. Marieschi, R. Bolpagni, A. Torelli**

In plant organisms, sulfur-mediated defense (SED) is crucial for limiting damage caused by heavy metals. Previous works, conducted on two strains of *Scenedesmus acutus*, Wt and chromium-tolerant (CrT), evidenced a transient tolerance increase following a period of sulfur starvation, related to the increase of sulfur uptake and assimilation, in both strains but significantly higher in the CrT strain leading to a higher cysteine content in this latter (Sardella et al. 2019).

Cysteine is synthesized by the enzymatic cysteine synthase complex (CSC), composed by a hexamer of Serine Acetyltransferase (SAT) and two dimers O-acetyl-Serine (Thiol) Lyase (OAS-TL) (Wirtz et al. 2006), present in different cell compartments with different role in cys homeostasis.

OAS-TL activity measured during Cr(VI) exposure preceded by 7-day preculture in standard (S-sufficient) or sulfur-deprived medium (S-replete), was strongly activated in both strains after S-starvation and dropped slowly in S-replete CrT strain exposed to chromium than in controls or in the Cr exposed Wt strain.

The significantly higher cysteine amount observed in the CrT strain during recovery after starvation was hypothesized to involve cytoplasmic rather than chloroplastic CSC complex (Sardella et al. 2019). This hypothesis poses however the question of sulfide production, since sulfite reductase (SIR) have apparent exclusively plastid localization. Extra plastidic sulfide production can be substained by OAS-TLs desulphydrase side activity (Riemenschneider et al. 2005) and from the activity of specific DES enzymes which can contribute to recycling organic sulfur. To verify possible differences between strains we measured L-Cys desulphydrase activity in the same conditions in which OAS-TL activity was assayed. Also in this case a 7 day S-starvation induced the increase of desulphydrase activity in both strain and a significantly slower decrease in the CrT strain exposed to 2 mg Cr(VI)/L. This trend clearly followed that of OAS-TL activity apparently confirming desulphydrase as OAS-TL side activity.

We further isolated a *S. acutus* sequence homologous to Arabidopsis D-Cys DES and analyzed its expression in response to S-starvation to verify if a different expression allowed to hypothesize a different sulfide production in the two strains. In Wt D-Cys DES is expressed at low levels in standard condition and is induced by S-deprivation, whereas in CrT DES levels are significantly higher than in Wt in standard medium and remains nearly stable in S-deprived medium.

A different relationship between D-Cys-DES and OAS-TL side activity in the two strains could underlie their different cysteine homeostasis and Cr(VI) tolerance. D-Cys DES activity remains however to be investigate to get more insight in extraplastidic sulfide production.

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## Effetti di composti fenolici presenti in Acque di Vegetazione sulle microalghe

**L. Mollo, F. Drigo, A. Norici**

La fitodepurazione di acque reflue attraverso microalghe è un processo biotecnologico che, attraverso il metabolismo mixotrofo di alcune specie algali e il loro elevato tasso di crescita, permette di abbassare i costi della coltivazione industriale di microalghe e, al contempo, depurare uno scarto potenzialmente inquinante.

Fra le varie tipologie di acque reflue di potenziale interesse per la coltivazione di microalghe vi sono le Acque di Vegetazione (AV), un sottoprodotto potenzialmente tossico (Sakarita et al., 2020) derivante dall'estrazione dell'olio d'oliva. Queste acque di scarto sono ricche in elementi minerali e materia organica (Komnitsas et al., 2012), ma la presenza di composti fenolici ne limita il loro utilizzo come mezzo di crescita per microalghe.

Le AV possono infatti contenere fino 1-2 g L<sup>-1</sup> (Deeb et al., 2012) di composti fenolici che, se rilasciati in modo incontrollato nell'ambiente, possono causare effetti dannosi per le comunità microbiche del suolo e delle acque (Bouarab-Chibane et al., 2019); una loro rimozione è quindi necessaria. Nel caso in cui si voglia percorrere la via sostenibile della fitodepurazione mediante microalghe, è necessario comprendere appieno l'effetto dei composti fenolici su questi organismi. Ad oggi, gli effetti dei fenoli delle AV sulle microalghe non sono del tutto noti (Lindner & Pleissner, 2019) e studi più approfonditi sono stati svolti solamente per composti fenolici di origine antropica e farmaceutica (Surkatti & Al-Zuhair, 2018).

In quest'ottica 10 diverse microalghe, scelte sulla base della loro origine filogenetica e presenti sia in mare che in acque dolci, sono state esposte a tre fenoli (tirosole, acido cumarico e acido caffeico) tipici delle AV. Sono stati quindi studiati i tassi di crescita e le rimozioni percentuali dei composti fenolici e si è valutata la presenza di risposte comuni alle specie in esame. Come già osservato, le risposte si sono rivelate specie-specifiche; tuttavia, è stata osservata una maggiore tolleranza, in termini di crescita, delle specie d'acqua dolce rispetto a quelle marine. Non è stata riscontrata una correlazione fra crescita e rimozione di composti fenolici, equivalente fra le due categorie, suggerendo quindi l'esistenza di meccanismi passivi di adsorbimento sulla parete cellulare (Wu et al., 2022).

Gli effetti citotossici e ossidativi dei fenoli sono stati inoltre studiati su *Dunaliella salina*, unica microalga marina in esame capace di sopravvivere ai tre composti fenolici: indice di riduzione, DPPH assay e Comet assay sono stati impiegati per studiare gli effetti dei fenoli sullo stato di ossidazione della cellula e a livello di DNA.

Ciò che è emerso è la comparsa di risposte rapide in seguito all'esposizione ai fenoli con successiva acclimatazione e riduzione della risposta nel tempo.

La comprensione degli effetti a livello cellulare dei composti fenolici apre le porte a una fitodepurazione più efficiente e a un utilizzo più conscio delle microalghe in questi processi biotecnologici.

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## Benthic diatom communities on seagrass and corals in Arabian Gulf: floristic analysis, population dynamics and estimate of contribute to the primary production of the basin

**C. Auciello, C. Pennesi, C. Ciniglia, M. Iovinella, L. Rabaoui, M. De Stefano**

Primary productivity generated by phanerogams, macroalgae, phytoplankton and microphytobenthos account for over 40% of entire world productivity (Duarte & Cebrih, 1996). Phytoplankton is considered the main global producer whereas macroalgae, distributed approximately for 1.8% of marine area, exhibit estimated values of primary productivity of  $2.55 \times 10^9$  tC/y (Charpy and Sournia, 1990). Primary productivity attributed to microphytobenthos (with an estimated distribution similar to those attributed to macroalgae) not exceed  $0.34 \times 10^9$  tC/y (Charpy and Sournia, 1990) this demonstrating a strong underestimation of the ecological contribution of microphytobenthic communities if we considering their wide distribution on all organic and inorganic substrates (Fe, 1971). All this is even truer considering that main composer of microphytobenthic communities are generally diatoms (Evrard, et al., 2012), microalgae characterized by a high photosynthetic efficiency and high rate of primary productivity (Falkowski & Raven, 1997). Therefore, we can assume that in order to study the contribution to net primary productivity of microphytobenthos in a basin, we could mainly study the primary productivity of its diatom communities. Our study, entirely based on scanning electron microscopy, aimed to decrypt and document the hidden biodiversity of diatoms associated to seagrasses and hard corals along the Saudi coasts of Arabian Gulf and to estimate their real contribute to the primary productivity of basin. Preliminary results demonstrated that both seagrasses and corals hosted benthic diatom communities who constituted the major element of their epibenthic microalgal flora. Significant differences in terms of cell density, species composition and community structure were observed between the diatom communities of seagrasses and corals and within the different sampling locations. In particular, cell densities of seagrass diatom communities are more than one hundred times higher than those of corals with mean values approximately of 6000-7000 cell/mm<sup>2</sup> in seagrass vs 100-300 cell/mm<sup>2</sup> in corals. Within the community, *Cocconeis* was the dominant genus represented by four species. The abundances are higher in the central stations of the gulf than in the north and south stations, most likely due to the particular environmental conditions that exist in these two areas of the gulf. The estimated primary productivity of the epiphytic diatom communities within the Arabian Gulf account for  $24 \times 10^6$  tC/y.

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## Potential of *Nannochloropsis oceanica* for bioremediation

**M. Blasio, G.V. de Klerk, P. Marotta, A. Cutignano, G. Trancone, G. Dupont, A. Sardo, P. De Luca, S. Papirio, S. Balzano**

Heavy Metals (HMs) are widespread in nature at trace concentrations, nevertheless, anthropogenic activities, including mining, agriculture, and manufacturing, cause an excessive discharge of HMs in the environment. Conventional technologies include chemical precipitation, electrochemical techniques, ion exchange, membrane filtration, coagulation and flocculation. In recent years, HM removal from wastewaters using living or dead organisms, including microalgae, has emerged as a valid alternative to conventional methods (Manikandan et al., 2022). The small size of unicellular organisms, including microalgae, leads to a great surface/volume ratio and a higher availability of HM binding sites on their cell surface compared to multicellular organisms (Cameron et al., 2018). In this study, we investigated the HM removal potential of *Nannochloropsis oceanica* CCMP1779, as well as two mutant strains engineered using CRISPR/Cas9 technology to knock out the polyketide synthase (PKS) gene. PKS is likely to be involved in the biosynthesis of the building blocks of the cell wall polymer algaenan. In detail, we evaluated the ability of *N. oceanica* wild-type and two mutant strains to grow for 10 days in the presence of chromium, copper, lead and zinc at concentrations similar to those observed in polluted hotspots of the Gulf of Naples. At late exponential phase, the biomass was harvested by centrifugation and HM concentrations were determined in both algal pellet and supernatant by inductively coupled plasma mass spectroscopy (ICP-MS). The results indicate that microalgal growth is poorly affected by the presence of HMs reaching a cellular concentration between 3 and 5 x 10<sup>7</sup> cell ml<sup>-1</sup>. In addition, the ICP-MS analysis highlighted an impressive ability of *Nannochloropsis* cultures to remove HMs from the environment. The two mutant strains, K09 and K059, exhibited a higher cellular content of chromium, zinc, and lead compared to the wild-type; the mutant K09 accumulated greater amounts of cadmium and copper compared to the wild type and the mutant K059. A fundamental role in HM removal is covered by functional groups present on microalgal cell wall surface. A putative modification in cell wall chemical composition occurring in mutant strains may result in a higher affinity toward HMs. Current results suggest a great potential of *Nannochloropsis oceanica* for the HM removal from contaminated sea water. In particular, mutants with putative alteration in cell wall chemistry exhibited a higher performance compared to the wild-type.

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## A Photo Guide of Harmful Algae along Coastal areas of Apulian Region

**S. D'Arpa, M.R. Vadrucci, P.C. Ciciriello, E. Stanca A. Pastorelli, N. Ungaro**

Harmful Algae Blooms (HABs) can cause serious negative impacts on the economy, fishery/seafood industry, environment, and even recreational activities. It should be stressed that some of these agents produce toxins that can cause human illness and even death. In particular, the presence of HABs species represent a great threat for bivalve aquaculture. The impact of their blooms is directly related to the harmfulness of the shellfish toxins they produce. Filter-feeding bivalves accumulate microalgal toxins, which can reach concentrations (regulatory levels) unsafe for human consumption and thus health and fisheries authorities have to implement management measures, including the closure of shellfish harvesting areas. The technical guidance for the application of EU Regulation identified the criteria for defining monitoring plans for the quality control of bivalve for human consumption and includes, among the parameters to be monitored, the presence of toxin-producer phytoplankton in the area of shellfish harvesting.

In the Apulia region a monitoring programme finalized to the assessment of the quality of marine water for bivalve aquaculture started at the beginning of 2021 (according to D.G.R n. 2014/2019).

The monitoring plans included the analysis of phytoplankton in a number of 40 sampling points among shellfish farms, relaying areas and natural beds of bivalves with a biweekly frequency.

Previous studies on HABs dynamics along the Apulian coasts and transitional waters showed the presence of phytoplankton toxin (ASP and DSP producers) and high-biomass producer (Roselli et al., submitted). Therefore, on the basis of previous knowledge and the new phytoplankton data collected, a photo guide of HABs species occurred in Apulia marine coastal areas (both toxin and high biomass producers) was developed with the following purposes:

- to support the technical staff of regional environmental agencies or students, as well as stakeholders and environmental managers in identifying, where possible, of phytoplankton species that may be harmful to human health or to productivity of the shellfish harvesting areas.
- to provide a practical approach which might facilitate the identification of phytoplankton organisms through morphological structures and characteristics visible mainly through optical light microscopy techniques (Bright field phase contrast, DIC and Epifluorescence)
- to provide an identification key of some genera that include HABs (Alexandrium, Dinophysis, Pseudo-nitzschia etc.) through a general schematic outlining of their major features.

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## Sessione Microalghe III

Moderatore: Marina Montresor



## Valutazione dell'effetto inibitorio di materiali legnosi modificati o trattati con impregnanti di nuova generazione sullo sviluppo della comunità microfitobentonica nella laguna di Grado (nord Adriatico)

**V. Natali, T. Cibic**

Qualsiasi substrato solido immerso in acqua marina o lagunare viene colonizzato dal biofilm microbico formato principalmente da batteri e diatomee. Per valutare come la formazione del biofilm risponda ad un possibile effetto inibitorio prodotto da materiali legnosi modificati o trattati con impregnanti, è stato condotto uno studio sperimentale nell'ambito del progetto DuraSoft finanziato dal programma interregionale Italia-Slovenia. A questo fine, sono stati preparati provini di abete bianco e abete rosso naturali per confrontarli con quelli opportunamente trattati con impregnanti di nuova formulazione (Silvanolin, rame etanoloammia), con prodotti già presenti sul mercato (CCB, rame-cromo-boro) o modificati termicamente per aumentarne la durezza. I provini di legno sono stati montati sui collettori e tenuti in acqua sempre alla stessa altezza (50 cm dal pelo dell'acqua, a prescindere dall'escursione di marea) mediante un sistema di boe di ancoraggio. In totale sono stati allestiti 24 collettori e posizionati in due siti della laguna di Grado in giugno 2021. I primi 12 sono stati posizionati nell'area "Schiusa" caratterizzata da elevato idrodinamismo, mentre i rimanenti 12 sono stati posizionati nell'area "Approdo" caratterizzata da condizioni idrodinamiche relativamente tranquille. In totale, sono stati immersi 144 provini, 72 per ciascuna area, 12 per ciascun tempo sperimentale, e recuperati dopo 6, 20 e 40 giorni per testare gli effetti a breve, medio e lungo periodo sull'abbondanza e composizione della comunità microalgale mediante diverse tecniche microscopiche (microscopia ottica, SEM, microscopia confocale a scansione laser). I risultati hanno evidenziato l'importanza dell'idrodinamismo dell'area: il minor idrodinamismo ha accelerato il processo di colonizzazione del biofilm microalgale, a prescindere dalla tipologia di trattamento applicato, con abbondanze cellulari fino a un ordine di grandezza maggiori. In condizioni di elevato idrodinamismo solo i trattamenti con Silvanolin hanno mantenuto l'efficacia inibente per tutta la durata dell'esperimento, invece l'efficacia degli altri trattamenti nell'inibire lo sviluppo del biofilm è scemata nel medio termine, dopo 20 giorni, o per effetto del dilavamento dei metalli pesanti oppure per il rilascio di composti nutritivi presenti negli impregnanti che hanno favorito la proliferazione microalgale. Focalizzandosi sul l'effetto inibente dei diversi trattamenti, i generi *Nitzschia* e *Amphora* sono risultati più tolleranti, presentando abbondanze maggiori nei trattamenti rispetto al legno naturale. Altre sono risultate sensibili, scomparendo del tutto nei trattamenti con gli impregnanti, come il genere *Achnanthes*. In generale, gli impregnanti testati hanno indotto una serie di modificazioni alla comunità microalgale, rispetto al controllo sono state osservate diatomee di dimensioni inferiori, con frustuli malformati e strategie di vita che permettono di contrastare l'effetto tossico indotto dalle sostanze contenute negli impregnanti.

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## Phytoplankton indicators for GES assessment: a case study in the N Adriatic Sea

**F. Neri, M. Ubaldi, T. Romagnoli, S. Accoroni, C. Totti**

Phytoplankton is considered as an optimal proxy to detect changes in the marine ecosystems, due to the fast turnover and the high sensitiveness to environmental conditions. For these reasons it is included as a descriptor for the assessment of the Good Environmental Status (GES) of pelagic habitats in the Marine Strategy Framework Directive, particularly at local scale. Effort must be done to test phytoplankton indicators at regional scale.

The aim of the study was to test several indicators using phytoplankton abundance data collected from 2008 to 2020 in 3 coastal stations of N Adriatic (Api, Senigallia and Portonovo).

Well-known indicators (i.e. Shannon diversity index and Pielou's evenness) were combined with newly ones (e.g. Rao index), to compare their efficiency. Multivariate statistical approaches (e.g. Non-Metric Multidimensional Scaling and clustering techniques) were used to look for patterns along the Mediterranean coasts. For each station, temporal changes were studied using the Local Contributions to Beta Diversity index (LCBD; a beta diversity measure) and the Importance Value Index (IVI) was calculated, revealing the taxa (genus) potentially responsible for the modification in the community structure.

This study will give information on the applicability of many indicators in the environmental assessment on a regional scale.

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## Espressione genica durante la formazione di spore nella diatomea marina *Chaetoceros socialis*

A. Pelusi, L. Ambrosino, M. Miralto, M.L. Chiusano, A. Rogato, M.I. Ferrante, **M. Montresor**

Molti organismi multicellulari ed unicellulari sono caratterizzati da periodi di quiescenza o dormienza durante i quali le attività metaboliche sono notevolmente ridotte. Tra le diatomee, microalghe unicellulari alla base delle reti trofiche acquatiche, diverse specie producono cellule dormienti (spore o 'resting cells') che possono sopravvivere nei sedimenti per lunghi periodi di tempo. Presenteremo i risultati di un primo studio sull'espressione genica durante il processo di formazione di spore in *Chaetoceros socialis*, una specie planctonica estremamente abbondante nei mari temperati. La formazione di spore è stata indotta da limitazione di azoto e l'esperimento di trascrittomico è stato condotto in condizioni di controllo (azoto non limitante) ed in tre momenti diversi durante la transizione da cellule vegetative a spore. Abbiamo rilevato *downregulation* di geni correlati alla fotosintesi e all'assimilazione dei nitrati, inclusi trasportatori di nitrati ad alta affinità (NTR). L'*upregulation* di altre vie cataboliche, come il ciclo dell'acido tricarbossilico, il ciclo del gliossilato e la beta-ossidazione, suggerisce che, durante il processo di formazione delle spore, questa diatomea potrebbe utilizzare i lipidi come fonte di energia. E' stata inoltre rilevata l'*upregulation* di una lipossigenasi e diverse aldeide deidrogenasi (ALDH) che supportano la presenza di ossilipine, metaboliti secondari con ruolo di mediatori chimici a livello intra-specifico. L'*upregulation* di geni coinvolti nella regolazione della dormienza in altri organismi (ad es. serina/treonina-protein chinasi TOR e il suo inibitore GATOR) rappresentano risultati interessanti che aprono la via a studi futuri. I risultati del nostro studio dimostrano che la transizione da una fase di crescita attiva ad una fase di dormienza è caratterizzata da marcati cambiamenti metabolici e forniscono evidenza della presenza di mediatori chimici che regolano la comunicazione fra le diverse cellule della popolazione.

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## Trait-based approach for rapid and effective characterization of phytoplankton patterns

**L. Roselli, S. Bevilacqua, A. Terlizzi**

Phytoplankton form the base of most aquatic food webs, accounting for approximately half of Earth's primary productivity. Also, such tiny microscopic organisms profoundly affect the biogeochemical cycling of many elements, such as carbon, nitrogen, and phosphorus. Phytoplankton determine the health of aquatic ecosystems, and have the potential, with harmful blooms, to reduce water quality and fishing yield. Because of the role that phytoplankton exert on the functioning of aquatic ecosystems, it is important to understand which factors govern the community assembly and dynamics. Human-induced pressures can alter phytoplankton community structure and, therefore, the need to understand the community reorganizations in the changing environment becomes even more pressing in the light of climate global change. For these reasons, phytoplankton assemblages are privileged descriptors of the ecological status of marine ecosystems and are regularly included in routine monitoring plans. Phytoplankton cell size and shape are key traits profoundly influence an organism's ecophysiological performance and evolutionary fitness, suggesting a link between morphology and diversity.

Trait-based approaches are increasingly used in phytoplankton ecology to explain and predict community organization along environmental gradients including human-induced ones. The high spatial and temporal variability of phytoplankton and the intrinsic difficulties of species identifications, however, combine in making reiterate assessments of this component of marine biota particularly demanding. Although with contrasting outcomes, coarse levels of taxonomic resolution (e.g., genus, family) or morpho-functional categories have been proposed to reduce identification efforts or to ease the analysis of phytoplankton assemblages for monitoring purposes. A major issue is that, in the absence of control for the loss of information associated to these alternative approaches, their application may lead to poor representations of genuine spatial and/or temporal patterns of assemblages in relation to natural and anthropogenic sources of variation. A new approach is proposed to reduce the efforts required to analyse phytoplankton assemblages that integrate morpho-functional classification of phytoplankton with the use of null models to estimate the consequent loss of information on species-level community patterns. Null models for information loss are built by randomly grouping the original species variables into a progressively decreasing number of groups, in order to identify the minimum number of aggregate variables needed to detect community patterns as at species level. Aggregate variables are then defined as morpho-functional groups, by grouping species on the basis of a combination of morpho-functional traits, including general taxonomy, cell size, shape, elongation and complexity. The approach has been applied to several case studies investigating the response of phytoplankton assemblages in marine and transitional water ecosystems under different environmental settings in areas spanning the world's ocean, including coral atolls, mangroves, estuaries, coastal lagoons and inlets. The approach allowed obtaining parsimonious sets of morpho-functional groups, which were suitable to detect changes in phytoplankton assemblage structure as at species level in all case studies. Trait-based approaches to phytoplankton research and monitoring are crucial to shed light on processes underlying phytoplankton community assembly and dynamics in the face of global change. In this perspective, our framework incorporates cost-effectiveness, instances from traditional monitoring programs aiming at the detection of community patterns, and the current need for a deeper understanding of functional responses of phytoplankton to environmental drivers.

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## Sessione Microalghe IV

Moderatore: Laura Pezolesi



## Dati preliminari su una microalga verde isolata dal Lido di Spina (Comacchio, Ferrara)

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Con il termine microalghe si intende un vasto gruppo di microrganismi fotosintetici appartenenti a taxa distinti, in grado di colonizzare svitati ambienti, anche quelli considerati estremi. Questi organismi possono, infatti, mettere in atto diversi adattamenti in risposta alle condizioni in cui si trovano e presentano, generalmente, un'elevata plasticità metabolica che gli permette di far fronte a cambiamenti anche repentini dell'ambiente circostante (Malcata *et al.*, 2018). Molte delle molecole prodotte dalle microalghe trovano impiego in vari ambiti di interesse umano, ad es. il settore agricolo, il cosmeceutico, il nutraceutico e della green energy (e.g., Levasseur *et al.*, 2020; Alvarez *et al.*, 2021; Kiran & Venkata Mohan, 2021), con il vantaggio che le sostanze prodotte dalle microalghe hanno generalmente nessuno o scarso impatto ambientale rispetto alle loro controparti di sintesi chimica. Inoltre, la produzione di microalghe richiede, solitamente, un limitato consumo di risorse e può essere condotta anche sfruttando sostanze di scarto (e.g. Shahid *et al.*, 2020).

In questo contesto è stato recentemente co-finanziato dal Fondo Sociale Europeo REACT EU – Programma Operativo Nazionale Ricerca e Innovazione 2014-2020, a titolarità del Ministero dell'Università e della Ricerca, un progetto dal titolo "Potenzialità biotecnologiche delle microalghe per la sostenibilità ambientale", il cui scopo è analizzare diversi ceppi microalgali, caratterizzarli e valutarne la crescita in laboratorio, per poterli impiegare in diversi settori di interesse antropico. All'interno del progetto si considereranno sia ceppi microalgali già presenti presso collezioni pubbliche o private, che ceppi isolati ex-novo dall'ambiente, con particolare attenzione all'ambiente marino.

Sono stati, quindi, effettuati campionamenti nella zona del Lido di Spina, Comacchio (FE), andando a prelevare campioni d'acqua sia lungo la costa che da bacini d'acqua interni. Una volta in laboratorio, i campioni d'acqua sono stati processati e utilizzati per l'isolamento di diversi ceppi microalgali. Tra i ceppi isolati è risultata particolarmente interessante una microalga verde coccoide, temporaneamente denominata ceppo KS2, presente in un campione d'acqua prelevato da uno dei bacini interni, della cui caratterizzazione verranno qui presentati i primi dati. La microalga è stata sottoposta ad un approccio di tipo polifasico, comprendente analisi molecolari basate sul gene 18S rRNA e sullo spaziatore intergenico trascritto 18S-28S ITS rRNA, analisi morfologiche ed ultrastrutturali, mediante microscopia ottica ed elettronica (SEM e TEM), e analisi biochimiche (es. pigmenti, polisaccaridi, proteine), nonché la valutazione della crescita del microorganismo in condizioni di laboratorio.

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## Effect of salinity on growth and microcystin production by the cyanobacterium *Plankthothrix rubescens*

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The cyanobacterium *Plankthothrix rubescens* is a common producer of cyanotoxins (mainly microcystins), which forms blooms in freshwaters. During algal blooms, microcystins can reach the sea and can be accumulated in bivalve molluscs with risks for consumption. In northern coast of Puglia (near the Lesina and Varano lakes), were found mussels that accumulated microcystins produced by *P. rubescens* at concentrations higher than tolerable daily intake (De Pace et al., 2014). In this study we tested the tolerance and microcystin production of *P. rubescens* to increasing salinity. One strain of *P. rubescens* was isolated from the lake of Castreccioni (Cingoli, MC) and exposed for one month to different salinities (0, 10, 20, 30) in batch cultures. Cell abundances and growth rate were obtained by optical density (OD650 e OD750) at the spectrophotometer and by counts through light microscope. Both methods showed that *P. rubescens* is unable to survive for more than 96 hours at salinity equal to or greater than 10. Anyway, the inhibition is not immediate, as for the first 4 days the abundances remained similar to the control. This implies that once the cells have reached the coasts, they remain vital enough to allow filter-feeding organisms to accumulate cells and toxins in their tissues. At the IZSUM, toxins were quantified in the cultures both in intracellular compartment and in culture medium (dissolved form) using LC-MS/MS. The most found microcystin was MC-RR desmethylated. Higher intracellular concentrations of the microcystins were observed at lower salinities (0 and 10), while the amount of the dissolved form was higher at salinities 20 and 30. Our results suggest that salinity stress could cause plasmolysis and release of toxins as already found by Rosen et al. (2018) for cyanobacteria. Monitoring of cyanotoxins in sea water and in bivalve molluscs is important for the public health.

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## Moschin Potential of a green algal strain collected from the Sarno River mouth for heavy metal bioremediation

**L. Barra, S. Balzano, M. Moros, A. Smerilli, E. Cavalletti, I. Percopo, P. Chiaiese, A. Sardo**

Heavy Metals (HMs) can induce both chronic and acute harmful effects on marine and freshwater biota and their presence in soils, sediments, and waters has been well documented (Yabanli et al. 2015). In this context, eukaryotic microalgae are actually considered as suitable microorganisms for bioremediation, since they (1) do not require organic substrates neither clean water to achieve fast growth, (2) they are able to uptake HMs and other pollutants from different environments, including wastewaters and contaminated sediments, and (3) they compartmentalize pollutants in vacuoles or other organelles minimizing their detrimental effects (Balzano et al., 2020). The Sarno River is a 30 km long freshwater stream located in Southern Italy that flows into the Gulf of Naples. Partially untreated industrial wastewaters, deriving from the tanning and canning industries, have been discharged directly to Sarno River or its tributaries for several decades. This led to significant pollution in hydrocarbons and HMs along the river as well as in the seawater basin in proximity of its mouth (Montuori et al., 2013). We aimed at determining the ability of a green microalgal strain, isolated from Sarno River Mouth, *Tetraselmis convolutae* strain BS14, to grow in artificially polluted seawater and to accumulate selected HMs within its biomass. We performed the molecular characterization of the species, the inhibition growth rate, and the analysis of Ion Coupled Plasma-Mass Spectrometry (ICP-MS) in presence of Cadmium (Cd), Lead (Pb) and Zinc (Zn). The metals were analyzed one at time, in order to investigate the potentiality of this green algal strain in the phyco-remediation process for future biotechnological application. *T. convolutae* BS14 performed a reasonably fast growth in the presence of cadmium (up to 50  $\mu\text{M}$ ), zinc (20  $\mu\text{M}$ ), and lead (50  $\mu\text{M}$ ) for seven days. Cell surface area slightly increased in the presence of HMs and cells were found to incorporate part of the HMs. The present study indicates the potential of *T. convolutae* BS14 for the bioremediation of HM contaminated waters.

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## Investigation of potentially toxic metabolites in a newly isolated freshwater cyanobacterium of the family Pseudanabaenaceae through a genomic approach

**M. Simonazzi, L. Pezzolesi, T.K. Shishido, D.P. Fewer, R. Pistocchi**

In the past decades, the proliferation of cyanobacteria in freshwater ecosystems has intensified, possibly as a consequence of climate changes and eutrophication. The ever-increasing presence of these phototrophic organisms in water bodies for human use (e.g. drinking water, recreational activities) is considered as an emerging health threat, since some cyanobacteria are able to produce harmful toxins, named after them cyanotoxins. So far, the discovery of cyanotoxins has been investigated after a human or animal intoxication event occurred, through a combination of chemical and biological analyses carried out on bloom samples or cultured organisms isolated from their natural environment. In recent years, the advances in genome sequencing and analysis have broadened our understanding and knowledge of prokaryotes such as cyanobacteria by in-depth investigations at genome level, thus expanding the potential for the discovery of unknown metabolites, including new toxic compounds.

In the present work, a cyanobacterium belonging to the family of Pseudanabaenaceae was isolated from a small lake that provides water for the local drinking water plant (Rimini, Emilia-Romagna, Italy). Aqueous cyanobacterial extracts were tested by *Artemia* sp. bioassay, showing high mortality rates of the individuals within 24h (98-100%). Chemical analyses (LC-MS) on methanolic extracts, and PCR amplification of genes involved in the synthesis of various cyanotoxins did not give any results, thus the production of known cyanotoxins in the strain was excluded. The genome of the strain was then sequenced by Illumina Miseq, obtaining a completeness of 99.64%. Genome analyses confirmed the absence of known cyanotoxins gene clusters, while revealed the presence of a region responsible for the synthesis of polyketides (i.e. trans-AT PKS), a highly diverse class of compounds both in terms of structure (i.e. alkaloid, macrolides, polyethers, aromatics) and biological activities (i.e. antibiotic, anticancer, antifungal), some of which considered involved in toxicity. The end product of this region has not been detected yet, and further analyses are needed to elucidate its structure and potential toxicological characteristics. Since the toxic effects were observed in aqueous extracts of the cyanobacterium, potential implication for the safety of drinking waters should be taken into consideration.

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## Organelle Phylogenomics unveil the diverging evolution of 6 Lineages in *Galdieria sulphuraria* (Cyanidiophyceae).

**M. Iovinella, C. Ciniglia, S.J. Davis**

Exploration of life in extreme environments allows the discovery of intriguing organisms with extraordinary biotechnological potential. An example of extreme environments is represented by hot springs, where harsh conditions (pH < 1; Temperature > 50°C; high concentrations of metals) are prohibitive for most living organisms, except for archaea, bacteria and a few eukaryotes like the unicellular red alga *Galdieria* (Iovinella et al., 2018). Phylogenetic analysis based on a few plastids and nuclear genes highlighted the intricate genetic structure of *Galdieria* and the hypothesis of diverging clades within the *G. sulphuraria* species (Toplin et al., 2008, Hsieh et al., 2015). To resolve enigmatic relationships between lineages, we used plastid and mitochondrial genome-scale data obtained from numerous strains from around the world. The resulting phylogenomic analysis identified: i) the divergence of each of the mitochondrial and plastid genomes into the same six clear lineages; ii) the independent evolution of the lineages; iii) the incongruent interlineages relationships between the genomes. Differential evolutionary pressure between the strains and the genomes were also highlighted by synonymous and non-synonymous substitutions.

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## Sessione Microalghe V

Moderatore: Chiara Pennesi



## ***Halomiconema metazoicum*, la storia di un cianobatterio, del suo ambiente e dei suoi vicini**

**M. Mutalipassi, V. Mazzella, N. Ruocco, V. Zupo**

I cianobatteri svolgono ruoli ecologici chiave in numerosi ambienti e mostrano un ampio adattamento ad innumerevoli differenti habitat. Molte specie producono molecole bioattive via secrezione costitutiva, in altri casi, alcune molecole prodotte sono invece metaboliti secondari attivati dal grazing (solitamente Wound-Activated Compounds). Sia le molecole costitutivamente espresse che quelle Wound-activated possono avere anche funzione di messaggio informativo di tipo chimico (infochimico). I nostri studi si sono focalizzati su un particolare cianobatterio del gruppo *Phormidium*-like, *Halomiconema metazoicum* C.Caroppo, P.Pagliara & P.Albertano, 2012, che possiede caratteristiche interessanti da un punto di vista ecologico e biotecnologico. E' stato identificato per la prima volta in simbiosi con la spugna *Petrosia ficiformis* (Poiret, 1789), con probabili ruoli antigrazing e azotofissazione (Pagliara et al., 2020). Nel 2016 è stato da noi isolato come colonia non associata a spugne e ritrovato come epifita di foglie di *Posidonia oceanica* (L.) Delile, 1813 (Ruocco et al., 2018). *Halomiconema metazoicum* è stato inoltre isolato nella colonna d'acqua testimoniando il comportamento quantomeno meroplanctonico della specie, suggerendo inoltre ruoli ecologici complessi in questi ambienti. Test comportamentali su alcuni invertebrati di fauna vagile hanno evidenziato come le specie campionate dalla prateria evitassero le aree arricchite di bouquet odorosi prodotti da *H. metazoicum*, riconoscendo gli infochimici del cianobatterio e la sua aggressività chimica (Zupo et al., 2016). Alcune evidenze suggeriscono come i bloom bentonici e planctonici possano essere dettati da fenomeni di allelopatia dettati da infochimici e quindi da comunicazione tra specie (Mutalipassi et al., 2021). Campionamenti hanno evidenziato come la presenza di alcuni cianobatteri meroplanctonici nella colonna d'acqua o nel benthos siano inversamente correlati con presenza o assenza di altri organismi, come *Ostreopsis ovata* Y.Fukuyo 1981, negli stessi compartimenti. Test da noi effettuati, a sostegno di questa tesi, dimostrano come molecole bioattive prodotte in certe condizioni dal cianobatterio provochino l'incistamento immediato di *O. ovata* senza però causarne la morte. Tali molecole hanno mostrato elevata tossicità su plutei di *Arbacia lixula* (Linnaeus, 1758) e *Paracentrotus lividus* (Lamarck, 1816), nonché su vari stadi di *Brachionus plicatilis* (Müller, 1786) (Mutalipassi et al., 2019, 2022). I mezzi di coltura sono stati anche testati su copepodi parassiti nonché su alcune linee cellulari (tumoral e non) umane, al fine di valutare il potenziale biotecnologico delle molecole costitutivamente prodotte. In conclusione, gli studi condotti dimostrano da un lato l'enorme ruolo svolto dai cianobatteri nelle comunità litorali, con un'ampia diversità di processi biochimici attivi e una vasta gamma di interazioni con altre specie; dall'altro lato dimostrano come, nonostante la lunga tradizione di screening e separazione di molecole bioattive, la biodiversità chimica e di specie dei cianobatteri è tuttora sottostimata, suggerendo grandi prospettive future per la drug discovery.

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## Induzione di stadi di resistenza nella diatomea marina *Thalassiosira rotula*

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Molte specie fitoplanctoniche, ed in particolare le diatomee, adottano la strategia di formare stadi di resistenza in risposta a condizioni sfavorevoli in termini di nutrienti, luce e temperatura, consentendo la sopravvivenza delle cellule e contribuendo al loro successo ecologico (McQuoid e Hobson, 1996). Si tratta infatti di uno stato reversibile dal quale le cellule sono in grado di riprendere la crescita vegetativa quando i fattori ambientali diventano nuovamente favorevoli. I meccanismi che sottendono la formazione di stadi di resistenza possono differire all'interno di uno stesso gruppo di organismi e anche nell'ambito dello stesso genere o specie; quindi, è necessaria una precisa descrizione di tali meccanismi a livello di specie. Abbiamo studiato l'induzione di forme di resistenza nella diatomea centrica *Thalassiosira rotula*, valutando le condizioni che ne inducono la formazione e la germinazione, con lo scopo finale di indentificarne i metaboliti responsabili con possibile interesse per scopi biotecnologici. *T. rotula* è una specie cosmopolita costiera, presente nel Golfo di Napoli, che spesso domina la comunità del fitoplancton per le sue grandi dimensioni e l'elevata concentrazione. Abbiamo stabilito le condizioni per indurre le cellule di *T. rotula* ad entrare in uno stato di dormienza valutando diverse concentrazioni di nutrienti (azoto, silice e ferro), di intensità luminosa, densità cellulare e livelli di ossigenazione. Una bassa disponibilità di azoto, una bassa densità cellulare iniziale e un buon livello di ossigenazione sono risultati i principali fattori che determinano l'induzione dello stato di dormienza in questa specie di diatomee. La germinazione è stata testata dopo diversi tempi di conservazione, dimostrando che queste cellule dormienti hanno una capacità di resistenza almeno fino a due mesi. L'osservazione al microscopio ottico e a fluorescenza ha mostrato che le forme di resistenza di *T. rotula* sono morfologicamente simili a quelle vegetative ma con un frustulo più spesso, un citoplasma condensato e addossato alle pareti e un livello di pigmentazione inferiore.

Questo studio preliminare fornisce la base per successivi studi volti ad identificare i meccanismi molecolari coinvolti nella formazione di stadi di resistenza in questa specie. A questo scopo, intendiamo valutare l'espressione differenziale dei geni coinvolti tramite analisi trascrittomica comparativa. Verrà inoltre studiato il possibile coinvolgimento di modificazioni epigenetiche nella transizione tra i due stadi.

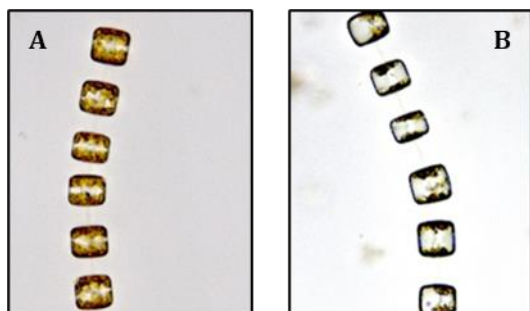


Figura 1 Immagini della diatomea *Thalassiosira rotula* in stato vegetative (A) and in stadio di resistenza (B) ottenute al microscopio ottico.

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Di Costanzo F., Di Dato V., van Zyl L.J., Cutignano A., Esposito F., Trindade M., Romano G. (2021). Three novel bacteria associated with two centric diatom species from the Mediterranean Sea, *Thalassiosira rotula* and *Skeletonema marinoi*. International Journal of Molecular Science, 22(24): 13199.

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## Production of the biopolymer polyhydroxybutyrate (PHB) from the Chlorophyceae *Desmodesmus communis*

**G. Zoffoli, C. Samorì, M. Simonazzi, R. Pistocchi, L. Pezzolesi**

The sustainability of plastic materials is a major focus of basic research, industry, government, and society to find substitutes to petroleum-based and non-biodegradable conventional plastics. Polyhydroxyalkanoates (PHAs) are promising alternatives that accumulate as energy and carbon storage material in various microorganisms, including bacteria and microalgae, being biodegradable and suitable for a wide variety of applications. The most prevalent and well-characterized biopolymer is the polyhydroxybutyrate (PHB), which belongs to the short-chain PHAs.

The present study was designed to evaluate algae-based PHB production using the Chlorophyceae *Desmodesmus communis* in two-phase nutritional mode of cultivation, namely a phototrophic growth phase (PGP) and a mixotrophic stress phase (MSP). Microalgal PHB accumulation was in fact driven by phosphate constraint, and organic carbon (i.e. sodium acetate) supply.

The highest PHB productivity (0.11 g PHB/g biomass/d; 0.015 g PHB/L/d), corresponding to 32.1% w/w of intracellular PHB, was observed for *D. communis* after 3 days of cultivation under mixotrophic conditions (e.g., low light, phosphorus-free medium, 1 g/L of sodium acetate). A scaled-up cultivation (10 L) was set up to evaluate the PHB yield in a semi-continuous system in relation to the biomass main composition. After 10 days of cultivation, a content of 37.9% w/w of proteins, 24.1% w/w of polysaccharides, 23.1% w/w of PHB, and 9.0% w/w of lipids was reached. As bacteria are traditionally the principal PHB producers, preliminary tests performed with axenic cultures were performed to understand the contribution of *D. communis* associated microbiota to the PHB production. Results highlighted as the achievement of a biomass dry weight of about 0.2-0.3 g/L is necessary to optimize PHB production from *D. communis* cells, resulting in a productivity up to 2- times higher than those reported for cyanobacterial species (Simonazzi et al., 2021) and comparable to the maximum value obtained for other Scenedesmaceae (García et al., 2020; Kumari et al., 2022). In addition, considering *D. communis* ability to bioremediate wastewaters, its exploitation in a circular economy perspective could be promising. Further investigations should be addressed to better understand the physiological mechanisms that allow this species to produce PHB, considering the importance of the associated microbiota.

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