Riunione Scientifica Annuale

Gruppo di Lavoro per l'Algologia

Società Botanica Italiana

Napoli, 27 - 28 ottobre 2023

Stazione Zoologica Anton Dohrn

Museo Darwin Dohrn (DaDoM)

Società Botanica Italiana
Gruppo di Algologia
Riunione Scientifica Annuale
Napoli, 27-28 ottobre 2023
Stazione Zoologica Anton Dohrn – Museo Darwin Dohrn, DaDoM

Comitato organizzatore:
Leonilde Roselli

Comitato Scientifico: Rossella Pistocchi, Anna Maria Mannino, Simona Armeli Minicanti, Giuseppina Alongi, Stefano Accoroni, Leonilde Roselli

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Si ringrazia:
Nikon Europe B.V. per aver patrocinato l'evento.

Riunione Scientifica Annuale
27-28 ottobre 2023, Napoli

PROGRAMMA
Venerdì, 27 ottobre 2023

8.30 Inizio registrazione dei partecipanti

9.15 Saluti di benvenuto e introduzione ai lavori

9:45 Relazione ad invito:

Prof. Charles-François Boudouresque (Institut Méditerranéen d'Océanologie, Marseille)

Seaweed: a modern and sassy look

I Sessione (10.30 – 11.00) Moderatrice: L. Roselli

S. Frasca, C. Conte, A. Alabiso, E. Apostolaki, L. Migliore
Contribution of microbial community to the long-term persistence of both alive and dead *Posidonia oceanica* 'matte'

M. Francavilla
Mediterranean Seaweed biorefinery for valuable compounds

11.00 - 11.30 Coffee break

II Sessione (11.30 – 12.30) Moderatrice: L. Pezzolesi

R. Trentin, E. Moschin, L. Custódio and I. Moro
Impact of Temperature on *Microglena antarctica* (Chlorophyceae): Insights into Growth, Metabolome, Lipid Profiles, and Photosynthetic Pigments

M. Minio, C. Gerotto
Effects of salinity and sulphur limitation on photosynthesis and cell composition in *Dunaliella salina*

M. Iovinella, C. Auciello, S.J. Davis, Ciniglia C.
Biosorption of Rare Earth Elements from electronic waste by *G. sulphuraria* (Cyanidiophytina, Rhodophyta)

J. Fabrello, V. Matozzo, E. Moschin, F. Dalla Vecchia, I. Moro
Effects of BPA analogs on the microalga *Phaeodactylum tricornutum* Bohlin

M.L. Madeo, P. Chaerle, O. Chepurnova, W. Vyverman, M. Montresor, M. Ferrari, T. Greca, L. Bruno, P. G. Romano, R. Cozza
Exploring cryopreservation techniques for the long-term storage of *Chaetoceros socialis* at different life cycle stages

12.30 – 14.00 Pausa pranzo

III Sessione (14.00 – 16.00) Moderatori: K. Sciuto – D. D'Alelio

A. Sfriso, A. Buosi, Y. Tomio, M. A. Wolf, A.-S. Juhmani , K. Sciuto, A.A. Sfriso
Impact of environmental parameters on macrophyte changes in TWS soft bottoms: The Venice Lagoon as study case.

V. Malavasi, N. La Rocca, N. Beltran-Sanz, S. Stocco, A. Bortolato, A. López-Chicheri, F. Maestre, F. Dal Grande
Dryland biobanking: preliminary data on the biodiversity of green algae in deserts worldwide

C. Micheli
Molteplici funzioni di *Chaetomorpha linum* (Chlorophyta, Cladophorales), un'alga poliedrica e cosmopolita.

C. Caroppo, A. Bergamasco, G. Caruso, F. Decembrini
Idrodinamismo e composizione specifica delle comunità fitoplanctoniche del Mar Adriatico meridionale

F. Neri, M. Ubaldi, T. Romagnoli, C. Totti, S. Accoroni
Interannual trend of *Ostreopsis* cf. *ovata* bloom in the Conero Riviera (northern Adriatic Sea)

A. Buosi, G. Silan, Y. Tomio, M.A. Wolf, A.A. Sfriso, A. Sfriso
Progetto Life Transfer, come strumento per il ripristino ambientale

A.A. Sfriso, K. Sciuto, M. Mistri, C. Munari, A-S. Jhumani, A. Buosi, Y. Tomio, A. Sfriso
Defining suitability of transplant sites in seagrass restoration

G. Bellanti, S. Bianchelli, F. Rindi
Can epiphytic vegetation be used as an indicator of the recovery state of *Cystoseira s.l.* forests? A study carried out along the Conero Riviera (N Adriatic).

G. Denti, A. Petrocelli, E. Cecere, F. Rubino, G. Fanelli, C. Richiardi, M. P. Adamo
Preliminary results of *Cymodocea nodosa* (Tracheophyta, Alismatales) monitoring in the Mar Piccolo of Taranto through in situ data and satellite images

16.00 – 16.30 Coffee break

IV Sessione (16.30 – 18.00) Moderatore: S. Savio

R. Ranaldi, L. Rugnini, F. Gabriele, C. Casieri, A. Canini, N. Spreti
Stones bioreceptivity: cyanobacterial biofilm growth and its removal with biocides encapsulated into alginate hydrogel

L. Rugnini, R. Ranaldi, F. Gabriele, C. Casieri, N. Spreti
From laboratory to in-situ application of alginate hydrogel encapsulating biocides on cultural heritage affected by biofilm growth

F. Di Costanzo, V. Di Dato, G. Romano
Approcci omici per l'identificazione di vie biosintetiche per composti bioattivi nella diatomea *Thalassiosira rotula*

M. Franchini, G. Xamin, M. Simonazzi, C. Samorì, R. Pistocchi, L. Pezzolesi
Investigations on the players involved in the production of polyhydroxybutyrate (PHB) by the Chlorophyta *Desmodesmus* communis under a mixotrophic cultivation regime

S. Savio, S. La Frazia, C. Rodolfo, R. Congestri
Anti-viral and anti-cancer potential of small metabolites from the mass cultivated diatoms *Staurosirella pinnata* and *Cylindrotheca closterium*

K. Sciuto, A.A. Sfriso, M.A. Wolf, A. Sfriso, E. Moschin, C. Munari, M. Mistri, I. Moro
Preliminary characterization of two *Spirulina* strains from the North Adriatic Sea (Italy)

M. Ferrari, R. Cozza, M. Marieschi, R. Ruotolo, A. Torelli
The role of Phytochelatin Synthase in the microalga *Scenedesmus acutus* M. (Sphaeropleales)

G. Furnari
Amenità ficologiche...e non solo!

18.15 ASSEMBLEA DEI SOCI

20.30 Pizza Sociale

Sabato, 28 ottobre 2023

9.00 Relazione ad invito:

Dr. Wiebe Kooistra (Stazione Zoologica Anton Dohrn)

Phytoplankton research at SZN: from the sea into the lab and back again

I Sessione (9.30 – 11.00) - Moderatore: S. Accoroni

A Mussi, T. Greca, M. Montresor, D. Sarno, W.H.C.F. Kooistra
Combining molecular, morphological and biogeographical approaches to characterize two potentially new species within the genus *Chaetoceros*

N. Romillac, G. Zampicinini, A. Zingone, D. Sarno
Insights into the ecology of cryptic phytoplankton species at LTER-MC (Gulf of Naples) using an 11 years-metabarcoding dataset

L. Russo, D. Bellardini, G. Zampicinini, F. Jordán, R. Congestri, D. D'Alelio
From metabarcoding time series to plankton food webs

A. Broccoli, M. T. Russo, D. Sarno, P. von Dassow, M. Montresor, M. I. Ferrante
From the exploration of diatom sex determination mechanisms to the detection of diatom sex events at sea

Y. Metti, G. Furnari, D. Serio
Analisi molecolari rivelano la presenza del genere *Corynecladia* (Rhodophyta, Rhodomelaceae) in Mediterraneo con due nuove specie: *C. millarii* sp. nov. and *C. mediterranea* sp. nov.

A. Tursi, A. Mincuzzi, A. Bottalico
First record of the invasive brown alga *Rugulopteryx okamurae* in the southern Adriatic Sea (Bari, Italy)

M.A. Wolf, K. Sciuto, A. Buosi, M. Orlando-Bonaca, A. Fortič, A. Sfriso
A new *Dasysiphonia* (Delesseriaceae, Rhodophyta) species discovered in the North Adriatic Sea (Mediterranean)

11.00– 11.30 Coffee break

II Sessione (11.30 – 13.00) - Moderatrice: L. Rugnini

G. Zoffoli, F. Guerrini, L. Pezzolesi, M. Cangini, S. Dall'Ara, R. Pistocchi
Paralytic shellfish poisoning (PSP) toxin profiles of *Alexandrium minutum* strains isolated from different Italian marine regions

M. Simonazzi, V. Bolletta, F. Guerrini, R. Pistocchi, L. Pezzolesi
Valorization of wastewaters from carp (*Cyprinus carpio*) farming: towards a sustainable circular economy model for algal cultivation and fish farming sectors

R. M. Sepe, I. Orefice, G. Romano, M. Montresor, V. Di Dato
Exploring diatom resting stages in Calabrian marine waters: biological diversity, chemical cues and biotechnological potential

F. Ortenzi, S. Savio, F. Montereali, A. Contaldo, A. Di Cave, L. Rugnini, S. Antonaroli, B. Morozzo Della Rocca, L. Bruno, R. Congestri
Mechanical stimulation: an innovative method to optimize microalgal-based bioproduction

L. Vitale, A. Fuentes-Lema, C. Sobrino, G. Romano, V. Di Dato
Prostaglandins biosynthetic pathway in diatoms under abiotic stresses

D. De Luca, R. Piredda, J. Troisi, S. Scamardella, P. Cennamo
The biodeterioration of Roman stuccoes in *laconicum* of the sector of Sosandra (archaeological site of Baia): a multi-omics approach

A. Di Cave, S. Savio, F. Ortenzi, L. Rugnini, L. Bruno, R. Congestri
Investigating the biostimulant potential of *Arthospira platensis* biomass on *Chicorium intybus*

13.00 Arrivederci!

Seaweed: a modern and sassy look

Charles-François Boudouresque, Institut Méditerranéen d'Océanologie, Marseille

Algae do not constitute a taxon, but a customary, artificial, polyphyletic group. In a way, algae don’t exist. Algae are often viewed with condescension by 'terrestrial botanists' as more or less primitive non-vascular plants. However, this is very inaccurate: algae can have a vascular apparatus and be as (and even more) evolved and complex as flowering plants.

I SESSIONE

Moderatrice: Leonilde Roselli

Contribution of microbial community to the long-term persistence of both alive and dead *Posidonia oceanica* '*matte*'

S. Frasca, C. Conte, A. Alabiso, E. Apostolaki, L. Migliore

In the Mediterranean Sea, the endemic seagrass *Posidonia oceanica* (L.) Delile provides several ecosystem services, including its role as a carbon sink. Besides the rich canopy, with leaves up to 1 mt long, *P. oceanica* develops a belowground structure called *matte*, consisting of lignified roots and rhizomes admixed with sediment, largely responsible for the blue carbon storing (Pergent-Martini et al., 2021). The *P. oceanica* matte is a thick and highly resistant structure able to persist for hundred years, even when the meadows die. This led to consider both alive and dead *matte* (*i.e.,* without foliar canopy) as an important marine long-term carbon sink (Apostolaki et al., 2022). Microbial community activities drive the *matte* decomposition process, depending on the oxic conditions of the *matte* layers and the refractory traits of the organic matter component. The putative roles of associated microorganisms on *matte* decomposition process are hitherto still unexplored, although they are the key factor in predicting *matte* carbon storage capacity, even in the context of climate change. To reduce this knowledge gap, it has been evaluated how the fungal taxonomic composition, and their putative function (besides the bacterial component) differs in the superficial and the bottom layer of alive and dead *matte* from a sea stretch of the Crete Island (Greece). Analyses were performed by ITS2 gene metabarcoding. The fungal colonizers were similar either in alive or dead *matte* and in their upper or bottom sections, due to the dominance of a putative *P. oceanica* specific fungal species: *Posidoniomyces atricolor*, which is considered responsible for the putative Dark Septate Endophyte (DSE) association, recently found in *P. oceanica* (Vohník et al., 2019). This fungus is the dominant colonizer of both matte layers notwithstanding the different environmental conditions, as oxygen is present in the upper layer, but it lacks in the bottom one. The role and capacity of *Posidoniomyces atricolor* will be discussed.

Letteratura citata

Apostolaki ET, Caviglia L, Santinelli V, Cundy AB, Tramati CD, Mazzola A, & Vizzini S. (2022) The importance of dead seagrass (*Posidonia oceanica*) matte as a biogeochemical sink. Frontiers in Marine Science 9:861998

Pergent-Martini C, Pergent G, Monnier B, Boudouresque CF, Mori C, & Valette-Sansevin A (2021) Contribution of *Posidonia oceanica* meadows in the context of climate change mitigation in the Mediterranean Sea. Marine Environment Research 165:105236.

Vohník, M., Borovec, O., Kolaříková, Z., Sudová, R., & Réblová, M. (2019). Extensive sampling and high-throughput sequencing reveal *Posidoniomyces atricolor* gen. et sp. nov.(Aigialaceae, Pleosporales) as the dominant root mycobiont of the dominant Mediterranean seagrass *Posidonia oceanica*. MycoKeys, 55, 59.

AUTORI

Sara Frasca (sara.frasca@students.uniroma2.eu), PhD Program in Evolutionary Biology and Ecology, Dept. of Biology, Tor Vergata University, Via della Ricerca Scientifica 00133, Rome (Italy)
Chiara Conte, Dept. of Biology, Tor Vergata University, Via della Ricerca Scientifica 00133, Rome (Italy)
Annamaria Alabiso, PhD School in Evolutionary Biology and Ecology, Dept. of Biology, Tor Vergata University, Via della Ricerca Scientifica 00133, Rome (Italy)
Eugenia Apostolaki, HCMR, Institute of Oceanography, Gournes 715 00, Crete (Greece)
Luciana Migliore, Dept. of Biology, Tor Vergata University, Via della Ricerca Scientifica 00133, Rome (Italy)

Mediterranean Seaweed biorefinery for valuable compounds

M. Francavilla

The European algae industry is a bio-based sector with a considerable potential to further develop and contribute to critical societal challenges such as the EU carbon neutrality, an innovative food system that ensures access to nutritious and sustainable food, and, ultimately, the support to a sustainable and circular European bioeconomy. The biorefinery approach (algae biofactory) is currently being investigated as a mean to increase the environmental sustainability and economic feasibility of existing conventional industrial processes. In considering the blue biorefinery concept, it is essential to focus on the sustainability aspect, which provides safety to workers and process/endproduct safety. Seaweed, among other biomass, represent an intriguing and challenging substrate that is studied and tested for biorefinery processes in our lab through a combination of chemical, thermochemical and biochemical processes. An overview of main research results referred to cascade process for the Mediterranean red seaweed *Gracilaria gracilis* conversion into high value products (algae biorefinery), will be provided and discussed.

AUTORI:
Matteo Francavilla (matteo.francavilla@unifg.it), STAR*Facility Centre, Department of Agriculture, Foods Natural Resources and Engineering (DAFNE), University of Foggia, Italy
Autore di riferimento: Matteo Francavilla

II SESSIONE

Moderatrice: Laura Pezzolesi

Impact of Temperature on *Microglena antarctica* (Chlorophyceae): Insights into Growth, Metabolome, Lipid Profiles, and Photosynthetic Pigments

R. Trentin, E. Moschin, L. Custódio, I. Moro

Cold–adapted microalgae are important contributors to primary productivity in alpine and polar regions representing a key element in polar food webs and playing a crucial role in global carbon fixation (Cvetkovska et al., 2022). Despite their important ecological functions in cold ecosystems, many aspects related to their biology, diversity and physiological and biochemical adaptations remain largely unknown (Trentin et al., 2022). This study aimed to explore the physiological and biochemical processes occurring in *Microglena antarctica* (Chlorophyceae) in response to changes in temperature. *M. antarctica* cultivated at three different temperatures (4°C, 8°C and 16°C) exhibited variations in growth patterns, metabolomes, fatty acid methyl esters (FAMEs) profile and photosynthetic pigment concentrations. Our results highlighted a decrease in growth at 16°C, confirming the cryophilic nature of this species. An untargeted metabolomics approach, coupling liquid chromatography (LC) with high resolution tandem mass spectrometry (MS/MS), was employed to study the chemical differences induced by temperature variations in the whole algal metabolome. To cope with the limited information regarding marine compounds in mass spectral libraries, we used novel *in–silico* tools to better understand the 'chemical dark matter' (Da Silva et al., 2015; Koester et al., 2022) in *M. antarctica* metabolome. Significant differences in *M. antarctica* annotated compounds, chemical classes and whole metabolomes were observed among 4, 8 and 16°C. Finally, targeted analyses were performed to evaluate changes in lipid profiles and photosynthetic pigment content. Higher percentages of polyunsaturated fatty acids (PUFAs) were observed at 4 and 8°C, while monounsaturated fatty acids (MUFAs) significantly increased at 16°C. A significant increase in chlorophyll *a* and carotenoid content was observed at 16°C. The present work highlights temperature–related responses in *M. antarctica* biochemical profile, combining untargeted and targeted approaches, and physiology, by growth analysis.

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Cvetkovska, M., Vakulenko, G., Smith, D.R., Zhang, X., Hüner, N.P.A. (2022). Temperature stress in psychrophilic green microalgae: Minireview. *Physiologia Plantarum* 174: e13811.

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Koester, I., Quinlan, Z.A., Nothias, L.F., White, M.E., Rabines, A., Petras, D., Brunson, J.K., Dührkop, K., Ludwig, M., Böcker, S., Azam, F., Allen, A.E., Dorrestein, P.C., Aluwihare, L.I. (2022). Illuminating the dark metabolome of *Pseudo-nitzschia*–microbiome associations. *Environmental Microbiology* 24: 5408–5424.

Trentin, R., Negrisolo, E., Moschin, E., Veronese, D., Cecchetto, M., Moro, I. (2022). *Microglena antarctica* sp. nov. a New Antarctic Green Alga from Inexpressible Island (Terra Nova Bay, Ross Sea) Revealed through an Integrative Approach. *Diversity*, 14: 337.

AUTORI

Riccardo Trentin (riccardo.trentin.2@studenti.unipd.it), Emanuela Moschin, Isabella Moro, Department of Biology, University of Padova, Via U. Bassi 58/B, 35131 Padova, Italy.
Luísa Custódio, Centre of Marine Sciences, Faculty of Sciences and Technology, University of Algarve, Ed. 7, Campus of Gambelas, 8005-139 Faro, Portugal.
Autore di riferimento: Isabella Moro

Effects of salinity and sulphur limitation on photosynthesis and cell composition in *Dunaliella salina*

M. Minio, C. Gerotto

Dunaliella salina is a wall-less Chlorophyta that dominates hypersaline lagoons, lakes and salt-saturated brines. Thanks to its ability to thrive in such environments, characterized by rapid shifts in salinity and nutrient depletion due to salts precipitation, *D. salina* has become a model organism for the study of stress responses (Giordano, et al., 2000; Polle, et al., 2020). *D. salina* is of biotechnological interest, for being intensively cultured for the commercial production of glycerol, that it accumulates as an osmoticum, and β-carotene (Monte, et al., 2020). In our study we are investigating on how *D. salina* responds to different salinities and low sulphate availability and how such changes impact photosynthesis, as sulphur limiting conditions are poorly studied in many organisms. We performed qualitative and quantitative assessment of pigments and *in vivo* chlorophyll fluorescence analysis, with initial results showing that *D. salina* cells effectively acclimate photosynthesis in response to environmental challenges and that the regulation of photosynthetic light reactions somewhat differs from that of plants and of other green microalgae. Analysis of the allocation of resources is also being performed through (i) FT-IR spectroscopy, to assess changes in the carbon allocation between the different macromolecular groups, and (ii) elemental analysis, to check for shifts in the ratios between macroelements in the cells, as a function of the growth conditions tested.

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Giordano M, Pezzoni V, Hell R. Strategies for the allocation of resources under sulfur limitation in the green alga Dunaliella salina. Plant Physiol. 2000 Oct;124(2):857-64. doi: 10.1104/pp.124.2.857. PMID: 11027733; PMCID: PMC59189.

Monte J, Ribeiro C, Parreira C, Costa L, Brive L, Casal S, Brazinha C, Crespo JG. Biorefinery of Dunaliella salina: Sustainable recovery of carotenoids, polar lipids and glycerol. Bioresour Technol. 2020 Feb;297:122509. doi: 10.1016/j.biortech.2019.122509. Epub 2019 Nov 30. PMID: 31812914.

Polle, Jürgen E. W., Calhoun, Sara, McKie-Krisberg, Zaid, Prochnik, Simon, Neofotis, Peter, Yim, Won C., Hathwaik, Leyla T., Jenkins, Jerry, Molina, Henrik, Bunkenborg, Jakob, Grigoriev, Igor V., Barry, Kerrie, Schmutz, Jeremy, Jin, EonSeon, Cushman, John C., and Magnusson, Jon K. Genomic adaptations of the green alga Dunaliella salina to life under high salinity. United States: N. p., 2020. Web. doi:10.1016/j.algal.2020.101990.

AUTORI

Miles Minio (m.minio@pm.univpm.it), Caterina Gerotto Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona.
Autore di riferimento: Miles Minio

Biosorption of Rare Earth Elements from electronic waste by *G. sulphuraria* (Cyanidiophytina, Rhodophyta)

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

Lanthanides are indispensable constituents of modern technologies and are often challenging to acquire from natural resources. The demand for REEs is so high that there is a clear need to develop efficient and eco-friendly recycling methods. Living and freeze-dried biomass of the polyextremophile *Galdieria sulphuraria* was employed to recover REEs both from synthetic solutions and from spent fluorescent lamps (FL) luminophores. In a preliminary set of tests, *G. sulphuraria* ACUF 427 living cells were exposed to single- and quaternary-metal aqueous solutions containing Yttrium, Cerium, Europium, and Terbium, at pH 2.5, 3.5, 4.5, and 5.5. By increasing the pH of the solutions, there was a significant surge in the aqueous removal performance of the strain; all four metal species were best removed at pH 5.5, with a maximum total bioremoval of 38.5 micromol/g dm. Based on these results, in the second set of experiments, lanthanides were extracted from the luminophores using sulphuric acid solutions according to standardised procedures, and the effect of biosorbent dosage (0.5-5 mg/ml) and biosorption time (5-60 minutes) were evaluated, by using freeze dried biomass. The content of individual REEs in the luminophores and the resulting algal biomass were determined using inductively coupled plasma mass spectrometry (ICP-MS). The most abundant REE in the luminophores was yttrium (287.42 mg/g dm, 91.60% of all REEs), followed by europium (20.98 mg/g, 6.69%); cerium, gadolinium, terbium and lanthanum was in trace. The best biosorption performances were achieved after 5 minutes and at the lowest biosorbent dosage (0.5 mg/mL). The highest total metal amount corresponded to 41.61 mg/g dried mass, and yttrium was the most adsorbed metal (34.59 mg/g dm, 82.88%), followed by cerium (4.01 mg/g); all other metals were less than 2 mg/g. The rapidity of the biosorption process and the low biosorbent dosage required confirmed this microalga as a promising material for creating an eco-sustainable protocol for recycling REEs.

AUTORI

Manuela Iovinella, Concetta Auciello, Claudia Ciniglia (Claudia.CINIGLIA@unicampania.it) Università degli Studi della Campania L. Vanvitelli, Caserta, Italia.
Seth J. Davis, University of York, York, UK
Autore di riferimento: Claudia Ciniglia

Effects of BPA analogs on the microalga *Phaeodactylum tricornutum* Bohlin

J. Fabrello, V. Matozzo, E. Moschin, F. Dalla Vecchia, I. Moro

Bisphenol A (BPA) analogs are emerging contaminants currently adopted as substitutes for BPA. It is known that BPA can cause toxic effects, mainly oxidative stress, on the reproductive system in both humans and animals (Matuszczak et al., 2019; Gassman et al., 2017). For these reasons BPA has been restricted in some industrial activities, such as in the manufacture of baby bottles in the USA, Canada, the EU, and Australia. Actually, at least 148 different BPA analogs are used, even if the main BPA analogs are BPAF, BPF and BPS. These analogs have many uses, such as plasticizer in polycarbonate plastic production, in thermal paper as a color developer, in epoxy resins, in medical equipment, and in consumer electronics (Vandenberg et al., 2007). In addition, their production and their environmental release is increasing, however data on the effects of BPA analogs are scarce. The aim of this study was to evaluate for the first time the biological effects of BPA analogs on a marine primary producer, the diatom *Phaeodactylum tricornutum* Bohlin. In details, we tested the three BPA analogs - BPAF, BPF, and BPS - and their mixture at the environmental relevant concentration of 300 ng/L. We evaluated the effects of these compounds on growth, biomarkers of oxidative stress and oxidative damage, and ultrastructure alterations through electron microscopy. Results indicated that BPA analogs reduced cell growth and increased the oxidative stress altering the activities of several antioxidant enzymes. Interestingly, we observed that the mixture caused higher alterations to the measured biochemical parameters in comparison to the single bisphenols. Furthermore, at the ultrastructural levels, we observed a general disorganization of the chloroplasts, with marked dilations of the thylakoid membranes and an increased vacuolization. In conclusion, the bisphenol A analogs tested in this survey can be harmful for the microalga *P. tricornutum*, as previously observed for BPA.

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AUTORI

Jacopo Fabrello (jacopo.fabrello@phd.unipd.it), Valerio Matozzo, Emanuela Moschin, Francesca Dalla Vecchia, Isabella Moro
Dipartimento di Biologia, Università degli Studi di Padova, Via Basssi 58/B, 35131 Padova.
Autore di riferimento: Jacopo Fabrello

Exploring cryopreservation techniques for the long-term storage of *Chaetoceros socialis* at different life cycle stages

M.L. Madeo, P. Chaerle, O. Chepurnova, W. Vyverman, M. Montresor, M. Ferrari, T. Greca, L. Bruno, G. Romano, R. Cozza

Marine diatoms represent a rich and variable genetic resource and, in the last decade, a rapid increase was seen in their biotechnology applications. Indeed, it's well known that most diatoms show fast growth and rapid response to different stressors, due to the plasticity of their genotype, which can be beneficial for the production of different metabolites of interest (Sharma et al., 2021). The development and marketing of biotechnological products therefore require the use of stable and well-kept of the most suitable strains. Strains are generally maintained by serial subcultures in which, over time, the reduction of size, the sexual reproduction and so genetic mutations may accumulate, modifying the physiology of the species, with the risk of losing important features for biotechnology approaches (Godhe & Rynearson, 2017; Bulankova et al., 2021). Therefore, alternative methods to subculturing, such as cryopreservation, are becoming increasingly important in algal culture collections (Stock et al., 2018). In this view, (cryo)preservation methods for the long-term storage of microalgae are needed, but at the same time, there is no universal protocol (Day, 2007). An alternative possibility for long-term storage of strains could involve the induction of resistance stages (spores or 'resting cells') that are part of the life cycle of many diatoms and that can remain viable in sediments for decades (Sanyal et al., 2021) followed by cryopreservation. In this work, we investigated several cryopreservation protocols for the diatom *Chaetoceros socialis* at 2 different stages of its life cycle, namely vegetative cells vs resistance stages (spores). The aim was to develop an effective cryopreservation protocol for *Chaetoceros socialis* through the evaluation of different cooling rates and times of equilibration in "DMSO", the most commonly used cryoprotective additive, to increase the post-thaw cell growth. To evaluate the post-thaw viability, we combined classical bright field microscopic analysis with Imaging Flow Cytometry. The main aim, next to following the recovery growth curve, was to observe possible phenotypical variations of the subcultures treated with different cryopreservation methods. The results show an earlier recovery of cryopreserved spores compared to vegetative cells; moreover, the cryopreserved spores showed higher viability compared to vegetative cells with a percentage ranging from 5% (after 7 days from thawing) up to 90% (after 14 days from thawing), while vegetative cells exhibit values of 5% (after 5 days from thawing) up to 15% (after 14 days from thawing). Overall, these first results indicate that for *Chaetoceros socialis* the different stages and technical strategies have a high influence on the successful cryopreservation of this diatom. The established protocols could be applied to long-term cryopreserve *Chaetoceros socialis* and perhaps other *Chaetoceros* species.

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AUTORI

Maria Letizia Madeo (marialetizia.madeo@unical.it), Leonardo, Bruno, Radiana, Cozza, Department of Biology, Ecology and Earth Sciences, University of Calabria, Via Ponte P. Bucci 6b, 87036 Arcavacata di Rende, Cosenza, Italy;
Peter Chaerle, Olga Chepurnova, Wim Vyverman, BCCM/DCG Diatoms Collection, Laboratory of Protistology and Aquatic Ecology, Department of Biology, Ghent University, Ghent, Belgium;
Michele, Ferrari Teresa Greca, Department of Biology, Ecology and Earth Sciences, University of Calabria, Via Ponte P. Bucci 6b, 87036 Arcavacata di Rende, Cosenza; Stazione Zoologica Anton Dohrn, Contrada Torre Spaccata 87071 Amendolara (CS), Italy;
Marina Montresor, Giovanna Romano, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy.
Autore di riferimento: Maria Letizia Madeo

III SESSIONE

Moderatori: Katia Sciuto e Domenico D'Alelio

Impact of environmental parameters on macrophyte changes in TWS soft bottoms: The Venice Lagoon as study case.

A. Sfriso, A. Buosi, Y. Tomio, M. A. Wolf, A.-S. Juhmani , K. Sciuto, A.A. Sfriso.

The lagoon of Venice is a large polyhedric basin composed by different microhabitats that represent the main environmental conditions of the Italian transitional water systems (TWS) (Sfriso et al., 2017). Macrophytes and the environmental parameters of the water column and surface sediments, sampled in late spring-early summer 2011, 2014, 2018 and 2021 in 87 stations spread in the entire lagoon, have been analyzed.
Globally, 187 macroalgal taxa were found, however the number yearly recorded ranged from 126 to 132 taxa with an annual species variation depending on weather conditions. On average, 32 taxa were found in 20-67% of the stations, whereas 55 in 0-1%. Among them, the number of non-indigenous species (NIS) increased from 2011 (11 taxa) to 2021 (18 taxa) for a total of 21 taxa, without affecting the biodiversity. At the same time the mean coverage of aquatic angiosperms increased significantly, ranging from 19.3% (56 Km^2) in 2011 to 34.9% (118 Km^2) in 2021, especially thanks to *Zostera noltei* and *Ruppia cirrhosa* recolonization (Sfriso et al., 2017).
The analysis of environmental parameters, carried out in 2021, highlighted the key role played by water transparency and salinity, which were strongly positively correlated to total number of taxa, to the presence of sensitive and crustose calcareous macroalgae of the genera *Hydrolithon*, *Pneophyllum* and *Melobesia* and to aquatic angiosperm cover, consisting mainly in *Cymodocea nodosa* and *Zostera marina*. In contrast, Chl-*a*, TSS, ammonium, nitrite, nitrate and silicate concentrations in water column, as well as moisture, phosphorus concentrations and Fines (fraction < 63 µm) in surface sediments, were strongly correlated to opportunistic species, especially *Ulva rigida* C. Agardh*, Gracilariopsis longissimima* (S. G. Gmelin) Steentoft *et al., Agardhiella subulata* (C. Agardh) Kraft *et* M. J. Wynne and *Solieria filiformis* (Kützing) P. W. Gabrielson. The correlation with the environmental parameters was extended also to 41 macroalgal taxa, i.e the most abundant taxa present in 20% of the considered stations.
The obtained results evidence that a constant monitoring of these environmental parameters can be very useful in order to predict the most probable evolution of aquatic vegetation in TWS, especially with regard to the presence of sensitive species and aquatic angiosperms.

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AUTHORS

Adriano Sfriso (sfrisoad@unive.it), Alessandro Buosi, Yari Tomio, Marion Adelheid Wolf, Department of Environmental Sciences, Informatics and Statistics (DAIS), University Ca' Foscari Venice, Via Torino 155, 30172 Mestre (Venice), Italy.
Abdul Salam Juhmani, Department of Biology and Biotechnology, Faculty of Science, The Hashemite University, Zarqa 13133, Jordan.
Katia Sciuto, Andrea Augusto Sfriso, Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Via L. Borsari 46, 44121 Ferrara, Italy.
Corresponding author: Adriano Sfriso

Dryland biobanking: preliminary data on the biodiversity of green algae in deserts worldwide

V. Malavasi, N. La Rocca, N. Beltran-Sanz, S. Stocco, A. Bortolato, A. López-Chicheri, F. Maestre, F. Dal Grande

Drylands are present on all inhabited continents and stretch across 41.3 % of the land surface on Earth. Deserts are extreme environments characterized by water scarcity, extreme temperatures, and radiation levels. Microorganisms constitute a considerable part of the earth's biodiversity. In detail, unicellular green algae play a crucial role within these communities in terms of primary production, nutrient cycle as well as formation and changes in soil structure. Despite this, phototrophic microalgae flora has remained one of the least studied components of microbial communities. The aim of this study is to investigate the algal biodiversity from a selection of dryland soils around the world. Specifically, we isolated and cultivated in vitro green microalgae from 23 soil samples representing 14 countries, collected within the framework of the BIODESERT project. Isolated strains were identified via DNA barcoding (18S rDNA and ITS2) and morphological observations. Currently, the stock culture strains at the University of Padua Botanic Garden are more than 200 isolates representing 35 algal genera. The genera *Muriella* (Trebouxiophyceae), *Myrmecia* (Trebouxiophyceae), *Pleurastrum* (Chlorophyceae), and *Klebsormidium* (Klebsormidiumphyceae) are the most abundant. Some of these strains will be tested for possible biotechnological applications, e.g., via competition experiments in a bioreactor environment. This initial screening of microalgal diversity in drylands and the establishment of a living collection of these organisms represent the first step towards the exploration of the genetic features of desert adaptations in microalgae.

AUTORI

Veronica Malavasi (veronica.malavasi80@gmail.com), Centro di Ateneo Orto Botanico, University of Padua, Via Orto Botanico 15, 35123 Padua, Italy; Department of Botany, Charles University, Benátská 2, CZ-12801 Praha 2, Czech Republic.
Nicoletta La Rocca Department of Biology, University of Padova, Via U. Bassi 58/B, Padua, Italy & National Biodiversity Future Center, Piazza Marina 61, 90133 Palermo, Italy
Núria Beltran-Sanz Centro di Ateneo Orto Botanico, University of Padua, Via Orto Botanico 15, 35123 Padua, Italy
Sofia Stocco, Alberto Bortolato Department of Biology, University of Padova, Padua, Italy
Alexandra López-Chicheri, University of Granada, Granada, Spain
Fernando Maestre, Departamento de Ecología, Universidad de Alicante, Alicante 03690, Spain
Francesco Dal Grande, Department of Biology, University of Padova & Centro di Ateneo Orto Botanico, University of Padua, Via Orto Botanico 15, 35123 Padua, Italy & National Biodiversity Future Center, Piazza Marina 61, 90133 Palermo, Italy
Autore di riferimento: Veronica Malavasi

Molteplici funzioni di *Chaetomorpha linum* (Chlorophyta, Cladophorales), un'alga poliedrica e cosmopolita.

C. Micheli

Chaetomorpha linum (Chlorophyta, Cladophorales) è una macroalga, poliedrica e cosmopolita ed è una delle specie dominanti nel Mediterraneo. L'alga si trova spesso in associazione con altre specie algali e fanerogame acquatiche, sia negli ambienti lagunari che nelle zone costiere: la sua distribuzione appare frequentemente sia nel Mar Piccolo di Taranto (Mar Ionio) che nella laguna di Orbetello (Mar Tirreno nord) dove, recentemente, ha raggiunto abbondanti quantità di biomassa tali da richiedere interventi di rimozione emergenziale.
C. linum ha un'ampia versatilità: partecipa all'importante funzione di decarbonizzazione, nel regolare il sequestro di carbonio e la conseguente purificazione dell'acqua, ed è ormai nota la sua capacità di eliminare nitrati e fosfati, come filtratore naturale negli acquari marini. *C. linum* è anche una risorsa per la produzione di biomolecole attive. Alcuni autori (Stabili *et al.,* 2000) hanno trovato nell'alga degli estratti lipidici con attività antibatterica contro il *Vibrio ordalii* e V. *vulnificus*, comuni patogeni in acquacultura, suggerendo il suo potenziale impiego nel controllo delle malattie dei pesci e dei crostacei (dovuto alle vibriosi) e nel ridurre il pericolo per la salute pubblica, relativo all'uso di antibiotici in acquacoltura. Recentemente è stato evidenziato in *C. linum* il suo valore aggiunto nella catena alimentare, proponendola come un buon ingrediente per la dieta Mediterranea (Stabili *et al.,* 2023).
E' comunque ormai noto che, se da un lato le macrofite sono considerate di vitale importanza nell'ecosistema marino per i loro innumerevoli servizi nel supportare l'habitat, rivestendo un ruolo prioritario nelle sue funzioni (ossigenazione e catena trofica), dall'altro l'elevata abbondanza diminuisce il beneficio quando, nel caso delle macroalghe, la crescita porta ad alte densità con un enorme sviluppo di biomassa che, in assenza di drenaggio ed opportune azioni, con la propria degradazione può portare all'eutrofizzazione dell'ecosistema. Ne è un esempio l'eutrofizzazione causata dall'alga nitrofila *Ulva* s.p. che negli anni 88-94 ha portato a gravi problemi nella laguna di Venezia, richiedendo forti interventi di recupero dell'ecosistema (Micheli *et al.,* 1995). A questo riguardo dobbiamo infatti considerare che, a differenza delle fanerogame acquatiche che rappresentano le specie climax, le macro-alghe che raggiungono grandi biomasse, rientrano nel circuito del riciclo restituendo sotto forma di degradazione, il contenuto di elementi come il C, N, P, S che portano l'ecosistema in una fase di distrofia.
La massiccia biomassa di *Chaetomorpha linum* (Müller) Kützing, che è sempre più presente lungo le zone costiere lagunari di Orbetello (Lenzi *et al.,* 2020), in entrambi i versanti di Levante e Ponente, sta rappresentando una significativa intrusione nel Mar Tirreno centrale tanto da richiamare l'interesse per il suo utilizzo suggerendo la possibilità di proporla come risorsa non convenzionale di bioenergia rinnovabile (Galletti *et al.,* 2019).
La novità di questo lavoro consiste quindi nel proporre una nuova scelta della tipologia di approccio ad impatto sull'ecosistema su cui si vuole agire. Quindi, una volta stabilite le caratteristiche fisiologiche e l'efficienza fotosintetica dell'alga, l'obiettivo sarà quello di ottimizzare le sue capacità di crescita in camere climatiche e vasche, per poter far fronte ad una disponibilità di biomassa costante, anche in assenza di emergenza ambientale dovuta all'eutrofizzazione.

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AUTORI

Carla Micheli (carla.micheli@enea.it), ENEA Agenzia Nazionale Italiana per le Nuove Tecnologie, l'Energia e lo Sviluppo Economico Sostenibile, Centro Ricerche Casaccia, Via Anguillarese 301, 00123 Roma.
Autore di riferimento: Carla Micheli

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Idrodinamismo e composizione specifica delle comunità fitoplanctoniche del Mar Adriatico meridionale

C. Caroppo, A. Bergamasco, G. Caruso, F. Decembrini

Negli ecosistemi marini costieri la composizione delle comunità fitoplanctoniche è influenzata dagli input continentali e dalle caratteristiche idrodinamiche delle masse di acque. Questo contributo descrive la composizione per taglia, la biomassa, l'abbondanza e la composizione specifica del fitoplancton del Golfo di Manfredonia (Mar Adriatico meridionale, Italia) nel periodo tardo primaverile. Quest'area è soggetta ad impatto antropogenico ed è influenzata dalla circolazione sia delle acque costiere provenienti dal mar Adriatico settentrionale sia da quelle aperte del Mar Ionio. Le analisi delle comunità sono state condotte su campioni raccolti con bottiglia Niskin. Considerando le proprietà termo-aline delle acque, tali campioni sono stati raggruppati in tre Cluster rappresentativi dei diversi Sistemi presenti nell'area di studio: Sistema Costiero (SC), Intermedio (SI) e Off-shore (SO).
Le abbondanze della componente di minori dimensioni, il pico-fitoplancton (0.2-3.0 μm), hanno raggiunto valori medi pari a 5,4 ± 3,8 × 10^6 cellule L^{-1} con concentrazioni cellulari decrescenti passando dal SC al SO, attraverso il SI. Il picofitoplancton si è confermato una componente importante delle comunità fitoplanctoniche del Golfo così come di altri siti costieri (Caroppo, 2015).
La frazione di Utermöhl (nano+micro-fitoplancton, rispettivamente 3-20 μm e 20-200 μm) è stata rappresentata da molti taxa e non sono stati rilevati fenomeni di dominanza. Dal punto di vista qualitativo, sono state identificate diverse associazioni in ognuno dei Sistemi analizzati. All'interno del SC, la comunità è risultata dominata da specie neritiche di maggiori dimensioni, come le diatomee *Cylindrotheca closterium*, *Pleurosigma* sp., *Navicula* sp. e *Nitzschia longissima*, e diverse specie di dinoflagellate. Questi taxa, con tassi di crescita relativamente bassi, sono tipici di una comunità che procede verso uno stadio maturo della successione *sensu* Margalef (1967) e sono ben adattati alla minore turbolenza tipica delle stazioni più interne del Golfo. Al contrario, nel SO si è osservata una associazione tipica del primo stadio della successione *sensu* Margalef (1967), prevalentemente nanoplanctonica come alcune specie appartenenti al genere *Chaetoceros* e piccole dinoflagellate (Gymnodiniales). Tra questi due Sistemi si trova il SI, probabilmente influenzato dal flusso superficiale delle acque provenienti dal Mare Adriatico Settentrionale (Corrente Adriatico Occidentale). Qui la biomassa fitoplanctonica (espressa in termini di clorofilla *a*), localizzata principalmente al DCM (Deep Chlorophyll Maximum), ha una concentrazione media doppia rispetto al SO e quasi un terzo inferiore al SC. Il SI è caratterizzato da una maggiore percentuale di picofitoplancton, che sostituisce il microfitoplancton, dominante nel SC. Per ciò che riguarda invece il nanoplancton, i valori di abbondanza riscontrati sono confrontabili in tutti i Sistemi esaminati, dimostrando la sua capacità di adattarsi a diverse condizioni idrologiche. Dal punto di vista qualitativo, nel SI sono presenti taxa riscontrati anche negli altri ambienti (SC e SO), con la presenza di specie tipiche del periodo primaverile nel Mare Adriatico (Decembrini et al. 2021).

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AUTORI

Carmela Caroppo (carmela.caroppo@irsa.cnr.it), Water Research Institute, National Research Council (CNR-IRSA), Section of Taranto, Via Roma 3, 74121 Taranto, Italy
Franco Decembrini, Gabriella Caruso, Institute of Polar Sciences, National Research Council (CNR-ISP), Section of Messina, Spianata S. Raineri 86, 98122 Messina, Italy
Alessandro Bergamasco, Institute of Marine Sciences, National Research Council (CNR-ISMAR), Section of Venice, Castello 2737/F, 30122 Venice, Italy
Autore di riferimento: Carmela Caroppo

Interannual trend of *Ostreopsis* cf. *ovata* bloom in the Conero Riviera (northern Adriatic Sea)

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

Harmful blooms of the toxic dinoflagellate *Ostreopsis* cf. *ovata* have been a recurrent phenomenon along the Mediterranean coasts in the last decades. Blooms of *Ostreopsis* cf. *ovata* along the Conero Riviera (northern Adriatic Sea) were recorded for the first time in 2006 and, since then, have occurred between the end of the summer and the beginning of the autumn. The aim of this study was to analyse the interannual trend of *Ostreopsis* cf. *ovata* blooms in the Conero Riviera (northern Adriatic Sea) and highlight if it is related to climate change predictors.
Data were collected in several years from 2007 to 2021. Time series decomposition and structural change point detection were performed to decompose the time series and detect changes in its structure. A linear model was then applied (using generalized least squares) to detect the trends before and after the change points. A hierarchical time series clustering was also applied. Mann-Kendall trend was performed on the decomposed temperature, nutrient concentration and N:P ratio.
A significant increasing trend in the magnitude of *Ostreopsis* phenomenon was observed up to year 2012 (148.06 ± 67.84 cells cm^{-2} per sampling period, $p<0.05$), then a stabilization at relatively low values was observed (19.39 ± 19.07 cells cm^{-2} per sampling time, $p>0.05$). In the study period, significant increasing and decreasing trends were detected for water temperature (tau = 0.32, $p<0.001$) and PO_4 (tau = -0.24, $p<0.001$), respectively. No significant trends were observed for DIN (tau = -0.01, $p>0.05$) and N:P ratio (tau = 0.11, $p>0.05$).
In conclusion, the trend of *Ostreopsis* cf. *ovata* in the study area does not follow the increase in water temperature and the decrease of PO_4, but rather recalls that of an invasive species.

AUTORI

Francesca Neri (f.neri@pm.univpm.it), Marika Ubaldi, Tiziana Romagnoli, Cecilia Totti, Stefano Accoroni, Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy
Autore di riferimento: Francesca Neri

Progetto Life Transfer, come strumento per il ripristino ambientale

A. Buosi, G. Silan, Y. Tomio, M.A. Wolf, A.A. Sfriso, A. Sfriso

Il Progetto LIFE TRANSFER (*Seagrass transplantation for transitional Ecosystem Recovery*, LIFE 19 NAT/IT/000264) ha lo scopo di migliorare lo stato di conservazione dell'Habitat 1150 (lagune costiere), attraverso il ripristino delle praterie a fanerogame acquatiche in alcune lagune del Delta del Po. Nel dettaglio, durante il periodo 2022-2023, sono stato eseguiti trapianti di zolle di 15 cm in diametro o rizomi di *Ruppia cirrhosa* (Petagna) Grande, *Zostera marina* Linnaeus, *Zostera noltei* Hornemann and *Cymodocea nodosa* (Ucria) Asherson in quattro stazioni situate nelle lagune di Goro (591 zolle), Fattibello (220), Barbamarco (354) e Caleri (463). Come è avvenuto nella parte settentrionale della laguna di Venezia (Sfriso *et al*., 2021), piccoli interventi diffusi di trapianto di fanerogame acquatiche in alcune aree mirate con condizioni ecologiche adatte favoriscono un'espansione naturale delle praterie, innescando a loro volta un miglioramento delle condizioni ambientali e di conseguenza un aumento dei servizi ecosistemici correlati (de los Santos *et al.*, 2020).
Nelle stesse stazioni sono stati condotti inoltre monitoraggi ambientali mensili, relativi alle matrici acqua, sedimento, particolato e valutato lo Stato Ecologico attraverso lo studio della diversità macroalgale, applicando l'indice MaQI (*Macrophyte Quality Index*) (Sfriso *et al.*, 2014).
I risultati preliminari dei monitoraggi mensili evidenziano una scarsa biodiversità macroalgale, con un massimo di 35 specie campionate nella stazione della laguna di Caleri (Gennaio-Giugno 2023) e un minimo di 16 specie nella stazione di Goro (Marzo 2022-Febbraio 2023), in cui è stata registrata l'assenza del *Phylum* Ochrophyta. In questa stazione, sono state registrate le biomasse macroalgali maggiori, con un valore medio pari a 1453 g fwt m^2, rappresentate principalmente da *Gracilariopsis vermiculophylla* Ohmi, *Gracilariopsis longissima* (S.G. Gmelin, Steentoft, L.M. Irvine & Farnham) e *Ulva australis* Areschoug. L'applicazione dell'indice di stato ecologico MaQI, ha mostrato un giudizio "*Poor*" nelle stazioni di Caleri, Barbamarco e Goro e un giudizio "*Bad*" nella stazione di Fattibello. In quest'ultima stazione sono state trovate alte concentrazioni di Chl-*a* totale (valore medio: 13.7±10.5µg L^{-1}) e TSS (*Total Suspended Solids*) (valore medio: 53.1±27.7 mg L^{-1}) e alti valori di DIN (*Dissolved Inorganic Nitrogen*) (valore medio di 35.8±52.7 µM) che riducevano sensibilmente la trasparenza e favorivano i bloom di fitoplancton.

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AUTORI

Alessandro Buosi (alessandro.buosi@unive.it), Giulia Silan, Yari Tomio, Marion Wolf, Adriano Sfriso, Dipartimento di Dipartimento di Scienze Ambientali, Informatica e Statistica, Università Ca' Foscari Venezia, Via Torino 155, 30172 - Venezia Mestre, Italy.
Andrea Augusto Sfriso, Department of Chemical Pharmaceutical and Agricultural Sciences, University of Ferrara, Via L. Borsari 46, 44121 Ferrara, Italy.
Autore di riferimento: Alessandro Buosi

Defining suitability of transplant sites in seagrass restoration

A.A. Sfriso, K. Sciuto, M. Mistri, C. Munari, A-S. Jhumani, A. Buosi, Y. Tomio, A. Sfriso

We address the critical issue of seagrass meadow decline in coastal and transitional water systems, primarily due to various human-induced pressures such as nutrient loading, pollution, and habitat destruction (Duarte et al., 2004). Consequently, efforts to restore and rehabilitate seagrass ecosystems have gained prominence, especially when natural recovery processes are compromised. These restoration activities involve multiple strategies, from establishing marine protected areas to substrate stabilization and especially as in our case transplanting seagrass species. Starting from the assumption that we aim to increase environmental quality and carbon sequestration in coastal marine areas through seagrass transplantation we come up against the limits dictated by the environmental conditions of the transplant recipient sites which are not always suitable to receive transplants. The existing literature on seagrass transplantation is vast, but the distinction between success and failure is often blurred. Clear thresholds for critical environmental conditions favouring successful transplantation are lacking. Therefore, we identified significant chemical, physical and biotic variables for water and sediment decisive for seagrass rooting and defined clear limit values that should be monitored before transplantation. Along with these we identified optimal seasons, techniques, and species for colonization. We focused on four key Mediterranean seagrass species of transitional water systems (*Cymodocea nodosa* (Ucria) Ascherson; *Zostera marina* Linnaeus, *Zostera noltei* Hornemann and *Ruppia cirrhosa* (Petagna) Grande) and competing fast growing pleustophytic macroalgae addressing the challenges of identifying suitable transplantation sites and techniques to perform a successful transplant.
Key parameters to consider in this evaluation were: water transparency, total chlorophyll-a and nitrates or dissolved inorganic nitrogen, for the water column; organic/inorganic phosphorus, organic carbon and Fines percent, for the sediments, and the presence/absence of sensitive macroalgae, especially epiphytic crustose coralline algae: *Pneophyllum fragile* Kützing*, Hydroliton boreale* (Foslie) Y. M. Chamberlain*, H. cruciatum* (Bressan) Y. M. Chamberlain*, H. farinosum* (J. V. Lamouroux) D. Penrose et Y. M. Chamberlain*, Melobesia membranacea* (Esper) J. V. Lamouroux. The latter displaying a monthly mean number of 2.4 species in the leaves of *C. nodosa* and *Z. marina* in optimal conditions*,* and no more of 0.6 in eutrophic areas, on annual basis. Water transparency (>98%) emerged as a decisive factor positively affecting macrophyte growth, whereas high Chl-a levels associated with phytoplankton had a detrimental effect by reducing light penetration. Similarly, high sediment organic phosphorus and total nitrogen content were associated with algal biomass presence and inversely with aquatic angiosperms. *Ruppia cirrhosa* showed distinct characteristics, not being affected by water transparency and phytoplankton and being positively correlated with Porg in the sediment, sediment moisture, and other sedimentary parameters associated with eutrophic areas. The species *Ruppia cirrhosa* can therefore be considered as a pioneer species for more eutrophicated and confined areas where other species would not survive, improving environmental quality compared to the presence of rapidly growing thionitrophilic macroalgae. So far transplantation by means of single rhizomes for the larger species (*Z. marina, C. nodosa*) and small bunches of rhizomes (10-15 rhizomes) for the smaller species (*Z. noltei, R. cirrhosa*) has given the best results thanks to the wider dispersion that this guarantee compared to sods (Sfriso et al. 2019). These findings provide insights into the critical parameters and tolerance ranges affecting aquatic angiosperm survival, which can inform conservation and restoration actors dealing with seagrass restoration in coastal ecosystems.

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AUTORI

Andrea Augusto Sfriso (sfrndr@unife.it), Katia Sciuto, Michele Mistri, Cristina Munari, Department of Chemical Pharmaceutical and Agricultural Sciences, University of Ferrara, Via L. Borsari 46, 44121 Ferrara, Italy.
Abdul Salam Juhmani, Department of Biology and Biotechnology, Faculty of Science, The Hashemite University, Zarqa 13133, Jordan.
Alessandro Buosi, Yari Tomio, Adriano Sfriso, Department of Environmental Sciences Informatics and Statistics, Ca' Foscari University of Venice, Via Torino 155, 30170 Mestre, Italy.
Autore di riferimento: Andrea Augusto Sfriso

Can epiphytic vegetation be used as an indicator of the recovery state of *Cystoseira s.l.* forests? A study carried out along the Conero Riviera (N Adriatic).

G. Bellanti, S. Bianchelli, F. Rindi

There is an increasing attention toward the state of macroalgal forests from both a conservation and restoration point of view. *Cystoseira s.l.* forests are considered habitats of great ecological relevance since the loss of these seaweeds, mainly caused by anthropogenic disturbances, may lead to the loss of biodiversity and ecosystem services. Interactions between habitat-forming macroalgae and their associated biota have a crucial ecological relevance which should be considered to plan conservation and restoration actions in coastal habitats. Despite of this, there is a major knowledge gap in this area, especially concerning the epiphytic communities. The identification of patterns in species composition and abundance of epiphytic communities associated to *Cystoseira s.l.* forests in different conditions of conservation (dense and well-preserved vs. fragmented), may allow the establishment of standardized indices which can act as early signals of the health status decline/recovery of the species, also on a large spatial scale.
In the period late spring/early summer 2023, the epiphytic vegetation of *Gongolaria barbata* was studied from 5 sites of the Conero Riviera hosting populations of this species in different health conditions: Due Sorelle, Sassi Neri, Spiaggia del Frate (well-preserved), Scalaccia and Scalinata del Passetto (fragmented). Branches were collected from 30 different thalli of *Gongolaria* using a nested sampling design (Fig. 1). Morphometric data of each branch were collected, and the associated epiphytic community was analyzed in terms of species composition and abundance (expressed as percentage cover for each species).
Overall, 21 macroalgal epiphytes were recorded, 5 of which were primary epiphytes (directly attached to *Gongolaria*) and 16 were also found as secondary and tertiary epiphytes. *Vertebrata fruticulosa* was present as primary epiphyte on all sampled branches. *Ceramium siliquosum* was the species most frequently found as secondary epiphyte, mostly growing on *Vertebrata*, and *Didymosporangium repens* was the species most frequently found as tertiary epiphyte, exclusively growing on *C. siliquosum*. Limited variation in species richness and total abundances was found within each site, whereas significant differences between sites were detected by Nested ANOVAs and PERMANOVAs. Post-hoc tests highlighted significant differences between Scalinata del Passetto and all other sites.
Overall, the results did not show clear differences between well-preserved and fragments forests, although the Scalinata del Passetto was clearly separated from the other sites. This site was the most urbanized, and more wave-exposed than the others; moreover, its *Gongolaria barbata* population was highly fragmented and characterized by a somewhat peculiar vegetative and reproductive phenology.
Further samplings, that will be carried out in different seasons, will allow a more detailed assessment of the differences in the epiphytic assemblages associated to the *Gongolaria* forests, and how they can be related to the health status of these communities.

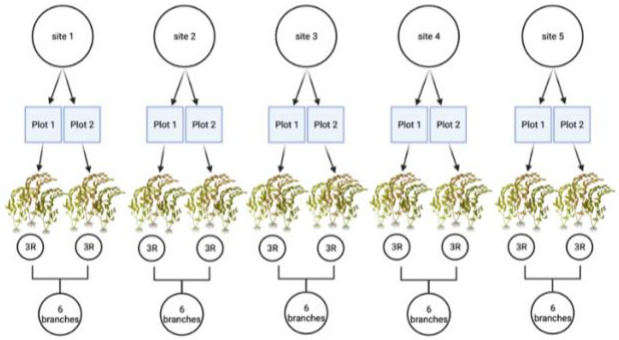

Fig.1 Nested sampling design implemented in the study.

AUTORI
Giulia Bellanti (g.bellanti@pm.univpm.it), Silvia Bianchelli, Fabio Rindi, Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy; National Biodiversity Future Centre, Palermo, Italy.
Autore di riferimento: Giulia Bellanti

Preliminary results of *Cymodocea nodosa* (Tracheophyta, Alismatales) monitoring in the Mar Piccolo of Taranto (southern Italy, Mediterranean Sea) through *in situ* data and satellite images

G. Denti, A. Petrocelli, E. Cecere, F. Rubino, G. Fanelli, C. Richiardi, M.P. Adamo

On the basis of the SPA-BIO protocol of the Barcelona Convention relating to specially protected areas and biodiversity, seagrass associations are priority for the conservation of "coastal lagoons" (habitat 1150*, Directive 92/43/EEC). In fact, aquatic phanerogams offer a remarkable set of ecosystem services (e.g., nursery areas for fish and invertebrates, sediment stabilization, nutrient recirculation) and are important indicators of ecological quality (Bonometto et al., 2018). Therefore, their continuous monitoring is advisable. In compliance with the Water Directive (2000/60/EC) and according to the protocols established by ISPRA, ARPA Puglia, since 2010, annually conducts the "Monitoring Service of Surface Water Bodies of the Apulia Region" that includes the Mar Piccolo of Taranto, a site of the Natura 2000 network and priority habitat according to Directive 92/43/EEC. For the assessment of the ecological status, the MaQI index (Macrophyte Quality Index) is one of the indices used, which considers the presence and percentage coverage of both macroalgae and angiosperms. According to the ARPA last results, the only phanerogam detected was *Cymodocea nodosa* (Ucria) Ascherson, with a coverage between 15% and 25%, while the ecological status of the basin ranged between "good" and "high". However, the Water Directive does not provide for the quantitative and phenological study of seagrasses. Therefore, within the project "M.I.A. RETE Natura 2000" funded by the Apulia Region, an innovative monitoring plan for the protection of the Mar Piccolo is going to be designed using quantitative data. A rapid monitoring method could be mapping by remote sensing (Traganos and Reinartz, 2018). In particular, the integration of data collected *in situ* and from remote sensing in the spectral range of the Vis-NIR-SWIR, through the application of data-driven supervised algorithms (e.g., SVM), allows the automatic mapping of aquatic vegetation in conditions of adequate transparency along the water column and for shallow waters (within 2 m) (Tarantino et al., 2019). A preliminary survey, conducted with ARA along the entire coast and in deeper areas, allowed to identify areas where the species is present. Successively, in the season of maximum growth, in four stations identified as the most representative, a quantitative study was conducted according to the protocols in force (Sfriso et al., 2001; Pergent et al., 2006). The quantitative data collected "at sea" are now being integrated with the information coming from satellite images acquired by the Sentinel-2 multispectral sensor of ESA (European Space Agency). As a result, distribution maps useful for a faster and cheaper space-time monitoring system will be provided.

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AUTORI

Giuseppe Denti, Antonella Petrocelli (antonella.petrocelli@irsa.cnr.it), Ester Cecere, Fernando Rubino, Giovanni Fanelli, Istituto di Ricerca Sulle Acque, Consiglio Nazionale delle Ricerche (IRSA-CNR), via Roma 3, 74123 Taranto.
Chiara Richiardi, Maria Patrizia Adamo, Istituto sull'Inquinamento Atmosferico, Consiglio Nazionale delle Ricerche (IIA-CNR), via Giovanni Amendola 173, 70125 Bari.
Autori di riferimento: Antonella Petrocelli, Maria Patrizia Adamo

Activities performed within the LTER network and the framework of: 1. POR PUGLIA FESR-FSE 2014/2020 - Axis VI, Action 6.5 "Interventions for protection and valorization of marine and terrestrial biodiversity" Sub Action 6.5.a, funded by the Apulian Region; 2. the Italian National Recovery and Resilience Plan (PNRR) funded by the Italian Ministry of University and Research, Mission 4, Component 2, "From research to business" NBFC, Investment 1.4, Project CN00000033.

IV SESSIONE

Moderatore: Saverio Savio

Stones bioreceptivity: cyanobacterial biofilm growth and its removal with biocides encapsulated into alginate hydrogel.

R. Ranaldi, L. Rugnini, F. Gabriele, C. Casieri, A. Canini, N. Spreti

The term "biodeterioration" is used when different substrates are subject to microbial attack and are responsible for any unwanted change in the properties of the material (Hueck, 1965). This phenomenon is sustained by abiotic factors such as the presence of water, light, temperature, humidity and also the stones bioreceptivity, that is the predisposition of material to be colonized by microorganisms (Guillitte, 1995).
In this research, five lithotypes typically employed in historical monuments have been selected to study the influence of stones bioreceptivity on cyanobacterial biofilm growth. These are Lecce stone, Serena stone, Peperino, Travertine and Granite. Several investigations have been performed to determine the properties of materials, including open porosity, hygroscopic properties and roughness parameters. Then, the stones parameters were correlated to the photosynthetic yields of the biofilms colonizing the different stones to propose a method to estimate stone bioreceptivity. After six months from the inoculation of cyanobacteria, the biofilms were treated using a hydrogel-biocide system as a support matrix to embed *Thymus vulgaris* essential oil (at 0.25 % or 0.1 %) or its main component thymol (at 0.18 % or 0.07 %). To evaluate the effect of the treatment, it has been used light and confocal laser scanning microscope, portable pulse amplitude modulated (Mini-PAM) fluorometer and colorimetric analyses, before and after the treatment. This step is of utmost importance because it allowed us to evaluate the effect of the hydrogel-biocide system also on the stones. In fact, restoration techniques commonly used for removal of the biological patinas from stone surfaces can induce damage to the material, by increasing its bioreceptivity. Moreover, despite the use of biocides being state regulated by UE n.528/2012, some toxic substances are still widely used by conservators against biodeteriogens of cultural heritage, due to the low cost and the short time required for the application. For these reasons, in recent years there has been an increase in the requests for natural biocides, safe for human health and the environment and, in this contest, the essential oils at low concentration have resulted as a safer and eco-friendly alternative to chemicals (Bruno *et al*., 2019; Rugnini *et al*., 2020; Ranaldi *et al*., 2022).
The collected results in this research suggested that among the lithotypes used, Lecce stone has the greatest bioreceptivity and the innovative hydrogel-biocide system containing *T. vulgaris* essential oil or thymol, is a sustainable cleaning protocol to counteract the biodeterioration of stone monuments subject to microbial attack that not induce any significant alteration to the lithic surfaces (Gabriele *et al*., 2023). Stones colonization by microorganisms and the efficacy of biocides are strictly connected to the features of the substratum and to its bioreceptivity. Ongoing researches aim to evaluate the stones bioreceptivity of different stones material colonized by natural biofilms sampled both in hypogean and outdoor environments.

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AUTORI

Roberta Ranaldi (roberta.ranaldi@alumni.uniroma2.eu), LBA-Laboratory of Biology of Algae, Dept. of Biology, University of Rome "Tor Vergata", Via Cracovia 1, I-00133, Rome, Italy. PhD program in Evolutionary Biology and Ecology, Dept. of Biology, University of Rome "Tor Vergata", Rome, Italy.
Lorenza Rugnini, LBA-Laboratory of Biology of Algae, Dept. Biology, University of Rome "Tor Vergata", Via Cracovia 1, 00133

Roma, Italy Dept. Physical and Chemical Sciences, University of Aquila, Via Vetoio, Coppito, I-67100, L'Aquila, Italy.
Antonella Canini, Dept. of Biology, University of Rome "Tor Vergata", Via della Ricerca Scientifica, 1, I-00133, Rome, Italy.
Francesco Gabriele, Cinzia Casieri and Nicoletta Spreti, Department of Physical and Chemical Sciences, University of L'Aquila, Via Vetoio – Coppito, I-67100, L'Aquila, Italy.
Autore di riferimento: Roberta Ranaldi

From laboratory to *in-situ* application of alginate hydrogel encapsulating biocides on cultural heritage affected by biofilm growth

L. Rugnini, R. Ranaldi, F. Gabriele, C. Casieri, N. Spreti

Conservation of cultural heritage assets is a very challenging matter aimed at reducing, as much as possible, the inevitable deterioration of works of art. Stone monuments in outdoor and indoor environments are subjected to biodeterioration due to the growth on their surfaces of biofilms responsible of aesthetic, physical and chemical damages (Warscheid and Braams, 2000; Sanmartín et al., 2021). Classical techniques used for the removal and eradication of these biological patinas from stone surfaces involve the use of brushes, pressurized water or chemical biocides. The former are mechanical brushes that remove the biofilms but can induce damages to the material by increasing its bioreceptivity; the latter are chemical methods including the use of biocides such as benzalkonium chloride, which kill microorganisms but are potentially toxic for operators and harmful to the environment. From these problems arose the need to develop innovative, eco-sustainable and safe solutions: at this purpose, increasing interest was addressed towards the application of phytochemical compound, among which essential oils (EOs) in aqueous solution (Rugnini et al., 2020; Favero-Longo et al., 2022; Ranaldi et al., 2022). Moreover, in recent years, the encapsulation of biocides in hydrogel matrices, has proven to be a very effective method of reducing the amounts of biocides required to achieve the same efficacy obtained when these compounds, either chemical or natural, were applied in solution at higher concentrations (Gabriele et al., 2021).
In this study, the efficacy of a hydrogel containing a natural biocide, thyme (*Thymus vulgaris*) essential oil (EO, 0.25% v/v), was compared with a hydrogel loaded with an oxidant biocide, sodium dichloisocyanurate (NaDCC; 0.8% v/v), in three selected areas of the Depero's mosaic, *Le Professioni e le Arti* (Rome, Italy). The mosaic was made with stones, glass and painted tiles and the entire site was restored under the supervision of Pantone Restauri S.r.l. and thanks to the authorization of Dr. Alessandra Montedoro, officer to the Ministero dei Beni Culturali. For this experimentation, the selected area (4 m, 16 m and 18 m) presented evident colonization by a microbial patina made of cyanobacteria, green microalgae, mosses and fungi, both on marble frame and mosaic tiles fixed in mortar surfaces. A simple method to prepare the biocidal hydrogels directly *in situ* and their easy application on large and vertical surfaces were developed. Before and after the treatment, macroscopic and microscopic observations and colorimetric measurements were performed to highlight the hydrogels' biocidal effectiveness in removing all colonizers. Only one treatment (lasting 24 hours) was enough to completely remove the microbial patina constituted of various prokaryotic and eukaryotic microorganisms. The efficacy of thyme EO was excellent and comparable to that obtained applying hydrogel containing NaDCC with the total disappearance of microorganisms without any aesthetic alteration of the substrates (Bruno et al., 2023). Moreover, the simplicity in the preparation of the hydrogel and in its application and removal, even on large surfaces, make this cleaning protocol very attractive for future restorations of the stone cultural heritage, and further studies are now ongoing in the Colosseum Arena (Rome).

This contribution is dedicated to the memory of Laura Bruno, our colleague and friend, who passed away in May 2023.

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AUTORI

Lorenza Rugnini (Lorenza.Rugnini@uniroma2.it), LBA-Laboratory of Biology of Algae, Dept. Biology, University of Rome "Tor Vergata", Via Cracovia 1, 00133 Roma, Italy. Dept. Physical and Chemical Sciences, University of Aquila, Via Vetoio, Coppito, I-67100, L'Aquila, Italy.

Roberta Ranaldi. LBA-Laboratory of Biology of Algae, Dept. Biology, University of Rome "Tor Vergata", Via Cracovia 1, 00133 Roma, Italy. Francesco Gabriele, Cinzia Casieri, Nicoletta Spreti. Dept. Physical and Chemical Sciences, University of Aquila, Via Vetoio, Coppito, I-67100, L'Aquila, Italy.

Autore di riferimento: Lorenza Rugnini

Approcci omici per l'identificazione di vie biosintetiche per composti bioattivi nella diatomea *Thalassiosira rotula*

F. Di Costanzo, V. Di Dato, G. Romano

Il successo evolutivo delle diatomee è dovuto in gran parte alla loro plasticità metabolica che consente loro di sopravvivere in condizioni di vita diverse e, talvolta, ostili attraverso la produzione di una varietà di molecole bioattive utilizzate per la difesa, la comunicazione e l'adattamento ai cambiamenti ambientali. Alcune di queste molecole hanno dimostrato di essere di potenziale interesse anche per applicazioni industriali, incluso il settore farmaceutico. Negli ultimi anni abbiamo studiato il potenziale biotecnologico della *Thalassiosira rotula*, una diatomea centrica raccolta nel Golfo di Napoli. Il sequenziamento e la successiva analisi del trascrittoma di questa microalga hanno permesso di identificare diverse vie metaboliche coinvolte nella produzione di molecole bioattive, ed in particolare: secologanina, polichetidi, prostaglandine e fitosteroli. Il livello di espressione dei geni di questi pathways è stato valutato in differenti fasi della crescita e in condizioni di stress (Di Dato *et al.,* 2019).
Inoltre, studiando la comunità batterica associata a *T. rotula* abbiamo rivelato l'esistenza di tre nuove specie, nei cui genomi sono stati identificati diversi cluster di geni biosintetici. Di particolare interesse è la via di biosintesi di peptidi non-ribosomiali/polichetide sintasi di tipo 1 (NRPS/T1PKS), potenzialmente responsabile della produzione di interessanti composti bioattivi (Di Costanzo *et al.,* 2021).
Complessivamente, i nostri risultati mostrano la presenza di vie biosintetiche funzionalmente attive nella specie cosmopolita *T. rotula* e nella comunità batterica associata, aprendo la strada all'utilizzo di questi microrganismi per futuri sviluppi biotecnologici.

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AUTORI

Federica Di Costanzo (federica.dicostanzo@szn.it), Valeria Di Dato, Giovanna Romano, Dipartimento di Biotechnologie Marine Ecosostenibili, Stazione Zoologica Anton Dohrn Napoli (SZN), Via Ammiraglio Ferdinando Acton 55, Giardini Molosiglio, 80133, Napoli, Italy.
Autore di riferimento: Federica Di Costanzo

Investigations on the players involved in the production of polyhydroxybutyrate (PHB) by the Chlorophyta *Desmodesmus communis* under a mixotrophic cultivation regime

M. Franchini, G. Xamin, M. Simonazzi, C. Samorì, R. Pistocchi, L. Pezzolesi

Polyhydroxyalkanoates (PHAs) are a family of biobased polymers that act as energy and carbon storage in various microorganisms, including bacteria and microalgae. Thanks to their biodegradability as well as their thermoplastic and elastomeric properties, PHAs represent a promising alternative to conventional fossil fuel-based plastics. Among these compounds, the prevalent and best characterized is the short-chain biopolymer polyhydroxybutyrate (PHB). Currently, most of the studies investigating the production of PHB are focused on heterotrophic bacteria, whereas little is known about its synthesis in microalgae.
In this study, algae-based PHB production was obtained in the Chlorophyta *Desmodesmus communis* grown under mixotrophic conditions in batch cultivation mode (e.g., low light, phosphorus-free medium, 1 g/L Sodium Acetate (NaOAc)). To evaluate PHB yields and biomass composition in a semi-continuous cultivation, *D. communis* cultivation was scaled-up to a 10 L system. Algal growth was monitored for 27 days: part of the culture was harvested every 3-4 days and replaced with phosphate-free fresh medium with NaOAc addition. A PHB content of 34% w/w was achieved on day 8, corresponding to a maximum PHB productivity of 0.10 g PHB/g biomass/d (or 0.011 g PHB/L/d), which increased up to 54% w/w on day 15. The obtained biomass had a content of about 30% w/w proteins, followed by 6% w/w polysaccharides, 11 % w/w lipids, thus proving to be valuable from a biorefinery perspective [Pezzolesi et al., 2023].
To better characterise the mechanism of PHB production in this specific context, the bacterial community associated with *D. communis* was also investigated with the final aim of shedding light on its contribution to the PHB accumulation process. A metabarcoding analysis of the bacterial strains present in this PHB-inducing system was performed at different time points (day 0, day 12 and day 22) of the algal mixotrophic growth, in order to assess the composition and dynamics of the bacterial community during the semi-continuous cultivation. Moreover, insight on the bacteria that are most strictly interacting with *D. communis* was gained performing the same molecular analysis on samples that had been treated with a mix of antibiotics.
In addition to this molecular approach, bacteria have also been isolated on solid medium and a first 16s sequencing identification has been carried out on culturable bacteria. This isolation and identification step sets the basis for possible artificial bacterial-algae consortia study and optimization.

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AUTORI

Martina Franchini (martina.franchini5@unibo.it), Giulia Xamin, Mara Simonazzi, Rossella Pistocchi, Laura Pezzolesi Department of Biological, Geological and Environmental Sciences (BiGeA), University of Bologna, via Sant'Alberto 163, 48123 Ravenna, Italy;
Chiara Samorì, Department of Chemistry "Giacomo Ciamician", University of Bologna, via S. Alberto 163, Ravenna, Italy
Chiara Samorì, Rossella Pistocchi, Laura Pezzolesi Interdepartmental Centre for Industrial Research in Renewable Resources, Environment, Sea and Energy (CIRI-FRAME), University of Bologna, Via S'Alberto 163, 48123 Ravenna, Italy.
Autore di riferimento: Martina Franchini

Anti-viral and anti-cancer potential of small metabolites from the mass cultivated diatoms *Staurosirella pinnata* and *Cylindrotheca closterium*

S. Savio, S. La Frazia, C. Rodolfo and R. Congestri

Diatoms interact with environmental biotic and abiotic factors through small metabolites with diverse ecological roles, including communication, stress response, and defence (Brown et al., 2019). These compounds exhibit remarkable chemical diversity, encompassing oxygenated polyunsaturated fatty acids (oxylipins), modified amino acids (domoic acid, mycosporine-like amino acids), the polysaccharide chrysolaminarin, and carotenoids like fucoxanthin and diadinoxanthin (Stonik and Stonik, 2015). Notably, these substances exhibit significant bioactivities against various human pathologies, indicating the potential of diatom bioactives for pharmaceutical development in the drug discovery process (Savio et al., 2021).
In this study, we investigate the anti-viral and anti-cancer potentials of small metabolites obtained fractionating the crude extracts of two field-isolated benthic diatoms, that were mass cultivated under controlled conditions (25 °C, irradiance of 80 µmol photons m^{-2} s^{-1} and 12:12h L/D cycle) in indoor photobioreactors (8 L): the brackish strain of *Staurosirella pinnata* (Ehrenberg) D.M. Williams & Round, isolated from biofilm living on sediments of the Cabras Lagoon in Sardinia, and the marine strain of *Cylindrotheca closterium* (Ehrenberg) Reimann & J.C. Lewin, isolated from net samples in Latium Region. The extraction was performed on lyophilized biomasses using 20% methanol solution, as previously outlined in Savio et al. (2020), while the fractionation procedure applied a molecular weight criterion followed by the implementation of a Solid Phase Extraction (SPE) using a hydrophobic-hydrophilic gradient of solvents. The obtained fractions, composed by small metabolites with a molecular weight under 3 kDa, were firstly tested for their anti-viral activity on Feline coronavirus (FCoV), Sendai paramyxovirus (SeV) and H1N1-Wisconsin Influenza A virus (WSN). Subsequently, the anti-cancer potential was assessed recording cell death induction levels on a set of human cell lines, including cancers, such as melanoma ($A375^{BRAFV600E}$, $CHL\text{-}1^{BRAFwt}$) and medulloblastoma (HDMB03 and its radiation resistant derivative, HDMB03-R), using normal keratynocites (HaCaT) as positive control.
Anti-viral assays indicated that small metabolite fractions from *S. pinnata* and *C. closterium* significantly influenced the replication of FCoV, SeV and WSN viruses. Indeed, administering fractions at 2.0 mg/mL on infected cells, led to a robust inhibition of virus replication, reaching up to 97% inhibition on CoV for *S. pinnata*, up to 85% on SeV for both diatom fractions, and up to 81% on WSN by *C. closterium*. Furthermore, cytomorphological analysis conducted on FCoV infected cells, revealed a cytoprotective activity by *S. pinnata*, as evident by the absence of cytomorphological aberrations typically induced by the virus infection.
Anti-cancer tests were performed using cytofluorimetric methods (FACS), revealing a strong selective cytotoxic effect of both small metabolite fractions on the melanoma cell lines. The percentage of cell death reached up to 70% for *S. pinnata* and 55% for *C. closterium* at the highest dose used (10 mg/mL), without significant detrimental effects on non-malignant keratinocytes. In addition, the fraction of *S. pinnata* also proved to be effective in inducing cell death on medulloblastoma cells, with a percentage of cell death levels up to 52% on the radiation resistant derivative HDMB03-R.
In conclusion, the reported results underscore the significant potential of the small metabolites derived from *S. pinnata* and *C. closterium*, positioning them as promising candidates for the development of anti-viral and anti-cancer drugs.

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AUTORI

Saverio Savio (saverio.savio@gmail.com), Simone La Frazia, Carlo Rodolfo and Roberta Congestri, Department of Biology, University of Rome 'Tor Vergata', Via della Ricerca Scientifica snc., 00133, Rome, Italy
Autore di riferimento: Saverio Savio

Preliminary characterization of two *Spirulina* strains from the North Adriatic Sea (Italy)

K. Sciuto, A.A. Sfriso, M.A. Wolf, A. Sfriso, E. Moschin, C. Munari, M. Mistri, I. Moro

Cyanobacteria and microalgae are photoxygenic microorganisms that can be found in different habitats worldwide, even those considered extreme. To cope with the typical environmental conditions where they live, but also to face sudden possible environmental changes, these microorganisms can adopt several strategies, including the production of bioactive compounds (e.g., Andersen, 1992; Whitton, 2012; Sciuto & Moro, 2015; Malcata *et al*., 2018). Many of the compounds produced by cyanobacteria and microalgae can be employed in different human fields (e.g., Levasseur *et al*., 2020; Alvarez *et al*., 2021; Kiran & Venkata Mohan, 2021), and projects aiming at finding new biomolecules and/or new exploitation ways of these photoxygenic microorganisms are increasing in the last years. Since the capability to produce some compounds by cyanobacteria and microalgae varies according to the taxa and there can be differences even among strains belonging to a same genus and/or species (e.g., Lauritano *et al*., 2016; Levasseur *et al.,* 2020), the correct identification and characterization of these photoxygenic microorganisms are at the base of every more practical study. Nevertheless, cyanobacterial and microalgal biodiversity and potentialities are still underestimated, in particular for some taxa (e.g., Gaignard *et al.,* 2019) and/or habitats (e.g., Jaspars *et al*., 2016; Cordeiro *et al*., 2020). In this context, the research project "Biotechnological potentials of microalgae for environmental sustainability" has been recently founded by the European Social Fund (ESF) - Italian National Operational Programme (NOP) on Research and Innovation 2014-2020, with the aim of isolating several cyanobacterial and microalgal strains from the environment and of characterizing them to understand their potentialities, with a focus on marine and transitional water habitats. Here we present the preliminary data obtained during the characterization of two *Spirulina* strains from the North Adriatic Sea (Italy). More precisely, the two cyanobacterial strains (temporarily tagged as strain KS5 and strain KS6) were sampled from a microbial biofilm growing on the glass walls of an artificial aquarium, filled in with water taken from Santa Maria del Mare (Venice Lagoon) and used to grow the seagrass *Cymodocea nodosa* (Ucria) Ascherson. In order to better identify and characterize these two cyanobacterial strains, molecular analyses were carried out, as well as morphological (light microscopy and scanning electron microscopy) and ultrastructural (transmission electron microscopy) observations. Biochemical analyses are, instead, still underway. Strain phylogeography and problems regarding the systematics of the genus *Spirulina* Turpin ex Gomont will also be discussed.

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AUTHORS

Katia Sciuto (katia.sciuto@unife.it), Andrea A. Sfriso, Cristina Munari, Michele Mistri, University of Ferrara, Department of Chemical, Pharmaceutical and Agricultural Sciences, via L. Borsari 46, 44121 Ferrara. Marion A. Wolf, Adriano Sfriso, Ca' Foscari University of Venice, Department of Environmental Sciences, Informatics and Statistics, via Torino 155, 30172 Venezia-Mestre. Emanuela Moschin, Isabella Moro, University of Padova, Department of Biology, via U. Bassi 58/B, 35131 Padova.
Autore di riferimento: Katia Sciuto

The role of Phytochelatin Synthase in the microalga *Scenedesmus acutus* M. (Sphaeropleales)

M. Ferrari, R. Cozza, M. Marieschi, R. Ruotolo, A. Torelli

Phytochelatins (PCs) are small cysteine-rich peptides that are not genetically encoded but are synthesized by Phytochelatin Synthase (PCS) in the presence of glutathione as substrate. The constitutive expression of PCS and the presence of homologues of the PCS gene(s) in plants growing in ecosystems geographically remote from metal-contaminated sites, as well as in representatives of various kingdoms of living organisms, suggest that PCS has a wide range of different functions (Clemens et al. 2009). However, the main function of PCs in plants is to immobilize, sequester, and detoxify metal ions (Shukla et al. 2013). The effect of metals on the activity of PCS can differ not only between species, but also between different isoforms of the enzyme within a species.
Although it is evident that chromium (Cr) ions can stimulate the formation of PCs in plants (Yu et al. 2018), there is no information on their presence in algae in response to Cr exposure.
In this study, two strains of the green alga *Scenedesmus acutus* with different Cr(VI) sensitivity, namely the wild type (wt) and the chromium-tolerant strain (Cr-t), were selected as a model algal species to increase the knowledge on the role of PCs in Cr responses in microalgae.
We previously reported the first evidence for a PCS gene (*SaPCS*) from the microalga *S. acutus*. More recently, we have also identified two *SaPCS* isoforms due to a putative alternative splicing.
To define the role of PCS in Cr detoxification, we analyzed the levels of *SaPCS* transcription, and the abundance of PCS by using RT-qPCR and Western blot, respectively, in both strains of *S. acutus* after 24h culture in standard and in Cr supplemented medium (1 and 2 mg Cr (VI)/l).
Given the relationship between sulfur (S) metabolism and Cr(VI) tolerance in *S. acutus* (Sardella et al. 2019), we also performed the same analyses in S-replete cells of both strains after medium renewal following S-starvation.
The results showed a different behavior between strains in the stress response. RT-qPCR analysis revealed an increase in *SaPCS* transcription after Cr(VI) stress and after medium renewal following S-starvation in wt cells. Instead, the Cr-t strain induced *SaPCS* transcription only under S-replete conditions.
Western blot analysis, performed with a polyclonal antibody raised against PCS of *Arabidopsis thaliana*, revealed an immunoreaction signal on two proteins with molecular weights of approximately 73 and 37 kDa. While the abundance of the 73 kDa protein did not change after the stresses studied in the two strains, the 37 kDa protein increased after S-starvation in both strains but in a significant manner only in wt.
Finally, a preliminary quantification of PC production was performed, through LC Mass analysis in the two strains in preculture with or without S and under 1 mg Cr(VI)/l, which showed no modulation of PC abundance in response to Cr(VI) stress and/or S deprivation. Nevertheless, the Cr-t strain maintained PCs level significantly higher than the wt at the end of S-starvation.
These observations suggest that PCS in *S. acutus* may not be involved in Cr(VI) detoxification, but may play a role in intracellular sulfur balance or in the cell homeostasis during stress.
The PC production in the other experimental conditions (i.e. exposures longer than those studied and different Cr(VI) concentrations) should be investigated to better clarify the role of the enzyme in Cr(VI) detoxification.

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AUTORI

Michele Ferrari (michele.ferrari@unical.it), Radiana Cozza, Department of Biology, Ecology and Earth Science, University of Calabria, Via P. Bucci, 87036 Rende (CS).
Matteo Marieschi, Roberta Ruotolo, Anna Torelli, Department of Life Sciences, University of Parma, Parco Area delle Scienze 11/A, 43124 Parma
Autore di riferimento: Michele Ferrari

Phytoplankton research at SZN: from the sea into the lab and back again

W.H.C.F. Kooistra

About 45 years ago, several SZN researchers teamed up to gather and share oceanographic and biological data for common research purposes. They assigned a station, called MareChiara, at the transition between coastal and offshore water in the Gulf of Naples (GoN); the result, the LTER project MareChiara. Phytoplankton diversity has been studied there ever since by means of cell counts in LM. Data remain comparable over the years by means of rigorously maintained protocols. But rigour sits in the way of change. So, since 2019, its sister project NEREA explores the use of innovative technologies (e.g., -omics) to study the GoN. Its stations, chosen along a gradient, include, besides MareChiara, the mouth of the Sarno and a canyon off Capri. In addition, links with SZN's Amendolara station and institutes elsewhere, and participation in global projects offer further opportunities for comparative research. So, how do all these initiatives advance our knowledge?
Physico-chemical data from the GoN show winter mixing (≥14 °C); early spring reduction of exchange and halocline creation through influx of nutrient-rich runoff; summer and early autumn thermocline (≤28 °C); and autumnal thermocline fading, vertical mixing and flushing (Kokoszka et al. 2022). This seasonality correlates with a recurrent succession of diverse communities of phytoplankton, protists, bacteria and animals. The observations enable formulation of hypotheses about the who, when, where and why of phytoplankton diversity and complexity, testable by means of experimental designs in the lab. In their turn, results of the lab experiments enable designing tools with which these hypotheses can then be tested *in situ* by means of observational and experimental approaches, e.g., in the project NEREA. One example regards *Chaetoceros*. Cell counts show high diversity and marked seasonality in this centric diatom genus, but there are also numerous unidentified morphs. Thus, many strains were established, especially of unidentified specimens. The result: apart from species already known for the GoN, several already described ones new for the GoN and ones new to science (Gaonkar et al. 2018). Their reference sequences were used to reveal their seasonality in metabarcode data from MareChiara (Gaonkar et al. 2020) and their biogeography in TARA Oceans and OSD data (De Luca et al. 2021). The result: many additional common, but unknown species. Another example regards *Pseudo-nitzschia multistriata*. This pennate diatom appeared in the LTER MC cell counts since Autumn 1996 and occurs nowadays from late summer into early winter. Cell length measurements in samples showed steady decreases followed by the appearance of a bimodal length distribution due to the appearance of large cells every second year, a pattern suggestive for a life cycle of two years (D'Alelio et al. 2010). The sexual cycle could also be studied in the lab, enabling finding out how partner recognition, meiosis, gamete fusion, and auxosporulation works, using experimental approaches and transcriptomics (Annunziata et al. 2022). Observing sex in situ is near-impossible with LM, but metabarcoding and LM reveal when the species occurs and metatranscriptomics of those same samples can then spot in the field those genes that according to the transcriptomics data are upregulated markedly or exclusively during sex. Similarly, a range of phenomena can be observed in field data and samples, explanations proposed - and tested - under controlled lab conditions, and then, with the proper tools also in situ (e.g., Santin et al. 2021). How do cells deal with nutrient scarcity? How do they respond to grazers? What entices them to go into senescence? Obtained results can be interconnected and brought into broader context thanks to the works of the many colleagues involved nowadays in LTER MC and NEREA. It requires cooperation and rapid sharing of data to learn a great many things about the ecosystem functioning in the GoN and in the global oceans.

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AUTORE

Wiebe H.C.F. Kooistra (kooistra@szn.it), Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italia

SESSIONE I

Moderatore: Stefano Accoroni

Combining molecular, morphological and biogeographical approaches to characterize two potentially new species within the genus *Chaetoceros*

A. Mussi, T. Greca, M. Montresor, D. Sarno, W.H.C.F. Kooistra

Among marine planktonic diatoms, *Chaetoceros* is arguably the most diverse genus, with well over 250 already described species, exhibiting high variety in cell morphology and ultrastructural details. The hallmark of this genus are the so-called setae, hollow silica tubes that link sibling cells forming chains of various length. *Chaetoceros* displays a worldwide distribution and represent an ideal target for ecological and evolutionary studies in marine phytoplankton. Multiple studies conducted in the Gulf of Naples (GoN) and elsewhere revealed marked cryptic diversity as well as several species new to science (Kooistra et al., 2010; Gaonkar et al., 2018; Li et al., 2017). From the net samples collected in the framework of the CRIMAC projects along the Calabrian Ionian coast, two strains were isolated and characterized combining molecular, morphological and ecological approaches. Amplification and sequencing of 28S rDNA and 18S rDNA markers, resolved the phylogenetic position of the specimens within the genus. In particular, one was found to belong to the *Chaetoceros lorenzianus* complex, and the other as a close relative of *Chaetoceros lauderi*. Morphological characterization was carried out using light microscopy, for the general chain and cells morphology, as well as scanning and transmission electron microscopy to observe the ultrastructure details of setae and valves, which are informative characters for species discrimination. Metabarcode data from the Long-Term Ecological Research station MareChiara (LTER-MC) in the GoN were used to detect the presence, abundance and distribution of these specimens along the seasonal cycles (Gaonkar et al., 2020), showing that they occur especially in the spring to summer period. Moreover, reference V4 sequences were blasted against the metabarcode database metaPR² (Vaulot et al., 2022) to map the distribution of the two species, revealing they are quite widespread, being found both in the Atlantic and Pacific oceans. All the information gathered was used to compare the new specimens with their closest relatives in order to understand if they could represent species new to science, or instead they just constitute geographical or ecological variants of already known species.

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Autori

Alessandra Mussi (alessandra.mussi@szn.it), Marina Montresor, Wiebe Kooistra, Dipartimento di Ecologia Marina Integrata (EMI), Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italia.
Teresa Greca Dipartimento di biologia, ecologia e scienze della terra (DiBEST), Università della Calabria, 87036 Arcavacata (CS), Italia; Dipartimento di Ecologia Marina Integrata (EMI), Stazione Zoologica Anton Dohrn, C. da Torre Spaccata, Località Torre Spaccata, 87071 Amendolara (CS), Italia.
Diana Sarno, Dipartimento di infrastrutture per la ricerca e le risorse in biologia marina (RIMAR), Stazione Zoologica Anton Dohrn, Villa Comunale 80121 Napoli, Italia.
Autore di riferimento: Alessandra Mussi

Insights into the ecology of cryptic phytoplankton species at LTER-MC (Gulf of Naples) using an 11 years-metabarcoding dataset

N. Romillac, G. Zampicinini, A. Zingone, D. Sarno

The description of the seasonality and ecological niche of phytoplankton species has been historically based on time series of light microscopy (LM) observations. However, identification in LM is not always possible for species of small size, species damaged by fixation and for cryptic species, which are morphologically identical. Recently, DNA metabarcoding has allowed the description of the whole planktonic community in great details but has been less applied to the study of single species.
Here, we use a decadal V4-18s rDNA metabarcoding dataset from the long-term ecological research site MareChiara (LTER-MC), located in the Gulf of Naples (Italy), to describe the seasonality and ecological niche of several phytoplankton taxa: the (pseudo)cryptic diatoms of the *Leptocylindrus* genus and of the *Chaetoceros curvisetus* complex, the small dinoflagellates of the *Heterocapsa* genus and the small Mamiellophyceae of the *Micromonas* genus.
Metabarcoding identified three species of *Leptocylindrus* lumped together in LM, *L. danicus* and *L. hargravesii*, distinguishable by ultrastructural features, and *L. aporus*, distinguishable by chloroplast shape. The last two species co-occurred in summer while the first one had a bi-modal distribution, being present in spring and autumn. All three *Leptocylindrus* species shared similar ecological niches. In the *Chaetoceros curvisetus* complex, three distinct lineages succeeded one another along the year and occupied different ecological niches. For the genus *Micromonas*, which is not detected in fixed material, an unexpectedly high number of recently described species (six) were found to live in sympatry in the Gulf of Naples. Most of them co-occurred in winter and shared similar ecological niches, while one species preferred the warm and stratified waters of late summer. Notably, *M. pusilla* (the only species known in the genus until 2016) had been previously reported in live-material studies from the area, but the taxon deserving this name did not show up in the metabarcoding data. In the genus *Heterocapsa*, metabarcoding showed at least three species corresponding to the late spring peak observed in LM, plus the regular occurrence of a winter *H. rotundata* which had gone unnoticed in LM. However, the species identification of *Heterocapsa* in metabarcoding was complicated by the poorly known taxonomy of the genus and by several species sharing the same sequence for the marker gene used in this study.
These results provide new insights into the ecology and distribution of some species not identified in LM. They also improve the knowledge on cryptic and pseudo-cryptic microalgal species previously obtained on a few-years study or with cultivation techniques, thus highlighting the value of metabarcoding studies at LTER sites. The results on the co-occurrence or niche separation among cryptic species contribute to the discussion on the mechanisms of speciation and co-existence of morphologically similar species and in general, on the factors shaping the ecological niche and temporal distribution of phytoplankton taxa.

AUTORI

Nicolas Romillac (nicolas.romillac@szn.it), Adriana Zingone, Diana Sarno, Research Infrastructure for Marine Biological Resources Department, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy
Gianpaolo Zampicinini, Integrative Marine Ecology Department, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy, Division of Oceanography, National Institute of Oceanography and Applied Geophysics - OGS
via Auguste Piccard 54, 34151 Trieste, Italy
Autore di riferimento: Nicolas Romillac

From metabarcoding time series to plankton food webs

L. Russo, D. Bellardini, G. Zampicinini, F. Jordán, R. Congestri, D. D'Alelio

The advent of metabarcoding (metaB) in aquatic ecology has provided a huge amount of information on plankton biodiversity worldwide. However, the large datasets obtained with that approach are still partially explored, especially for what concerns the study of trophic interactions and food webs. In this study, we analysed a planktonic metaB dataset (90% protists, 10% metazoa) obtained by high-throughput sequencing (HTS) of PCR amplicons of the V4 and V9 regions of the 18S ribosomal RNA gene, that allowed a high taxonomic resolution of plankton at the genus level, from 48 samples collected over three years (January 2011-December 2013) at the Long-Term Ecological Research station MareChiara (LTER-MC) in the Gulf of Naples (Russo et al., 2023). Our aim was to analyse the metaB dataset and to integrate it within an ecological framework in order to describe the link between plankton diversity and food-web structure. Specifically, we derived co-occurrence networks (correlations of co-occurring taxa over time) from the metaB time series and identified putative trophic interactions among co-occurrences based on information about plankton (body size and trophic habit) retrieved in the literature, then we converted the co-occurrence networks obtained into a conceptual models of food web. The model showed structural properties resembling ecological processes, such as trophic hierarchy and modularity that were driven by dimensional differences between predator and prey. Moreover, thanks to the high taxonomic resolution of metaB analysis, we also analysed the role of planktonic organisms in maintaining network modularity, evidencing a crucial role of planktonic microalgae. Indeed, we found that micro- and meso-planktonic mixotrophic and heterotrophic microalgae occupied distinct modules of the network, suggesting trophic niche partitioning, whereas the nanoplanktonic primary producers worked as fundamental connectors between larger predators (and different modules).

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AUTORI

Luca Russo (luca.russo@students.uniroma2.eu), Ph.D. Program in Evolutionary Biology and Ecology, Department of Biology, University of Rome 'Tor Vergata', Roma, Italy.
Daniele Bellardini, Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Naples, Italy - DiSTAV. Department of Earth, Environment and Life Sciences, University of Genoa, Genoa, Italy.
Giampaolo Zampicinini, Department of Oceanography, National Institute of Oceanography and Applied Geophysics, Trieste, Italy.
Ferenc Jordán, Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy
Roberta Congestri, Laboratory of Biology of Algae, Department of Biology, University of Rome 'Tor Vergata', Rome, Italy.
Domenico D'Alelio, Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Naples, Italy.
Autore di riferimento: Luca Russo

From the exploration of diatom sex determination mechanisms to the detection of diatom sex events at sea

A. Broccoli, M. T. Russo, D. Sarno, P. von Dassow, M. Montresor, M. I. Ferrante

Sexual reproduction is a conserved feature in eukaryotes. Many different sex-determination systems have evolved in different lineages. In unicellular organisms, including diatoms, key primary producers in the marine environment, opposite sexes are called Mating Types (MT). In 2018[1], the first diatom MT-determining gene was identified in the planktonic species *Pseudo-nitzschia multistriata*: expression of the gene *MRP3* defines the MT+. *MRP3* regulates the expression of four downstream genes, *MRP1* and *MRP2* which are upregulated in MT+, and *MRM1* and *MRM2* in MT-. Currently, no other sex-determining genes are known for other diatom species.
In this project, we first asked if this simple system is conserved among diatoms, focusing attention on the species-rich genus *Pseudo-nitzschia.* MT+ and MT- strains for *Pseudo-nitzschia* species are being isolated from the Gulf of Naples and the Chilean coasts, then a transcriptomic approach will be applied to compare their gene expression profiles and the genomes of two selected species will be sequenced.
Differential gene expression analysis between opposite MTs is currently ongoing for *P. arenysensis* and preliminary analyses suggest conservation of *MRP2, MRP3* and *MRM2* and their regulation. The next goal will be the definition of the precise sex determination mechanism. The isolation of strains for additional species is in progress. Sexual reproduction has consequences for the evolutionary trajectories of diatom lineages, as well as direct, bottom-up ecological consequences.
Sex events are rare and difficult to detect in the natural environment using classical approaches. The genes identified in this project should represent suitable markers to detect diatom sexual reproduction in the environment. In this respect, we will explore the metatranscriptomic data produced within the worldwide survey TARA Oceans (http://www.taraoceans-dataportal.org/) and the Neapolitan NEREA (https://www.nerea - observatory.org/), with the aim to reveal where and how frequently this key phase of diatom life cycles occurs. Molecular tools to trace sexual events at sea could represent a remarkable advance in understanding diatom ecology.

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AUTORI

Andea Broccoli (andrea.broccoli@szn.it), Monia Teresa Russo, Diana Sarno, Marina Montresor, Maria Immacolata Ferrante, Stazione Zoologica Anton Dohrn, Naples, Italy;
Peter von Dassow, Facultad de Ciencias Biológicas, Instituto Milenio de Oceanografía, Pontificia Universidad Católica de Chile, Santiago, Chile;
Maria Immacolata Ferrante, National Institute of Oceanography and Applied Geophysics, Triest, Italy.
Autore di riferimento: Andrea Broccoli

Analisi molecolari rivelano la presenza del genere *Corynecladia* (Rhodophyta, Rhodomelaceae) in Mediterraneo con due nuove specie: *C. millarii* sp. nov. and *C. mediterranea* sp. nov.

Y. Metti, G. Furnari, D. Serio

I generi appartenenti al *Laurencia* complex, fino ad oggi segnalati in Mediterraneo, sono *Laurencia* J.V. Lamouroux (con 8 specie e 1 sottospecie), *Laurenciella* Cassano, Gil Rodríguez, Sentíes, Díaz-Larrea, M.C. Oliveira *et* M.T. Fujii (1 specie), *Osmundea* Stackhouse (7 specie) e *Palisada* (Yamada) K.W. Nam (5 specie).
In questo studio viene segnalato per la prima volta in Europa, il genere *Corynecladia* J. Agardh (Metti *et al*., in press), fino ad oggi endemico dell'Australia (Metti *et al*., 2015; Cassano *et al*., 2019), con due nuove specie: *Corynecladia millarii* sp. nov. e *C. mediterranea* sp. nov..
Lo studio è basato su esemplari raccolti lungo le coste siciliane nell'ambito della revisione delle specie mediterranee appartenenti al *Laurencia* complex e in particolare su esemplari morfologicamente simili a *Laurenciella marilzae* (Gil-Rodríguez, Sentíes, Díaz-Larrea, Cassano & M. T. Fujii) Gil-Rodríguez, Sentíes, Díaz-Larrea, Cassano & M. T. Fujii (Serio *et al*. 2000; Furnari *et al*., 2001; Serio *et al*., 2020).
L'analisi dei campioni è stata effettuata utilizzando sia osservazioni sui caratteri morfologici, anatomici e riproduttivi sia analisi filogenetiche delle sequenze nucleotidiche dei cloroplasti (rbcL) e dei mitocondri (COI-5P). L'analisi molecolare degli esemplari mediterranei ha consentito di distinguere due nuove specie all'interno del clade che raggruppa le specie del genere *Corynecladia*. Dal punto di vista morfologico esse differiscono dai generi *Laurencia* and *Laurenciella*, per la posizione profonda delle sinapsi secondarie tra le cellule corticali, dalle altre specie dello stesso genere, per l'assenza di corticazione secondaria e di granuli d'amido nelle cellule midollari e tra loro solo per le sequenze molecolari diverse.

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AUTORI

Yola Metti, Australian Institute of Botanical Science, Botanic Gardens of Sydney, 362 Narellan Rd, Mount Annan NSW 2567, Australia.
Giovanni Furnari, Donatella Serio (d.serio@unict.it), Dipartimento di Scienze Biologiche, Geologiche e Ambientali dell'Università di Catania, Via Empedocle, 58 – 95128 Catania.
Autore di riferimento: Donatella Serio

First record of the invasive brown alga *Rugulopteryx okamurae* in the southern Adriatic Sea (Bari, Italy)

A. Tursi, A. Mincuzzi, A. Bottalico

The invasive brown alga *Rugulopteryx okamurae* (E. Y. Dawson) I. K. Hwang, W. J. Lee & H. S. Kim, native to the western Pacific Ocean, was first reported in the Mediterranean Sea in the Thau Lagoon (France) (Verlaque *et al*. 2009). Since its introduction, this species has rapidly spread along western Mediterranean coasts, suffocating native communities and leading to massive losses in the biodiversity of coastal zones (García-Gómez *et al*. 2020). The success of *R. okamurae* can be attributed to biochemical defences against herbivores, such as sulfuric acid in vacuoles and high concentrations of dilkamural, as well as high fiber content acting as physical barriers against grazing (Casal-Porras *et al*., 2021).
Rugulopteryx okamurae was spotted along the coast of Bari (Italy, southern Adriatic Sea) in April 2023, in a portual area intensively urbanized and with several organic inputs. Then, surveys were performed to collect specimens for morpho-anatomical observations and molecular confirmation by the *psbA* and *rbcL* gene portions.
The morphology and anatomy of the collected samples fitted with those reported in literature for *R. okamurae* (Hwang *et al.*, 2009; Terradas-Fernández *et al.*, 2023). In particular, in cross section, the median part of the thallus was characterized by 2-3-(4) layers of medullar cells near the margin, becoming monostromatic in the central medulla, Molecular results of the *psbA* and *rbcL* gene portions confirmed the attribution to *R. okamurae*. This species has recently been reported along the north-western coast of Sicily, in the Gulf of Palermo (Bellissimo *et al.*, 2023). Therefore, our record is currently the easternmost of the Mediterranean Sea.
Further surveys, including periodic underwater monitoring, will be carried out to implement the knowledge about the distribution of the species along the coast of Bari, also aiming at assessing the effects of this species on the native photophilic communities.

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AUTORI

Andrea Tursi, Antonella Bottalico (antonella.bottalico@uniba.it), Department of Biosciences, Biotechnologies and Environment (DBBA), University of Bari "Aldo Moro", Via Orabona 4, 70126, Bari, Italy.
Annamaria Mincuzzi, annamaria.mincuzzi@uniba.it, Department of Soil, Plant and Food Sciences. University of Bari "Aldo Moro", Via Amendola 126, 70126, Bari, Italy.
Autore di riferimento: Antonella Bottalico

A new *Dasysiphonia* (Delesseriaceae, Rhodophyta) species discovered in the North Adriatic Sea (Mediterranean)

M.A. Wolf, K. Sciuto, A. Buosi, M. Orlando-Bonaca, A. Fortič, A. Sfriso

Dasysiphonia I.K. Lee & J.A. West is a genus of the family Delesseriaceae (Rhodophyta) including 9 taxonomically accepted species (Guiry and Guiry, 2023). Among these, only one is currently reported in the Adriatic Sea (Mediterranean): *Dasysiphonia japonica* (Yendo) H.-S. Kim. This invasive red seaweed is native to the Hokkaido Island (Guiry and Guiry, 2023), one of the major oyster production areas in Japan (about 700 tons a year; Hasegawa *et al.*, 2015). The species was first recorded in Europe near an oyster culture area in Brittany (France) in 1984 and in Galicia (Spain) in 1988 (Sjøtun *et al*., 2008). The introduction into Europe and its dispersal in the Mediterranean is most likely due to its association with the Pacific oyster *Magallana gigas* (Thunberg, 1793) (Sjøtun *et al*., 2008). This seaweed is spreading aggressively in coastal waters with blooms that have been associated with fish die-offs (Fofonoff *et al.*, 2018).

In this study we reported the first finding of another species belonging to the genus *Dasysiphonia* in the Mediterranean Sea. Samplings of this taxon were carried out by the Marine Biology Station Piran of the National Institute of Biology in Slovenian coastal waters, and thalli of the putative new species were analyzed both by molecular and morphological methods and compared with the other known taxa. Samples of the invasive *D. japonica* were collected in different sites of the Venice Lagoon (Italy) during monitoring campaigns of the project MoVEco to assess the ecological status of the lagoon in the framework of the European Water Directive (2000/60/EC). In the phylogenetic reconstruction, based on the plastid *rbcL* gene, the Slovenian samples were well distinct from all the species included in the genus *Dasysiphonia* as well as from those which were recently transferred from the sister genus *Dasya* (Cassidy *et al.*, 2022). The morphological characters were analyzed and compared with those of the species *D. japonica* in order to estimate the invasive potential of this new taxon.

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AUTORI

Marion Adelheid Wolf (marion.wolf@unive.it), Alessandro Buosi, Department of Environmental Sciences, Informatics and Statistics (DAIS), University Ca' Foscari Venice, Via Torino 155, 30172 Mestre (Venice), Italy.
Katia Sciuto, Department of Chemical and Pharmaceutical Sciences, University of Ferrara, Via Fossato di Mortara 17, 44121 Ferrara, Italy.
Martina Orlando Bonaca, Marine Biology Station Piran, National Institute of Biology (NIB), Fornače 41, 6330 Piran, Slovenia.
Ana Fortič, Marine Biology Station Piran, National Institute of Biology (NIB), Fornače 41, 6330 Piran, Slovenia.
Adriano Sfriso, Department of Environmental Sciences, Informatics and Statistics (DAIS), University Ca' Foscari Venice, Via Torino 155, 30172 Mestre (Venice), Italy.
Autore di riferimento: Marion Adelheid Wolf

SESSIONE II

Moderatrice: Lorenza Rugnini

Paralytic shellfish poisoning (PSP) toxin profiles of *Alexandrium minutum* strains isolated from different Italian marine regions

G. Zoffoli, F. Guerrini, L. Pezzolesi, M. Cangini, S. Dall'Ara, R. Pistocchi

Alexandrium minutum is a globally distributed dinoflagellate known for producing paralytic shellfish toxins (PSTs), which can threaten human and ecosystem health. Its presence is closely monitored and there is strong evidence that its blooms are expanding into previously unobserved locations.
In Italy, *A. minutum* periodically causes blooms in the Northern Adriatic Sea and in the coastal waters of Sardinia, Sicily, and Calabria. Intoxications due to paralytic shellfish poisoning (PSP) associated with these blooms have never represented a serious problem for the Northern Adriatic Sea, except for a few isolated cases (Honsell et al., 1995; Milandri et al., 2008). On the contrary, along the coasts of Sardinia, PST concentrations even higher than the regulatory limit have been regularly found in bivalve molluscs since 2002, despite the low concentration of *A. minutum* cells in the water column (Bazzoni et al., 2020). This is not the case for the Ionian and Tyrrhenian coasts of Sicily and Calabria, where *A. minutum* causes rather large blooms that can be detected for the water discoloration but which often go uncharacterized, due to the lack of regular monitoring of areas not subjected to shellfish production (Costa et al., 2021; Pistocchi et al., 2021).
In this work, a comparison was made between different strains of *A. minutum* isolated from different Italian marine regions during some of the above-mentioned blooming events. Some of them were previously analysed; however, this study applied an updated and optimised method for the extraction and analysis of PSTs in algal matrix, developed at the Marine Research Centre of Cesenatico (CRM). PST levels in mussels during the same events were also evaluated.
The first strain was isolated from the Gulf of Trieste in 1994, corresponding to the first toxic event caused by PSTs in the North Adriatic Sea (Honsell et al., 1995). Five strains were isolated in 2018 from the Jonian coast of Calabria at Rocella Jonica, where there are no mussel farms, but two successive bloom events, the first in 2018 and the second in 2020, caused a yellow/brown water discoloration (Pistocchi et al., 2021). Additionally, eight strains of *A. minutum* isolated between February and April 2023 from the Gulf of Oristano in Sardinia, where blooms of this dinoflagellate have been occurring for years (Lorenzoni et al., 2023), were considered.
All the strains presented a common profile characterised by GTX1-4, GTX2-3 and neoSTX. The ones from Calabria contained the lowest levels of STXs (28 fgSTXs/cell for the most toxic strain), followed by the strains isolated from the Gulf of Oristano, Sardinia (243 fgSTXs/cell for the most toxic strain). All these strains were dominated by GTX1-4. *A. minutum* strain isolated from the Gulf of Trieste, despite almost 30 years in culture, was the one that maintained the highest STXs values (535 fgSTXs/cell), with an unusual prevalence of GTX2-3.
A. minutum blooms are historically rare. However, there has been an increase in the occurrence of intense toxic blooms, particularly along the coasts of southern Italy and the islands. This is a cause for concern and highlights the need for increased monitoring and surveillance.

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AUTORI

Giorgia Zoffoli (giorgia.zoffoli3@unibo.it), Franca Guerrini, Laura Pezzolesi, Rossella Pistocchi, Department of Biological, Geological and Environmental Sciences, University of Bologna Ravenna Campus, Via Sant'Alberto 163, 48123 Ravenna
Monica Cangini, Sonia Dall'Ara, National Reference Laboratory for Marine Biotoxins (FCRM), Viale Vespucci 2, 47042 Cesenatico (FC)
Autore di riferimento: Giorgia Zoffoli

Valorization of wastewaters from carp (*Cyprinus carpio*) farming: towards a sustainable circular economy model for algal cultivation and fish farming sectors

M. Simonazzi, V. Bolletta, F. Guerrini, R. Pistocchi, L. Pezzolesi

In recent years, algal cultivation has gained greater attention thanks to its many benefits. Microalgae can produce valuable compounds such as pigments, proteins, polysaccharides, lipids and essential fatty acids, which are exploitable in different economic sectors (e.g., feed and food, cosmetics, agriculture). Nonetheless, many factors can negatively affect the cost of large-scale production of microalgae, namely energy, harvesting, and water and nutrients supply. Balancing the pros and cons of algal cultivation can be challenging and several approaches have been suggested to solve this bottleneck. In this context, models based on circular economy can bring great benefit to all levels, by simultaneously reducing the costs associated with algae cultivation and enhancing the re-use of industrial wastes. Among emerging economic sectors, aquaponics (i.e., the integration of aquaculture and fish farming with plant cultivation) may represent an attractive option for algal cultivation, as nutrient-rich wastewaters generated by the fish can sustain both plant and algal growth.
In the present study, wastewater from a "Koi" carp (*Cyprinus carpio*) aquaponics farming was preliminary assessed as a feasible growth medium for algae. A 10-days batch cultivation was set up using filtered aquaponics water (8 µm) and two Chlorophyta were tested, i.e., *Chlorella vulgaris* and *Desmodesmus communis*, each proven to be suitable candidates for phycodepuration and attractive for their high-valuable biomass composition. *C. vulgaris* and *D. communis* grew in this wastewater without further nutrient replacement, both reaching a maximum dry weight of 0.8-1.0 g L^{-1}. Phosphates (20-25 mg L^{-1}) were completely removed by both Chlorophyceae within 5 days, while nitrates had been halved but were still abundantly available at the end of the cultivation period, passing from 400-450 to 200-250 mg L^{-1}. Afterwards, the potential of aquaponics' wastewater as algal growth medium was assessed in semi-continuous operative mode, following the cultivation of *D. communis* as an emerging candidate for aquafeed applications and previously proven to produce, under conditions of low light and with an organic carbon source (mixotrophy), the biopolymer polyhydroxybutyrate (PHB), a valuable polyester possessing thermoplastic and elastomeric properties (Pezzolesi et al., 2023). The semi-continuous cultivation was followed for 43 days in 1 L glass bottles (triplicates). About one-third of culture was harvested every 4-7 days for biomass composition analyses and replaced with fresh filtered wastewater. In parallel, sub-cultures of *D. communis* were prepared by taking aliquots from the bottles between one biomass harvest and another, i.e., when phosphates were limited; these sub-cultures were subjected to mixotrophic conditions (low light and sodium acetate supply) to also test the potential accumulation of PHB. Throughout the semi-continuous cultivation, the biomass yield did not decline and remained in the range 0.2-0.5 g L^{-1}, as well as algal photosynthetic efficiency (0.7-0.8 and 0.4-0.5, maximum and effective quantum yield, respectively), suggesting that the aquaponics' water did not affect *D. communis* physiology while sustaining its growth. The algal biomass composition did not vary, maintaining a high protein content (>30%dw). On the contrary, in sub-cultures placed under mixotrophic conditions, PHB was not detected (<3%dw), suggesting that more specific growth conditions are required to boost the synthesis of this compound.
In conclusion, these first evidences suggest that wastewater generated from aquaponics could support algal growth without the need to supply further nutrients while producing valuable protein-rich algal biomass from a waste product. From a circular economy perspective, the exploitation of aquaponics-derived algal biomass into other economic sectors, e.g. aquafeed, should be encouraged. Finally, further investigations should be addressed to fully understand the scalability and economic sustainability of the process in a real-world scenario.

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AUTORI

Mara Simonazzi (mara.simonazzi2@unibo.it), Laura Pezzolesi, Franca Guerrini, Rossella Pistocchi, Department of Biological, Geological and Environmental Sciences-BiGeA, University of Bologna, Via Sant'Alberto, 163, 48123 Ravenna (RA), Italy
Valentina Bolletta, Mj Energy Srl Societa' Agricola, Contrada S.S. Crocifisso, 22, 62010 Treia (MC), Italy
Autore di riferimento: Mara Simonazzi

Exploring diatom resting stages in Calabrian marine waters: biological diversity, chemical cues and biotechnological potential

R. M. Sepe, I. Orefice, G. Romano, M. Montresor, V. Di Dato

Diatoms are amongst the most diversified single-celled microalgae, broadly present in freshwater and marine ecosystems. They play a fundamental role in the functioning of global ecosystems by producing bioactive secondary metabolites to cope with the challenges of the adverse marine environments, and, for this, they have a great potential for biotechnological applications. Diatoms have complex life cycles with distinct phases: active growth, dormant or quiescent stages, and sexual reproduction. Particularly interesting is the transition to the quiescent/resting phase that allows long-term persistence letting the survival of species under unfavourable environmental conditions, contributing in maintaining biological diversity and ecosystem recovery from perturbations. The transitions between a vegetative phase to the dormant one and vice versa play a fundamental role in the dynamics of diatom populations, but the factors and mechanisms that determine them are still largely unknown (Pelusi et Al. 2023). In addition, information on the species with this distinctive life-cycle trait is extremely scant in the Mediterranean Sea.
In this project we focus on the formation of resting stages of the diatom *Thalassiosira rotula*, a Mediterranean species with high biotechnological potential (Di Dato et Al. 2019).
The scientific objectives of the project are:

- assess and compare the diversity of *T. rotula* clones producing resting stages along the Calabria coast and in the Gulf of Naples;
- investigate the factors that trigger the formation of these stages;
- identify the transcripts and the secondary metabolites involved in the formation of resting stages;
- test possible epigenetic mechanisms involved in the transition from vegetative cells to resting stages.

For this purpose, we have set up the conditions to induce the *T. rotula* cells in a dormant status playing with different factors. Nitrogen depletion, an initial low cell density and a good level of oxygenation resulted to be the major conditions triggering the induction of the resting status in this diatom species.
This project brings together basic research in diatoms with important ecological implications and biotechnological exploitation, since secondary metabolites, eventually identified, could be further tested for their bioactivity and for possible pharmaceutical applications.

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AUTORI

Rosa Maria Sepe (rosamaria.sepe@szn.it), Ida Orefice, Giovanna Romano, Marina Montresor, Valeria Di Dato, Stazione Zoologica Anton Dohrn, Naples, Italy, Marine Biotechnology Department (RMS, IO, GR, VDD)/Integrative Marine Ecology Department (MM)
Autore di riferimento: Rosa Maria Sepe

Mechanical stimulation: an innovative method to optimize microalgal-based bioproduction

F. Ortenzi, S. Savio, F. Montereali, A. Contaldo, A. Di Cave, L. Rugnini, S. Antonaroli, B. Morozzo Della Rocca, L. Bruno and R. Congestri

Mechanical wave cycle, i.e. pressure variation in microbial culture media, has been shown to enhance productivity of heterotrophic microorganisms, *Escherichia coli* and *Saccharomyces cerevisae*, and also that of root cell line of the plant *Panax ginseng.* In detail, mechanical stimulation irradiated by an ultrasonic bath setting increased *E. coli* cell abundance, *S. cerevisae* growth rate and biomass and the yield of saponins in *P. ginseng* root cells (Chisti, 2003). As for microalgae, this kind of culture stimulation was evaluated in the model green alga *Chlamydomonas reinhardtii*, where the loss of flagella and calcium influx induction were observed, although no details on biomass and bioproduct yields were reported (Min *et al.,* 2014).
In this work we aimed to develop an *ad hoc* growth system, a sono-photobioreactor (S-PBR) small scale prototype, to deliver mechanical stimuli to selected green algal cultures and to evaluate the effect on biomass growth and macromolecule composition (proteins, carbohydrates and lipids). The S-PBR platform is made of a 1L flask connected to a transducer of ultrasounds controlled by a wave generator. The platform delivers mono-frequency and modulable mechanical stimuli in the culture media of one strain of the species *Desmodesmus* sp. (VRUC 281) and of the commercial *Chlorella vulgaris* CCAP 211/12. Before mechanical stimulation, cultures (1L) of the two strains were set up using intensively cultivated biomass (stationary phase) inside 3L systems and kept at controlled conditions 24°C, 40 µmol photon/m^2/s, L/D 12/12 h. Then, a 96 h mechanical cycle was applied and cultures' parameters measured in 2 mL samples collected every 24 h (for optical density and pigments) and every 48 h (for fresh and dry weight). At the end of the experiment, the biomasses were harvested, and the macromolecule contents evaluated: proteins according to Bradford method (Bradford, 1976), carbohydrates according to Dubois method (Dubois *et al.,* 1951) and lipids according to Ehinmen and colleagues (2010).
Data suggested a trade-off effect of the ultrasound-induced stimulation. Indeed, high stimulus intensity enhanced biomass production but reduced macromolecule content in both strains, while at low intensities opposite effects were recorded. High intensity stimuli resulted in final biomass increase in both strains with values up to 28% (DW, g/L), for *Desmodesmus* sp., while low intensity stimuli enhanced macromolecule accumulation.
Our preliminary results confirm the possibility to exploit mechanical stimuli in microalgae bioproduction and its application to structurally/phylogenetically diverse organisms is foreseen to better study microalgae responses in the context of algae biomass application.

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AUTORI

Federico Ortenzi (fed.ort@icloud.com) and Alberta Di Cave, PhD Program in Evolutionary Biology and Ecology, Laboratory of Biology of Algae, Dept. of Biology, University of Rome 'Tor Vergata', Via della Ricerca Scientifica 00133 Roma
Saverio Savio, Lorenza Rugnini, Roberta Congestri and Laura Bruno, Laboratory of Biology of Algae, Dept. of Biology, University of Rome 'Tor Vergata', Via della Ricerca Scientifica 00133 Roma
Blasco Morozzo Della Rocca, Dept. of Biology, University of Rome 'Tor Vergata', Via della Ricerca Scientifica 00133 Roma
Simonetta Antonaroli, Dept of Chemical Sciences and Technology, University of Rome 'Tor Vergata', Via della Ricerca Scientifica 00133 Roma Scientifica 00133 Roma
Alessandro Contaldo, Federico Montereali, Yeastime Srl, Via Plutarco 48, 00012 Roma
Autore di riferimento: Federico Ortenzi

Prostaglandins biosynthetic pathway in diatoms under abiotic stresses

L. Vitale, A. Fuentes-Lema, C. Sobrino, G. Romano, V. Di Dato

Diatoms are a diverse group of marine and freshwater microorganisms and well-known producers of polyunsaturated fatty acids (PUFAs). They represent a successful example of organisms capable to adapt to various ecological habitats with high rates of survival to different types of abiotic stresses. This makes diatoms an attractive model to study fatty acid metabolism.
Prostaglandins (PGs), lipid mediators derived from 20-carbon PUFAs, were recently found in marine microalgae, and their biosynthetic pathway was characterized (Di Dato *et al*, 2017).
These molecules are well studied in mammals, where they control several physiological and pathological processes and are key players in the inflammatory response. In marine microorganisms, their role is still unknown. Though, the conservation of the pathway and the similarity of the aminoacidic sequences among marine and mammal-representative cyclooxygenase (COX-1) suggests an equally fundamental role of PGs in marine microorganisms with relevant potential for pharmaceutical applications.
PGs have been hypothesized to act as chemical mediators during stress-induced response in marine organisms (Di Costanzo *et al*, 2019). In a scenario of climate change with high-rate increase in ocean acidification, elevated temperatures and light intensities our research aim is to investigate the regulation of PGs biosynthetic pathway in cultures of two clones of the marine diatom *Skeletonema marinoi* exposed to different concentrations of abiotic stresses. We growth the cultures under high or low temperature values, high or low light irradiation and under highCO_2 influx. By sampling every three hours along the light part of the day cycle we saw a significant variation in the expression of the enzymes in the PG pathway depending from both the clone and the day time.
From a biotechnological perspective, PGs are often employed in clinical setting for their therapeutic properties, but their full potential is still to be explored. A better understanding of PGs biosynthetic pathway regulation will also lead to the exploitation of diatoms as microbial cell factories for the production of such valuable compounds.

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AUTORI

Laura Vitale, Antonio Fuentes-Lema, Cristina Sobrino, Giovanna Romano, Valeria Di Dato (valeria.didato@szn.it), Stazione Zoologica Anton Dohrn, Naples, Italy, Marine Biotechnology Department (LV, GR, VDD); UVigo Marine Research Centre, Lagoas Marcosende Campus, 36310 Vigo, Spain (AFL, CS).
Autore di riferimento: Valeria Di Dato

The biodeterioration of Roman stuccoes in *laconicum* of the sector of Sosandra (archaeological site of Baia): a multi-omics approach

D. De Luca, R. Piredda, J. Troisi, S. Scamardella, P. Cennamo

Stuccoes are traditional decorative elements of ceilings and vaults of buildings and villas of the Roman age. Before the discovery of Nero's *Domus Aurea* in Rome in the XV century, the only evidence of these artworks came from written accounts, like Pliny the Elder's *Naturalis Historia* (Gapper, 1999). Due to their fragile nature, very few stuccoes survived intact or almost intact to present days and, in rarest cases, preserving the original colours. In addition, the exposure to abiotic and biotic agents after excavation alter both their structure and aesthetics, making stuccoes even more fragile. In recent years, the study of biodeterioration has been fostered by the introduction of -omics techniques (Beata, 2020), which allow non only the taxonomic characterization of microbial communities involved in the colonization (metabarcoding and metagenomics) but also their metabolic activity (metatranscriptomics, metabolomics, proteomics). In this study (funded by the PNRR Project PE 0000020 CHANGES), we used a multi-omics approach combining 16S and 18S metabarcoding and untargeted metabolomics to assess the taxonomy and metabolite profile of the prokaryotic and eukaryotic community involved in the biodeterioration of Roman stuccoes in the archaeological site of Baia (Campania region, Italy). Furthermore, we tested the efficacy of essential oil extracts for the removal of biological patinas. We analyzed seven biofilm samples from stuccoes collected on the ceiling of the *laconicum* (a private environment for sauna) in the Sector of Sosandra and found that four of them consisted almost exclusively of prokaryotes, while the other three of eukaryotes. Green algae (Chlorophyta) were the dominant eukaryotic group in all samples except one, and represented by Picocystophyceae (*Picocystis salinarum*), Trebouxiophyceae (*Pseudostichococcus* sp. and *Symbiochloris* sp.), and Ulvophyceae (*Ctenocladus circinnatus* and *Trentepohlia* sp.); other eukaryotes were fungi (Ascomycota), arthropods, and bryophytes. The most abundant metabolites were chlorophyll a, xanthophylls (lutein), products of fermentative processes (lactic acid, 2-hydroxyisocaproic acid, and sorbose), amino acids and several organic acids. This data suggested an active acid metabolism, especially concerning the prokaryotic community. Lastly, the treatment with a blend of essential oils proved to be effective for the removal of biological patinas from stuccoes.

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AUTORI

Daniele De Luca (daniele.deluca@szn.it), Department of Biology, University of Naples Federico II, via Foria 223, Naples, Italy. Present address: Department of Research Infrastructure for Marine Biological Resources, Stazione Zoologica Anton Dohrn, Villa Comunale, Naples, Italy.

Roberta Piredda, Department of Veterinary Medicine, University of Bari Aldo Moro, Piazza Umberto I, Valenzano, Italy.

Jacopo Troisi, Department of Chemistry and Biology "A. Zambelli", University of Salerno, Via Giovanni Paolo II 132, Fisciano, Salerno, Italy.

Sara Scamardella, Department of Humanities, Suor Orsola Benincasa University, via Santa Caterina da Siena 37, Naples, Italy.

Paola Cennamo (paola.cennamo@unisob.na.it), Department of Humanities, Suor Orsola Benincasa University, via Santa Caterina da Siena 37, Naples, Italy.

Autori di riferimento: Daniele De Luca, Paola Cennamo

Investigating the biostimulant potential of *Arthospira platensis* biomass on *Cichorium intybus*

A. Di Cave, S. Savio, F. Ortenzi, L. Rugnini, L. Bruno, R. Congestri

Climate change and resource scarcity pose significant challenges for agriculture. Meeting current food demands has led to the reliance on agrochemicals, intensive tillage, and over-irrigation, resulting in pollution, greenhouse gas emissions and ecosystem degradation. To cope and mitigate this impact, considerable interest is now directed towards the development of plant biostimulants: a category of products with the ability to enhance plant growth, nutrient use efficiency, qualitative traits of the final product and to stimulate plant response to abiotic or biotic stresses (du Jardin, 2015). In this context, mass-cultivated microalgae and cyanobacteria are importance sources for the production of plant biostimulants, thanks to their capacity to produce a wide range of bioactive compounds with promising bioactivities on edible plants. In addition, cultivating these organisms in controlled systems allows for the precise tuning of the biochemical composition of the biomass, thereby standardizing production of commercial biostimulants (Santini *et al*., 2021).
The main objective of this research is to explore the biostimulant properties of the biomass of the cyanobacterium *Arthrospira platensis* Gomont cultivated at industrial scale in the North of Italy. For this purpose, we tested the leftover biomass (POST), resulted at the end of phycocyanin extraction, activity on *Cichorium intybus* L. and compared data with those obtained using the pre-extraction (PRE) biomass.
Both PRE and POST biomasses were firstly lyophilized, resuspended in distilled water and chemically characterized, for the macromolecular content, using FTIR and spectrophotometric methods. This preliminary analysis showed that POST biomass was composed by 15% of carbohydrates, 8% of lipids and 50% of proteins, in addition we revealed the presence of important amino acids, that are precursors of plant hormones, such as auxin.
Analyses of the biostimulant activity of PRE and POST biomass, started with testing potential adverse effects on *C. intybus* seeds germination using four concentrations (2.5, 5.0, 7.5, 10.0 mg/mL). After 6-days, we found a slight delay in the germination rate for the seeds treated with the highest concentrations, but no difference was observed in the number of germinated seeds, indicating the absence of toxic effects of the leftover biomass. Subsequently, we investigated the effect of the PRE and POST biomasses treating plants of *C. intybus* and analyzing the morphometry (shoot, leaf, and root lengths), fresh weight and pigment contents, using plants treated with a commercial fertilizer and distilled water as positive and negative controls, respectively. These tests were conducted under two experimental conditions: one in the laboratory, under controlled conditions, and the other at the greenhouse of the Botanical Garden of the University of Rome 'Tor Vergata' in spring.
Overall results indicated that the POST biomass was able to exert a better effect on plants, possibly due to the extraction process itself, which could contribute to augmenting the bioavailability of cyanobacterial metabolites. This experimental set up is currently being applied using *Trichormus variabilis* (Kützing ex Bornet & Flahault) Komárek & Anagnostidis residual biomass to compare the datasets obtained.
Further tests will also focus on different microalgae products, such as the extracts and leftover biomass of two diatoms currently mass cultivated and analyzed for their bioactivity potential. Additionally, the effect of cyanobacterial and microalgae derivatives on the plants' rhizosphere will be investigated using community metabolism (Biolog Ecoplate) and NGS (Next Generation Sequencing) analyses.

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AUTORI

Alberta Di Cave (alberta.dicave@gmail.com), Federico Ortenzi, PhD Program in Evolutionary Biology and Ecology, Laboratorio Biologia delle Alghe, Dipartimento Biologia, Università di Roma Tor Vergata, Via della Ricerca Scientifica 00133 Roma, Italy.
Saverio Savio, Lorenza Rugnini, Laura Bruno, Roberta Congestri, Laboratorio Biologia delle Alghe, Dipartimento Biologia, Università di Roma Tor Vergata, Via della Ricerca Scientifica 00133 Roma. Italy.
Autore di riferimento: Alberta Di Cave