# RIUNIONE SCIENTIFICA 2020

# GRUPPO DI ALGOLOGIA

# Società Botanica Italiana

Tenuta in forma telematica

20 novembre 2020

# **PROGRAMMA E RIASSUNTI**

A cura di:

Fabio Rindi

Comitato scientifico:

Stefano Accoroni, Giuseppina Alongi, Simona Armeli Minicante, Roberta Congestri, Anna Maria Mannino e Fabio Rindi

Organizzazione telematica della riunione:

Giuseppina Alongi

# PROGRAMMA
## VENERDÌ 20 NOVEMBRE

9:15 **Introduzione ai lavori – Rossella Pistocchi e Direttivo del Gruppo di Algologia**

9:30 S. Armeli Minicante
**Il patrimonio algale degli Erbari italiani: primi risultati del censimento**

9:45 S. Casabianca, S. Capellacci, M.G. Giacobbe, C. Dell'Aversano, L. Tartaglione, F. Varriale, R. Narizzano, F. Risso, P. Moretto, A. Dagnino, R. Bertolotto, E. Barbone, N. Ungaro, A. Penna
**Plastic-associated harmful phytoplankton assemblages in marine environment**

10:00 S. Heesch, M.D. Guiry, W.A. Nelson, F. Rindi
**The brown algal genus *Cladostephus* (Sphacelariales): a taxonomic reassessment based on a polyphasic approach clarifies species circumscription and nomenclature**

10:15 F. Misurale, V. Giussani, L. Pezzolesi, R. Pistocchi, F. Colonna, R.M. Bertolotto, A. Novellino, A. Pagano, S. Alloisio
**A multidisciplinary approach for a fast HAB monitoring and a comprehensive evaluation of the risks on human and environment health**

10:30 V. Malavasi, K. Sciuto, M.A. Wolf, S. Soru, M. Secci, P. Addis, A. Sfriso
**First assessment of algal diversity in Santa Gilla lagoon (Sardinia, Italy) in the framework of the aquaculture industry**

10:45 N. Caputo, F. Guerrini, S. Vanucci, L. Pezzolesi, R. Pistocchi
**Reddish-brown bloom of the dinoflagellate *Prorocentrum cordatum* in the brackish lake "Lago delle Nazioni" (Comacchio, FE)**

11:00 ***Pausa***

11:30 F. Cipolletta, F. Vidussi, M.C. Buia, L. Longobardi, B. Mostajir, D. Sarno
**Effects of a simulated heat wave on the phytoplankton assemblage of the Thau Lagoon (France)**

11:45 D. Lenzo, L. Pezzolesi, A. Pasteris, M. Colangelo, F. Rindi, R. Pistocchi

**Ecological implication of allelopathic aldehydes produced by marine algae**

12:00 L. Longobardi, D. Sarno, L. Dubroca, A. Zingone

**Clocks in the ocean: phytoplankton periodicity in a highly variable environment**

12:15 S. Farrotti, S. Savio, A. Amati, K. Krasojevic, N. Perini, F. Costa, L. Migliore, R. Congestri

**A microbial consortium as biofilter to upcycle dishwasher wastewater**

12:30 G. Vaccarisi, S. Falsini, L. Lazzara, C. Nuccio, E. Corti, G. Paoletti, A. Papini

**Recupero della forma spiralata da quella lineare da parte di *Arthrospira platensis* Gomont dopo il reintegro dei nutrienti**

12:45 D. Serio, G. Furnari, R. Sanfilippo, J. Neiva

**"*Cystoseira*" *hyblaea* Giaccone (Ochrophyta, Fucales), a little known but probably widely distributed species in the Mediterranean Sea**

13:00 ***Pausa***

14:30 S. Fasiello, C. Garrido Perez

**Growth rate of *Tetraselmis chuii* cultured in different concentration of nitrate and phosphate: effects on the biomass productivity and compounds**

14:45 D. Spagnuolo, A. Manghisi, M. Morabito, R.M. Byeng, G. Genovese

**Potential uses of *Asparagopsis* species for methane reduction in intensive farming**

15:00 S. Giulietti, M. Ubaldi, T. Romagnoli, S. Accoroni, C. Totti

**Morphology and phylogeny of a new pennate planktonic diatom species from the northwestern Adriatic Sea: *Nitzschia gobbii* sp. nov.**

15:15 A. Guzzon, E. Bellini, L. Rugnini, L. Bruno

**Effect of abiotic factors on photosynthetic performance of two strains of Cyanobacteria**

15:30 A. Pelusi, A. Godhe, M.I. Ferrante, M. Ribera d'Alcalà, K. Thamatrakoln, K. Bidle, M. Montresor

**High cell density and viral infection trigger formation of resting stages in the marine diatom *Chaetoceros socialis***

15:45 M.R. Vadrucci, L. Roselli, A. Pastorelli

**PhytoNumb3rs: An easy-to-use computer toolkit for counting microalgae by the Utermöhl method**

16:00 ***Pausa***

16:30 L.G. Costanzo, G. Marletta, G. Alongi

**Preliminary observations on the coralligenous macro-phytobenthos in the Marine Protected Area Isole Ciclopi (eastern coast of Sicily)**

16:45 C. Gerotto, D. Pousa Kurpan Nogueira, A. Norici, M. Giordano

**Diversity of Sulfur metabolism in marine microalgae**

17:00 M. Palmieri, M.R. Di Cicco, C. Lubritto, C. Ciniglia

**Use of *Galdieria sulphuraria* (Cyanidiophytina, Rhodophyta) in recovering rare-earth elements from fluorescent lamps (FL).**

17:15 A. Petrocelli, G. Alabiso, G. Portacci, P. Ricci, R. Carlucci, E. Cecere

**Effectiveness of long-term observations for the behavior assessment of a potentially invasive non-indigenous species (NIS) in the Mar Piccolo of Taranto (northern Ionian Sea, Mediterranean Sea)**

17:30 S. Savio, L. Lvova, R. Paolesse, G. Persichetti, G. Testa, R. Bernini, R. Congestri

**'Sensorial' systems and microalgae affairs**

17:45 G. Furnari

**Amenità algologiche e non solo…..**

18:00 **Comunicazioni al Gruppo e saluti – Rossella Pistocchi**

# RIASSUNTI

# Il patrimonio algale degli Erbari italiani: primi risultati del censimento

**S. Armeli Minicante**

La biodiversità vegetale è sicuramente documentata dalle collezioni e dagli esemplari depositati negli erbari, i quali costituiscono una notevole e insostituibile fonte di informazione sulle specie e sul loro habitat, contribuendo altresì ad un'ampia gamma di studi quali tassonomia, sistematica, morfologia, fenologia, biodiversità, paleobiologia, ecologia ed etnobotanica. Una monografia sul patrimonio algologico italiano è stata presentata in occasione del *2nd European Phycological Congress* a Montecatini Terme da parte del Gruppo di Lavoro di Algologia della S.B.I. (Abdelahad, 1999). Successivamente, una panoramica degli Erbari italiani in possesso di collezioni algali è stata realizzata da Giaccone *et al.* (2008), nella quale venivano segnalati 17 Erbari italiani contenenti collezioni algali. Nel 2017 è stato effettuato un censimento delle collezioni algali presenti negli Erbari italiani al fine di aggiornare e approfondire lo stato del patrimonio algologico. Il questionario per la raccolta dati è stato inviato a 78 Erbari, utilizzando gli indirizzi ricevuti in carico dal Gruppo di Algologia, la rete CoRIMBo (Coordinamento della Rete Italiana dei Musei Botanici) e i dati presenti sul portale CollMap dell'ANMS (Associazione Nazionale Musei Scientifici). Ad oggi, 31 Erbari (di cui 27 registrati nell'*Index Herbariorum*) hanno fornito informazioni sulle collezioni algali di cui sono in possesso. Da una prima stima, il patrimonio algale risulta composto da circa 112.500 campioni storici (raccolti tra il 1600 e il 1950) e circa 42.000 campioni moderni (raccolti dal 1950 ad oggi), per un totale di circa 154.500 campioni. In molti Erbari sono presenti collezioni originali e campioni tipo, mentre 142 sono gli Autori riconducibili alle principali collezioni.

**Letteratura citata**

Abdelahad N. (1999). Il patrimonio algologico italiano. Officine Grafiche Borgia. I.G.E.A., Roma.

Giaccone T., Catra M., Serio D., Giaccone G. (2008). A review of Mediterranean macrophytobenthos collections present in Italy: a contribution to the Mediterranean Initiative on Taxonomy. Chemistry and Ecology, 24: 175-184.

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# Plastic-associated harmful phytoplankton assemblages in marine environment

**S. Casabianca, S. Capellacci, M.G. Giacobbe, C. Dell'Aversano, L. Tartaglione, F. Varriale, R. Narizzano, F. Risso, P. Moretto, A. Dagnino, R. Bertolotto, E. Barbone, N. Ungaro, A. Penna**

Among marine debris categories originating from sea-based and land-based sources, there is the plastic debris. Floating plastics are a durable and persistent substratum for the bio-adhesion of micro- and macro-organisms enabling the dispersal from native to new habitats (Eriksen *et al*., 2014). Plastic debris has been considered to play a role in the dispersal of toxic compounds having implications for humans and marine organisms across the marine food web. In previous studies, the harmful dinoflagellates *Alexandrium* sp., *Coolia* sp. and *Ostreopsis* sp. were recorded on plastics floating in coastal waters of the Mediterranean Sea (Zettler *et al*., 2013; Masó *et al*., 2016). As plastics may act as a vehicle for toxins to enter the food web, this study aims to identify and quantify target harmful microalgal taxa potentially attached to micro and macro plastics collected in coastal and off-shore waters of the Mediterranean Sea using molecular qPCR-based assays. Forty-two plastic samples were positive for the presence of Dinophyceae and Bacillariophyceae by qPCR assay. Genus and species-specific investigations indicated that sixteen plastic samples supported the presence of the toxic and allochthonous *Alexandrium pacificum* (highest abundance of 30 cells $cm^{-2}$), nine samples were positive for the presence of *A. minutum* (highest abundance of 73 cells $cm^{-2}$) and nine samples were positive for the presence of *Ostreopsis* cf. *ovata* (highest abundance of 259 cells $cm^{-2}$). Finally, the genus *Pseudo-nitzschia* was present on almost all plastic samples with abundance values ranging from 1 to 6.6 x $10^3$ cells $cm^{-2}$. Moreover, the application of FTIR spectroscopy allowed the identification of plastic polymers of the collected samples. The amount of sampled microplastics corresponding to 64% was non-polar (polyethylene and polypropylene) with a typical crystalline structure. The remaining 36% had a certain level of polarity with a typical amorphous structure. Ten cultured strains of *A. pacificum* isolated from the surface of plastic debris were analysed by liquid chromatography-high resolution multiple stage mass spectrometry (LC-HRMS) and they all produced PST (paralytic shellfish toxin) with a total toxin content in the range of 35 - 6032 fg $cell^{-1}$. Furthermore, the rate of adhesion by several target microalgal species was measured *in vitro*, demonstrating the capacity of plastic substrates to be rapidly colonized by microalgae. In particular, the dinoflagellate *A. pacificum* appeared to be the species that adhered most rapidly to the plastics, followed by the diatoms *Skeletonema marinoi* and *Pseudo-nitzschia multistriata*. The species that adhered at the slowest rate were *A. minutum* and *O.* cf. *ovata*. This study provided new information regarding the quantification of noxious microbiota attached to the plastics floating in the Mediterranean Sea based on molecular qPCR analysis. These findings illustrate the potential risk of dispersal of noxious microalgal assemblages associated with plastic pollution. Toxins produced by microalgae attached to the plastic surface can be transferred by the grazing of organisms of lower trophic levels (such as zooplankton) to organisms of higher trophic levels representing contaminated seafood for humans. In view of these recent findings, measures to prevent and manage plastic pollution are urgently needed.

**Letteratura citata**

Eriksen M., Lebreton L.C., Carson H.S., Thiel M., Moore C.J., Borerro J.C., Galgani F., Ryan P.G., Reisser J. (2014). Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS One, 9: e111913.

Masó M., Fortuño J.M., De Juan S., Demestre M. (2016). Microfouling communities from pelagic and benthic marine plastic debris sampled across Mediterranean coastal waters. Scientia Marina, 80: 117-127.

Zettler E.R., Mincer T.J., Amaral-Zettler L.A. (2013). Life in the 'plastisphere': microbial communities on plastic marine debris. Environmental Science & Technology, 47: 7137-7146.

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# The brown algal genus *Cladostephus* (Sphacelariales): a taxonomic reassessment based on a polyphasic approach clarifies species circumscription and nomenclature.

**S. Heesch, M.D. Guiry, W.A. Nelson, F. Rindi**

*Cladostephus* is globally distributed in temperate regions, where it occurs in intertidal and shallow subtidal habitats. The genus is easily recognized due to its pseudoparenchymatous erect axes bearing whorls of determinate branches. Despite such a distinctive habit, species circumscription in *Cladostephus* has long been uncertain. The lectotype species, *C. spongiosus* (Hudson) C.Agardh, is widely distributed in the North Atlantic and has a spongy habit, in which the whorls are not clearly separated and the branches form a dense, continuous cover. Another entity widely distributed in Europe was originally described as a separate species, *C. verticillatus* (Lightfoot) Lyngbye; this alga bears distinctly separated whorls and has been generally considered a form of *C. spongiosus* [*C. spongiosus* f. *verticillatus* (Lightfoot) Prud'homme]. However, it was unclear whether the distinction between these entities is genotypic or phenotypic. Several other species and subspecific taxa have been described from the southern hemisphere as well as the Mediterranean Sea, but their taxonomic identities are currently uncertain. Species circumscription and distribution in *Cladostephus* were reassessed based on morphological observations and DNA sequence data (sequences of the plastid *rbc*L and *psb*C genes, the mitochondrial COI-5P gene and the nuclear ITS nrDNA) obtained from specimens collected from a wide geographical range. Phylogenetic analyses separated our *Cladostephus* samples into three well-supported clades. One of these corresponded to *C. spongiosus* and its distribution was limited to the Atlantic coast of western Europe. Verticillate samples formed a separate clade and had a cosmopolitan distribution, spanning both the Northern and Southern hemispheres. Based on phylogenetic distinctness and pairwise sequence distances, we conclude that this entity represents a separate species, for which the name *C. hirsutus* (Linnaeus) Boudouresque & M. Perret is available. Analysis of sequence data available for this species revealed that the haplotypic diversity of *C. hirsutus* is highest in the Mediterranean Sea. The third clade consisted of specimens collected from the Southern Hemisphere. These specimens had a bushy habit, a generally smaller size and straighter whorled branches than *C. hirsutus* and *C. spongiosus*. Comparisons with descriptions and images of type specimens suggest that this southern entity conforms most closely to the description of *Cladostephus australis* Kützing, *nom. illeg*., which has been renamed as *C. kuetzingii* Heesch, Rindi & W.A.Nelson.

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# A multidisciplinary approach for a fast HAB monitoring and a comprehensive evaluation of the risks on human and environment health

**F. Misurale, V. Giussani, L. Pezzolesi, R. Pistocchi, F. Colonna, R.M. Bertolotto, A. Novellino, A. Pagano, S. Alloisio**

Harmful algal blooms (HAB), typical of warm tropical seas, are getting more and more frequent in temperate latitudes. Blooms can be harmful for the environment, human health and aquatic life due to the production of noxious toxins and the consequences of accumulated biomass (e.g. oxygen depletion). In the Ligurian Sea, the most serious sanitary problems occurred in 2005 and 2006 when *Ostreopsis* cf. *ovata* algal blooms caused respiratory, skin and eyes ailments to people exposed to marine aerosol leading, in some cases, to hospitalization (Ciminiello *et al.*, 2006). Since then, a monitoring program started along the Ligurian coast (north-western Mediterranean), where time series of cell abundances have been collected for several sites. As far as the studies on the action mechanism are concerned, almost all studies refer to the commercial toxin standards and no robust evidence is available on the effects of the exposure to the real toxins' mixture released from algae. In this context, the ongoing project @lgaWarning (EuroTrans-Bio 12$^{th}$ Transnational Call) aims to improve the HAB monitoring strategy by achieving the following objectives: creating a portable device to analyze environmental samples directly in the field using a smartphone; optimizing a miniaturized fluorescence based analyzer that allows the automatic classification of algal blooms; conducting a comprehensive in vitro/in vivo toxicological study on the effect of the entire toxin mixture produced by selected dinoflagellates in order to evaluate their risks on human and environment health. To achieve these objectives, four target species were selected among those widespread in the Ligurian Sea: two toxic species, *Ostreopsis* cf *ovata* and *Prorocentrum lima*; *Coolia monotis,* suspected to produce toxins; and the non-toxic *Scrippsiella trochoidea*. In order to preserve all compounds produced by the algae, experimental treatments were prepared using a non-chemical extraction protocol based on cell lysis. To evaluate the ecotoxicological effect of these species, we exposed nauplii of *Artemia franciscana* to the different treatments for 48 h and evaluated their mortality rate. To evaluate human risk, we performed irritation tests by exposing commercial 3D cultured human skin and cornea for 1h and 4h as indicated by OECD guidelines. Then, we performed neurotoxicity tests on cultured mouse cortical neuronal networks by means of MTT assay and electrophysiological evaluation using a Multi Electrode Array (MEAs) based approach. Results show a high mortality rate in *A. franciscana* after 48h for both *O.* cf *ovata* ($LC_{50}$=104,5cell/mL) and *P. lima* ($LC_{50}$=2356,3cell/mL), confirming the strong toxic potential of the two species and validating the treatment preparation protocol, since the results agree with literature reports (Faimali *et al*., 2012). Surprisingly, skin and eye irritation tests did not show any significant effect, suggesting that other cellular pathways may be involved. Conversely, MEAs-based analysis showed a significant inhibition of spontaneous electrical activity at low concentrations after exposure to *O.* cf *ovata* ($IC50_{MFR}$ = 4,5cell/mL) and *P. lima* ($IC50_{MFR}$ = 30cell/mL), followed by cell death only at high concentration exposure. Finally, the exposure of *Coolia monotis* and *Scrippsiella trochoidea* did not show any effect in any of the tests considered, confirming that both species are non-toxic.

**Letteratura citata**

Ciminiello P., Dell'Aversano C., Fattorusso E., Forino M., Magno G.S., Tartaglione L., Grillo C., Melchiorre, N. (2006). The Genoa 2005 outbreak. Determination of putative palytoxin in mediterranean *Ostreopsis ovata* by a new liquid chromatography tandem mass spectrometry method. Analytical Chemistry, 78: 6153–6159.

Faimali M., Giussani V., Piazza V., Garaventa F., Corrà C., Asnaghi V., Privitera D., Gallus L., Cattaneo-Vietti R., Mangialajo L., Chiantore M. (2012). Toxic effects of harmful benthic dinoflagellate *Ostreopsis ovata* on invertebrate and vertebrate marine organisms. Marine Environmental Research, 76: 97–107.

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# First assessment of algal diversity in Santa Gilla lagoon (Sardinia, Italy) in the framework of the aquaculture industry

**V. Malavasi, K. Sciuto, M. A. Wolf, S. Soru, M. Secci, P. Addis, A. Sfriso**

The lagoon of Santa Gilla is Sardinia's largest coastal wetland, surrounded by port facilities, large industrial complexes and the metropolitan city of Cagliari. The lagoon covers an area of about 1300 hectares and it is located along the Southern coast of Sardinia. In its southern part, the lagoon is connected to the sea through a channel, while in the northern part it receives two freshwater inflows from the Flumini Mannu and Cixerri rivers. The lagoon is a unique natural water body, characterized by large fluctuations of salinity and temperature. While the water quality and the macrozoobenthic community have been studied (Cabiddu *et al.*, 2014), up to now the algal diversity has been poorly investigated (Cottiglia, 1995). The European Community has recognized this area, named "Stagno di Cagliari, Saline di Macchiareddu, Laguna di Santa Gilla" (ITB040023), as a Site of Community Importance (SCI). The present work is part of a study conducted in the area for the purposes of Integrated Multi-Trophic Aquaculture (IMTA). The accurate knowledge of Santa Gilla macroalgal diversity has been the focus of a pilot study aimed at identifying the effects of different algal feeding regimes on the nutritional profile of the edible sea urchin *Paracentrotus lividus* (Lamarck) and it can be useful for other similar researches connected with the aquaculture industry. Preliminary investigations on the macroalgal flora were carried out on specimens collected from three different sampling sites located on the west coast of the Gulf of Cagliari, where fishing is currently allowed. Sampling of water and macroalgae was carried out in March, July, and October 2020. Physical-chemical parameters (temperature, pH, salinity, dissolved oxygen and conductivity) were also measured with a multiprobe. A combination of morphological and molecular approaches was adopted for the identification of the macroalgal specimens. *Ectocarpus* cf. *siliculosus* Dillwyn (Lyngbye) (Ochrophyta) and *Gracilaria gracilis* (Stackhouse) M. Steentoft *et al*. (Rhodophyta) were identified based on morphological characters. A DNA barcoding approach, based on the *tuf*a marker, was used for the green macroalgae, revealing the presence of different *Ulva* species (e.g., *Ulva compressa* Linnaeus*, Ulva flexuosa* Wulfen*, Ulva californica* Wille*, Ulva linza* Linnaeus). In addition, the genera *Ulvella* P. Crouan & H. Crouan and *Blidingia* Kylin were also detected. This study is a first contribution to the knowledge of the macroalgal community in Santa Gilla lagoon and represents the starting point for further investigations in this direction.

**Letteratura citata**

Cabiddu S., Culurgioni J., Palma, F., Soldovilla G., Atzori G. (2014). The macrozoobenthic community of the Santa Gilla lagoon (Southern Sardinia, Italy). Transitional Waters Bulletin, 8: 73-83.

Cottiglia M. (1995). The Santa Gilla lagoon. Rendiconti del Seminario della Facoltà di Scienze dell'Università di Cagliari, 65: 15–19.

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# *Reddish-brown* bloom of the dinoflagellate *Prorocentrum cordatum* in the brackish lake "Lago delle Nazioni" (Comacchio, FE)

**N. Caputo, F. Guerrini, S. Vanucci, L. Pezzolesi, R. Pistocchi.**

Transitional Waters (TWs) include lagoons, saline lakes, river estuaries and deltas, and are highly dynamic and heterogeneous systems, widely distributed in the Italian Peninsula and strongly used for human exploitation. The monitoring program proposed by the European Water Framework Directive (WFD, 2000/60/EEC) represents an important assessment of the ecological status, and an increased knowledge of the existing variability is a key point for the conservation and the management of these aquatic systems. The TWs investigated in this study are the Sacca di Goro and the Lago delle Nazioni (Ferrara, Emilia-Romagna). The Lago delle Nazioni (44°46′10.74″N, 12°14′30.39″E) is a semi-artificial big brackish lake, located besides the seaside town of Lido delle Nazioni. In the '60s, the basin was exploited for touristic and sporting purposes; to date it hosts experimental breedings of juveniles of the clam *Ruditapes philippinarum* (Mollusca, Bivalvia, Veneridae). The lake's water monitoring raised outstanding interest along the study, especially in terms of trophic index and quali-quantitive composition of phytoplankton assemblages. During the last winter (December 2019-January 2020) an exponential reddish-brown discoloration of the water caused by an intense bloom of the dinoflagellate *Prorocentrum cordatum* was observed. The cells reached a density of $4.7 \times 10^7$ cells $L^{-1}$, which amounted to 99% of the phytoplankton community, and corresponded to a Chl *a* peak of 251 µg $L^{-1}$. As shown by water analysis, the bloom occurred with low concentrations of N and P (avg. 5.5 µg $L^{-1}$ and 5.7 µg $L^{-1}$, respectively), resulting in a N:P ratio far lower than the Redfield one, ranging from 0.3 to 5. The evident lack of dissolved nitrogen led to hypothesize that mixotrophy and, presumably, organic matter inputs from the sediment or the land, could play an important role in driving the bloom. Since the last decades of the 20$^{th}$ century, *P. cordatum* has been linked to harmful effects on bivalves (Leibovitz *et al*., 1984); moreover, recent studies have reported the presence of tetrodotoxins (TTXs) in European bivalve molluscs in correlation with blooms of *Prorocentrum* (Vlamis *et al*., 2015). Therefore, this strain was isolated in the laboratory and thereafter LC-MS analysed by the Marine Research Centre of Cesenatico, with the result that no TTXs were detected. In addition, no toxic effects and mortality for juvenile shellfish were reported; on the contrary, a positive empirical correlation between the presence of *P. cordatum* and the health of *R. philippinarum* was noted during the winter bloom. Further studies are in progress to evaluate the algal life cycle and the potential relationship with other microorganisms; at the same time the lake's monitoring program should be pursued to better understand the phytoplankton community's dynamics and occurrence in time.

## Letteratura citata

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# Effects of a simulated heat wave on the phytoplankton assemblage of the Thau Lagoon (France)

**F. Cipolletta, F. Vidussi, M.C. Buia, L. Longobardi, B. Mostajir, D. Sarno**

Climate change has led to the worldwide increase in persistence, occurrence and severity of extreme weather events such as heat waves, causing disturbance to the ecosystems (Houghton *et al.*, 2001). Phytoplankton communities, which play a major role in global biogeochemical cycles and contribute to about 50% of the global net primary production (Field *et al*., 1998), appear to be sensitive to climate change (Behrenfeld *et al*., 2006). However, understanding of how individual species may respond to climate change is still limited. The present study is part of the interdisciplinary research "Summer in Spring. Heat wave effects on Mediterranean Plankton communities: Resistance, Resilience and Recovery", carried out in the Thau Lagoon (South of France) from the 24th of May to the 12th of June 2019 in the frame of the Transnational Access of AQUACOSM project. Six *in situ* mesocosms were filled with 2260 L of natural lagoon water. To mimic a heat wave, three mesocosms (Heated) were warmed to 3°C above the temperature of the remaining mesocosms, which followed ambient water temperature (Controls). After ten days, the treatment was stopped and all mesocosms followed natural water temperature variations for the remaining 10 days. All mesocosms were sampled every two days to analyze phytoplankton abundance and species composition. Overall, a total of 28 phytoplankton taxa were identified. In both Control and Heated mesocosms, phytoplankton assemblages were strongly dominated by small undetermined phytoflagellates (<10 µm). Diatoms, mainly represented by *Cylindrotheca closterium*, constituted about 47% of the total phytoplankton abundance at the beginning of the experiment, but declined after few days (day 5). Dinoflagellates, especially small undetermined naked and thecate forms (<15 µm), represented on average only 1.4% of the total abundance. Although the two conditions were not associated with a significant difference in total phytoplankton abundance, our results showed that warming can affect phytoplankton communities acting in different ways on the abundance of individual species. Indeed, in Heated mesocosms, apparently similar flagellate taxa showed higher (positive response, e.g., *Pseudoscourfieldia marina*), lower (negative response, e.g., *Ollicola vangoorii*) or comparable abundance (neutral response, e.g., *Dinobryon faculiferum*) than in Control mesocosms. The variety of the responses reflects the biological and ecological diversity of species that co-occur in a natural phytoplankton community and highlights the importance of proper biodiversity assessments. Spring heat waves could have species-specific and even unexpected effects on community structure with potential cascading consequences for marine food webs and carbon flow.

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# Ecological implication of allelopathic aldehydes produced by marine algae

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Allelopathic interactions are likely to play an important role in species' successions. Field and laboratory studies have been shown that marine algae, particularly diatoms, produce allelopathic compounds with high structural variability, including alkaloids, phenols, organic acids, cyclic peptides, and polyunsaturated aldehydes (PUAs) (Pezzolesi *et al*., 2017). PUAs derive from the oxidation of fatty acids and are characterized by different lengths of the carbon chains and number of unsaturations. Negative effects on the reproduction of marine organisms exposed to PUAs have been observed, including predators such as copepods (e.g. reduction in survival, egg production and hatching success) (Ianora *et al*., 2003); additionally, changes in growth, cell membrane permeability, and cell morphology in phytoplankton organisms exposed to PUAs have been reported (Pezzolesi *et al*., 2017; Gallina *et al*., 2016; Ianora and Miralto, 2010). Little information is available on the production of these compounds by macroalgae and on their effect on microphytobenthic and meiobenthic communities, as most of the studies have been performed in planktonic environments. This study focuses on the benthic community, with the aim to investigate the involvement of macroalgae in terms of PUAs production and their role in the interaction with meio- and phytobenthos. Samples were collected from May to September 2018 in a semi-enclosed and shallow rocky area at Passetto (Ancona, northern Adriatic Sea, Italy), characterized by a rich phytobenthic community and annual blooms of a toxic dinoflagellate (i.e. *Ostreopsis* cf. *ovata*). Two macroalgae, *Cystoseira compressa* (CC) and *Dictyopteris polypodioides* (DP), were sampled and quali-quantitative analysed for PUAs' profile and their microphyto- and meiobenthic communities. PUAs results showed differences between the two algae. In particular a higher concentration of PUAs was detected in DP, with a peak in May, when values of 225.5 µg/g of fresh weight were measured for DP and 17.1 µg/g of fresh weight for CC. At the same time, some long-chain compounds, such as hexadeca-trienal (C16:3), hexadeca-tetraenal (C16:4), tetradeca-pentaenal (C14:5) were produced by DP, while CC produced mainly a short-chain PUA (i.e. hexadienal; C6:2). The microphytobenthic community was more abundant on DP than on CC; for both macroalgae diatoms represented the main microalgal group throughout the study period. Indeed, the most abundant diatom group on DP was represented by pennate species, mostly belonging to Naviculales (60% of the entire diatom assemblage), while on CC the most abundant species belonged to the orders Licmophorales (33%), Bacillariales (14%) and Naviculales (50%). Dinoflagellates were present in low concentrations, except during the bloom of the dinoflagellate *Ostreopsis* (at the end of August 2018). In the meiobenthic community, the main taxa (nauplii, nematodes and copepods) and the 12 species of harpacticoid copepods identified showed different temporal patterns, resulting more abundant on DP than on CC, as for the microphytobenthos. The observed patterns could be explained on one hand by the different morphological complexity of the two macroalgae, and on the other hand by the concentrations of the different allelopathic compounds (e.g. PUAs) produced. In fact, as observed in this study, in CC more microalgal species were reported but with a lower average density, while in DP there was a higher average density but, specifically, only some species, such as *Navicula* spp., *Cylindrotheca* spp., and *Nitzschia* spp occurred in large amounts. These results highlight how these compounds could act more qualitatively by selecting the species rather than quantitatively on the entire community, affecting the dynamics and composition of the meio- and phytobenthic communities.

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# Clocks in the ocean: phytoplankton periodicity in a highly variable environment

**L. Longobardi, D. Sarno, L. Dubroca, A. Zingone**

Phytoplankton algae are a fundamental component of marine systems. They are involved in the global regulation and functioning of biogeochemical and trophic processes, and are strictly connected to human health and well-being through the provision of essential ecosystem services. Despite their fundamental importance, there is still no broad consensus on the mechanisms underlying their seasonal and interannual variability, while even less is known about the ecology of individual species and their response to climate variability given the scarcity of comprehensive long-term observation sets. Here, by taking advantage of high-frequency oceanographic and biological data collected over more than 25 years in a coastal pelagic Mediterranean site, we have investigated different aspects of community and individual species' ecology, with a particular emphasis on the environmental factors and the mechanisms underlying phytoplankton phenology. The results highlighted an impressive regularity in the annual occurrence of phytoplankton community and individual taxa, which translated in a considerable stability of community composition despite a highly variable environment. Light was the predominant factor regulating species turnover and replacement, and seemed to regulate endogenous biological processes associated to species-specific phenological patterns. Overall, the results of this study highlight the considerable resilience and the active role that phytoplankton plays under different environmental constraints, which contrasts the view of these organisms as passively undergoing external changes that occur at different temporal scales in their habitat, and show how, under certain conditions, endogenous biological processes prevail over environmental forcing.

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# A microbial consortium as biofilter to upcycle dishwasher wastewater

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Wastewater (WW) is known to be a widely available and valuable resource that can be used to provide ecological benefits, reduce water demand and increase water supplies (Mo & Zhang, 2013). WW resource recovery technologies have been extensively elaborated by the scientific community and biological WW treatment is among the most important biotechnological applications in which, as drivers of the key processes, microorganisms are central to its success (Daims *et al*., 2006). Recent biological filtering and bioremediation strategies are based on the synergistic relationship between photosynthetic and heterotrophic microorganisms, the so-called 'microbial consortia'. These consortia are effective biofilters to treat WW and proved to be a more sustainable (Posadas *et al*., 2017) and efficient treatment approach for pollutant removal and biofiltration ability (Gonçalves *et al*., 2017) than conventional strategies (e.g. activated sludge systems). In this work we assembled a biofilter as a suspended biofilm to treat dishwasher WW. This biofilter is the core of an integrated system (Zero Mile System®) devoted to reusing and upcycling of reconditioned wastewater, partly in subsequent dishwasher cycles and partly into a vertical garden for plant food cultivation. The microbial consortium consists of: (i) the photosynthetic, filamentous cyanobacterium, *Trichormus variabilis*, a biofilm forming and EPS producing laboratory domesticated strain (Di Pippo *et al*., 2012; Bellini *et al*., 2018) and (ii) three selected heterotrophic, aerobic, bacteria isolated from the dishwasher WW itself, identified by Sanger sequencing as: *Acinetobacter, Aeromonas* and *Exiguobacterium* spp.. *T. variabilis* constitutes an active mucous scaffold and produces oxygen used by the heterotrophic counterpart of the consortium that attack and consume the organic load of the dishwasher WW mineralizing it. To produce the microbial consortium, the bacterial strains isolated were tested with *T. variabilis* in co-culture experiments (from *one-to-one* tests to the final *one-to-three* test). The survival and growth of *T. variabilis* in all co-cultures were evaluated spectrophotometrically and by morphological observations using light and confocal laser scanning microscopy. The efficiency of the biofilter was evaluated as its ability to reduce the concentration of total nitrogen and phosphorus in the wastewater; finally, the trial included also metagenomic analysis in Next Generation Sequencing (NGS) to evaluate the composition of the community associated with the wastewater, the cyanobacterium monoculture and the *one-to-one* co-cultures. The engineered consortium thrives in the wastewater much better than *T. variabilis* alone, efficiently stripping N and P in a short time, a pivotal step for the reuse and saving of water in household appliances.

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# Recupero della forma spiralata da quella lineare da parte di *Arthrospira platensis* Gomont dopo il reintegro dei nutrienti

**G. Vaccarisi, S. Falsini, L. Lazzara, C. Nuccio, E. Corti, G. Paoletti, A. Papini**

La morfologia tipica che contraddistingue *A. platensis* dagli altri cianobatteri è caratterizzata da filamenti spiralati (o tricomi), composti da cellule cilindriche, ma lo stress ambientale (temperatura, pH, luce e disponibilità di nutrienti) può indurre un cambiamento morfologico al cianobatterio linearizzandolo. *A. platensis* Gomont è un cianobatterio filamentoso, pluricellulare, fotoautotrofo, non eterocistico, che abita corpi d'acqua tropicali e subtropicali con un alto livello di bicarbonato, carbonato e PH alcalino (Tomaselli, 1987). Principalmente cresce nel lago Ciad in Africa e nel lago Texcoco in Messico. La Spirulina è considerata un "super-food" a causa dei suoi elevati componenti nutrizionali, in particolare l'alto contenuto proteico. La sua coltivazione è riconosciuta in tutto il mondo e, ad oggi, il 70% del mercato della Spirulina è per consumo umano (Karssa *et al.,* 2018). Questa sperimentazione ha lo scopo di analizzare la crescita e variazione morfologica di *A. platensis* nel mezzo di coltura fresco Zarrouk, dopo essere stata prelevata dal terreno esausto, a bassa concentrazione di nutrienti. Per l'analisi sono state utilizzate tecniche di microscopia ottica ed elettronica.
Dall'analisi al microscopio ottico è emerso, nel corso del tempo, un incremento del tasso di crescita di *A. platensis*. In particolar modo, una volta reinserita in ambiente ottimale per la sua crescita, riprende l'attività di spiralizzazione che nel terreno esausto aveva ormai perso. Inoltre, è stato rilevato un allungamento dei tricomi lineari, probabilmente perché questi non andavano incontro a divisione cellulare, che in *A. platensis* avviene mediante frammentazione del filamento in seguito a necrosi di cellule intercalari, i necridi. Dall'analisi al microscopio elettronico a trasmissione si è ricavata l'ultrastruttura del cianobatterio: parete cellulare, citoplasma con aree elettrontrasparenti per la presenza di vacuoli di gas e aree elettrondense con ribosomi, carbossisomi e tilacoidi dilatati ai quali sono associati i pigmenti fotosintetici. In conclusione, quando *A. platensis* proveniente da culture impoverite di nutrienti e in larga parte in forma linearizzata viene reinserita nel mezzo Zarrouk fresco, appare non soltanto nella sua tipica forma spiralata, ma anche in quella lineare che continua ad aumentare di numero. Le possibili ragioni potrebbero essere legate o a mutazione, o effetti epigenetici. Una ipotesi è che la struttura del peptidoglicano potrebbe essere modificata impedendole così di formare la piega settale, che conferisce la tipica forma a spirale. La ridotta frammentazione nei filamenti lineari potrebbe essere indotta da una carenza di nutrienti, come fosforo e zolfo, portando così a un allungamento di questi tricomi.

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# *"Cystoseira" hyblaea* Giaccone (Ochrophyta, Fucales), a little known but probably widely distributed species in the Mediterranean Sea

**D. Serio, G. Furnari, R. Sanfilippo, J. Neiva**

*"Cystoseira" hyblaea* was described by Giaccone (1986) based on specimens collected from infralittoral rocky substrates at Punta D'Aliga (Ragusa), along the southeastern coast of Sicily. As remarked by Cormaci *et al*. (2012), this species was no longer recorded after its description. Nevertheless, "*C." hyblaea* has recently been found in the Tunisian coasts by Bouafif *et al*. (2016), who collected some specimens in 2014 at Kelibia, at the depth of 0.2-1.5 m on semi-exposed rocky shores. During a recent study of species of the "*Cystoseira* complex" from the Sicily coasts, a "*C." hyblaea* community associated with biogenic crusts of the reef-building worm *Sabellaria alveolata* (Linnaeus, 1767) (Polychaeta: Sabellariidae) was found on shallow infralittoral bottoms of the southeastern coast near "Isola delle Correnti" (Syracuse). The thalli of "*C." hyblaea* show the same characters as those from Tunisia reported by Bouafif *et al*. (2016), while they only partially correspond to Giaccone's (1986) description. Therefore, a comparative study between our thalli and Giaccone's Herbarium specimens held in the Herbarium of the Department of Biological, Geological and Environmental Sciences of the University of Catania (CAT, *ex* "Herbarium Giaccone") was made, in order to better characterize the species. Taking into account the paper by Orellana *et al.* (2019), molecular studies were also undertaken in order to establish to which genus of the *Cystoseira* complex such a rare species should be referred to. Our plants are caespitose, attached to the substrate by a robust and compact basal disc, 3-5 cm in diameter. Cauloids (4-20) are erect, up to 30 cm high and 3-5 mm in diameter. The apex of cauloids is smooth and slightly protruding, never surrounded by spiniform appendages, sometimes surrounded by short cylindrical non-spiny young primary branches. Primary branches are cylindrical, bearing cylindrical branches of superior order with a subequal development that gives the thallus a cupressoid habit. Tophules and aerocysts are absent. Receptacles are terminal, cylindrical-ovoid to clavate, 0.5-1 cm long and 1 mm wide, with deciduous spinous appendages; conceptacles are subspherical and located at the base of spinous deciduous appendages. Thalli show the maximum vegetative and reproductive development in winter. The above characters, including the slightly prominent and smooth apex of cauloids, were observed in Giaccone's Herbarium specimens. Therefore, a discrepancy does exist between original material and what stated by Giaccone in his diagnosis of the species: *"apice densis spinulis ornato haud satis saliente..."*. Since according to Giaccone's (1986) diagnosis, *C. hyblaea* is similar to *C. crinita* [=*Carpodesmia crinita* (Duby) Orellana & Sansón] in habit but "*ab ipsa apicibus...praecipue differens*", we checked Herbarium specimens of *Carpodesmia crinita* (labelled as *Cystoseira crinita*) held in CAT and in particular those collected by Pizzuto during the study of the "*C. crinita*" community from "Isola delle Correnti" (Pizzuto, 1999). This study showed that Pizzuto's specimens should be referred to "*C." hyblaea*. The occurrence of "*C." hyblaea* in Sicily outside its type locality is confirmed. The "*C." hyblaea* community was found in association with a bioconstruction of *Sabellaria alveolata*. It should be noted that, "*C." hyblaea* as well as *S. alveolata* represent first reports from the study area.

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# Growth rate of *Tetraselmis chuii* cultured in different concentration of nitrate and phosphate: effects on the biomass productivity and compounds.

**Fasiello S., Garrido Perez C.**

The bioremediation of wastewater using microalgae overcomes problems related to the physical and chemical treatment of wastewater. Proliferation of aquaculture is generating high amounts of wastewater containing a huge amount of nitrogen, phosphorus and other substances that can cause environmental pollution, and microalgae can absorb these compounds and convert them into useful biomass. The current study was motivated by the increasing potential of microalgae biomass and the implementation of environmentally friendly tools in treating aquaculture wastewater. The aim of this work was to study the effects of N:P ratio variation on the growth rate and biochemical components (carbohydrates, lipids and proteins) of the marine microalga *Tetraselmis chuii* (Butcher, 1959) cultured under five different growth conditions combining f/2 medium (Guillard, 1975) and sea bass aquaculture wastewater nutrients. The cultures reached the stationary phase in different days after the start (from 8 to 21 days). The tests ended when the stationary phase was reached. The suitable N:P ratio for achieving the optimum biomass production for *T. chuii* ranged between 8 and 12. Biomass lipid, protein and carbohydrate content depend on the aging cultures. The lipid content reaches a maximum value of 39.5% for *T. chuii* cultured in aquaculture sea bass wastewater plus f/2 medium. The results indicate that aquaculture wastewater can be re-used as a possible source of low-cost nutrient to culture *T. chuii* for live feed utilization in aquaculture. More detailed studies are needed to improve or give a fruitful solution to utilize the aquaculture wastewater for mass production of microalgae.

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# Potential uses of *Asparagopsis* species for methane reduction in intensive farming

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Methane ($CH_4$) is a greenhouse gas with a global warming power 25 times higher than carbon dioxide. In the last decade, literature data reported that agriculture and waste management contributed 62% of global $CH_4$ emissions (Kirschke *et al*., 2013) by ruminant enteric fermentation. Enteric $CH_4$ is a consequence of anaerobic fermentation of feed organic matter by a methanogenic archaea community. Diet manipulation is regarded as the most direct and effective approach to lowering methane ($CH_4$) emissions from ruminant production systems (Brooke *et al*., 2020). Use of an antimethanogenic compound in the diet of farmed ruminants could reduce greenhouse-gas emissions. Some chemical additives have been shown to decrease $CH_4$ production from livestock (Li *et al*., 2018) and reductions of microbial community. Macroalgae are used in the nutraceutical and health field and have demonstrated antibacterial, anti-viral, antioxidant, antiprotozoal, antifungal or anti-inflammatory properties (Genovese *et al*., 2013). Antimethanogenic compounds are also known to exist naturally in macroalgae in varying concentrations, as showed in red marine macroalgae belonging to the family Bonnemassoniaceae. *Asparagopsis* species produce haloforms and dihalomethanes (Paul and Pohnert, 2011) in specialized cells called "gland cells", as a natural defense against disease and marine herbivory (Paul *et al*., 2006). These molecules include bromoform, bromochloromethane and dibromochloromethane, which exhibit a potential natural effect as $CH_4$ inhibitor. Bromoform is believed to be the principal compound in red algae because it is able to reduce $CH_4$ emissions, although other compounds such as dibromochloromethane and dibromoacetic acid have also been detected at lower concentrations (Machado *et al*., 2018). The bioactive molecules from *Asparagopsis* have been identified and shown to reduce $CH_4$ production, more than in other species (*Zonaria farlowii*, Phaeophyceae) that have also bromorganic compounds (Brooke *et al*., 2020). Recent *in vitro* and *in vivo* studies suggest that the two species *Asparagopsis taxiformis* and *A. armata* have the potential to reduce methane ($CH_4$) production when added to grass-based diets, but their efficacy is unclear. However, there is much variability in the antimethanogenic power between species of macroalgae among animal species such as dairy cattle, beef cattle, and sheep (Roque *et al*., 2019). The objective of this research is to quantify the effects of macroalgae supplementation in beef cattle diets on enteric methane production, rumen fermentation characteristics, and microbial diversity changes; so as to exploit an invasive macroalgal species in the Strait of Messina.

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# Morphology and phylogeny of a new pennate planktonic diatom species from the northwestern Adriatic Sea: *Nitzschia gobbii* sp. nov.

**S. Giulietti, M. Ubaldi, T. Romagnoli, S. Accoroni, C. Totti**

*Nitzschia* Hassall is a widely distributed genus of pennate diatoms consisting of 829 species (Guiry and Guiry 2020). Species of this genus are very common in diverse aquatic habitats (i.e. freshwater, estuaries, coastal and marine waters) (Trobajo *et al.,* 2004; Rovira *et al.,* 2012a), with higher diversity in the microphytobenthos than in phytoplankton communities. Several *Nitzschia* species are good indicators of environmental conditions such as heavy metal contamination (Tlili *et al.,* 2011) and hydrological dynamics (Rovira *et al.,* 2012a; Rovira *et al*., 2012b). However, this genus is also known for the problematic taxonomy due to the numerous varieties and forms whose status has not been reassessed. Through an integrated approach based on microscopy and molecular analyses, we describe *Nitzschia gobbii* sp. nov. from phytoplankton of the coastal site SG1 of the LTER Senigallia transect. In the analysis of the long-term phytoplankton data, a morphotype resembling the *Nitzschiella* section (Hasle, 1964) was often observed in the phytoplankton community, possibly sometimes misidentified as *Cylindrotheca closterium*. In this study strains of this morphotype isolated from net samples were analyzed using both light and electron microscopy combined with molecular analysis based on LSU and ITS rDNA, leading to the description of a new species. Cells of *Nitzschia gobbii* sp. nov. (Apical Axis (AA) from 38.7 to 53.1 µm, Transapical Axis (TA) from 0.79 to 2.51 µm) showed an expanded central part occupying 1/2–1/3 of the total length and fine projections towards apexes. In valve view, the raphe was present on the same side on both valves, with the central nodule always present. The mantle was short, without evident structures. Striae were uniseriate from 38 to 42 in 5 µm and incomplete. Fibulae occurred with higher density on the projections (from 10 to 43 in 10 µm) than in the central valve (from 4 to 20 in 10 µm). Furthermore, fibulae were unequally distributed between the two valves of the same frustule. In girdle view, two (rarely three) cingular bands were observed (band striae from 56 to 64 in 10 µm): the valvocopula and the second cingular band had identical pattern with two rows of areolae which had hymen highly perforated. These peculiar features, in combination with the molecular analysis based on LSU and ITS rDNA, allow us to describe *Nitzschia gobbii* sp. nov. within the paraphyletic group of *Nitzschia*.

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# Effect of abiotic factors on photosynthetic performance of two strains of Cyanobacteria

**A. Guzzon, E. Bellini, L. Rugnini, L. Bruno**

Photosynthetic activity of cyanobacteria in culture has been investigated for decades, with great efforts devoted to understanding the impact of abiotic factors, primarily light intensity and temperature, on the photosynthetic process. To date, however, limited information is available on the influence of medium mixing, despite its pivotal role in gas and nutrient exchange, essential for proper functioning of the photosynthetic system and, consequently, growth. At the same time, abiotic stressors may affect photosynthesis. For instance, cadmium ($Cd^{2+}$) can replace magnesium ($Mg^{2+}$) both in RuBisCo catalytic centre and Chlorophyll *a*. In the latter case, damage to the affected organism originates from degradation of this pigment that bleaches easily, but also from its unsuitability for photosynthesis (Küpper and Andresen, 2016). In this regard, two cyanobacterial strains (*Gloeobacter violaceus* PCC 7421 and *Nostoc* sp. PCC 7120) were batch cultured under controlled light and temperature conditions in 1) absence of mixing, 2) with magnetic stirring at 120 rpm, 3) with air bubbling. During the entire experiment, growth parameters (OD665nm and Chl *a*) were evaluated and correlated with specific photosynthetic parameters measured by Pulse Amplitude Modulated (PAM) fluorescence. In detail, photosynthetic quantum yield of light acclimated-samples (ΔF/Fm') was measured and Rapid Light Curves (RLCs) were performed for estimating relative maximum Electron Transport Rate ($rel.ETR_{max}$), photosynthetic efficiency (α) and Saturating Irradiance ($I_k$) (Genty *et al.,* 1989; Henley, 1993). At the end of experiment, Photochemical Quenching (qP) and Non-Photochemical Quenching (NPQ) were investigated (Oxborough and Baker, 1997; Hendrickson *et al.,* 2004). Furthermore, a parallel experiment was conducted on air-bubbling cultures of *Nostoc* sp. with the purpose of evaluating photosynthetic performance under different Cd-treatments (10, 20 and 36 μM). Culture mixing affected growth and photosynthesis of the two strains tested. For Chl *a* of *G. violaceus* cultures, no significant differences were observed among treatments, while Chl *a* content of *Nostoc* sp. static and stirred samples was significantly higher than air-bubbled ones. Photosynthesis was negatively affected in air-bubbling treatment, as indicated by significant reduction of quantum yield and RLCs parameters. Consistently, NPQ showed differences among culture mixing conditions, with a decline of the amount of regulated energy dissipation from absence of mixing to increased agitation. Heavy metal addition negatively impacted photosynthesis of *Nostoc* sp. bubbling cultures as highlighted by significant lower values of quantum yield and RLCs parameters compared to control cultures, with a more pronounced decrease at the highest $Cd^{2+}$ concentration. This study provided preliminary data on the effect on cyanobacteria photosynthetic performance of culture medium mixing, that, together with light intensity and temperature, is among the key abiotic factors extensively investigated to improve photosynthetic biomass production and extraction of high-value compounds.

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# High cell density and viral infection trigger formation of resting stages in the marine diatom *Chaetoceros socialis*

**A. Pelusi, A. Godhe, M.I. Ferrante, M. Ribera d'Alcalà, K. Thamatrakoln, K. Bidle, M. Montresor**

Many diatoms have heteromorphic life cycles that include the formation of resting stages (von Dassow and Montresor, 2011). These stages can increase the range of conditions in which species can survive, thus ensuring their persistence in time with important ecological implications for population dynamics. Although common to many species, cues and modality of resting stage formation remain unclear. Nitrogen starvation is reported as the most effective one in the laboratory; however, the link between spore formation and nitrogen depletion in the natural environment remains elusive. Using the widespread marine diatom *Chaetoceros socialis* as model species, we tested additional factors that may play a role in inducing spore formation. Even though nitrogen depletion was confirmed to be the most effective trigger in laboratory conditions, resting stages were formed also when cell density was high but nutrients were not limiting and when cultures were grown in a culture medium conditioned by high cell density. This suggests the presence of chemical signals that induce the transition between different life stages. *Chaetoceros socialis* can be infected by a small RNA virus and we also tested if viral attack can induce the formation of resting spores. Indeed, significantly higher numbers of spores were produced in virus-infected cultures as compared to the non-infected control. Biological interactions, both between cells of the same species and between diatom cells and the virus, can play an important role in regulating the life cycle of this species with important ecological implications.

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# PhytoNumb3rs: An easy-to-use computer toolkit for counting microalgae by the Utermöhl method.

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Phytoplankton constitutes a biological quality element that is used to assess the ecological health status of water bodies as well as changes occurring under a range of environmental conditions including anthropogenic pressures. Demographic traits such as presence/absence, abundance and biomass have traditionally been included in directives and monitoring programs as descriptors of the ecological status of aquatic ecosystems (Water Framework Directive EU/2000/60, Marine Strategy Directive EU/2008/56). Even though the Utermöhl method (Utermöhl, 1958) is the most widely adopted method to determine the abundance of phytoplankton assemblages, the procedures used for phytoplankton analysis vary widely between research and monitoring groups, despite the numerous efforts to standardise phytoplankton data. Such data may need to be used several decades after they were first gathered by users that did not participate in their production; a fundamental effort is thus required in order to ensure data harmonisation and consistency of time series, facilitating data accessibility and comparability. In order to facilitate reliable evaluation of the changes in phytoplankton data, as well as the comparison of phytoplankton data collected at different temporal and spatial scales, quality assurance and quality control procedures are crucial. For example, the data are manually reported into computer spreadsheets, which takes additional time and introduces possible typing errors. In addition, the collected data need to be stored in flat files or spreadsheets with minimal formal structure and few metadata. We developed PhytoNumb3rs, a tool designed to support phytoplankton analysts and researchers during each step of the analysis from the compilation of the raw data sheet to the computation of abundance to the structuring of the dataset in a given format. The PhytoNumb3rs counting toolkit is based on the Utermöhl method (UNI EN 15204, 2006). However, besides being time-consuming and labour-intensive, the procedure described in the UNI EN 15204 (2006) guidance also suffers from a lack of detail concerning the counting analysis. For example, the information about how analysts should choose the counting strategy, how many cells per sample should be counted and how uncertainty should be estimated are purely generic. The innovative aspect of PhytoNumb3rs is that it aims to provide a guidance on microalgal analysis, including statistical procedures for the design and the optimization of methods to analyze each single sample. In addition, we have developed a tool for microscopic analysis of phytoplankton that makes it easier to calculate cell density and to incorporate qualitative and quantitative aspects concerning data precision, bias and method sensitivity. It represents a first step towards harmonization of data and the promotion of a standardized procedure for data management that will save time during data entry and storage. The use of PhytoNumb3rs would greatly enhance information exchange within and between environmental protection agencies and scientific research institutions, improving the accessibility of the phytoplankton databases.

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# Preliminary observations on the coralligenous macrophytobenthos in the Marine Protected Area Isole Ciclopi (eastern coast of Sicily)

**L.G. Costanzo, G. Marletta, G. Alongi**

Coralligenous macroalgal assemblages are a peculiar feature of deep subtidal systems in the Mediterranean Sea, which are represented by calcareous structures consisting of encrusting Rhodophyta belonging to the orders Corallinales and Hapalidiales (Garrabou and Ballesteros, 2000; Ballesteros, 2006). These bioconstructions are characterized by a dynamic balance of building processes by calcareous organisms (principally encrusting coralline algae) and biological eroding processes (Garrabou and Ballesteros, 2000). However, this balance can be easily disturbed by several types of stressors. Thus, the maintenance of stable environmental conditions is extremely important for the survival of this habitat (Piazzi *et al.*, 2012). The coralligenous macroalgal assemblages of the Ionian coast of Sicily (Italy) have been poorly investigated. In fact, the last floristic studies on this habitat date back to the seventies (Furnari and Scammacca, 1970; Furnari *et al.*, 1977) and to 2001 (Giaccone and Pizzuto, 2001). In the framework of the programme "Progetto Operativo di Monitoraggio (P.O.M.)" of the EU Marine Strategy Directive (MSFD), a study was carried out on the coralligenous habitat of the Marine Protected Area (MPA) Isole Ciclopi (central-eastern coast of Sicily) in summer/autumn 2018. Through ROV surveys and sample analysis, it was observed that the coralligenous assemblages of the MPA Isole Ciclopi (namely the two facies *Eunicella cavolinii* and *Lithophyllo-Halimedetum tunae*) were well-structured, especially the encrusting Rhodophyta, which showed a high coverage degree. The comparison of previous studies with current data showed that, in approximately 50 years, the local biodiversity of coralligenous macroalgal assemblages has increased. This increase in biodiversity could be related to the "reserve effect", due to the establishment of the MPA in 1989. Moreover, over the past 50 years, a change in the flora of the coralligenous habitat of the MPA has occurred. Indeed, there was a decrease of the Ochrophyta percentage, that could be indicative of a rise in the sedimentation rate. Furthermore, the number of Atlantic and Pantropical species increased, while the number of Mediterranean species, which are characteristic of deep subtidal environments, decreased. These variations might be attributed to a rise in the seawater temperature, which promotes the establishment of warm-water species. Indeed, since the end of the 1980s, the Mediterranean Sea has undergone a regime shift in the atmospheric, hydrological, and ecological systems (Conversi *et al.*, 2010). In conclusion, the present study allowed to gain an updated knowledge of the flora associated with the coralligenous habitat along the Ionian coast of Sicily. The high level of biodiversity and coverage of calcareous Rhodophyta suggests that the coralligenous assemblages of the MPA are well-structured and healthy.

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# Diversity of Sulfur metabolism in marine microalgae

**C. Gerotto, D. Pousa Kurpan Nogueira, A. Norici, M. Giordano**

Photosynthetic organisms often face non optimal environmental conditions and their productivity and survival rely on their ability to acclimate to environmental challenges. Sulfur (S) is an essential macronutrient in algae. It is acquired by cells as sulfate and assimilated as sulfide in an energy demanding reductive process. Despite the central role of S in multiple cellular processes, e.g. by allowing redox regulation of enzymes, our knowledge on regulation of S metabolism is still fragmentary. Most available information refers to plants while very little is known for algae. Further, S is often excluded from stoichiometry studies, possibly because in today's oceans sulfate concentration is rarely limiting for the growth of phytoplankton. Yet, changes in the availability of other resources, as other nutrients or light, may affect and reduce S assimilation. We are investigating S metabolism in microalgae of different phylogeny by identifying sequences of putative S metabolism enzymes and by evaluating physiological responses of algae under S-limitation. *In silico* analyses of S metabolism enzymes suggest that different species may display a diversified regulation of S metabolism. Characterization of cells growth and organic composition under S limitation revealed that S limitation occurs under markedly different sulfate concentrations, in line with the change in species dominance during the history of oceans. Indeed, the increase of oceanic sulfate concentrations at the boundary between the Paleozoic and the Mesozoic eras matched with a transition in species dominance in marine phytoplankton. Green algae and cyanobacteria, characterized by relatively low S cell quotas, were prominent when S availability was lower (Paleozoic), whereas red lineage algae, with higher S cell quota, are more abundant in today´s oceans.

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# Use of *Galdieria sulphuraria* (Cyanidiophytina, Rhodophyta) in recovering rare-earth elements from fluorescent lamps (FL).

**M. Palmieri, M.R. Di Cicco, C. Lubritto, C. Ciniglia**

The disposal and recovery of waste electrical and electronic equipment (WEEE) is a problem that governments are considering, and affects the entire population, since it shows critical issues from an economic, environmental and health point of view (European Commission, 2014). In the last decade, recovery of metals, especially of rare earth elements (REEs), using bioprocess technology has been one of the most promising technologies due to low management costs and low environmental impact (Pollmann *et al.,* 2018). *Galdieria sulphuraria* is a polyextremophilic red alga able to proliferate in strongly acidic, naturally metal-rich environments, which developed unique mechanisms of metal tolerance (Iovinella *et al.,* 2018; Minoda *et al.,* 2015). This microalga was employed in a set of experiments to test the capacity of simultaneous removal of rare metals from a mixed solution (cerium, europium, yttrium and terbium); different growth conditions were tested, being *G. sulphuraria* able to multiplicate under autotrophy, mixotrophy, and heterotrophy. Results were comparable to literature information: *G. sulphuraria* bioaccumulated all tested metals, with a prevalence of yttrium, followed by other tested metals. Mixotrophic growth on sucrose as source of carbon was the best condition for metal uptake. The algal physiological state was monitored through growth curves, chlorophyll and phycocyanin production, and ammonium and phosphate uptake. Surprisingly, growth of cultures was enhanced, as well as pigments accumulation. The most abundant element that was accumulated in the algal biomass was confirmed to be yttrium, followed by europium and lanthanum.

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# Effectiveness of long-term observations for the behaviour assessment of a potentially invasive non-indigenous species (NIS) in the Mar Piccolo of Taranto (northern Ionian Sea, Mediterranean Sea)

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Long Term Ecological Research (LTER), which proceeds across many years, is an all-important activity in following the course of changes in ecological systems (Kratz *et al*., 2013). The Mar Piccolo of Taranto is a transitional water system (TWS) in the northern Ionian Sea included into the European LTER network (LTER_EU_IT_095) on account of the availability of historical sets of both biological and chemical-physical data. A recent assessment of changes in the macrophytobenthic assemblages imputable to human pressures (Petrocelli *et al*., 2019) was carried out together with a study on the opposite fate of two non-indigenous species (NIS), *Undaria pinnatifida* (Harvey) Suringar and *Hypnea cornuta* (Kützing) J. Agardh, introduced in the basin (Cecere *et al*., 2016). Both studies highlighted the need of effectiveness in the preparation of management plans that include indications from long-term observations. For this reason, a decennial study was carried out to follow the course of another NIS, *Grateloupia turuturu* Yamada, which is considered one of the worst invasive seaweeds on global scale due to its fast spread and deep alteration of the hosting ecosystem. In particular, G. turuturu was recorded for the first time in the Mar Piccolo of Taranto in February 2007 (Cecere *et al*., 2011) and from October 2008 to December 2018, monthly in situ measurements of thalli number and length were performed on its population, settled on plastic nets commonly used in mussel farming and abandoned on the Mar Piccolo bottom. Each month, four randomly selected squares (20 cm side) were sampled. Size and density data were statistically analyzed to outline the changes occurred in the population structure. Both sets were correlated with long term temperature data to detect its possible effect on the species establishment and spread. A seasonal growth cycle was detected for *G. turuturu* in the Mar Piccolo. Plantlets appeared in autumn-early winter (i.e. October-January) and showed a continuous recruitment, with adult thalli present until late spring (i.e. May-June). Neither plantlets or erect thalli were ever recorded between July and September, but only crustose thalli firmly wrapped around the plastic nets. No gametophytic thalli were observed. A marked reduction in the growth cycle duration was assessed starting from 2009. Both size and density values were the highest in the coldest months (i.e. February and March), even though no significant correlation was detected between *G. turuturu* density and temperature. A significant density decrease was observed throughout the study period, while the size reduction was not significant. This study showed that *G. turuturu* has not become invasive in the basin to date. It can be considered well established, even though a marked regression of its population occurred throughout the investigated period, as well as the loss of sexual reproduction, observed in 2007. Notwithstanding both species were native of Pacific cold temperate waters, *G. turuturu* seemed to have adapted to the high summer temperatures recorded in the study area, contrarily to *U. pinnatifida*, which after its introduction completely disappeared in ten years (Cecere *et al*., 2016). Most probably, G. turuturu exploits a better survival strategy through the production of crustose thalli, which behave as resting structures during the adverse season but maintain the capacity of budding when environmental conditions become more favourable. However, the lack of sexual reproduction most likely reduced its potential invasiveness.

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# ‘Sensorial’ systems and microalgae affairs

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The potential of sensors to monitor microalgal growth provides real-time systems for quality control in biomass cultivation processes (Havlik *et al*., 2013) as well as for the monitoring of microalgal population changes in natural environments where early warning of bloom outbreaks can be crucial (Gardner *et al*., 2000; Lvova *et al*., 2016). Here, we report on the use of two sensory systems: **i**) an Electronic Tongue (E-Tongue), based on an array of seven metal semiconductor sensors, with pronounced sensitivity towards cations, anions and RedOX species, used to detect the co-presence of an intensively cultivated diatom and a cyanobacterial contaminant **ii**) a portable sensor, based on fluorescence spectroscopy, used to discriminate between the fluorescence signals, after UV excitation, of a set of cyanobacterial and microalgal cultures, representative of major phytoplankters in nature. The E-Tongue approach was applied to distinguish the variations in *Phaeodactylum tricornutum* Bohlin culture ‘environment’, over time, either in monoculture conditions or in co-culture with a unicellular, undetermined, cyanobacterium that we isolated as contaminant in an indoor photobioreactor. Results showed that signal intensities, recorded analysing the cultivation media of the mono-and co-cultures, allowed us to detect changes in the chemical composition of the media over the growth of each culture. Specific signal patterns for the cyanobacterium, the diatom *P. tricornutum* and for their co-cultures were identified and PCA (Principal Component Analysis) showed differences in cultures over the whole growth period for each culture tested. These results suggest that E-Tongue can be a fast and indirect tool to monitor microalgal culture purity, opening up new perspectives for the management of intensive microalgae cultivation. The portable spectroscopic sensor, based on multi-wavelength excitation and fluorescence detection, was used to register the *in vivo* spectra at 265, 275 and 405 nm of 12 strains cultivated at lab conditions, belonging to Cyanobacteria, Bacillariophyta, Chlorophyta, and Haptophyta. Spectrum analysis showed that the emitting light at 265 and 275 nm allowed to excite a set of cellular metabolites, while 405 nm was able to efficiently excite chlorophylls. Overall, these data were used to obtain a spectral fingerprint of each strain analysed and were used as reference for *in situ* monitoring. Indeed, the portable sensor was applied to monitor water from the aquaponic farm of the Institut National de la Recherche Agronomique-Pisciculture Expérimentale Inra des Monts d’Arrée (INRA-PEIMA), subject to the proliferation of cyanobacteria. Each chosen excitation wavelength provided specific signal and information: 275 nm was used to excite the “tryptophan like” molecules, useful for the estimation of the whole biomass and for the organic matter present in the samples, while the wavelength of 405 nm excited chlorophyll *a*, highlighting the abundance of the phytoplankton community.

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