Riunioni scientifiche dei Gruppi di Lavoro

e delle Sezioni Regionali della

Società Botanica Italiana onlus

Mini lavori della Riunione scientifica del Gruppo di Lavoro per l'Algologia

(a cura R. Pistocchi)

10-11 novembre 2017, Trieste

In copertina: aggregati di cellule di *Ostreopsis* cf. *ovata* (Dinophyceae) isolate nel Golfo di Trieste, con abbondante mucillagine formata da filamenti di tricocisti e polisaccaridi extracellulari (Golfo di Trieste)
foto di Giorgio Honsell

On the cover: aggregates of *Ostreopsis* cf. *ovata* (Dinophyceae) cells, isolated in the Gulf of Trieste, showing abundant mucilage composed of trichocyst filaments and extracellular polysaccharides (Gulf of Trieste)
photo by Giorgio Honsell

Factors affecting growth and toxin production in *Prorocentrum hoffmannianum* (Dinophyceae) in Florida Keys

S. Accoroni, M. Ceci, C. Dell'Aversano, L. Tartaglione, T. Romagnoli, C. Totti

Prorocentrum hoffmannianum M.A.Faust is a benthic dinoflagellate that produces biotoxins which are causative of diarrhetic shellfish poisoning (DPS) in warm marine waters. The occurrence of this dinoflagellate was reported only in tropical areas (Belize, Florida, Mexico, Brazil, northern Australia and Hainan Island, China).
In this study, we investigated the effect temperature and nutrient depletion on *P. hoffmannianum* isolated in from field samples (Florida Keys, Florida, USA) in 2014.
Monoclonal cultures were prepared by isolating single cells by the capillary pipette method. Cultures were maintained at a temperature of 21±0.1 or 26±0.1 °C and a light intensity of 90–100 µmol m^{-2} s^{-1} with light-dark cycle 12-12 h. For each temperature, *P. hoffmannianum* were grown in f/4 medium (as control), N-free and P-free medium in triplicate. Subsamples (2 ml) were sampled every 2 days for 30 days. Cell counts were made following the Utermöhl sedimentation method, through epifluorescence microscopy.
Both temperature and nutrient condition significantly affected growth rates and maximum yield of *P. hoffmannianum* showing the maximum values recorded in high temperature and replete medium. Production of okadaic acid was induced in all conditions (min 50±46 pg/cell; max 643±189 pg/cell), showing values up to 1 order of magnitude higher than that observed in other DSP species.
Toxin concentration (both on a per cell and a biovolume basis) resulted enhanced at 26 °C and in P depletion, corroborating the knowledge that toxin production is modulated by cell physiological conditions, which are affected by a wide spectrum of factors, including several environmental stressors such as nutrient availability.
Toxin fraction released in the growth medium was neglectable. No okadaic acid esters were detected in this strain of *P. hoffmannianum.*

AUTORI

Stefano Accoroni (s.accoroni@univpm.it), Martina Ceci, Tiziana Romagnoli, Cecilia Totti, Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy
Carmela Dell'Aversano, Luciana Tartaglione, Dipartimento di Farmacia, Scuola di Medicina, Università degli Studi di Napoli "Federico II", Via D. Montesano 49, 80131 Napoli, Italy
Autore di riferimento: Stefano Accoroni

Ostreopsis ovata Fukuyo 1981 along the eastern Adriatic coast

A. Baričević, N. Kužat, M. Smodlaka Tanković, D. Marić Pfannkuchen, M. Pfannkuchen

Ostreopsis spp. are benthic dinoflagellates that are known to cause harmful algae blooms. They are known producers of a range of palytoxins and ovatoxins. During bloom events those toxins can be found in high concentrations in benthic biofilms, in the water column as well as in coastal aerosols. *Ostreopsis* spp. distribution and related harmful algae blooms are well documented along the western Adriatic coast and parts of the western Mediterranean. Little was known so far about its distribution along the eastern Adriatic coast which presents very different ecological conditions with respect to the western Adriatic coast. A high resolution sampling regime along the eastern coast of the northern Adriatic revealed a dense population structure and high abundances along that coast line. Samples from the middle and southern Adriatic confirmed the presence of *Ostreopsis ovata* along the entire eastern Adriatic coast. Our results demonstrate, that oligotrophic rocky shores can be a very suitable habitat for *O. ovata*. Massive bloom events with record abundances and toxin concentrations are reported. The population along the north-eastern Adriatic coast share one ITS1-5.8S-ITS2 genotype with most of the populations recorded around the Mediterranean.

AUTORI

Ana Baričević (ruso@cim.irb.hr), Nataša Kužat, Mirta Smodlaka Tanković, Daniela Marić Pfannkuchen, Martin Pfannkuchen, Center for Marine Research, Ruđer Bošković Institute, G. Paliaga 5, Rovinj, Croatia
Autore di riferimento: Ana Baričević

Hydrogel scaffolds from *Trichormus variabilis* (Nostocales, Cyanobacteria) exopolymers

E. Bellini, M. Ciocci, S. Savio, S. Antonaroli, D. Seliktar, S. Melino, R. Congestri

Cyanobacterial Extracellular Polymeric Substances (EPS) are highly complex and variable compounds exhibiting hydrophilic and anionic properties. EPS can comprise heteropolysaccharides, of six or more sugar units, proteins, nucleic acids and lipids. EPS highly retentive properties make them promising for producing hydrogel scaffolds for drug delivery and tissue engineering.
Released-EPS (R-EPS) were obtained by intensive indoor cultivation of a strain, VRUC 168, of the cyanobacterium *Trichormus variabilis* (Kützing ex Bornet & Flahault) Komárek & Anagnostidis, isolated from sediments of a Mediterranean coastal lagoon, in polyethylene bags (10L; BG11 medium; 25 °C; L-D 12-12h; irradiance 80 µmolphotons/m^2/s^1). At stationary phase, day 20, the culture medium was collected and R-EPS concentrated by centrifugation and rotary evaporation. R-EPS were then precipitated in cold ethanol and the pellet freeze-dried. This material was characterized by RP-HPLC, spectroscopic analyses, cytochemical staining, BCA and phenol-sulphuric acid assays.
R-EPS were used to produce hybrid hydrogels (EPS-Hys) for detoxification enzyme- and stem cells- carrier systems. To this end, a specific protocol for the synthesis of the EPS-hydrogel through photopolymerization was developed. Hydrogel stability, resistance to hydrolysis and mechanical properties were also assessed. Thus, hybrid hydrogels were tested for enzymatic encapsulation using thiosulfate-cyanide sulfur transferase (TST) and 3D scaffolding for human cardiac mesenchymal stem cells (cMSCs).
Mass cultures of *T. variabilis* reached productivity of 0.039±0.001 g(DW)$L^{-1}d^{-1}$ and the R-EPS yield was of 2 gDW. Characterization of this EPS fraction revealed the presence of carbohydrates with sulfated and carboxylic groups. FT-IR spectrum showed peaks within 2900 and 3450 cm^{-1}, attributable to -CH and –OH group vibrations, and a peak around 1600 cm^{-1} attributable to -COOH group vibrations.
The fabricated hybrid hydrogels (EPS-Hys) resulted more stable and resistant to dehydration and spontaneous hydrolysis, at 37 °C, than PEGDa-Hys and rheological analysis of hybrid hydrogels evidenced that cyanobacterial EPS improved hydrogel mechanical performances.
Enzymatic encapsulation test showed that TST activity was preserved in the hydrogel at 37 °C. 3D scaffold application of the novel hybrid EPS-Hy showed effective adherence and growth of cMSCs cells, with an increase in cell viability after 3-day growth, suggesting a non-cytotoxic effect of the EPS-Hys on the stem cells. Results showed that *T. variabilis* S-EPS are a sustainable source of biomaterials adaptable to a large variety of biotechnological applications.

AUTORI
Erika Bellini (erikabellini1990@gmail.com), Saverio Savio, Roberta Congestri, Dipartimento di Biologia, Università di Roma 'Tor Vergata', Via Cracovia, 00133 Roma, Italia,
Matteo Ciocci, Simonetta Antonaroli, Sonia Melino, Dipartimento di Scienza e Tecnologie Chimiche, Università di Roma 'Tor Vergata', Via Cracovia, 00133 Roma, Italia
Dror Seliktar, Dipartimento di Ingegneria Biomedica, Technion Israel Institute of Technology, 3200003 Haifa, Israele
Autore di riferimento: Erika Bellini

Two new species of the genus *Ceramium* Roth (Ceramiales, Rhodophyta) from the Venice Lagoon (Italy)

V.M. Betto, M.A. Wolf, A. Buosi, K. Sciuto, I. Moro, C.A. Maggs, A. Sfriso

The cosmopolitan genus *Ceramium* Roth (Ceramiaceae), with about 213 currently accepted species (and infraspecific) names, is one of the largest groups in the Rhodophyta. This taxon is characterized by cylindrical or slightly compressed thalli, whose axial cells can be incompletely to completely covered by cortical cells, with alternate to pseudo-dichotomous branching, and straight to inrolled apices. It typically occurs in eulittoral or shallow subtidal habitats and its worldwide distribution is often connected with naval traffics. In fact, *Ceramium* thalli are often carried by hull fouling.
The nomenclature and the taxonomy of this genus are still in a state of chaos. Taxonomic problems are, indeed, tied to a high degree of variations in the morphological characters classically used in species recognition: presence of cortical spines, numbers of periaxial cells, developmental patterns of the corticating filaments, branching pattern, and tetrasporangial features. At the same time, the identification of new species is often complicated by their small sizes and epiphytic life style, that make them cryptic.
Culture studies, suggesting a strong influence of the environment on morphology, and the use of molecular tools have questioned the validity of morphological features to discriminate different *Ceramium* species. In particular, as the inventory of *Ceramium* species in some areas, has been only based on morphological observations, more and more authors have started using molecular markers (e.g. *rbc*L gene and *rbc*L-*rbc*S intergenic spacer, SSU rDNA, ITS2) to determine species diversity.
Here we characterize different isolates, sampled in transitional waters of the Venice Lagoon (Italy), in order to better investigate presence and biodiversity of *Ceramium* in these areas. Through accurate morphological observations and phylogenetic analyses, based on the plastid ribulose-1,5-bisphosphate carboxylase/oxygenase gene (*rbc*L) as molecular marker, we identify two distinct *Ceramium* taxonomic entities, never reported before in this environment according to the available checklists. The validity of the two entities as recognizable species is also discussed.

AUTORI

Viola M. Betto (838243@stud.unive.it), Marion A. Wolf, Alessandro Buosi, Adriano Sfriso, Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice, Via Torino 155, 30172 Venice, Italy
Katia Sciuto, Isabella Moro, Department of Biology, University of Padova, Via U. Bassi 58/B, 35131 Padova, Italy
Christine A. Maggs, Bournemouth University, Fern Barrow, Poole, Dorset, BH12 5BB, United Kingdom
Autore di riferimento: Viola M. Betto

Monitoraggio del fitoplancton lungo la costa laziale (Tirreno centrale) negli ultimi quindici anni

I. Bianco, E. Viaggiu, R. Congestri

Le attuali conoscenze della dinamica del fitoplancton marino nelle acque costiere del Lazio sono scarse. Poche sono infatti le pubblicazioni, risalenti ad oltre un decennio fa, incentrate sulla composizione della comunità fitoplanctonica e sulle sue variazioni spazio-temporali nelle acque superficiali, entro i 500 m dalla costa. I lavori nati dalla collaborazione tra l'Agenzia Regionale di Protezione dell'Ambiente, ARPA Lazio e il Laboratorio di Biologia delle Alghe dell'Università di Roma "Tor Vergata", hanno portato ad un primo aggiornamento dei popolamenti fitoplanctonici con l'identificazione di oltre 250 *taxa*, comprendenti specie tossiche, identificati con tecniche di microscopia ottica ed elettronica (Bianco et al 2006, Congestri et al. 2004, 2006). Il presente lavoro ha permesso di riprendere lo studio della componente fitoplanctonica del Lazio mediante la revisione e l'allineamento dei dati raccolti dal 2002 al 2016 nei programmi nazionali di monitoraggio, ai sensi della L. 979/82, del D.Lgs 152/2006 (direttiva quadro sulle acque 2000/60/CE) e del D.Lgs 116/2008 (direttiva sulle acque di balneazione 2006/7/CE), a cui sono stati aggiunti i dati prodotti nell'ambito della nuova attività di monitoraggio effettuata in ottemperanza al D.Lgs.190/2010 (direttiva sulla strategia marina 2008/56/CE). Tale programma, a tutt'oggi in corso, è stato avviato nel luglio 2015 e prevede un campionamento bimestrale lungo 4 transetti, ciascuno comprendente 3 stazioni di campionamento. L'aspetto innovativo di questo monitoraggio è la stima quali-quantitativa del fitoplancton anche in acque profonde in corrispondenza del DCM (Deep Chlorophyll Maximum) e più a largo, ovvero in stazioni poste a 3, 6 e 12 miglia nautiche dalla costa.
Le analisi dell'intero set di dati, che annovera oltre un migliaio di campioni, hanno mostrato preliminarmente un aumento del numero di *taxa* con oltre 350 record e hanno confermato la presenza di dinoflagellati potenzialmente tossici, come *Alexandrium minutum*, *Dinophysis caudata*, *Dinophysis sacculus*, *Lingulodinium polyedrum*, *Phalacroma rotundatum*, *Ostreopsis ovata*, *Prorocentrum lima*, *P. minimum* e varie specie di diatomee appartenenti al genere *Pseudo-nitzschia* già registrati prima del 2004. Inoltre è stata rilevata la presenza della rafidioficea ittiotossica *Chattonella subsalsa* e di altri dinoflagellati potenzialmente tossici, come *Dinophysis rapa*, *Gonyaulax spinifera*, *Karenia* cf. *brevis*, *K.* cf. *mikimotoi*, *K.* cf. *papillonacea* e *Protoceratium reticulatum*, osservati a partire dal 2005. Specie ritenute non indigene, come la diatomea *Pseudo-nitzschia multistriata* e il dinoflagellato bentonico *Ostreopsis ovata*, sono occasionalmente presenti nei campioni, quest'ultimo in corrispondenza di eventi di fioritura estiva. Infine, in relazione ai campioni prelevati nel monitoraggio per la strategia marina, sono stati rilevati, nelle stazioni più a largo, un numero ridotto di *taxa* e abbondanze minori con un contributo rilevante del gruppo atassonomico di nanoflagellati.

Letteratura citata

Bianco I, Congestri R et al. (2006) Fioriture di microalghe potenzialmente tossiche lungo le coste laziali. Biologia Marina Mediterranea 13 (1): 947-950.

Congestri R, Bianco I, Albertano P (2004) Potentially Toxic Thecate Dinoflagellates of Middle Tyrrhenian Coastal Waters (Mediterranean Sea)". In: Steidinger KA, Landsberg JH, Tomas C, Vargo GA (Eds) Proceedings of the Xth International Conference on Harmful Algae. Florida Fish and Wildlife Conservation Commission and Intergovernmental Commission of UNESCO.

Congestri R, Bianco I et al. (2006) Il fitoplancton delle coste laziali dal 1997 a oggi: struttura della comunità, taxa dominanti e specie tossiche. Biologia Marina Mediterranea 13 (1): 54-60.

AUTORI

Ilen Bianco (ilen.bianco@arpalazio.gov.it), Dipartimento di Biologia, Università di Roma "Tor Vergata", Via della Ricerca Scientifica, 00133 e ARPA Lazio Roma Servizio Risorse Idriche e Naturali, Unità Acque Superficiali e Sotterranee, Via Giuseppe Saredo 52, 00173 Roma

Emanuela Viaggiu, Roberta Congestri, Dipartimento di Biologia, Università di Roma "Tor Vergata", Via della Ricerca Scientifica, 00133 e AlgaRes s.r.l., Spin off dell'Università di Roma "Tor Vergata", c/o il Parco Scientifico, Via della Ricerca Scientifica, 00133 Roma

Autore di riferimento: Ilen Bianco

Applicazione di oli essenziali come metodo non-invasivo per il controllo del biodeterioramento di beni culturali in pietra

L. Bruno, V. Valle, A. Gismondi, G. Di Marco, A. Canini

Il valore e la durabilità nel tempo dei beni culturali di tutto il mondo sono minacciati dal fenomeno del biodeterioramento, ovvero 'la perdita irreversibile di valore e/o informazione di un oggetto d'arte in seguito all'attacco di organismi viventi' come definito da Urzì e Krumbein (1994). In particolare, in presenza di luce naturale o artificiale e determinate condizioni ambientali, le superfici esposte di monumenti in pietra sono interessate dallo sviluppo di biofilm fototrofi. Questi biofilm sono comunità complesse immerse in una matrice polisaccaridica e formate principalmente da cianobatteri in associazione con batteri, microalghe, muschi e funghi, che sono in grado di formare patine colorate su vari tipi di substrato in ambienti aperti o confinati (Fig. 1) come cripte, chiese e ipogei (Albertano 2012, Rossi et al. 2012, Bruno, Valle, 2017).

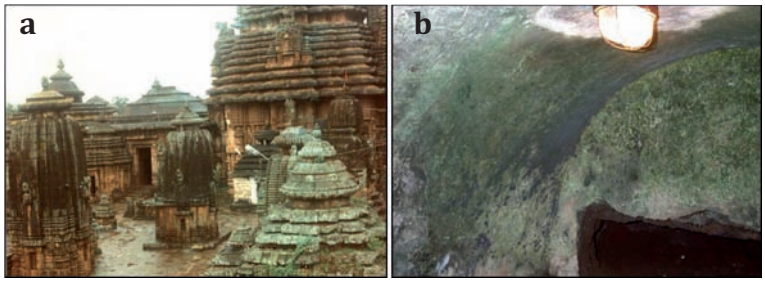

Fig. 1
Monumenti in pietra interessati dal biodeterioramento: a) Complesso dei templi Lingaraj a Bhubaneswar, Orissa, India; b) Cubicolo illuminato da luce artificiale nelle Catacombe di Domitilla, Roma, Italia.

La presenza di questi biofilm sul bene culturale determina un danno estetico per l'alterazione dell'aspetto del bene stesso e a lungo termine può essere responsabile anche di danni strutturali che ne compromettono la stabilità e quindi la durabilità nel tempo. I metodi convenzionali utilizzati dai restauratori per l'eradicazione di questi biofilm dalle superfici di pregio consistono in spazzolature meccaniche accoppiate all'impiego di biocidi; questi metodi risultano deleteri per i substrati, pericolosi per l'ambiente e dannosi per la salute umana (Urzì et al. 2016). Da qui la necessità di ricercare nuove soluzioni non-invasive per il substrato e anche eco-compatibili e non dannose per l'uomo (Bruno et al. 2014a, b, Hsieh et al. 2014, Bruno, Valle 2017, Ruffolo et al. 2017). Recentemente diversi olii essenziali, risultati non citotossici per le cellule umane e ampiamente impiegati per scopi medicinali (Gismondi et al. 2014, Giovannini et al. 2016), si sono dimostrati efficaci contro funghi e batteri presenti su beni culturali in pietra e legno (Stupar et al. 2014). Con l'obiettivo di sviluppare nuove strategie per il problema del biodeterioramento dei beni culturali e progettare un protocollo di facile utilizzo per il restauro dei patrimoni culturali in pietra, è stata quindi testata la potenzialità di due oli essenziali di poter essere utilizzati come 'biocidi verdi'. A tal fine sono state allestite piastre contenenti terreno minerale BG11 agarizzato (Rippka et al. 1979) su cui sono stati fatti crescere biofilm fototrofi costituiti da cianobatteri isolati dalle Catacombe Romane (Fig. 2). Gli oli essenziali distillati da *Lavandula angustifolia* Mill. e *Thymus vulgaris* L. sono stati testati, singolarmente e in combinazione, in diverse concentrazioni, sui biofilm cresciuti su piastra.

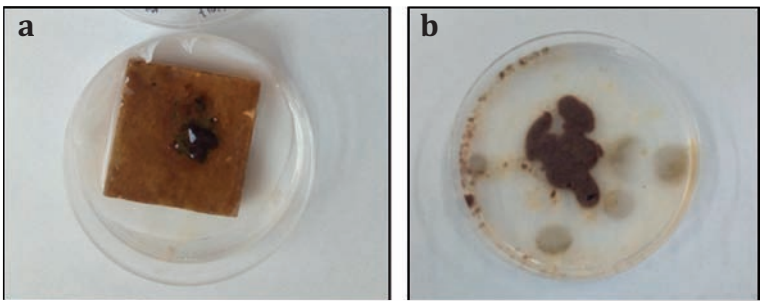

Fig. 2
I biofilm a cianobatteri utilizzati per testare l'efficacia degli oli essenziali come biocidi sono stati fatti crescere su a) pietre calcaree e b) piastre agarizzate.

L'effetto degli oli essenziali sui cianobatteri è stato monitorato mediante il controllo visivo della crescita del biofilm su agar e la valutazione della loro attività fotosintetica mediante il fluorimetro portatile mini-PAM. Poiché gli oli essenziali impiegati in questo studio erano di origine commerciale, i loro profili chimici sono stati studiati, in dettaglio, tramite gas cromatografia associata a spettrometria di massa (GC-MS). I profili GC-MS hanno mostrato come entrambi gli oli essenziali presentassero un elevato numero di metaboliti secondari vegetali dalle note proprietà antimicrobiche (come canfora, timolo) (Falcone et al. 2005, Chen et al. 2013). Al fine di valutare se questi oli essenziali potessero essere utilizzati anche su materiale di pregio senza indurre alterazioni al substrato, è stato anche valutato l'effetto della loro applicazione su pietre calcaree. I risultati ottenuti

hanno evidenziato che gli oli essenziali erano in grado di ridurre efficacemente la crescita del biofilm in coltura diminuendo la loro attività fotosintetica; l'applicazione sulle pietre calcaree non ne alterava l'aspetto.
In conclusione, lo studio ha dimostrato la fattibilità dell'impiego di oli essenziali come nuovi biocidi per il restauro di beni culturali.

Letteratura citata

Albertano P (2012) Cyanobacterial biofilms in monuments and caves. In: Whitton BA (Ed) Ecology of Cyanobacteria II: Their Diversity in Space and Time: 317 e 343. Springer, Netherlands.

Bruno L, Bellezza S, De Leo F, Urzi C (2014a) A study for monitoring and conservation in the roman catacombs of St. Callistus and Domitilla, Rome (Italy) (Chapter 5). In: Saiz-Jimenez C (Ed) The Conservation of Subterranean Cultural Heritage: 37 e 44. CRC Press. ISBN 978-1-138-02694-0.

Bruno L, Ficorella I, Valentini F, Quici L, Keshari N, Adhikary SP (2014b) Characterization of phototrophic biofilms deteriorating Indian stone monuments, their response to heat stress and development of a non-invasive remediation strategy. In: MA Rogerio-Candelera (Ed) Science, Technology and Cultural Heritage: 205-210. CRC Press/Balkema, The Netherlands. ISBN 978-1-138-02744-2.

Bruno L, Valle V (2017) Effect of white and monochromatic lights on cyanobacteria and biofilms from Roman Catacombs. International Biodeterioration and Biodegradation 123: 286-295.

Chen W, Vermaak I, Viljoen A (2013) Camphor—a fumigant during the black death and a coveted fragrant wood in ancient Egypt and Babylon—a review. Molecules 18(5): 5434-5454.

Falcone P, Speranza B, Del Nobile MA, Corbo MR, Sinigaglia M (2005) A study on the antimicrobial activity of thymol intended as a natural preservative. Journal of food protection 68(8): 1664-1670.

Giovannini D, Gismondi A, Basso A, Canuti L, Braglia R, Canini A, Mariani F, Cappelli G (2016) *Lavandula angustifolia* Mill. essential oil exerts antibacterial and anti-inflammatory effect in macrophage mediated immune response to *Staphylococcus aureus*. Immunological Investigations 45 (1): 11-28.

Gismondi A, Canuti L, Grispo M, Canini A (2014) Biochemical composition and antioxidant properties of *Lavandula angustifolia* Miller essential oil are shielded by propolis against UV radiations. Photochemistry and Photobiology 90 (3): 702-708 – [Erratum, Photochemistry and Photobiology 90 (5): 1214].

Hsieh P, Pedersen JZ, Bruno L (2014) Photoinhibition of Cyanobacteria and its Application in Cultural Heritage Conservation. Photochemistry and Photobiology 90: 533-543.

Rippka R, Deruelles J, Waterbury B, Herdman M, Stanier RY (1979) Generic assignments, strains histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology 111: 1-61.

Rossi F, Micheletti E, Bruno L, Adhikary SP, Albertano P, De Philippis R, (2012) Characteristics and role of the exocellular polysaccharides produced by five cyanobacteria isolated from phototrophic biofilms growing on stone monuments. Biofouling: The Journal of Bioadhesion and Biofilm Research 28 (2): 215-224.

Ruffolo SA, De Leo F, Ricca M, Arcudi A, Silvestri C, Bruno L, Urzì C, La Russa MF (2017) Medium-term in situ experiment by using organic biocides and titanium dioxide for the mitigation of microbial colonization on stone surfaces. International Biodeterioration and Biodegradation 123: 17-26.

Stupar M, Lj Grbić M, Džamić A, Unković N, Ristić M, Jelikić A, Vukojević J (2014) Antifungal activity of selected essential oils and biocide benzalkonium chloride against the fungi isolated from cultural heritage objects. South African Journal of Botany 93: 118–124.

Urzi C, De Leo F, Krakova L, Pangallo D, Bruno L (2016) Effects of biocide treatments on the biofilm community in Domitilla's catacombs in Rome. Science of Total Environment 572: 252–262.

Urzì C, Krumbein WE (1994) Microbiological impacts on the cultural heritage. In: Krumbein WE, Brimblecombe P, Cosgrove DE, Staniforth S (Eds) Durability and Change: The Science Responsibility, and Cost of Sustaining Cultural Heritage: 107 e 135. Wiley J & Sons.

AUTORI

Laura Bruno (laura.bruno@uniroma2.it), Veronica Valle, Dipartimento di Biologia, Università degli Studi di Roma 'Tor Vergata', Via Cracovia 1, 00133 Roma, Italia; CUC, Centro Universitario Cattolico
Angelo Gismondi, Gabriele Di Marco, Antonella Canini, Dipartimento di Biologia, Università degli Studi di Roma 'Tor Vergata', Via della Ricerca Scientifica snc, 00133 Roma, Italia
Autore di riferimento: Laura Bruno

Harmful Algal dynamic in the gulf of Trieste during the last 30 years

M. Cabrini, D. Fornasaro, M. Kralj

A 30-year (1986 – 2015) time series of data on phytoplankton abundances, collected in the Long Term Ecological Research (LTER) site in the Gulf of Trieste (North Adriatic Sea), has been analysed. A particular focus was put on the dynamics of potentially toxic species occurrence. The possible effects on economic activities based on marine living resources, such as mussel farming, a traditionally well-developed sector in the Gulf of Trieste, were also valuated. During these decades several changes in phytoplankton abundances and communities composition were observed. An evident short-term as well as a interannual variability, with a range of more than one order of magnitude, characterized the 30 year – time of phytoplankton abundances in the Gulf of Trieste. The timing of interannual variability was different for total phytoplankton, diatoms and dinoflagellates, the latter two representing the main groups evaluated in this study. Total phytoplankton started to decline in the mid '90s, reaching the lowest abundances in 2006 and 2007, while a new increase was recorded after 2008, with several cases of abundances even higher than during the '80s and '90s. The diatoms time – series was characterised by a pronounced decrease in the mid '90s and by a prolonged one in the mid 2000, followed by an increase after 2009, with abundances exceeding 5 millions cells/l. Dinoflagellates abundances were characterised by a prolonged decrease from 2003 to 2010, followed by an increase in abundances up to values higher than during the beginning of the time series. The recent increase of dinoflagellates is of particular concern since the class includes also toxin-producing genera, which may adversely affect aquaculture and shellfish - farming activities located in the area. Considering dinoflagellates composition, some potentially toxic species were reported. Mostly of the recorded species belong to *Dinophysis* and *Alexandrium* genera, which caused contamination of mussels in the Gulf of Trieste since 1989. *Dinophysis caudata, D. sacculus*, *D. fortii*, undetermined *Alexandrium* species, *Gonyaulax polygramma* and *Lingulodinium polyedrum* are the most frequent taxa observed in all the samples collected. Considering the number of cases per year, both *Dinophysis* and *Alexandrium* genera increased in frequency after 2003. The highest frequency of *Dinophysis* occurrence resulted concomitant with episodic alerts due to the presence of biotoxins, leading to the bans of shellfish harvesting. Biotoxin emergencies are among the more important threats to productivity indicated by local mussel farmers and the improved knowledge on their temporal trends and triggering factors are highly needed to protect public health and shellfish farming activities against economic losses.

AUTORI

Marina Cabrini (mcabrini@inogs.it), Daniela Fornasaro, Martina Kralj, OGS- Istituto Nazionale di Oceanografia e di Geofisica Sperimentale, Via A Piccard 54, 34151 Trieste, Italy
Autore di riferimento: Marina Cabrini

Dynamics of the phytoplankton communities in the Mar Piccolo of Taranto, an Italian Long-Term Ecosystem Research (LTER-Italy) site

C. Caroppo, F. Rubino

The Mar Piccolo of Taranto (Northern Ionian Sea), one of the most studied coastal areas in Italy, is a Long-Term Ecosystem Research site (LTER_EU_IT_095).
Phytoplankton data, both as active stages in the water column and cysts in the sediments, have been collected since 1991 (with gaps). Notwithstanding a reduction of nutrients caused by a a relocation of wastewater inputs during the early 2000s, phytoplankton biomass (in terms of chlorophyll *a*) did not show significant variations over the years. However, other relevant changes occurred such as a reduction of the community size, due to a shift of dominance from diatoms to nanoflagellates, an increased duration of secondary blooms (beside the spring one), and the appearance of a diversified dinoflagellate community, comprised mixotrophic and heterotrophic species. An increase of the pico-sized planktonic component has also been recently detected. Moreover, wastewater diversion did not preserve the Mar Piccolo from harmful algal blooms and about 25 harmful species were monitored throughout the years.
These signals are in some ways mirrored in the sediments. In fact, the resting stages communities, during the last 20 years passed through many changes in their structure, even though calcareous dinocysts produced by Thoracosphaeraceae remained dominant. During the last 5 years, in particular, resting spores of some diatoms of the genus *Chaetoceros* were observed, together with the increase and new findings of some heterotrophic species, apparently a signal of the changes occurred in the Mar Piccolo. This change has been registered also with the disappearing of species, e.g. *Protoperidinium pentagonum* and *P. divaricatum*, whose cysts were regularly observed in surface sediments, even with low densities.

AUTORI

Carmela Caroppo (carmela.caroppo@irsa.cnr.it), Fernando Rubino, CNR - Istituto di Ricerca sulle Acque, S.S. Talassografico di Taranto, Via Roma 3, 74123 Taranto
Autore di riferimento: Carmela Caroppo

Microphytobenthic community structure in four Po Delta lagoons subjected to natural and anthropogenic disturbance

T. Cibic

Benthic communities inhabiting the sediments of the Po Delta lagoons are subjected to multiple impacts. Besides those of natural origin, as the stress caused by the alternation of flood river conditions with the lean-season flow, several anthropogenic ones are present, e.g. excessive organic and nutrient loads deriving from urban and industrial discharges, and agricultural activities as well as high contamination levels. To investigate how the benthic communities respond to these disturbances, a scientific survey was carried out in May 2016 in the framework of the Project RITMARE. Four coastal lagoons of the Po River were chosen: two with more marine features (Scardovari and Caleri) and two more directly affected by the Po River freshwater (Canarin and Vallona-Marinetta). A greater freshwater input was mirrored in higher overall microphytobenthos (MPB) abundances in Marinetta-Vallona and Canarin (81600±22735 and 81200±50938 cells cm^{-3}, respectively) compared to Scardovari and Caleri (42350±19131 and 48700±10086 cells cm^{-3}, respectively). The MPB community was dominated by diatoms (88.12±5.21%). In Marinetta-Vallona and Canarin, the higher relative abundace (RA) of Chlorophyceae, Cyanobacteria and freshwater planktonic diatoms was a clear signature of the major river inflow. Cluster and nMDS analyses highlighted different communities both at the intra-lagoonal level (among stations within the same lagoon), and inter-lagoonal level (among the four lagoons). Indeed, as revealed by diversity indices, the MPB community was more biodiverse at stations closest to the sea due to the concomitant presence of marine and brackish species, compared to the innermost stations where the lowest biodiversity was observed. Under organic enrichment conditions, as those encountered in Marinetta-Vallona due to intensive clam farming, *Nitzschia tryblionella* that prefers high organic loads was the most represented species (RA=19.1%).The benthic diatom community was also clearly affected by contamination levels. In Canarin, the most contaminated lagoon, diatom living forms that are scarcely or not at all associated with the sediments prevailed over the typical benthic forms, suggesting a negative influence attributed to contaminants accumulated in the sediments.

AUTORE

Tamara Cibic (tcibic@inogs.it), Sezione di Oceanografia, Istituto Nazionale di Oceanografia e di Geofisica Sperimentale - OGS,Via A. Piccard 54, 34151 Trieste
Autore di riferimento: Tamara Cibic

Flora marina bentonica del Mediterraneo: Rhodophyta (*Rhodymeniophycidae* escluse)

M. Cormaci, G. Furnari, G. Alongi

Dopo una breve introduzione sui caratteri generali delle Rhodophyta (Alghe Rosse) e sulla loro classificazione, distribuzione geografica ed ecologia, viene riportata una chiave d'identificazione dei generi e dei *taxa* specifici e infraspecifici macroalgali di questo Phylum [ad esclusione delle *Rhodymeniophycidae* che saranno oggetto di altro contributo] presenti in Mediterraneo [Mar Nero escluso].
A seguito di una revisione tassonomico-nomenclaturale, 120 *taxa* a livello specifico e infraspecifico sono stati accettati e trattati. 44 generi, 15 famiglie, 12 ordini, 3 sottoclassi, 4 classi e 2 subphyla sono rappresentati. Inoltre 61 *taxa*, a livello specifico e infraspecifico, sono stati considerati *taxa excludenda*, di cui 18 da confermare in Mediterraneo; 90 *taxa*, a livello specifico e infraspecifico, e 1 a livello di genere, sono considerati *taxa inquirenda*; 29 *taxa*, a livello specifico e infraspecifico, sono risultati nomi illegittimi; 33 *taxa*, a livello specifico e infraspecifico, e 1 a livello di famiglia, sono risultati invalidi; infine sono state individuate 17 combinazioni invalide, mentre vengono proposte 4 nuove combinazioni e un nome nuovo.
L'aggiornamento tassonomico segue i più recenti lavori basati sull'analisi molecolare. Di ogni *taxon* viene data una breve descrizione preceduta da alcuni riferimenti bibliografici riportanti sue illustrazioni e/o la sua distribuzione in Mediterraneo. Inoltre, la trattazione della maggior parte dei *taxa* è arricchita da note a supporto delle sinonimie indicate, o delle scelte tassonomiche seguite, o delle motivazioni per cui il *taxon* è stato considerato *inquirendum* o *excludendum*. Il lavoro è completato da un glossario di 148 voci, da un indice di tutti i *taxa* citati nel testo e da 52 tavole.

AUTORI

Mario Cormaci, Giovanni Furnari, Giuseppina Alongi (g.alongi@unict.it), Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Catania, Sezione di Biologia Vegetale, Via A. Longo 19, 95130 Catania
Autore di riferimento: Giuseppina Alongi

Tracking the arrival and spread of non-indigenous macroalgae in New Zealand

R. D'Archino, W. Nelson, K. Neill, J. Sutherland

New Zealand has an effective Biosecurity System that helps to protect New Zealand's economy, environment and human health from harmful organisms like pests and diseases. In the marine environment, control of pests/diseases is problematic and preventing the introduction of non-indigenous species is very difficult. Nevertheless, since 2002 New Zealand's Ministry for Primary Industries (MPI) has funded a national program of marine surveillance at the 11 most heavily-used ports and marinas to detect non-indigenous species. Approximately 3000 survey locations at these 'high-risk' sites are checked twice a year, through a variety of survey methods. Suspected non-indigenous specimens, or organisms whose identity is unknown, collected during these surveys are sent to the Marine Invasives Taxonomy Service (MITS) for taxonomic identification. The Biosecurity System depends on the ability to distinguish between indigenous and non-indigenous species. The identification of macroalgae is particularly challenging as the New Zealand flora is still far from being fully documented. Species of macroalgae were being recognized as introduced to New Zealand waters from the 1970s onwards, and in 1983, Adams assembled the first checklist of 14 marine macroalgae that she hypothesized were non-native species, based on their distribution patterns. Currently, 48 algal species are considered non-native to New Zealand (13 brown, 23 reds and 12 green algae), with about half of these being recognized as such in the last decade. Some of these introduced species have a restricted distribution in New Zealand while others (e.g., the Asian kelp *Undaria pinnatifida* (Harvey) Suringar or *Grateloupia turuturu* Yamada), are widespread. Particularly challenging are species belong to *Polysiphonia sensu lato* or *Ulva* spp. that are common on vessels, and require DNA analysis for precise identification. Some examples of past and current research on macroalgae will be presented.

AUTORI

Roberta D'Archino (roberta.darchino@niwa.co.nz), Wendy Nelson, Kate Neill, Judy Sutherland, National Institute of Water and Atmospheric Research Ltd, Private Bag 14-901, Wellington 6241, New Zealand
Wendy Nelson, School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand
Autore di riferimento: Roberta D'Archino

A journey of discoveries: New Zealand Kallymeniaceae

R. D'Archino

The seaweeds of New Zealand have always challenged taxonomists. Part of the problem is that concepts and names of algae from other parts of the world have been used. Also New Zealand includes diverse geographic regions from the subantarctic islands to subtropical waters. Over the last 10 years we have realized that the Kallymeniaceae of New Zealand are more diverse and unique than we ever could have imagined. New genera have been established, *Psaromenia, Stauromenia, Judithia, Wendya*, *Blastophyllis* and *Zuccarelloa*, to accommodate species previously assigned to other genera, but the great challenges are still ahead; the delimitation of the genus *Callophyllis* and the increasing number of new discoveries. The number of taxa increased from 14 in 2007 (6 genera) to 18 in 2017 (9 genera), but, including the undescribed taxa, currently we have identified 37 species (23 genera). Some species names have been used largely in the past eg. *Callophyllis ornata* rarely collected from its type locality - Auckland Is, but this name has been applied to numerous specimens with wide ranging morphologies. Kallymeniaceae are notoriously difficult to identify as critical distinguishing characters frequently are based on fertile material at the right stage of maturity. Often our samples are sterile or come from remote locations (eg. subantarctic islands, Three Kings, deep water), where only occasional collections have been made, and for some of these undescribed taxa only few specimens are available. Recently, great diversity has been uncovered in Australia, increasing the number of Kallymeniaceae genera to 43, and while it was expected these two countries would share common taxa, it seems that New Zealand has very unique Kallymeniaceae.

AUTORE

Roberta D'Archino (roberta.darchino@niwa.co.nz), National Institute of Water and Atmospheric Research Ltd, Private Bag 14-901, Wellington 6241, New Zealand
Autore di riferimento: Roberta D'Archino

A multi-gene phylogeny to uncover species diversity in the planktonic diatom genus *Chaetoceros* Ehrenberg, 1844

D. De Luca, D. Sarno, W. H. C. F. Kooistra

The genus *Chaetoceros* (Ehrenberg, 1844) is arguably the most species-rich among planktonic diatoms, with more than 200 taxonomically accepted species distributed in upwelling regions and coastal areas all over the globe. Its species are recognized easily because they possess hollow spines, called *setae*. However, species identification is less easy since morphology is variable and affected by culture conditions and environmental factors. Previous analyses resulted in the characterization of hundreds of *Chaetoceros* strains by means of morphological (light, scanning and transmission electron microscopy) and genetic (D1-D4 region of 28S rDNA, 18S rDNA) data. Results show that several terminal clades (operational taxonomic units or species) are still unknown to science and that many morphologically defined species actually consist of species complexes (e.g. *C. affinis*, *C. curvisetus*, *C. lorenzianus*, *C. socialis*). Here we present a multi-gene phylogeny of *Chaetoceros* using nuclear (18S and 28S rDNA), chloroplast (rbcL and psbA) and mitochondrial (COI) genes to assess if the signal obtained from the evolutionary history of organelle genes is congruent with the nuclear ones. Preliminary results show that the phylogenies are, indeed, congruent. Some discrepancies exist in species complexes, with *rbc*L being more effective in discriminating closely related species than *psb*A. Also bootstrap support to internal nodes was higher for *rbc*L than for *psb*A, probably because of a higher substitution rate of the former gene. The COI tree topology differed markedly from that of the other gene trees, mostly due to the high substitution rate of the marker. The latter aspect makes this gene region a good marker for population level inferences or barcoding approaches.

AUTORI

Daniele De Luca (daniele.deluca@szn.it), Diana Sarno, Wiebe H.C.F. Kooistra, Integrative Marine Ecology Department, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli
Autore di riferimento: Wiebe H.C.F. Kooistra

Survey on dinoflagellate cysts in recent marine sediments of the port of Trieste (Northern Adriatic Sea)

E. Di Poi, A. Beran, M. Cabrini

In coastal and temperate shallow waters, dinoflagellates can produce benthic resting stages to overcome unfavorable periods, and germinate in the water column once favorable environmental conditions are re-established. Studies on the composition, abundance and distribution of cysts in surface sediments of semi-enclosed bays such as ports can provide valuable information both to detect potential harmful and non-indigenous species and to foresee dinoflagellate dynamics inside the phytoplankton community. To this aim, a monitoring programme was carried out in the port of Trieste within the framework of the European IPA project BALMAS (Ballast Water Management System for Adriatic Sea Protection). In May 2014 and March 2015, 4 sites (Ts1-2-3-4) characterized by maritime traffic and anthropic pressures and a reference site (C1) located in the Marine Reserve of Miramare (Gulf of Trieste) were sampled. Surface sediments were collected by means of a box corer and samples consequently processed. The identification of the resting stages was based on the shape and morphology of cyst bodies (Matsuoka, Fukuyo 2000) using the inverted microscope at 20x objective. Furthermore, to verify the taxonomic identification, some selected cysts were isolated and induced to germinate.
In this study, a total of 38 dinoflagellate taxa were recognized. Cyst assemblage resulted dominated by *Gonyaulacales* and calcareous *Peridiniales*, mainly represented by the potentially toxic *Lingulodinium polyedrum* and by resting cysts of the genus *Alexandrium* in the size 20 µm. In May 2014 cysts varied from a minimum of 17 to a maximum of 232 cells g^{-1} of dry weight detected respectively at C1 and Ts4. In March 2015, cysts ranged from 21 to 60 cells g^{-1} of dry weight respectively at Ts2 and Ts4. The latter site Ts4 recorded the higher cyst deposits detected in the port. From the germination experiments carried out on the cysts collected during the second survey (March 2015), a new record for the northern Adriatic, the species *Alexandrium margalefii*, was developed. Other potentially toxic morphotypes of the genus *Alexandrium* identified in both campaigns were already renowned in this area. Compared to previous few studies conducted in the Gulf of Trieste, the number of taxa and the most representative morphotypes were comparable to past studies whereas abundances were much lower than past monitoring. Our results remark the importance of these stages of dinoflagellate life cycle, and the necessity to intensify investigation especially in areas at high risk such as ports.

Letteratura citata

Matsuoka K, Fukuyo Y (2000) Technical guide for modern dinoflagellate cyst study. WESTPAC-HAB/WESTPAC-IOC. 29 pp.

AUTORI

Elena Di Poi (edipoi@inogs.it), Alfred Beran, Marina Cabrini, OGS-Istituto Nazionale di Oceanografia e di Geofisica Sperimentale, Via Auguste Piccard 54, Trieste, Italy
Autore di riferimento: Elena Di Poi

The potential role of phytoplankton in traps of carnivorous bladderworts (Utricularia, Lentibulariaceae)

N.T.W. Ellwood, R. Congestri, S. Ceschin

Carnivorous aquatic plants of the genus *Utricularia* (Lentibulariaceae) are well known to capture small aquatic invertebrates, using structures called bladders. But information is coming to the fore suggesting that *Utricularia* could be re-classified as an omnivore as phytoplankton species can make up a major proportion of bladder contents. It is not fully understood if phytoplankton cells are preyed upon or if they are taken in merely as a consequence when invertebrate prey triggers the bladder. Past studies have indicated that both scenarios could be possible. Some studies now also suggest that a mutual-symbiotic relationship exists between *Utricularia* and the community in the within the bladders. The present study is to determine a potential role for phytoplankton in nutrient supply to *Utricularia*.
The identification and quantification of phytoplankton species were carried out on the contents of bladders sampled from the carnivorous bladderwort *Utricularia australis*. Samples of three full *Utricularia* filaments were taken from 18 central Italian sites and the contents of 10 or 15 randomly chosen bladders were observed. These data were compared to the external phytoplankton community and water chemico-physical and nutrient variables. Every bladder was shown to contain some phytoplankton cells, with findings showing species spread across 6 divisions (Chlorophyta, Bacillariophyta, Pyrrophyta, Euglenophyta, Cryptophyta, Cyanophyta). Significant relationships of phytoplankton species assemblages within the bladders and those in the ambient water were found. Also in the majority of bladders there were a high proportion of live compared to dead cells. Many bladders had phytoplankton-only communities. It was not possible to say for certain if phytoplankton was preyed upon or not, or if there was a mutualistic-symbiotic relationship, but the presence of many living cells could suggests the latter option is possible. The findings here imply that the bladder phytoplankton community is a consequence of the ambient community given the relationships between them. The presence of phytoplankton-only contents in many bladders implies possible automatic - rather than prey - triggered bladder functioning.

AUTORI

Neil Thomas William Ellwood (ellwood@uniroma3.it), Roberta Congestri, Simona Ceschin, Department of Science, University of Rome 'Roma Tre', Viale G. Marconi 446, 00146 Roma, Italy
Neil Thomas William Ellwood, Roberta Congestri, Department of Biology University of Rome 'Tor Vergata', Via Cracovia 1, 00133 Rome, Italy
Autore di riferimento: Neil T.W. Ellwood

Gene expression changes in diatoms in response to external stimuli

M.I. Ferrante, A. Amato, G. Dell'Aquila, M. Ribera d'Alcalà, R. Sanges, D. Iudicone

Signal transduction mechanisms are still poorly defined in marine phytoplankton, including diatoms. Yet, understanding how cells sense and integrate environmental signals is fundamental to explain population dynamics, community interactions and ecosystem functioning. We use several approaches, mostly based on transcriptomics and loss of function strategies, to study molecular changes occurring in diatom cells upon reception of external stimuli, such as changes in turbulence, or chemical cues produced during sexual reproduction or grazing, using dedicated, custom-made instrumentation.
I will present data on diatom responses to turbulence. It is known that diatoms thrive in turbulent environments, however, despite several experimental and numerical studies, if and how diatoms may profit from turbulence is still an open question. One of the leading arguments is that turbulence favours nutrient uptake. To study this process, we designed and built the TURBOGEN, a prototypic instrument that can generate natural levels of microscale turbulence. We tested several diatom species using TURBOGEN, and demonstrated that diatoms actively respond to turbulence in non-limiting nutrient conditions by tuning their chain length. Differential expression analyses were conducted to study the transcriptional response to turbulence of *Chaetoceros decipiens*, one of the species displaying obvious morphological changes. *C. decipiens* responded to turbulence by activating energy storage pathways like fatty acid biosynthesis. In addition, in experiments lasting 12 days, we observed that in turbulence *C. decipiens* continued to take up phosphorus and carbon even when silicon was depleted. These findings indicate that turbulence affects diatoms in a more sophisticated fashion than what was accepted so far.

AUTORI

Maria Immacolata Ferrante (mariella.ferrante@szn.it), Alberto Amato, Gianluca Dell'Aquila, Maurizio Ribera d'Alcalà, Remo Sanges, Daniele Iudicone, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli
Autore di riferimento: Maria Immacolata Ferrante

A putative phytochelatin is involved in Cr(VI) tolerance in strict relation with sulphur metabolism in *Scenedesmus acutus* (Chlorophyceae)

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

Metal contamination is a serious environmental problem that affects life forms and changes the natural microbiota of aquatic ecosystems.
Chromium (Cr) is one of several heavy metals (HM) that causes serious environmental contamination in soil, sediments, and groundwater. Cr can exist in the environment as Cr(III) or Cr(VI), and particularly Cr(VI) form is extremely toxic, mutagenic, and carcinogenic.
Microalgae resistance and/or detoxification to heavy metals may occur through several mechanisms: binding to the cell wall; low plasma membrane permeability; active extrusion; biotransformation; compartmentalization of HM into vacuoles and other intracellular organelles; complexation with chelating agents, such as non-proteinaceous compounds (as malate, citrate, and polyphosphates) or metal-binding proteins, such as metallothioneins and phytochelatins. Phytochelatins (PCs) are heavy metals chelating cysteine rich polypeptides with the general structure $(\gamma\text{-Glu-Cys})_n$-Gly. Phytochelatins are produced by the enzyme Phytochelatin Synthase (PC-synthase) which uses glutathione (GSH) as its substrate. It is now well established that glutathione (GSH) and PCs are a central component of the plant defence system against various forms of stresses.
Several physiological studies in plants, indicated the role of PCs in the homeostasis and detoxification of toxic metals including Cr. However, synthesis of PCs has received little attention in algal cells and no data regarding Cr-induced PCs synthesis in microalgae exist.
In this work, we reported the first evidence of a PCs synthase gene from the microalga *Scenedesmus acutus.* Using degenerate primers, we amplified a partial cDNA fragment of nearly 1300 bp (*SaPCS*). The deduced protein shows a high identity homology (46%-74%) with other PCs synthase of algae and plants.
In order to define its role in Cr detoxification, we analysed by RT- PCR the level of *SaPCS* transcription in two strains of *S. acutus* with different chromium sensitivity (Cr tolerant strain vs wild type) after 24h culture in standard and in Cr supplemented medium (1mg/l Cr (VI)). Since the pathway of Cr (VI) translocation is an active mechanism involving transporter of essential nutrients such as phosphate and sulphate, we also evaluated the role of PCs synthase in the two different *S. acutus* strains after sulphur starvation.
Our preliminary data indicate that in the Cr-tolerant strain *SaPCS* trascription is induced following Cr(VI) treatment both in S-sufficient and in S-replete condition whereas in the wild type the induction is significantly enhanced by sulphur starvation. These observations indicate that in *S. acutus* PC synthase is not only involved in Cr detoxification, but can also play a role in the balance the intracellular sulphur.

AUTORI
Michele Ferrari, Radiana Cozza, Dipartimento di Biologia, Ecologia e Scienze della Terra, Università della Calabria, 87036 Rende (CS)
Michele Ferrari, Matteo Marieschi, Anna Torelli (anna.torelli@unipr.it), Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Università degli Studi di Parma, 43124 Parma
Autore di riferimento: Anna Torelli

Monitoring the cyanobacteria species biodiversity and growth during the maturation of Euganean Thermal Muds

B. Gris, F. Caldara, L. Treu, R. Zampieri, N. La Rocca

The Euganean Thermal District is the oldest and largest thermal center in Europe and represents a reference in mud-based thermal treatments. The mature mud is the result of traditional methods, handed down over the centuries up to our days. During the mud maturation process, we can observe the formation of a biofilm, mainly consisting of cyanobacteria. They are suggested to produce active molecules conferring the therapeutic properties of the mud. Among them, *Phormidium* ETS-05, an endemic species firstly isolated from the Euganean District, is well known for the production of galactosyldiacylglycerols with anti-inflammatory effects (Bruno et al. 2005, doi: 10.1016/j.ejphar.2005.09.023), leading to the achievement of the European Patent on the active principles of the Euganean Thermal Muds in 2013 (EP1571203). Recently, another endemic species, *Cyanobacterium aponinum* ETS-03, has been studied for its ability to produce polysaccharides with immunostimulating effects (Gudmundsdottir et al. 2015, doi: 10.1016/j.imlet.2014.11.008; Gris et al. 2017, doi: 10.1007/s10811-017-1133-3). A raw protocol, called "Disciplinare per la Tutela del Marchio Collettivo di Origine del Fango del Bacino Termale Euganeo" has been written to formalize the traditional methods. Therefore, the scientific bases and a deep knowledge of all elements influencing the mature mud formation are still missing.
In the first part of this study, the maturation process of 2 thermal SPAs has been monitored every 15 days for 2 months, following the cyanobacteria population growth and composition as function of time and environmental parameters. In the second part, the presence and the total amount of the Patent target species, *Phormidium* ETS-05, in mature muds of 33 different thermal SPA, has been investigated as an indicator of the correct maturation process.
Results showed that, during maturation, the maintenance of temperature in a proper constant range is one of the crucial aspects to consider to have a proper final mature mud. The observations and the analysis of mature muds of different SPAs revealed that the temperature influences both the final concentration and the cyanobacteria population composition. The target species *Phormidium* ETS-05 was present in almost all the monitored SPAs, and represented always at least the 40% of the total cyanobacteria population.

Letteratura citata

Bruno A, Rossi C, Marcolongo G, Di Lena A, Venzo A, Berrie CP, Corda D (2005) Selective in vivo anti-inflammatory action of the galactolipid monogalactosyldiacylglycero. European Journal of Pharmacology 524(1-3): 159-68. doi:10.1016/j.ejphar.2005.09.023

Gris B, Sforza E, Morosinotto T, Bertucco A, La Rocca N (2017) Influence of light and temperature on growth and high-value molecules productivity from *Cyanobacterium aponinum*. Journal of Applied Phycology 29(4): 1781-1790. doi: 10.1007/s10811-017-1133-3

Gudmundsdottir AB, Omarsdottir S, Brynjolfsdottir A, Paulsen BS, Olafsdottir ES, Freysdottir J (2015) Exopolysaccharides from Cyanobacterium aponinum from the Blue Lagoon in Iceland increase IL-10 secretion by human dendritic cells and their ability to reduce the IL-17+RORγt+/IL-10+FoxP3+ ratio in CD4+ T cells**.** Immunology letters 163(2): 157-162. doi: 10.1016/j.imlet.2014.11.008

AUTORI

Barbara Gris (barbara.gris@phd.unipd.it), Raffaella Zampieri, Nicoletta La Rocca, Dipartimento di Biologia, Università degli Studi di Padova, Via U. Bassi 58/b, 35131 Padova
Fabrizio Caldara, Centro Studi Termali Pietro d'Abano, Largo Marconi 8, 35031 Abano Terme
Laura Treu, Dipartimento di Ingegneria Ambientale, Technical University of Denmark, Miljøvej 2800 Kgs., Lyngby, Denmark.
Autore di riferimento: Barbara Gris

Ten years of research on *Ostreopsis* cf. *ovata* (Dinophyceae) in the Gulf of Trieste: an interdisciplinary approach

G. Honsell, C. Dell'Aversano, L. Tartaglione, M. Pelin, A. Penna, S. Sosa, A. Tubaro

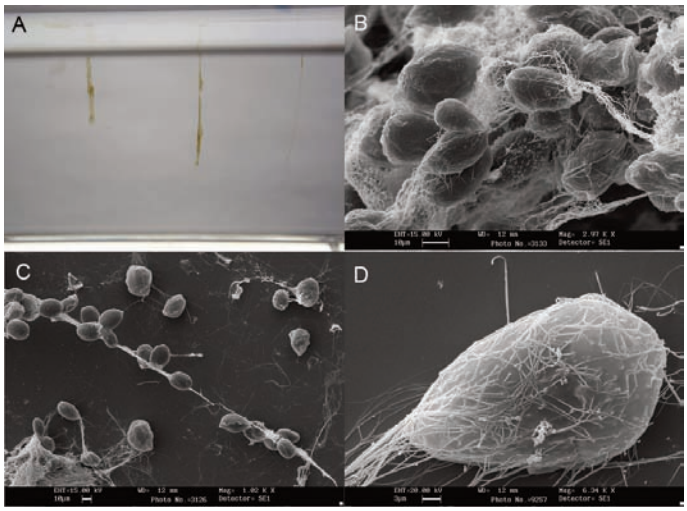

Fig. 1
Ostreopsis cf. *ovata* cultures from the Gulf of Trieste. A. Cells in a culture flask forming filamentous floating aggregates departing from water surface. B. Scanning electron microscope (SEM) image of cell aggregates with high amounts of mucilage formed by filaments and amorphous material. C. SEM image of a mucilage filament connecting more cells together. D. SEM image of a cell extruding numerous trichocysts joining together at the ventral end of cell.

Since 2000s, benthic blooms of the harmful dinoflagellate *Ostreopsis* cf. *ovata* have been occurring with increasing frequency and extension along the Mediterranean coasts. Concomitantly to these events, episodes of human adverse effects associated mainly with cutaneous and inhalational exposure to marine aerosols and/or seawater have been recorded. Although high levels of *Ostreopsis* toxins have been detected in edible marine organisms, no human cases of seafood poisoning ascribed to these compounds have been documented, so far. Mediterranean strains of *O.* cf. *ovata* have been found to produce palytoxin (PLTX) analogues, with ovatoxin-a (OVTX-a) as the major toxin, in addition to other ovatoxins and low amounts of isobaric palytoxin. In the Gulf of Trieste, *O.* cf. *ovata* was first recorded in 2006, but the real beginning of an extensive research on this organism dates back to 2009 when a bloom occurred near the small beach of Canovella de' Zoppoli (Trieste). This improved the collaboration within a research team of biologists, chemists and toxicologists for an interdisciplinary study providing new insights on different features of this organism: from taxonomy to cell ultrastructure, genetic and molecular characterization, toxin profile, intracellular localization of toxins and other metabolites by immunochemistry and/or Raman spectroscopy. A short review of the results obtained is given.

Taxonomic identification: *Ostreopsis* cells of the Gulf of Trieste were identified as *Ostreopsis* cf. *ovata* by light microscopy and molecular analysis. Final alignment of *Ostreopsis* sequences with the Genbank sequence database of *Ostreopsis* species confirmed that the *Ostreopsis* isolates belonged to the *O.* cf. *ovata* Atlantic/Mediterranean clade (Honsell et al. 2011).

Cell structure: new ultrastructural features were described by scanning and transmission electron microscopy. *Ostreopsis* mucilage appeared to be formed by a network of trichocysts extruded through thecal pores and by amorphous polysaccharidic material deriving from mucocysts (Fig. 1). A role of trichocysts as fibrillar component of mucilage seems to be a specific feature of *Ostreopsis*, not observed in other benthic dinoflagellates like *Coolia monotis* or *Prorocentrum lima.* Two different types of plastids were found in all stages of cell growth: large elongated chloroplasts with stacked thylakoids in peripheral cytoplasm and small round proplastid-like structures in the central part of the cell. A considerable part of cytoplasm was occupied by neutral lipid droplets. Raman spectroscopy showed also the presence of poly-unsaturated lipids, concentrated in spots at the cell border (Honsell et al. 2013).

Toxin analysis: several palytoxin-like compounds (ovatoxin-a,-b,-c,-d,-e) were identified by LC- HRMS in natural populations, ovatoxin-a being the most abundant (Honsell et al. 2011). In culture, the highest toxin content was reached during the senescent phase (Honsell et al. 2013).

Immunocytochemistry: the presence of PLTX-like compounds in *O.* cf. *ovata* cells was investigated by immunocytochemistry, using monoclonal or polyclonal anti-PLTX antibodies. Both antibodies reactions localized PLTX-like compounds in a cytoplasmic network made of filaments and small dots surrounding chloroplasts and other organelles, and in numerous brighter dots in the peripheral cytoplasm (Honsell et al. 2011).

Detection methods: a panel of different detection methods was set up and evaluated for their suitability to quantify PLTXs in microalgal samples and mussels. A LC-HRMS method was developed for identification, structural characterization and quantitation of the entire array of toxins produced by *O.* cf. *ovata* in algal pellets (Ciminiello et al. 2012), seawater, and marine aerosols (Tartaglione et al. 2016). Combination of solid phase extraction with

LC-HRMS detection proved able also to determine the presence of ovatoxins in mussels at level as low as 15 g/kg, well below the challenging maximum permitted level of PLTX recommended by EFSA of 30 g/kg (Ciminiello et al. 2015). An indirect sandwich ELISA was initially developed using murine monoclonal and rabbit polyclonal antibodies against PLTX. The good sensitivity (limit of detection, LOD, and quantitation, LOQ, of 1.1 and 2.2 ng/mL, respectively), its good accuracy and reproducibility demonstrated its suitability to detect PLTX, also in contaminated mussels (LOQ = 11 µg/kg) below the limits suggested by the European Food Safety Authority (Boscolo et al. 2013).
The sensitivity of the sandwich ELISA was further improved by an ultrasensitive electrochemiluminescence-based sensor, taking advantage of the specificity provided by anti-PLTX antibodies, the good conductive properties of carbon nanotubes, and the excellent sensitivity achieved by a luminescence-based transducer. The sensor was able to produce a concentration-dependent light signal, allowing PLTX quantification in mussels, with a LOQ of 2.2 µg/kg of mussel meat, more than 2 orders of magnitude more sensitive than that of the commonly used detection techniques, such as LC-MS/MS (Zamolo et al. 2012).

Letteratura citata

Boscolo S, Pelin M, De Bortoli M, Fontanive G, Barreras A, Berti F, Sosa S, Chaloin O, Bianco A, Yasumoto T, Prato M, Poli M, Tubaro A (2013) A sandwich ELISA assay for the quantitation of palytoxin and its analogs in natural samples. Environmental Science & Technology 47(4): 2034-2042.

Ciminiello P, Dell'Aversano C, Dello Iacovo, Fattorusso E, Forino M, Grauso L, Tartaglione L (2012) High resolution LC-MSn fragmentation pattern of palytoxin as template to gain new insights into ovatoxin-a structure. The key role of calcium in MS behavior of palytoxins. Journal of the American Society for Mass Spectrometry 23 (5): 952-963.

Ciminiello P, Dell'Aversano C, Dello Iacovo, Forino M, Tartaglione L (2015) Liquid chromatography-high resolution mass spectrometry for palytoxins in mussels. Analytical and Bioanalytical Chemistry 407: 1463-1473.

Honsell G, Bonifacio A, De Bortoli M, Penna A, Battocchi C, Ciminiello P, Dell'Aversano C, Fattorusso E, Sosa S, Yasumoto T, Tubaro A (2013) New insights on cytological and metabolic features of *Ostreopsis* cf. *ovata* Fukuyo (Dinophyceae): a multidisciplinary approach. PLoS One 8: e57291.

Honsell G, De Bortoli M, Boscolo S, Dell'Aversano C, Battocchi C, Fontanive G, Penna A, Berti F, Sosa S, Yasumoto T, Ciminiello P, Poli M, Tubaro A (2011) Harmful dinoflagellate *Ostreopsis* cf. *ovata* Fukuyo: detection of ovatoxins in field samples and cell immunolocalization using antipalytoxin antibodies. Environmental Science & Technology 45: 7051–7059.

Tartaglione L, Dell'Aversano C, Mazzeo A, Forino M, Wieringa A, Ciminiello P (2016) Determination of palytoxins in soft coral and seawater from a home aquarium. Comparison between *Palythoa* and *Ostreopsis*-related inhalatory poisonings. Environmental Science & Technology 50: 1023-30.

Zamolo VA, Valenti G, Venturelli E, Chaloin O, Marcaccio M, Boscolo S, Castagnola V, Sosa S, Berti F, Fontanive G, Poli M, Tubaro A, Bianco A, Paolucci F, Prato M (2012) Highly sensitive electrochemiluminescent nanobiosensor for the detection of palytoxin. ACS Nano 6: 7989–97.

AUTORI

Giorgio Honsell (giorgio.honsell@uniud.it), Dipartimento di Scienze AgroAlimentari, Ambientali e Animali, Università di Udine, Via delle Scienze 91-93, 33100 Udine
Carmen Dell'Aversano, Luciana Tartaglione, Dipartimento di Farmacia, Università di Napoli Federico II, Via D. Montesano 49, 80131 Napoli
Antonella Penna, Dipartimento di Scienze Biomolecolari, Università di Urbino, Viale Trieste 296, 61121 Pesaro
Marco Pelin, Silvio Sosa, Aurelia Tubaro, Dipartimento di Scienze della Vita, Università di Trieste, Via Alfonso Valerio 6, 34127 Trieste
Autore di riferimento: Giorgio Honsell

Physiological adaptation within the Chaetoceraceae to the ecological challenges in the northern Adriatic

N. Kužat, D. Maric Pfannkuchen, M. Smodlaka, A. Baricevic, E. Pustijanac, B. Gasparovic, T. Novak, I. Ivancic, M. Pfannkuchen

The northern Adriatic is a suitable ecosystem for observing phytoplankton under a variety of different ecological conditions. This shallow basin is characterized by a multitude of steep and quickly changing gradients (e.g. nutrients), both temporal and spatial. The current systems and the major freshwater input (River Po) generate strong gradients in nutrient concentrations with an expressed N/P imbalance and sustained phosphate limitation. The genus Chatoceros is among the most diverse and species rich diatoms often dominating the phytoplankton community in the Adriatic Sea. There is a big number of species belonging to this genus with different physiology, seasonality and ecology.
We analyzed species from the genus *Chaetoceros,* with a special focus on their metabolic reaction towards phosphate limitation. Here we report data on species-specific growth rates under different nutrient regimes, phosphate uptake rates, alkaline phosphatase activity, localization and activation patterns and characteristics of alkaline phosphatase activity. Our results demonstrate a high interspecific variation in metabolic responses to phosphate limitation in sympatric congeneric species. Ecological characteristics and hence significance and function appears hence to be defined on the species level and appears far from homogeneous within genera. This results in a highly structured planktonic ecosystem that allows for a high level of sympatric congeneric species diversity.

AUTORI

Nataša Kuzat (nkuzat@irb.hr), Daniela Maric Pfannkuchen, Mirta Smodlaka, Ana Baricevic, Ingrid Ivancic, Martin Pfannkuchen, Center for marine research, Ruđer Bošković Institute, G. Paliaga 5, Rovinj, Croatia,
Emina Pustijanac, Juraj Dobrila University of Pula, Zagrebačka 30, Pula, Croatia
Blazenka Gasparovic, Tihana Novak, Ruđer Bošković Institute, Division for Marine and Environmental Research, Bijenička 54, Zagreb, Croatia
Autore di riferimento: Nataša Kužat

Citizens and scientists work together to monitor marine alien macrophytes

A.M. Mannino, P. Balistreri, A. Deidun

The introduction of non-indigenous species (NIS) is an ongoing phenomenon which has been pointed out as a major threat to biodiversity at different levels (Wallentinus, Nyberg 2007, Katsanevakis et al. 2014, Vergés et al. 2016). NIS may in time become invasive (Invasive Alien Species "IAS") and may cause biodiversity loss and ecosystem service changes (Brunel et al. 2013, Giakoumi 2014, Vergés et al. 2016). The Mediterranean Sea is an important hotspot for marine NIS (ca. 1,000 such species recorded to date, Zenetos et al. 2012, Galil et al. 2015, Verlaque et al. 2015). To reduce the risk of future IAS introduction and to better understand their invasive potential and spread dynamics, monitoring and surveillance plans are required. The creation of permanent alarm systems and public awareness campaigns are crucial for reducing the risk of IAS introduction. Since intensive monitoring programs could be very expensive, citizen science, involving citizens (e.g. tourists, fishermen, divers) in the collection of data, could be a useful tool for providing data on IAS, that would otherwise be impossible to collect because of limitations on time and resources. Citizen science is having an increasing success worldwide. Citizen science projects has rapidly and enormously increased in recent years (Conrad, Hilchey 2011), also thanks to the wide availability of mobile technologies and internet access that enable an easy and cheap way to communicate, share and interchange data. The value of citizen science has been widely recognized. Of course, in order to be used for scientific purposes and management decisions, the collected data need appropriate quality assurance measures such as validation and verification by taxonomic experts. We report on the experience of two citizen science projects: the Project "*Caulerpa cylindracea* – Egadi Islands" and the Project "Invasive Algae", included within the "Seawatchers" platform. The first one, sponsored by the STEBICEF Department of the University of Palermo and by the Egadi Islands Marine Protected Area (MPA), aimed at creating a database on the spread dynamics of *Caulerpa cylindracea* Sonder within the Egadi Islands MPA (western coast of Sicily, Tyrrhenian Sea). Among IAS, *C. cylindracea*, introduced from Australia and New Caledonia (Belton et al. 2014), has raised serious concern due to its ascertained impact on Mediterranean communities (Klein, Verlaque 2008, Papini et al. 2013, Katsanevakis et al. 2014). The project, presented during the International Congress GeoSub2016 (Mannino et al. 2016), allowed to gather 156 sightings, mainly recorded by citizens (Fig. 1). Useful information on the behaviour strategies of the alga was also collected. The second one, coordinated by the Institute of Marine Sciences of Barcelona (CSIC, Spain), collects data on 10 marine IAS (*Acrothamnion preissii* (Sonder) E.M. Wollaston, *Asparagopsis armata* Harvey, *A. taxiformis* (Delile) Trevisan, *C. cylindracea*, *C. taxifolia* (M. Vahl) C. Agardh, *Codium fragile* subsp. *fragile* (Suringar) Hariot, *Halimeda incrassate* (J. Ellis) J.V. Lamouroux, *Lophocladia lallemandii* (Montagne) F. Schmitz, *Stypopodium schimperi* (Kützing) Verlaque & Boudouresque, *Womersleyella setacea* (Hollenberg) R.E.Norris). These projects highlight how important the contribution of citizen science campaigns is for collecting new data and information on marine NIS and to significantly improve the efficacy of monitoring and surveillance plans. Moreover, in areas particularly vulnerable to biological invasions, such as Sicily, they represent an opportunity to promote the creation of early-warning systems, and an effective tool in the management of present and future introductions of NIS within the Mediterranean Sea.

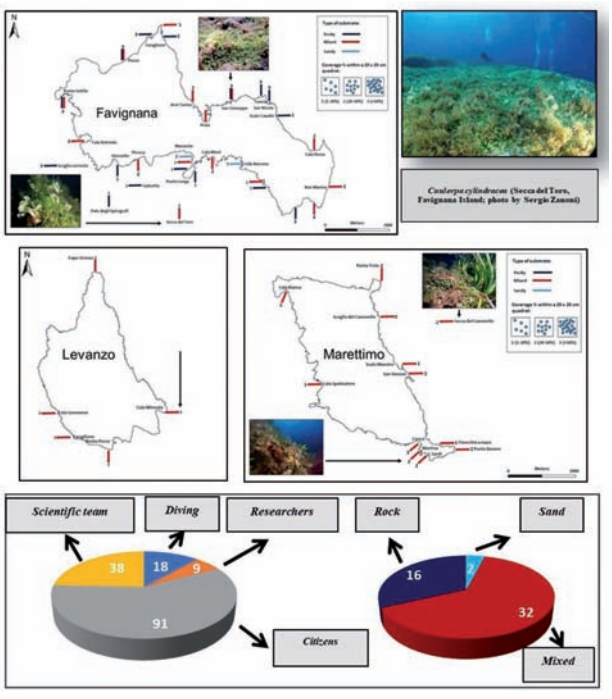

Fig. 1
Main results of the project "*Caulerpa cylindracea* – Egadi Islands": sightings recorded in the main islands, groups of volunteers involved in the project, substrates colonized by *Caulerpa cylindracea.*

Letteratura citata

Belton GS, Prud'homme van Reine WF, Huisman JM, Draisma SGA, Gurgel CFD (2014) Resolving phenotypic plasticity and species designation in the morphologically challenging *Caulerpa racemosa–peltata* complex (Chlorophyta, Caulerpaceae). Journal of Phycology 50: 32-54.

Brunel S, Fernández-Galiano E, Genovesi P, Vernon HH, Kueffer C, Richardson DM (2013) Invasive alien species: a growing but neglected threat? In: Late lessons from early warning: science, precaution, innovation. Lessons for preventing harm. EEA Report 1/2013, Copenhagen, p. 518–540.

Conrad CC, Hilchey KG (2011) A review of citizen science and community-based environmental monitoring: issues and opportunities. Environmental Monitoring and Assessment 176: 273-291.

Galil B, Boero F, Fraschetti S, Piraino S, Campbell M, Hewitt C, Carlton J, Cook E, Jelmert A, Macpherson E, Marchini A, Occhipinti-Ambrogi A, Mckenzie C, Minchin D, Ojaveer H, Olenin S, Ruiz G (2015). The enlargement of the Suez Canal and introduction of non-indigenous species to the Mediterranean Sea. Limnology and Oceanography Bulletin 24: 41-43.

Giakoumi S (2014) Distribution patterns of the invasive herbivore *Siganus luridus* (Rüppell, 1829) and its relation to native benthic communities in the central Aegean Sea, Northeastern Mediterranean. Marine Ecology 35: 96-105.

Katsanevakis S, Coll M, Piroddi C, Steenbeek J, Ben Rais Lasram F, Zenetos A, Cardoso AC (2014) Invading the Mediterranean Sea: biodiversity patterns shaped by human activities. Frontiers in Marine Science 1: 32. doi:10.3389/fmars.2014.00032

Klein J, Verlaque M (2008) The *Caulerpa racemosa* invasion: a critical review. Marine Pollution Bulletin 56: 205-225.

Mannino AM, Donati S, Balistreri P (2016) The Project "*Caulerpa cylindracea* in the Egadi Islands": citizens and scientists working together to monitor marine alien species. Biodiversity Journal 7 (4): 907-912.

Papini A, Mosti S, Santosuosso U (2013) Tracking the origin of the invading *Caulerpa* (Caulerpales, Chlorophyta) with Geographic Profiling, a criminological technique for a killer alga. Biological Invasions 15: 1613-1621.

Vergés A, Doropoulos C, Malcolm HA, Skye M, Garcia-Pizá M, Marzinelli EM, Campbell AH, Ballesteros E, Hoey AS, Vila-Concejo A, Bozec YM, Steinberg PD (2016) Long-term empirical evidence of ocean warming leading to tropicalization of fish communities, increased herbivory, and loss of kelp. Proceedings of the National Academy of Sciences U.S.A. 113: 13791-13796.

Verlaque M, Ruitton S, Mineur F, Boudouresque CF (2015) Vol. 4 Macrophytes. In: Briand F (Ed) CIESM Atlas of exotic species in the Mediterranean. CIESM publ., Monaco. 1–364 pp.

Wallentinus I, Nyberg CD (2007) Introduced marine organisms as habitats modifiers. Marine Pollution Bulletin 55: 323-332.

Zenetos A, Gofas S, Morri C, Rosso A, Violanti D, García Raso JE, Çinar ME, Almogi-Labin A, Ates As, Azzurro E, Ballesteros E, Bianchi Cn, Bilecenoglu M, Gambi Mc, Giangrande A, Gravili C, Hyams-Kaphzan O, Karachle Pk, Katsanevakis S, Lipej L, Mastrototaro F, Mineur F, Pancucci-Papadopoulou Ma, Ramos Esplá A, Salas C , San Martín G, A. Sfriso A, Streftaris N, Verlaque M (2012) Alien species in the Mediterranean Sea by 2012. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD). Part 2. Patterns in introduction trends and pathways. Mediterranean Marine Science 13: 328-352.

AUTORI

Anna Maria Mannino (annamaria.mannino@unipa.it), Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche, Sezione di Botanica e Ecologia vegetale, Università di Palermo, Via Archirafi 38, 90123 Palermo
Paolo Balistreri, Vicolo Giotto 6, 91023 Favignana
Alan Deidun, Department of Geosciences, University of Malta, Msida, Malta
Autore di riferimento: Anna Maria Mannino

In vitro isolation of anti-*Alexandrium minutum* nanobodies from a pre-immune library

E. Mazzega, A. Beran, M. Cabrini, A. de Marco

At the present, the identification of planktonic species in coastal water mostly relies on light microscopy observations, only in some cases through PCR analysis and for specific morphological analyses by electronic microscopy. The analyses are performed by highly trained personnel and require lab equipment and long time in order to get the results. The increasing danger represented by Harmful Algal Blooms (HABs) and the new legislation on ballast water management urge faster and possibly easy-to-perform diagnostic methods able to guarantee sensitivity and reliability. Immuno-reagents for microalgae capture would have the requisites of specificity necessary for developing semi-automatic sensitive biosensors to be operated on-site by generic personnel. This alternative is at the moment neglected because of the elevated costs for monoclonal antibody selection and production.
Here we demonstrate for the first time the feasibility to recover nanobodies (VHHs) selective for native surface epitopes of *Alexandrium minutum* by direct whole cell biopanning using a pre-immune phage display library. The recombinant nature of VHHs enabled their bacterial expression as eGFP fluorescent reagents. We demonstrated that these reagents are directly suitable for fluorescence microscopy and flow cytometry, but could be used also on solid surfaces such as ELISA plates. The isolated fluorescent nanobodies showed no cross-reactivity with other microalgae of the Collection of Sea Microorganisms (COSMI) available at INOGS such as *Scrippsiella lachrymose* (COSMI n: 1049), *Prorocentrum minimum* (COSMI n. 1034), *Pleurochrysis cf. roscoffensis* (COSMI n. 5005), Cryptophycean *sp*. 2 (COSMI n. 3002) and *Phaeodactylum tricornutum* (COSMI n. 2007). They were able to bind selectively the target cells in both fixed and fresh samples with minimal processing, demonstrating the advantage of these reagents over conventional IgGs. The establishment of the protocol represents a proof-of-principle that can be applied to further species. It is possible to isolate monoclonal nanobodies in 1:3 of the time and at a cost roughly 1:10 of that necessary to select a monoclonal antibody by hybridoma technique. The production costs are between 10 and 100 times lower. Furthermore, nanobodies are easy to engineer and, therefore, optimal material for biotechnological applications (functionalization of solid surfaces, nanoparticles, sensor chips).

AUTORI

Elisa Mazzega (elisa.mazzega@ung.si), Ario de Marco, Laboratory for Environmental and life Sciences, University of Nova Gorica, Glavni Trg 8, Vipava – Slovenia.
Alfred Beran, Marina Cabrini, OGS- Istituto Nazionale di Oceanografia e di Geofisica Sperimentale, Via A Piccard 54, 34151 Trieste, Italy
Autore di riferimento: Elisa Mazzega

Assessment of *Posidonia oceanica* (L.) Delile (1813) meadows along the Italian coasts: an *excursus* of 25 years

C. Micheli

The aim of this study was to estimate time series of data of *Posidonia oceanica* (L.) Delile (1813) meadows growing along the Italian islands and in the northern-central Tyrrhenian coasts, during 25 years (1991-2016) of monitoring studies.
During the years we have assessed the ecological status of the species integrating the knowledge acquired about genetic, biomass, density and morphological features of the populations in relation to different natural and anthropogenic impacts and various substrate composition (rocky, sand and matte) (Micheli et al. 2018). *P. oceanica* meadows growing along the coastline are under continuous natural and anthropogenic pressure where the way meadow health correlates with its genetic and genotypic diversity (Micheli et al. 2005, 2015).
Previous studies have revealed the important role of currents (Micheli et al. 2010a, Rotini et al. 2011) and during 2003–2004, when a massive fruiting event occurred; fruits were collected from plants at Monterosso al Mare (meadow) and compared with stranded fruits sampled in front of the meadow and down current in Tuscany along 80 km of the coast (Aliani et al. 2006, Micheli et al. 2010a). After their growth in culture, the plants were analyzed by RAPD molecular markers. Cluster analysis of similarity showed four distinct genetic populations revealing their provenience.
Moreover, due to different anthropogenic impacts, such as a nourishment work, changes in the ecological structure of the *P. oceanica* meadows, at Monterosso a mare (Ligurian Gulf) were observed over ten years (Micheli et al. 2012).
Recently we have identified new diagnostic tools for effective monitoring of the conservation status of *P. oceanica* beds by applying innovative methodologies like remote sensing techniques (Micheli et al. 2010b, Borfecchia et al. 2013a,b,), and using a multidisciplinary method as a suite of indicators to highlight the seasonal variation of biomass, phenols and the main biotic parameters as well as pigment concentration of seagrass meadows (Rotini et al. 2013).
Knowledge of the ecological structure of meadows is considered a pre-requisite for many multidisciplinary programs as well as the marine energy projects by which the renewable wave energy resource can be exploited in the Mediterranean (Borfecchia et al., 2016 Micheli et al. 2017).
Assessment of *P. oceanica* meadows along the coasts allowed us to evaluate the role of the species in sustaining the primary production and the trophic level in the Mediterranean.

Letteratura citata

Aliani S, Gasparini GP, Micheli C, Molcard A, Peirano A (2006) Can southern meadows of the Mediterranean seagrass *Posidonia oceanica* (L) Delile supply northern one? A multidisciplinary approach in Ligurian sea. Biologia Marina Mediterranea 13: 14-18.

Borfecchia F, De Cecco L, Martini S, Ceriola G, Bollanos S, Vlachopoulos G, Valiante LM, Belmonte A, Micheli C (2013a) *Posidonia oceanica* genetic and biometry mapping through high-resolution satellite spectral vegetation indices and sea-truth calibration. Internation Journal of Remote Sensing 34: 4680-4701.

Borfecchia F, Micheli C, Belmonte A, De Cecco L, Sannino G, Bracco G, Mattiazzo G, Struglia MV (2016) Impact of ISWEC sea wave energy converter on *Posidonia oceanica* meadows assessed by satellite remote sensing in the coastal areas of Pantelleria island. Geophysical Research Abstract. EGU General Assembly 2016, 18: 16787.

Borfecchia F, Micheli C, Carli F, Cognetti De Martis S, Gnisci V, Piermattei V, Belmonte A, De Cecco L, Martini S, Marcelli M (2013b) Mapping spatial patterns of *Posidonia oceanica* meadows by means of Daedalus ATM airborne sensor in the coastal area of Civitavecchia (central Tyrrhenian). Remote Sensing 5: 4877-4899.

Micheli C, Borfecchia F, De Cecco L, Belmonte A, Piermattei V, Marcelli M (2018) Effects of Anthropogenic Pressure on *Posidonia oceanica* (L) Delile (1813) Ecosystems. Geophysical Research Abstract. EGU General Assembly 2018, 20: 3470-6.

Micheli C, Borfecchia F, De Cecco L, Martini S, Ceriola G, Bollanos S, Vlachopoulos G, Valiante L, Fresi E, Campbell G (2010b) Seagrass Monitoring by Remote Sensing in the Context of Biodiversity Conservation. Rapports et Proces-Verbaux des Réunions Commission international pour l'Exploration Scientifique de la Mer Méditerranée 39: 778.

Micheli C, Cupido R, Lombardi C, Belmonte A, Peirano A (2012) Changes in genetic structure of *Posidonia oceanica* at Monterosso al Mare (Ligurian Sea) and its resilience over a decade (1998-2009). Environmental Management 50: 598-606.

Micheli C, De Cecco L, Belmonte A, Struglia MV, Sannino G, Borfecchia F (2017) Monitoring *Posidonia oceanica* (L.) Delile (1813) meadows at Pantelleria island (Strait of Sicily) by traditional and innovative methodologies. Biologia Marina Mediterranea 24: 154-155.

Micheli C, D'Esposito D, Belmonte A, Peirano A, Valiante LM, Procaccini G (2015) Genetic diversity and structure in two protected *Posidonia oceanica* meadows. Marine Environmental Research 109: 124-131.

Micheli C, Paganin P, Peirano A, Caye C, Meinesz A, Bianchi CN (2005) Genetic variability of *Posidonia oceanica* in relation to local factors and biogeographic patterns. Aquatic Botany 82: 210-221.
Micheli C, Spinosa F, Aliani S, Gasparini GP, Molcard A, Peirano A (2010a) Genetic input by *Posidonia oceanica* (L) Delile fruits dispersed by currents in the Ligurian Sea. Plant Biosystems 144: 333-339.
Rotini A, Belmonte A, Barrote I, Micheli C, Peirano A, Santos RO, Silva J, Migliore L (2013) Effectiveness and consistency of a suite of descriptors for assessing the ecological status of seagrass meadows (*Posidonia oceanica)*. Estuarine coastal and Shelf Science 130: 252-259.
Rotini A, Micheli C, Valiante LM, Migliore L (2011) Assessment of *Posidonia oceanica* (L.) Delile conservation status by standard and putative approaches: the case study of Santa Marinella meadow (Italy, W Mediterranean). Open Journal of Ecology 1: 48-56.

AUTORE

Carla Micheli (carla.micheli@enea.it). ENEA Agenzia Nazionale per le Nuove tecnologie, l'Energia e lo Sviluppo Economico Sostenibile. Dipartimento Tecnologie per l'Energia. Laboratorio di Biomasse e Biotecnologie per l'Energia (DTE BBC-BBE). Centro Ricerche Casaccia. Via Anguillarese 301/00123, Santa Maria Di Galeria, Roma
Autore di riferimento: Carla Micheli

Towards the assessment of the diversity of the dinoflagellate genus *Tripos*: single cell approach

S. Mordret, T. Mollica, E. Biffali, M. Montresor, R. Pannone, R. Piredda, W.H.C.F. Kooistra, D. Sarno

The dinoflagellate genus *Tripos* (previously included in *Ceratium*) represents a significant component of marine microplankton. The genus includes more than 120 morphologically described species and infraspecific taxa, such as forms and varieties, found from polar to tropical areas. The genus has been suggested as indicator of ocean warming because of its taxonomic richness, wide geographical distribution and geographical patterning of its taxa related to temperature. However, the great variability of shapes described even within a single strain, coupled with the lack of clear discriminating characters, makes challenging species circumscription based on morphological criteria.
Using a single-cell approach, 52 *Tripos* cells isolated from the Gulf of Naples were characterized from the molecular (28S and 18S) and morphological point of view. In most cases, sequences obtained from morphologically identical cells clustered in the same clade. The 28S phylogeny showed a better capacity to discriminate different species than 18S, but also suggested the existence of cryptic diversity in the genus. As an example, cells morphologically identified as *T. trichoceros* clustered into two different clades.
Taxonomically validated sequences were added to the existing reference database DinoREF to improve the interpretation of metabarcoding data from a time series collected in the Gulf of Naples (LTER MC, 48 dates in 3 years).
I will present preliminary results and discuss problems and future possible approaches to study the diversity of this important but poorly known genus.

AUTORI

Solenn Mordret, Thomas Mollica, Elio Biffali, Marina Montresor, Raimondo Pannone, Roberta Piredda, Wiebe H.C.F. Kooistra, Diana Sarno (diana.sarno@szn.it), Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy
Autore di riferimento: Diana Sarno

BiOLEAP Biotechnological optimization of light use efficiency in algae photobioreactors

T. Morosinotto

New renewable energy source are highly needed to compensate exhausting fossil fuels reserves and reduce greenhouse gases emissions. Some species of algae have an interesting potential as feedstock for the production of biodiesel thanks to their ability to accumulate large amount of lipids. Strong research efforts are however needed to fulfil this potential and address many issues involving optimization of cultivation systems, biomass harvesting and algae genetic improvement. This proposal aims to address one of these issues, the optimization of algae light use efficiency. Light, in fact, provides the energy supporting algae growth and must be exploited with the highest possible efficiency to achieve sufficient productivity and make their cultivation competitive.
Algae efficiency in converting solar radiation, however, depends on many environmental factors, including light intensity, temperature, nutrient and CO2 availability. Optimizing microalgae productivity in such a complex environment hinges on our ability to describe, in a quantitative manner, the effect of these various parameters as well as their mutual interactions. Application of computational models that are capable of quantitative predictions can prove especially useful in identifying which parameters have the largest impact on productivity, thereby providing a mean for enhancing growth through design and operational changes. This information has now been employed to select *Nannochloropsis gaditana* strains with altered regulation of photosynthesis that showed improved productivity in lab scale photobioreactors.
Light influence on algae metabolism has also been investigated using a combination of genomic, transcriptomic and metabolomics analyses. These evidenced how light availability in *N. gaditana* modulates the carbon partitioning and TAG biosynthesis by affecting the transport of reduced carbon in and out of the chloroplast.

AUTORE

Tomas Morosinotto (tomas.morosinotto@unipd.it), Dipartimento di Biologia, Università di Padova, Via U. Bassi 58/B, 35121 Padova
Autore di riferimento: Tomas Morosinotto

Phytoplankton diversity in Adriatic ports

P. Mozetič, M. Cangini, J. Francé, M. Bastianini, F. Bernardi Aubry, M. Bužančić, M. Cabrini, F. Cerino, M. Čalić, R. D'Adamo, D. Drakulović, S. Finotto, D. Fornasaro, F. Grilli, R. Kraus, N. Kužat, D. Marić Pfannkuchen, Ž. Ninčević Gladan, M. Pompei, A. Rotter, I. Servadei, S. Skejić

Besides being a highly sensitive ecosystem, the Adriatic Sea is also an important seaway for international and local shipping, which poses a serious risk on the transfer of harmful aquatic organisms and pathogens (HAOP) by ships. Monitoring programs and surveillance activities would be of limited efficacy if carried out on a local scale only. The first step in setting up a common Adriatic approach towards unwanted threats to the environment from the transfer of HAOP was to prepare an inventory of organisms in ports. Phytoplankton diversity was determined through the port baseline survey in twelve Adriatic ports in years 2011, 2014 and 2015. Phytoplanktonic HAOP were identified as species, either native or non-indigenous (NIS) that can trigger harmful algal blooms (HAB). A list of 689 taxa was prepared and among them 52 were classified as HAB and five as NIS. Records of toxigenic NIS (*Pseudo-nitzschia multistriata*, *Ostreopsis* species including *O.* cf. *ovata*) indicate that the intrusion of non-native invasive phytoplankton species has already occurred in some Adriatic ports. The seasonal occurrence and abundance of HAOP offers a solid baseline for a monitoring design in ports in order to prevent possible expansion of HAOP outside their native region.

AUTORI

Patricija Mozetič (patricija.mozetic@nib.si), Janja Francé, Ana Rotter, National Institute of Biology, Marine Biology Station, Fornače 41, 6330 Piran, Slovenia
Monica Cangini, Marinella Pompei, Irene Servadei, Fondazione Centro Ricerche Marine, National Reference Laboratory on Marine Biotoxins, V.le A. Vespucci 2, 47042 Cesenatico (FC)
Mauro Bastianini, Fabrizio Bernardi Aubry, Stefania Finotto, Institute of Marine Sciences, National Research Council, Castello 2737/f, 30122 Venezia
Mia Bužančić, Živana Ninčević Gladan, Sanda Skejić, Institute of Ocean ography and Fisheries, Šetalište I. Meštrovića 63, 21000 Split, Croatia
Marina Cabrini, Federica Cerino, Daniela Fornasaro, Oceanography Division, Istituto Nazionale di Oceanografia e di Geofisica Sperimentale - OGS, Via Piccard 54, 34151 Trieste
Marijeta Čalić, University of Dubrovnik, Institute for Marine and Coastal Research, Kneza Damjana Jude 12, 20000 Dubrovnik, Croatia
Raffaele D'Adamo, Institute of Marine Sciences, National Research Council, Via Pola 4, 71010 Lesina (FG)
Dragana Drakulović, Institute of Marine Biology, University of Montenegro, Dobrota bb, 85330 Kotor, Montenegro
Federica Grilli, Institute of Marine Sciences, National Research Council, Largo Fiera della Pesca 2, 60125 Ancona
Romina Kraus, Nataša Kužat, Daniela Marić Pfannkuchen, Ruđer Bošković, Institute, Center for Marine Research, Giordano Paliaga 5, 52210 Rovinj, Croatia
Autore di riferimento: Patricija Mozetič

Different features of the ultrastructure of autophagy in algae

A. Papini, C. Tani, P. Di Falco, G. Wolswijk, U. Santosuosso, C. Nuccio, L. Lazzara, R. Ballini

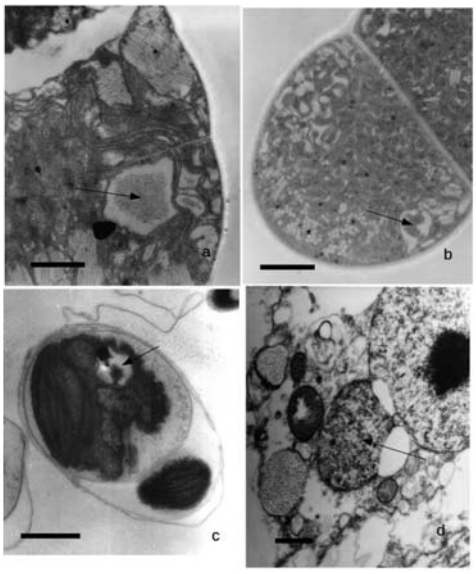

Fig. 1
1a: *Arthrospira platensis*. A large area containing granular material at low electron density is surrounded by membrane (arrow). Also the gas vesicles (asterisk) appear to be surrounded by a limiting membrane. Bar = 800 nm. 1b: *A. platensis*. A portion of cytoplasm with the same electron density of the rest of the cytoplasm is surrounded by thylakoidal membranes (arrow). Some gas vesicles appear fragmented (asterisk). Bar = 2 µm. 1c: *Dichtiosphaerium* cfr. *Ehrenber-gianum.* A vacuole (arrow) is enveloping a portion of cytoplasms and a lipid droplet is forming in the same organelle. Bar = 800 nm. 1d: *Heterosigma hakashiwo* . A portion (arrow) is detaching (blebbing) from the main part of the nucleus. Bar = 200 nm.

The eukaryotic algae do not represent a monophyletic group, since some clades of algae derived from primary endosymbiosis and others from secondary endosymbiosis. For this reason, the cell machinery does not work the same in every group. Concordantly, also autophagy showed quite variable features from the archaeplastida group (red algae, Glaucophyta, green algae and land plants) with respect, for instance, to the heterokont algae. Some example are presented.
Some doubt arises also about the origin of autophagy, since this phenomenon is typical of eukaryotes and currently no evidence of sequence similarity to autophagy-related eukaryotic genes has been found in prokaryotes genomes. As a matter of fact, autophagy consists in an activity of intracellular membranes and, by definition, prokaryotes should not have autonomous membranes with respect to the plasmamembrane. Nevertheless, cyanobacteria have a very developed membrane system, mainly devoted to photosinthesis, but also to the formation of gas vesicles. In cyanobacteria some open space, apparently surrounded by membranes, and containing granular material can be observed (Dvorak et al. 2015, Capelli et al. 2017), while there is evidence of protein recycling, particularly Rubisco. In *Arthrospira platensis* Gomont, some cytoplasm space containing granular material appear to get surrounded apparently by thylakoids and then to reduce their electron density (Fig. 1a and 1b). This observation may be a first hint to explain the strange autophagic activity performed by plastids in some plants (van Doorn, Papini 2013, Papini et al. 2014).
While in green algae the ultrastructural features are relatively similar to autophagy in land plants as in *Dichtiosphaerium* cfr. *ehrenbergianum* Nägeli (Fig. 1c), in the more distantly related *Heterosigma hakashiwo* (Hada) Hada ex Hara & Chihara (Raphidophyceae), belonging to one of the secondary endosymbiosis clades, autophagy assumes more extended features in the cytoplasm, particularly in case of starvation (Papini et al. 2017) and shows even evidence of nuclear blebbing (Fig. 1d), that is a typical feature of programmed cell death in metazoans.

Letteratura citata

Capelli C, Ballot A, Cerasino L, Papini A, Salmaso N (2017) Biogeography of bloom-forming microcystin producing and non-toxigenic populations of *Dolichospermum lemmermannii* (Cyanobacteria). Harmful Algae 67: 1-12.

Doorn WG van, Papini A (2013) Ultrastructure of autophagy in plant cells: a review. Autophagy 9(12): 1922-1936.

Dvorak P, Poulıckova A, Hasler P, Belli M, Casamatta DA, Papini A (2015) Species concepts and speciation factors in cyanobacteria, with connection to the problems of diversity and classification. Biodiversity and Conservation 24(4): 739-757.

Papini A, Fani F, Belli M, Niccolai C, Tani C, Di Falco P, Nuccio C, Lazzara L (2017) Structural and ultrastructure changes show an increase of amoeboid forms in *Heterosigma akashiwo* (Raphidophyceae), during recovery after nutrient depletion. Plant Biosystems 151(6): 965-973.

Papini A, Mosti S, van Doorn W G (2014) Classical macroautophagy in *Lobivia rauschii* (Cactaceae) and possible plastidial autophagy in *Tillandsia albida* (Bromeliaceae) tapetum cells. Protoplasma 251(3): 719-725.

AUTORI

Alessio Papini (alpapini@unifi.it), Corrado Tani, Pietro Di Falco, Giovanna Wolswijk, Caterina Nuccio, Luigi Lazzara, Raffaello Ballini, Department of Biology, University of Florence, Via Micheli 1-3, Firenze, 50121 Italy

Ugo Santosuosso, Department of Clinical and experimental Medicine, Università di Firenze, Largo Brambilla 3, 50134 Firenze, Italy

Autore di riferimento: Alessio Papini

Speciation by time in diatoms: the case of *Pseudo-nitzschia allochrona* sp. nov., a cryptic species in the *P. delicatissima*-complex

I. Percopo, D. Sarno, M.V. Ruggiero, R. Rossi, R. Piredda, A. Zingone

A new non-toxic species of the marine diatom genus *Pseudo-nitzschia*, *P. allochrona*, was isolated and cultivated from the Gulf of Naples and the Ionian Sea. Molecular differences in four phylogenetic markers (18S rDNA, LSU rDNA, ITS, *rbc*L) as well as the ITS2 secondary structure indicated this species to be distinctly separated from other congeneric species in the *P. delicatissima*-complex, some of which, i.e. *P. arenysensis*, *P. dolorosa* and *P. delicatissima* sensu stricto (s.s.), are very closely related to *P. allochrona* and occur in sympatry with it. The shape and ultrastructure of *P. allochrona* did not show any perceivable differences from those of *P. arenysensis* and *P. delicatissima.* Mating experiments showed sexual separation from the most closely related, *P. arenysensis*, definitely supporting the establishment of a new species. More than 60 strains of *P. allochrona* were isolated over the years from the LTER-MC station in the GON, and they were invariably recovered from the beginning of the summer throughout autumn. In contrast, *P. arenysensis*, *P. delicatissima* s.s. and *P. dolorosa* were recurrently found during the early spring annual peak. This clear separation over the annual cycle, also confirmed by metabarcoding data, may imply ecophysiological and functional differences between *P. allochrona* and the congeneric species. These results point at a possible mechanisms of speciation by time, whereby blooms of sympatric populations in different periods of the year may bring about sexual isolation and give rise to new taxa. As commonly accepted in the rest of the vegetal and animal realms, phenological patterns in diatoms are probably the result of genetic circannual clock modulated by environmental factors. Therefore, phenological signatures could be considered among phenotypic characters to be used in species delimitation.

AUTORI

Isabella Percopo, Diana Sarno, Maria Valeria Ruggiero, Roberta Piredda, Adriana Zingone (zingone@szn.it), Integrative Marine Ecology Department, Stazione Zoologica Anton Dohrn, Villa Comunale 80121, Napoli, Italy
Rachele Rossi, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Via Salute 2, 80055 Portici, Italy
Autore di riferimento: Adriana Zingone

Effect of CO_2 supply on the growth and nutrient uptake in microalgae cultures for industrial applications

L. Pezzolesi, F. Guerrini, S. Vanucci, R. Pistocchi

Atmospheric CO_2 concentration has been rising since the Industrial Revolution and is predicted to double by the end of this century (IPCC 2014). Microalgae play an important role in the carbon cycle of marine ecosystems and their responses to elevated atmospheric CO_2 are of considerable interest (Li et al. 2013). The effects of CO_2 enrichment are variable and may depend on the availability of nutrients, since CO_2 supply leads to a higher growth rate and to an increased demand for nutrients.

CO_2 released by industrial activities contributes substantially to increase the atmospheric CO_2 level; thus it is becoming more and more important to find alternatives to control its release in the atmosphere (Beneman, Hughes 1997). Flue gases produced by processes such as anaerobic digestion, for instance, could be potentially used for microalgae culturing (de Godos et al. 2014), thus preventing their release and allowing the transformation of polluting gas fluxes into new and valuable microalgae products.

In this study physiological responses to CO_2 supply in two microalgal species that have potential industrial applications were investigated. In particular *Desmodesmus communis,* a green alga widely used for phycoremediation due to its high resistance to chemical and environmental stress, and the diatom *Phaeodactylum tricornutum*, a PUFAs producer widely used in aquaculture, were studied using 1 L batch cultures (Fig. 1) or 70 L photobioreactors (Figg. 2, 3). Flue gases obtained by anaerobic digestion and enriched in CO_2 by the removal of the biogas fraction were also tested for microalgae culturing.

Results showed a marked increase of the growth and productivity in cultures with CO_2 addition respect to the ones grown with only air.

Moreover, a different use of macronutrients by algal cells and changes in cellular composition were observed when CO_2 was supplied. Preliminary experiments performed using flue gases supplied to *P. tricornutum* cultures reported no inhibition of the algal growth and promising results attesting their potential use to cultivate microalgae.

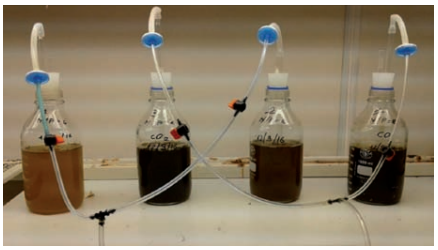

Fig. 1
Batch cultures (1 L) of the diatom *Phaeodactylum tricornutum.*

Fig. 2
Desmodesmus communis cultivation in 70 L photobioreactors.

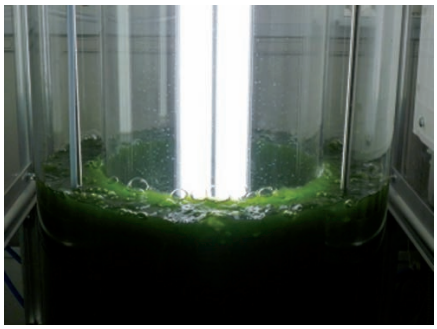

Fig. 3
CO_2 insufflation in a 70 L photobioreactor used for *Desmodesmus communis* cultivation.

Letteratura citata

Beneman JR, Hughes E (1997) Biological fossil CO_2 mitigation, Energy Convers. Management 38: 467-473.

Godos I de, Mendoza JL, Acién FG, Molina E, Banks CJ, Heaven S, Rogalla F (2014) Evaluation of carbon dioxide mass transfer in raceway reactors for microalgae culture using flue gases. Bioresource Technology 153: 307-314.

IPCC (2014) Climate change 2014: synthesis report. In: Core Writing Team, Pachauri RK, Meyer LA(Eds). Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change. Geneva, IPCC 2014. 151 pp. https://www.ipcc.ch/report/ar5/syr/

Li S, Luo S, Guo R (2013) Efficiency of CO_2 fixation by microalgae in a closed raceway pond. Bioresource Technology 136: 267-272.

AUTORI

Laura Pezzolesi (laura.pezzolesi@unibo.it), Franca Guerrini, Rossella Pistocchi, Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Bologna, Via Sant'Alberto 163, 48123 Ravenna

Silvana Vanucci, Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, Università di Messina, Viale Ferdinando Stagno d'Alcontres 31, 98166 S. Agata, Messina

Autore di riferimento: Laura Pezzolesi

The ecology of the genus *Leptocylindrus* in a highly structured ecosystem, the northern Adriatic

D.M. Pfannkuchen, N. Kuzat, J. Godrijan, M. Smodlaka, A. Baricevic, E. Pustijanac, R. Jahn, I. Ivancic, M. Pfannkuchen

The marine diatom genus *Leptocylindrus* is a major component of phytoplankton blooms in coastal ecosystems worldwide; however, little is known about the ecology and meticulous species composition of this genus in the Adriatic Sea. Although *Leptocylindrus danicus* and *L. minimus* have been reported from numerous studies conducted in Adriatic, there has been neither unequivocal species identification nor focused examination of the temporal abundance of *Leptocylindrus* in this region. In a recent reappraisal of *Leptocylindrus* by Nanjappa et al. (2013) five species and one new genus were identified using both morphological and molecular markers. We here describe the temporal distribution of the genus *Leptocylindrus* based on 45 years of records, revealing that this diatom is a key component of the seasonal phytoplankton cycle, with greatest abundance in the spring and autumn. Using light and scanning electron microscopy as well as genetic analysis based on the nuclear-encoded rDNA regions, our study unambiguously revealed three species new for the Adriatic Sea, *Leptocylindrus hargravesii*, *L. convexus* and *L. aporus.* Furthermore, we investigated the growth, auxospore and resting spore formation of one of the *Leptocylindrus* species under different nutrient regimes. The ecology, physiology and evolution of this significant diatom genus should be further investigated.

Letteratura citata

Nanjappa D, Kooistra WHCF, Zingone A (2013) A reappraisal of the genus *Leptocylindrus* (Bacillariophyta), with the addition of three species and the erection of *Tenuicylindrus* gen.nov. Journal of Phycology 49(5): 917-936.

AUTORI

Daniela Maric Pfannkuchen (Daniela.Maric@irb.hr), Nataša Kuzat, Mirta T. Smodlaka, Ana Baricevic, Ingrid Ivancic, Martin Pfannkuchen, Center for marine research, Ruđer Bošković Institute, G.Paliaga 5, Rovinj, Croatia
J. Godrijan, Division for Marine and Environmental Research, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia
Emina Pustijanac, University Jurja Dobrile, G. Paliaga 5, Pula, Croatia
Regina Jahn, Botanischer Garden und Botanisches Museum Berlin-Dahlem, Freie Universitat Berlin, Konigin-Luise-Str.6-8, Berlin, Germany
Autore di riferimento: Daniela Maric Pfannkuchen

Canopy-forming algae and biodiversity: the structure and large-scale spatial variability of macroalgal assemblages associated to *Cystoseira* beds

L. Piazzi, S. Pinna, G. Ceccherelli

In the Mediterranean Sea, *Cystoseira* species are the most important canopy-forming algae in shallow rocky bottoms. The aim of the present study is to contribute to the knowledge of the structure of the *Cystoseira*-dominated assemblages through the assessment of a large-scale study. Specifically, we tested the hypothesis that the number of taxa, the abundance and the structure of epiphytic and epilithic macroalgae associated to the *Cystoseira* beds changed among different spatial scales. A hierarchical sampling design in a total of five sites across the Mediterranean Sea was used. A total of 118 taxa (species and genus level) associated to *Cystoseira* beds were identified. The Rhodophyta *Polysiphonia subulifera* and *Haliptilon virgatum* and the Ochrophyta *Sphacelaria cirrosa* and *Dictyota* spp were the most common epiphytic macroalgal taxa. The Rhodophyta *Laurencia obtusa*, and the Ochrophyta *Padina pavonica* and *Halopteris scoparia* were among the most abundant no-epiphytic macroalgae. The occurrence of small and large scale variability was highlighted, but the different assemblages composing the whole macro community associated to *Cystoseira* showed different patterns of spatial variability. The relative importance of the different scales of spatial variability highlighted can be of practical use to optimize sampling designs focused on the ecological problems of this habitat and monitoring plans.

AUTORI

Luigi Piazzi (lpiazzi@uniss.it), Stefania Pinna, Giulia Ceccherelli, Dipartimento di Scienze della Natura e del Territorio, Università di Sassari, Via Piandanna 4, 07100 Sassari, Italy
Autore di riferimento: Luigi Piazzi

Phytoplankton stable carbon isotopes as a tool to monitor CO_2 leakage at Carbon Capture and Storage sites

F. Relitti, N. Ogrinc, M. Giani, F. Cerino, C. De Vittor, L. Urbini, B. Krajnc, P. Del Negro

Carbon capture and storage technology (CCS) is expected to play a key role among strategies for the mitigation of climate change by reducing CO_2 emissions into atmosphere from fossil fuel combustion. Although a well-engineered storage site is not expected to leak, the risk of failure of CO_2 containment and the subsequent environmental impact is a major issue for the acceptance of this approach. This study aims to the evaluation of stable carbon isotope analysis as a tool for effective early warning of CO_2 migration from CCS, since different carbon sources have specific $\delta^{13}C$ (usually lower values in anthropogenic emissions than in natural CO_2).
Two culture experiments were conducted within 2L-photobioreactors under controlled conditions (light, temperature). In each experiment, the diatom *Thalassiosira rotula* was grown in two different media: one prepared with natural seawater and the other with artificial seawater, whose carbonate system was derived from anthropogenic CO_2. Both media were equally supplemented with nutrients (silicate, nitrate and phosphate), metals and vitamins. Daily variations of $\delta^{13}C$ in dissolved inorganic carbon (DIC) and in diatoms were analysed in order to study the phytoplankton response.
According to the experiments results, differences exist in microalgae isotope composition between natural and anthropogenic conditions. In natural medium, the diatoms $\delta^{13}C$ values did not show important deviations from the starting value (−24.4 ± 0.3‰), whereas, in the anthropogenic CO_2 condition, the uptake of ^{13}C-depleted DIC (−44.7 ± 0.8‰) resulted in the decreasing values of microalgae carbon isotope composition (until −44.4‰). The rapid change in phytoplankton isotope composition confirms the method tested as reliable for identifying different CO_2 sources.

AUTORI

Federica Relitti (frelitti@inogs.it), Michele Giani, Federica Cerino, Cinzia De Vittor, Lidia Urbini, Paola Del Negro, Istituto Nazionale di Oceanografia e di Geofisica Sperimentale – OGS, Sezione Oceanografia, Via Auguste Piccard 54, 34151 Trieste, Italy
Federica Relitti, Dipartimento di Scienze della Vita, Università degli Studi di Trieste, Via Licio Giorgieri 1, 34127 Trieste, Italy
Nives Ogrinc, Bor Krajnc, Jožef Stefan Institute, Department of Environmental Sciences, Jamova cesta 39, 1000 Ljubljana, Slovenia
Autore di riferimento: Federica Relitti

Phytoplankton traits, functional groups and community organization

L. Roselli, E. Litchman

A central goal in ecology is to understand and predict the effects of environmental change on ecological communities and the consequences of community change for ecosystem functioning. Searching for common patterns and decoding underlying mechanisms from those patterns is how scientists can contribute to sustainable ecosystem management and conservation. Trait-based approaches have become increasingly successful in community ecology in general and phytoplankton communities in particular. Phytoplankton communities have a rich history as model systems in community ecology and are ideally suited for applying and further developing trait-based concepts. Understanding trait changes is key to better forecasting community responses to environmental impacts, including anthropogenic global change. In the context of maintenance of biodiversity and ecological functions, microbial ecologists face the challenge of linking individual level variability in functional traits to larger scale ecosystem processes. Phytoplankton cell size and shape are key traits under selection by environmental filters and species interactions. Here, we explore how size and shape enter the diversity game. How does taxonomic and morpho-functional community structure vary at different spatial scales? What are the potential drivers shaping the structure of phytoplankton communities? We explore these questions by looking at the individual level variability in taxonomic and morphological traits in a biogeographical snapshot of natural phytoplankton communities in coastal ecosystems around the globe. Species diversity was more variable than trait diversity from local to global spatial scales. We suggest that structural organization of phytoplankton communities in coastal ecosystems may follow a hierarchical pattern of trait organization, where a different combination of multiple functional traits may represent effective strategies and promote success under given environmental conditions as a resolution of Hutchinson's paradox.

AUTORI

Leonilde Roselli (l.roselli@arpa.puglia.it), Arpa Puglia, Dipartimento di Lecce, Via Miglietta, 73100 Lecce
Elena Litchman, Kellogg Biological Station and Department of Integrative Biology, Michigan State University, Hickory Corners, MI, 49060 USA
Autore di riferimento: Leonilde Roselli

Un approccio integrato per la rimozione di fosforo e metalli pesanti accoppiata alla produzione di lipidi ad opera della microalga verde *Desmodesmus* sp.

L. Rugnini, G. Costa, R. Congestri, S. Antonaroli, L. Bruno

La qualità dei corpi idrici superficiali è fortemente influenzata dalle attività antropiche che causano emissioni diffuse e / o puntiformi di inquinanti organici e inorganici. Gli effluenti ricchi di nutrienti come l'azoto (N) e il fosforo (P) possono dar luogo a fenomeni come l'eutrofizzazione (Schindler et al. 2008), mentre la presenza di metalli può rappresentare un serio rischio sia per la salute umana che per l'ecosistema (Torres et al. 2017). Le microalghe sono in grado di sottrarre i nutrienti in eccesso nelle acque reflue e di resistere alla presenza di metalli tossici accumulandoli all'interno delle cellule, caratteristica che ne permette l'utilizzo nel settore del biorimedio (Gismondi et al. 2016, Rugnini et al., 2017, 2018). L'impiego di microalghe per il trattamento delle acque reflue permette, inoltre, di ottenere una biomassa che può essere, ad esempio, impiegata per la produzione di biocarburanti come biodiesel, etanolo, bioidrogeno (Bruno et al. 2012, Bux, Chisti 2016, Gismondi et al. 2016). In questo lavoro è stata valutata la possibilità di utilizzare la microalga verde *Desmodesmus* sp. (Fig. 1) sia per il biorimedio delle acque reflue dall'eccesso di P e metalli pesanti quali rame (Cu) e nichel (Ni), sia per la produzione di biodiesel.

Fig. 1
Immagine al microscopio ottico di cenobi di *Desmodesmus* sp.

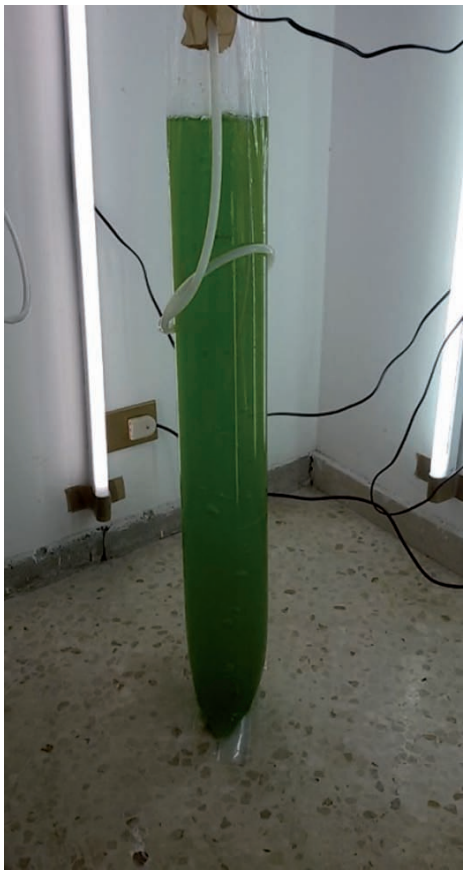

Fig. 2
Fotobioreattore da 10 L costituito da buste di polietilene impiegato per gli esperimenti indicati come *Run 1* e *Run 2*.

Sono stati effettuati due esperimenti di crescita in coltura, indicati come *Run 1* e *Run 2,* in fotobioreattori a basso costo della capacità 10 L (Fig. 2) utilizzando come mezzo di coltura il terreno BG11 (Rippka et al. 1979) modificato per il contenuto di P che era pari a 4.55 mg L^{-1} per simulare le concentrazioni osservate in effluenti di impianti di depurazione civili o industriali (Water Environment Federation 2010, Cai et al. 2013). Inoltre, nel *Run 2*, la coltura di *Desmodesmus* sp. è stata anche esposta a una soluzione bimetallica di Cu e Ni e dopo 2 giorni di esposizione/contatto è stata valutata la capacità di biosorbimento delle cellule. La biomassa ottenuta in entrambi i *Run* è stata sottoposta ad analisi della capacità di rimuovere il P e ad estrazione dei lipidi, che successivamente sono stati analizzati mediante gas-cromatografia per la caratterizzazione degli esteri metilici degli acidi grassi (FAME).

I risultati hanno dimostrato che *Desmodesmus* sp. è in grado di rimuovere tra il 96 e il 100% del P presente, impiegando 21 giorni nel *Run 1* e 14 giorni nel *Run 2*, mentre sono stati sufficienti solo 2 giorni di contatto microalga-metallo per la rimozione del 94% del Cu totale e l'85% di Ni (Rugnini et al. 2018). Molti studi (Kumar et al. 2015, Rugnini et al. 2017) riportano che in soluzioni multi-metalliche che contengono Cu e Ni l'affinità per il Cu è sempre maggiore a quella del Ni, come ottenuto anche in questo studio, probabilmente a causa di una più forte interazione tra ione metallico e gruppi carbossilati della parete cellulare delle microalghe. È noto come il contenuto lipidico delle alghe dipenda da diversi fattori, come la disponibilità dei nutrienti nel mezzo di coltura, la salinità, l'intensità luminosa, e la presenza di metalli e altri contaminanti che possono indurre stress ossidativi causa di accumulo di lipidi come meccanismo di difesa (Torres et al. 2017). In questo studio, l'estrazione e lo studio dei profili lipidici hanno rivelato che la presenza di metalli ha diminuito la resa lipidica, ma, secondo gli standard europei (EN 14214) e statu-

nitensi (ASTM-D6751), la qualità del biodiesel ottenuto dalla biomassa esposta ai metalli (*Run 2)* era superiore a quella del biodiesel ottenuto dalla biomassa non esposta (*Run 1)*, poiché risultava maggiore la quantità di lipidi saturi e monoinsaturi accumulati.
Questo studio ha dimostrato, quindi, l'efficacia dell'utilizzo della microalga verde *Desmodesmus* sp. per la rimozione di contaminanti come P, Cu e Ni. Tale applicazione può essere integrata con successo all'uso della biomassa ottenuta come materia prima per la produzione di biocarburanti, offrendo una possibile soluzione alternativa a più problemi ambientali.

Letteratura citata

Bruno L, Di Pippo F, Antonaroli S, Gismondi A, Valentini C, Albertano P (2012) Characterization for biofilm-forming cyanobacteria for biomass and lipid production. Journal of Applied Microbiology 113: 1052–1064.
Bux F, Chisti Y (Eds) (2016) Algae Biotechnology Products and Processes. Springer.
Cai T, Park SY, Li Y (2013) Nutrient recovery from wastewater streams by microalgae: Status and prospects. Renewable & Sustainable Energy Reviews 19: 360-369.
Gismondi A, Di Pippo F, Bruno L, Antonaroli S, Congestri R (2016) Phosphorus removal coupled to bioenergy production by three cyanobacterial isolates in a biofilm dynamic growth system. International Journal of Phytoremediation. DOI: 10.1080/15226514.2016.1156640.
Kumar KS, Dahms HU, Won EJ, Lee JS, Shin KH (2015) Microalgae - A promising tool for heavy metal remediation. Ecotoxicology and Environmental Safety 113: 329-352.
Rippka R, Deruelles J, Waterbury J B, Herdman M, Stanier RY (1979) Generic Assignments, Strain Histories and Properties of Pure Cultures of Cyanobacteria. Journal of General Microbiology 111: 1-61.
Rugnini L, Costa G, Congestri R, Antonaroli S, Sanità di Toppi L, Bruno L (2018) Phosphorus and metal removal combined with lipid production by the green microalga *Desmodesmus* sp.: An integrated approach. Plant Physiology and Biochemistry 125: 45-51.
Rugnini L, Costa G, Congestri R, Bruno L (2017) Testing of two different strains of green microalgae for Cu and Ni removal from aqueous media. Science of the Total Environment 601–602: 959–967.
Schindler DW, Hecky RE, Findlay DL, Stainton MP, Parker BR, Paterson MJ, Beaty KG, Lyng M, Kasian SEM (2008) Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. Proceedings of the National Academy of Science (PNAS) 105(32): 11254-11258.
Torres EM, Hess D, McNeil BT, Guy T, Quinn JC (2017) Impact of inorganic contaminants on microalgae productivity and bioremediation potential. Ecotoxicology and Environmental Safety 139: 367–376.
Water Environment Federation (2010) Nutrient Removal. WEF Manual of Practise No.34. WEF Press, Virginia. 450 pp.

AUTORI

Rugnini Lorenza (Lorenza.Rugnini@uniroma2.it), Roberta Congestri, Laura Bruno, Dipartimento di Biologia, +390672595989, Università di Roma "Tor Vergata", Via Cracovia 1, 00133 Roma, Italia
Giulia Costa, Laboratorio di Ingegneria Ambientale, Dipartimento di Ingegneria Civile e Ingegneria Informatica, Università di Roma "Tor Vergata", Via del Politecnico, 00133 Roma, Italia
Simonetta Antonaroli, Dipartimento di Chimica, Università di Roma "Tor Vergata", Via della Ricerca Scientifica snc, 00133 Roma, Italia

Autore di riferimento: Lorenza Rugnini

The use of Marine Resources for Restoration and Conservation of Artworks

U. Santamaria, F. Morresi, F. Fratini, S. Bani

In the conservation of artifacts the use of natural materials has been increasing in recent year, especially at Vatican Museums, thanks to the work of the Scientific Laboratories. In many cases, natural materials are progressively replacing synthetic ones, allowing less invasive interventions on artworks and a minor impact on operators and the Environment. The emergence of these new restoration methodologies represents, thus, a real change in approaches, leading restorers towards the principle of sustainability in the practice of conservation. In the restoration Laboratories of Vatican Museums, chemists, naturalists, biologists, and restorers work together to find new restoration solutions through the use of natural materials. In this context, marine resources represent an extraordinary source of innovative products to be tested in the field of Cultural Heritage. Among the natural materials for consolidation, "Funori", a polysaccharide extracted from the red alga *Gloiopeltis furcata* (Postels & Ruprecht) J.Agardh of the Pacific Ocean is now commonly used. The matrix from which the Funori is obtained is soluble in hot water and consists of a mixture of polysaccharides, sulphates, lipids, proteins, salts, and dyes (Izumi 1971). *G. furcata* is purified in laboratory and its extract is used by restorers on paper, wood, metal, and wall paintings (Michel 2011). At the same time, studies and experiments on the red Mediterranean alga *Sphaerococcus coronopifolius* Stackhouse, are ongoing at Vatican Museums. Several tests have shown that the extract obtained from *S. coronopifolius* has properties similar to those of Funori. Moreover, it seems to may assure a better yield in terms of pH, conductivity and adhesiveness (Fratini et al 2016). Recent studies have demonstrated that the species reproduces on the Lazio coasts trough a direct-type of life history, ensuring a less variability in the composition and yield of the natural products synthesized by it (Abdelahad et al 2016). Finally, the use of *Posidonia oceanica* (L.) Delile for restoration purposes is to be also remarked (Lomoro et al 2011). The egocropiles of this marine plant are treated in laboratory and used as stucco for woodwork. Tests and applications on *P. oceanica* are still ongoing at the Scientific Laboratories (Minnini et al. 2009, Parente et al. 2009).

Letteratura citata

Abdelahad N, Barbato F, O'Heir S, Fratini F, Velletta A, Ninivaggi L, Alfinito S (2016), Reproduction of *Sphaerococcus coronopifolius (Gigartinales,* Rhodophyta*)* in natural populations of the Lazio coasts (central Italy) and in culture*.* Cryptogamie, Algologie 37 (4): 265.272. Doi/10.7872/crya/v37.iss4.2016.265.

Fratini F, Rivière C, Santamaria U (2016), *Sphaerococcus coronopifolius* (Rhodophyta*, Gigartinales*): a Mediteranean red alga with potential and applications in restoration*.* Natural product research, DOI: 10.1080/14786419.2016.1163552

Izumi K (1971) Chemical heterogeneity of anhydrogalactose containing polysaccharides from *Gloiopeltis furcata.* Agric ultural and Biological Chemistry 35: 633-657.

Lomoro A, Grieco F, Attanasio C, Guido M (2011) Recupero e valorizzazione di residui vegetali spiaggiati di *Posidonia oceanica* e *Cymodocea nodosa.* In: Atti dei Seminari: Rivoluzione Ecoindustriale, 9-12 Novembre 2011 - eco-logicasrl.it, Bari.

Michel F (2011) Funori and JunFunori: two related consolidant with surprising properties*.* In: Proceedings of Symposium 2011-Adhesives and consolidants for Conservation: 1-14. October 17 to 21, 2011, Ottawa. Information archived on the web by the Canadian Conservation Institute.

Mininni M, Santamaria P (2009) I tanti usi dei residui di posidonia spiaggiata. In "Il caso dei residui spiaggiati di *Posidonia oceanica*: da rifiuto a risorsa". Levante Editori, Bari.

Parente A, Cocozza C, Signore A, Santamaria P (2009) Caratterizzazione chimico-fisica dei residui di *Posidonia* spiaggiata. In: https://www.researchgate.net/publication/267210052.

AUTORI

Ulderico Santamaria, Fabio Morresi, Stefania Bani, Scientific Laboratories of Vatican Museums, 00120 Città del Vaticano, Roma

Filippo Fratini (filippofratini.f@libero.it), Freelance Marine Biologist, 00124 Roma, Italy

Autore di riferimento: Filippo Fratini

Acclimatazione all'acidificazione in Caulerpa *cylindracea* Sonder

A. Santin, E. Moschin, M. Lorenti, M.C. Buia, I. Moro

Il continuo aumento di gas serra, in particolare di anidride carbonica (CO_2) rilasciata nell'atmosfera dalle crescenti attività antropiche attraverso l'uso di combustibili fossili, è causa di importanti cambiamenti climatici i cui effetti più eclatanti si manifestano in un aumento della temperatura e in una devastante siccità (IPCC, 2014). Nell'ambiente marino il riscaldamento si sovrappone ad un altro effetto, l'acidificazione degli oceani, dal momento che un terzo dell'anidride carbonica emessa nell'atmosfera viene assorbita dalle grandi masse d'acqua che ricoprono il nostro pianeta (Sabine et al. 2004).
Mentre i primi studi sugli effetti dell'acidificazione sono stati condotti per lo più in laboratorio o in mesocosmi semi-naturali utilizzando organismi con alti tassi di crescita e brevi cicli vitali (Kim et al. 2016), negli ultimi dieci anni l'attenzione si è spostata verso quei siti marini naturalmente acidificati dalla presenza di emissioni gassose di varia natura e che perciò possono rappresentare laboratori naturali in cui validare la coerenza dei risultati ottenuti in sistemi artificiali (Fabry et al. 2008, Koch et al. 2013, Kroeker et al. 2013).
L'obiettivo di questa ricerca è stato quello di studiare le risposte di *Caulerpa cylindracea* Sonder a variazioni di pH. Questa specie invasiva di origine tropicale, grazie alla sua elevata plasticità, è in grado di colonizzare vari habitat con condizioni di luce e temperatura estremamente varie (Raniello et al. 2006, Flagella et al. 2008).
Per valutare la risposta della macrofita all'acidificazione, sono stati condotti *in situ* trapianti incrociati di talli tra aree a pH 8.01 e aree a pH 7.50, nel sito del Castello Aragonese di Ischia (Napoli), dove emissioni naturali di CO_2 creano un gradiente di acidità compreso tra 8.1 e 6.7 (Hall-Spencer et al. 2008, Porzio et al. 2011, 2013). Gli effetti legati alle variazioni di pH ambientale sulla specie in esame sono stati studiati al microscopio ottico ed elettronico a trasmissione. È stata inoltre valutata l'efficienza fotosintetica attraverso variazioni dell'attività fotochimica (Diving PAM) e del contenuto in pigmenti. Sono state analizzate le risposte ai cambiamenti di pH a breve termine (3, 7, 14 giorni dal trapianto) e la capacità di recupero dopo il riposizionamento dei talli alle condizioni di origine, per valutare l'eventuale reversibilità delle modificazioni subite. Inoltre, il confronto tra le due popolazioni naturali a pH 7,50 e a pH 8,01 ha permesso di valutare gli effetti su lunga scala temporale.
Mentre i risultati relativi agli effetti su lunga scala temporale non hanno evidenziato importanti alterazioni fisiologiche e ultrastrutturali, nel breve periodo sono state riscontrate modificazioni significative. I talli trapiantati da pH 7.50 a 8.01, infatti, hanno risposto positivamente all'aumento del pH, mostrando un aumento progressivo del numero di cloroplasti all'aumentare del tempo di esposizione alla nuova condizione ambientale, un aumento del rapporto Chl a/Chl b e una diminuzione del contenuto in carotenoidi dal 3° al 14° giorno dal trapianto. Inoltre, è stato riscontrato un miglioramento dell'efficienza fotosintetica ed una diminuzione della dissipazione termica dell'energia di eccitazione, segnali questi di un passaggio verso un ambiente favorevole.
Al contrario, il trasferimento da pH 8.01 a pH 7.50 ha innescato risposte negative evidenziate dalla diminuzione nel numero di cloroplasti, dall'aumento delle dilatazioni tra le membrane tilacoidali e da importanti accumuli di amido. Queste variazioni ultrastrutturali sono state confermate dalla diminuita efficienza fotosintetica, dall'aumento dei carotenoidi e del rendimento non fotochimico e dalla diminuzione delle clorofille, sottolineando uno stress per la macroalga.
D'altra parte, il fatto che *C. cylindracea* sia presente naturalmente in siti a pH 8,01 e a pH 7,05, ci porta a supporre che le risposte allo stress da acidificazione messe in atto nel breve periodo siano transitorie e che tale specie potrebbe acclimatarsi al progressivo acidificarsi dei mari, nello scenario dei cambiamenti climatici in atto.

Letteratura citata

Fabry VJ, Seibel BA, Feely RA, Orr JC (2008) Impacts of ocean acidification on marine fauna and ecosystem processes. ICES Journal of Marine Science: Journal du Conseil 65: 414-432.
Flagella MM, Lorenti M, Buia MC (2008) Temperature response in a shallow Mediterranean population of *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta) and a possible strategy of season anticipation. Botanica Marina 51: 278-284.
Hall-Spencer JM, Rodolfo-Metalpa R, Martin S, Ransome E, Fine M, Turner SM, Rowley SJ, Tedesco D, Buia MC (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. Nature 454: 96-99.
IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team RK Pachauri and LA Meyer (eds)]. IPCC, Geneva, Switzerland. 151 pp.
Kim JH, Kang EJ, Edwards MS, Lee K, Jeong HJ, Kim HY (2016) Species-specific responses of temperate macroalgae with different photosynthetic strategies to ocean acidification: a mesocosm study. Algae 31 (3): 243-256.
Koch M, Bowes G, Ross C, Zhang X-H (2013) Climate change and ocean acidification effects on seagrasses and marine macroalgae. Global Change Biology 19:103-132.
Kroeker KJ, Micheli F, Gambi MC (2013) Ocean acidification causes ecosystem shifts via altered competitive interactions. Nature Climate Change 3: 156-159.

Porzio l, Buia MC, Spencer-Hall J (2011) Effects of acidification on macroalgal communities. Experimental Marine Biology and Ecology 400: 278-287.
Porzio L, Garrard SL, Buia MC (2013) The effect of ocean acidification on early algal colonization stages at natural CO_2 vents. Marine Biology 160: 2247-2259.
Raniello R, Lorenti M, Brunet C, Buia MC (2006) Photoacclimatation of the invasive alga *Caulerpa racemosa* var. *cylindracea* to depth and daylight patterns and a putative new role for siphonoxanthin. Marine Ecology 27 (1): 20-30.
Sabine CL, Feely RA, Gruber N, Key RM, Lee K, Bullister JL, Wanninkhof R, Wong CS, Wallace DWR, Tilbrook B, Millero FJ, Peng TH, Kozyr A, Ono T, Rios AF (2004) The oceanic sink for anthropogenic CO_2. Science 305: 367-371.

AUTORI

Anna Santin, Emanuela Moschin, Isabella Moro (isabella.moro@unipd.it), Dipartimento di Biologia, Università di Padova, Via U. Bassi 58B, 35121 Padova
Maurizio Lorenti, Maria Cristina Buia (mariacristina.buia@szn.it), Centro di Villa Dohrn-Ecologia del Benthos, Dipartimento di Ecologia Marina Integrata, Stazione Zoologica A. Dohrn, Punta San Pietro, 80077 Ischia (Napoli)
Autori di riferimento: Maria Cristina Buia, Isabella Moro

Intensive cultivation of *Staurosirella pinnata* (Bacillariophyceae) for mycosporine-like aminoacid characterization and bioactivity on melanoma cell line

S. Savio, E. Bellini, C. Rodolfo, L. Piredda, L. Canuti, A. Canini, R. Congestri

Mycosporine-like amino acids (MAAs) are a class of small, water-soluble molecules known for their capability of absorbing UV radiation and widespread across a variety of algae and cyanobacteria. Some red algae MAAs showed bioactivity with potential in drug discovery, but no evidence yet exist on MAA production, composition and potential application in cultured diatoms.
In this work, one brackish strain of *Staurosirella pinnata* (Ehrenberg) D.M. Williams & Round, VRUC 290, was mass cultivated in indoor polyethylene photobioreactors (10 L) to produce biomass for MAA extraction using methanol/water (20:80 v/v). Extracts were then characterized using LC-MS and subsequently analyzed for their anti-oxidant activity with 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and, finally, tested for their effect on melanoma cells (CHL-1) by flow cytometry.
Chromatographic profiles allowed identification of four different mycosporines, two of which mycosporine-glycine and euhalothece, present in relatively high amounts compared to concentrations recorded for other two diatom strains *Phaeodactylum tricornutum* and *Thalassiosira weissflogii* (SAG Culture Collection) cultivated in the same conditions in our laboratory. The radical scavenging activity of *S. pinnata* extract was assessed by DPPH assay showing high anti-oxidant activity, with an IC_{50} of 15.0 +/- 0.1 µg.
Bioactivity of the extract was then tested on human melanoma CHL-1 cells and cytofluorimetric analyses (Fluorescence-Activated Cell Sorting) evidenced a strong cytotoxic effect of the after 24h and 48h treatments. Finally, alteration of cell cycle was observed after 24 and 48h treatments. CHL-1cells showed an increase in cells in G1 and G2/M phases while a decrease of cells in S phase was measured. These results suggest an anti-proliferative effect of the *S. pinnata* extract on the melanoma cell line.

AUTORI

Saverio Savio (saverio.savio@gmail.com), Erika Bellini, Roberta Congestri, Dipartimento di Biologia, Università degli Studi di Roma 'Tor Vergata', Via Cracovia, 00133 Roma
Carlo Rodolfo, Lucia Piredda, Lorena Canuti, Antonella Canini, Dipartimento di Biologia, Università degli Studi di Roma 'Tor Vergata', Via della Ricerca Scientifica, 00133 Roma
Autore di riferimento: Saverio Savio

Success of aquatic angiosperm transplantation in the northern Venice Lagoon

A. Sfriso, A. Buosi, C. Facca, P. Franzoi, L. Scapin, M. Zucchetta, A. Bonometto, R. Boscolo, F. Rampazzo, F. Oselladore, E. Ponis

The project SeResto (LIFE12 NAT/IT/000331) aimed at the recovering of the northern part of the Venice Lagoon by transplantation of aquatic angiosperms. The main anthropogenic impacts that affected the lagoon in the second part of the last century reducing aquatic angiosperm prairies, since the mid-2000s declined. The banning of phosphorus from the formulation of detergents (October 1988), the installation of urban waste water treatment plants in the hinterland and a general management of nutrient inputs in the lagoon, triggered a progressive reduction of the trophic conditions and blooms of Ulvaceae restoring conditions suitable for plant recruitment.

The objective was to trigger the natural re-colonization in a confined area where plant presence was negligible by mean of widespread, manual and low-cost transplantations of small sods or single rhizomes, mainly of *Zostera marina* and *Z. noltei,* avoiding any impact on the shallow bottoms. Aquatic angiosperms are environmental engineers which provide many environmental services and suitable conditions for benthic, fish and bird fauna. Their presence is the first condition for habitat recovering and the restoration of pristine or almost pristine environments.

After three years from the beginning of the project ca. 44700 rhizomes have been transplanted in 35 sites placed in an area of ca. 36 km^2. Transplantations have been successful in 32 sites with a mean coverage of 60% and many areas have been completely colonized. However, results were very different among sites and were negatively affected by raining conditions and water temperature whose extreme values can severely hamper their spread. Despite the different and unpredictable climatic conditions now aquatic angiosperms have colonized all the bottoms close to salt marshes and the canal edges. They are self-sustaining and expand exponentially because they produce a high number of seeds that colonize all the suitable areas. If extreme climatic changes are not taking place and other human impacts will be not occur, in a few years the environmental conditions of the northern lagoon will be restored as before the anthropogenic impacts.

AUTORI

Adriano Sfriso (sfrisoad@unive.it), Alessandro Buosi, Chiara Facca, Piero Franzoi, Luca Scapin, Matteo Zucchetta, Department of Environmental Sciences, Informatics & Statistics, Via Torino 155, 30172 Mestre-Venezia, Italy

Andrea Bonometto, Rossella Boscolo, Federico Rampazzo, Federica Oselladore, Emanuele Ponis, Italian National Institute for Environmental Protection and Research, ISPRA, Loc. Brondolo, 30015 Chioggia (Venezia), Italy

Autore di riferimento: Adriano Sfriso

Investigating silver nanoparticles toxicity on marine macroalgae (Ulva rigida) using biochemical and physiological biomarkers

A.A. Sfriso, A.-S. Juhmani

Keywords: biomarkers, silver nanoparticles, Ulva rigida, Venice Lagoon

Despite the high ecological and economical importance of transitional environments and their high productivity, they represent a sink for many pollutants including metals. The steady increase in the production and release of products, containing silver nanoparticles (AgNP), could lead to an increasing dispersion and diffusion of silver into transitional environments. In this study, in vitro experiments were conducted to examine the toxicity of AgNP using the marine macroalga Ulva rigida C. Agardh, the most common species in transitional environments. The toxicity of both AgNP and aqueous silver (AgNO3) was estimated based on the implementation of both biochemical (lipid peroxidation) and physiological (primary production) biomarkers with conditions similar to the Venice Lagoon environment.
The primary production showed an adverse effect of AgNP starting from 0.1 ppm reaching complete inhibition at 5ppm. Conversely, AgNO3 displayed a severe inhibition of primary production in U. rigida already at 0.01 ppm.
The lipid peroxidation, measured by malondialdehyde levels, showed that the lowest observed stress effect was at 1 ppm of AgNP. The oxidative stress generated by AgNP steadily increased up to a concentration of 30 ppm resulting in membrane disruption. In contrast, the oxidative stress generated by AgNO3 was less significant, proving that AgNO3 was very toxic (as measured by primary production) but did not produce stress at cellular level. The silver bioaccumulated by U. rigida from an AgNP solution of 1 ppm reached 8.5 mg Kg-1fw and the bioaccumulation reached a saturation point at 11.3 mg Kg-1 fw from a 5ppm AgNP solution.
The concentration of silver in the water column don't exceed the ppt range, which is not toxic for U. rigida, but the reported concentrations for benthic marine organisms from the Venice Lagoon were in the ppm range. These concentrations of AgNP were found to be high enough when bioaccumulated to elicit a phytotoxic effect.

AUTORI

Andrea A. Sfriso (asfriso@hotmail.it), Ca' Foscari University of Venice, Department of Molecular Sciences and Nanosystems, Dorsoduro 3246, 30123 Venezia
Abdul-Salam Juhmani, Ca' Foscari University of Venice, Department of Environmental Sciences Informatics and Statistics Dorsoduro 3246, 30123 Venezia
Autore di riferimento: Andrea A. Sfriso

Acidification and temperature effects on the gene expression of the coccolithophore *Pleurochrysis pseudoroscoffensis*

F. Spazzali, M. Gerdol, F. Cerino, M. Antonioli, A. Beran, A. Pallavicini, M. Cabrini

Coccolithophores are nanoplanktonic microalgae characterized by an exo-skeleton composed of minute calcified plates and play an important role in control of climate changes because they are involved in the sulfur cycle for the production of dimethylsulfide as well as in the carbon cycle for the photosynthesis and calcification processes. Recent studies on the ocean acidification due to the carbon dioxide increase in the atmosphere have provided evidence on a possible impact on coccolithophore calcification.
The aim of this study was: a) to investigate possible effects on gene expression of the coccolithophore *Pleurochrysis pseudoroscoffensis* due to changes in pCO_2 and temperature; b) to assemble de novo the genome and transcriptome of the coccolithophore.
In the framework of the project Acid.it (Costruzione di conoscenze e di strumenti a supporto della definizione di strategie di mitigazione ed adattamento agli effetti dell'acidificazione marina, con particolare riferimento ai mari italiani), culture experiments were performed in triplicate in 2.5 L-photobioreactors testing four pCO_2/temperature conditions: control (21 °C and 400 ppm CO_2), high temperature (25 °C and 400 ppm CO_2), high pCO_2 (21 °C and 700 ppm CO_2) and high temperature and pCO_2 (25 °C and 700 ppm CO_2). Samples were collected at two different timing: after 11 and 20 days. The RNA from samples was isolated with a commercial kit, and the cDNA library were prepared for the sequencing with the Illumina Hiseq 3000/4000 technology. The reads obtained from the sequencing were trimmed and assembled with the Trinity software, the trascriptome is about 51000 contigs. The functional annotation was made with the software Trinotate, and for evaluate differentially expressed genes was used the program CLC Genomics Workbench.
Considering the gene expression, the only effect of CO_2 did not result in significant variations, whereas the only temperature had a more significant effect. However, these two factors seemed to show a combined effect, with a strong gene up-regulation and down-regulation at the end of the culture. The genes down regulated were related to protein transcription and translation, so to presume a complete stop of the protein synthesis. Among the upregulated genes, there were some involved in several metabolic pathways mainly related to nitrogen synthesis, but also genes involved in the coccolithogenesis and a carbonic anhydrase, suggesting a possible alteration of the production of the calcium carbonate skeleton.
Instead, for the genome we isolated the DNA and sent it to the sequencer senter, where they prepare the library and sequenced them with the Illumina Hiseq 3000/4000 technology. The reads were then assembled with four different software (CLC G.W., Soap, Spades2 and Abyss). The better output, based on different parameters was the one with Abyss, and with another program (Jellyfish) we were able to estimate the genome size which is about 213Mb.

AUTORI

Francesca Spazzali (francesca.spazzali@gmail.com), Marco Gerdol, Alfredo Pallavicini, Department of Life Science, University of Trieste, Via Giorgieri 5, Trieste 34127, Italy
Federica Cerino, M. Antonioli, Alfred Beran, Oceanography Section, Istituto Nazionale di Oceanografia e di Geofisica Sperimentale, Borgo Grotta Gigante 42/c, 34010 Sgonico, Trieste
Marina Cabrini (mcabrini@inogs.it), OGS, Via Auguste Piccard 54, 34151 Trieste, Italy
Autori di riferimento: Francesca Spazzali, Marina Cabrini

The Ecology of One Cosmopolitan, One Newly Introduced and One Occasionally Advected Species from the Genus *Skeletonema* in a Highly Structured Ecosystem, the Northern Adriatic

M. Smodlaka Tanković, D. Marić Pfannkuchen, J. Godrijan, A. Baričević, N. Kužat, T. Djakovac, E. Pustijanac, R. Jahn, M. Pfannkuchen

The diatom genus *Skeletonema* is globally distributed and often an important constituent of the phytoplankton community. In the marine phytoplankton of the northern Adriatic Sea, we found three species of the genus *Skeletonema*: *Skeletonema menzelii*, *S. marinoi* and *S. grevillei*. Making use of the steep ecological gradients that characterize the northern Adriatic, along which we could observe those species, we report here on the ecological circumstances under which those species thrive and how their respective populations are globally connected. This is the first detailed ecological study for the species *S. grevillei*. This study is also the first report for *S. grevillei* for the Adriatic Sea and Mediterranean, together with additional electron microscopic details on fresh in situ samples for this species. *S. marinoi* appears to clearly prefer strong freshwater influence and high nutrient concentrations delivered by low salinity waters. It can outcompete other diatom species and dominate microphytoplankton blooms. *S. grevillei* on the other hand appears to thrive in high nutrient concentrations triggered by water column mixing. It also appears to prefer higher salinity waters and coastal embayments. Genetic analysis of *S. grevillei* demonstrated a peculiar dissimilarity with isolates from coastal waters off Yemen, India, Oman and China. However, a closely related sequence was isolated from coastal waters off Japan. These results indicate that *S. grevillei* is an introduced species, possibly transported by ballast waters. *S. menzelii* is a sporadic visitor in the northern Adriatic, advected from rather oligotrophic middle Adriatic waters and never dominates the phytoplankton community in the northern Adriatic.

AUTORI

Mirta Smodlaka Tanković (mirta@cim.irh.hr), Daniela Marić Pfannkuchen, Ana Baričević, Nataša Kužat, Tamara Djakovac, Martin Pfannkuchen, Center for Marine Research, Ruđer Bošković Institute, G. Paliaga 5, Rovinj, Croatia
Jelena Godrijan, Division for Marine and Environmental Research, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia
Emina Pustijanac, University Jurja Dobrile, G. Paliaga 5, Pula, Croatia
Regina Jahn, Botanischer Garden und Botanisches Museum Berlin-Dahlem, Freie Universitat Berlin, Konigin-Luise-Str.6-8, Berlin, Germany
Autore di riferimento: Mirta Smodlaka Tanković

Preliminary study of primary production and nutrient uptake by two seagrasses (*Zostera marina* and *Zostera noltei*) in the Venice Lagoon

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

Seagrass meadows are among the most productive ecosystems on Earth, that provide high-value ecosystem service and contribute to carbon sequestration. Nutrient and light availability are the primary physical factors controlling seagrass growth. The median carbon, nitrogen and phosphorus contents in seagrass leaves translate into a median atomic C:N:P ratio of 432:20:1. If we compare these values with the Redfield ratio for the production of organic matter by phytoplankton (C:N:P 106:16:1), we can deduce that seagrasses need about a quartet of the nitrogen and the phosphorus that are necessary for phytoplankton.
This is the first study *in situ* of seagrass primary production and nutrient uptake from the water column in the Venice Lagoon. We tested the Net Community Production (NCP) and the nutrient uptake (nitrogen and phosphorus) of *Zostera marina* and *Z. noltei* through benthic incubation chambers. This work was carried out in summer (July and August 2017). The result showed that the NCP of *Z. marina* decreased from July (mean 0.108±0.02 mmoli m^2 h^{-1} g(ftw)$^{-1}$) to August (mean 0.053±0.08 mmoli m^{-2} h^{-1} g(ftw)$^{-1}$), according to the growth rates of this specie. Moreover, the comparison of *Z. marina* and *Z. noltei* NCP, during the August sampling, highlighted that the species with the highest NCP was *Z. noltei*, with a mean of 0.709±0.35 mmoli m^{-2} h^{-1} g(ftw)$^{-1}$ against a mean of 0.108±0.02 mmoli m^{-2} h^{-1} g(ftw)$^{-1}$ of *Z. marina*. Indeed, in this month, the growth rates of *Z. noltei* increased while that of *Z. marina* decreased.
Finally, this study displayed that the nutrient uptake for both the species was higher for ammonium, with a mean of 17.1 nM h^{-1} g(ftw)$^{-1}$ for *Z. marina* and a mean of 23.5 nM h^{-1} g(ftw)$^{-1}$ for *Z. noltei*, while the phosphorus and nitrate uptake were lower. In particular, the mean for both species of phosphorus was 5.2 nM h^{-1} g(ftw)$^{-1}$ and of nitrate was 1.1 nM h^{-1} g(ftw)$^{-1}$.

AUTORI

Yari Tomio (yari.tomio@unive.it), Alessandro Buosi, Marion Adelheid Wolf, Adriano Sfriso, Department of Environmental Sciences, Informatics & Statistics (DAIS), University Ca' Foscari Venice, Via Torino 155, 30127 Mestre-Venezia, Italy
Autore di riferimento: Yari Tomio

Application of taxonomic and morpho-functional properties of phytoplankton communities to water quality assessment for artificial lakes in the Mediterranean Ecoregion

M.R. Vadrucci, E. Barbone, N. Ungaro, A. Romano, R. Bucci

Starting in September 2010, a monitoring program was developed for evaluating the water quality in six artificial lakes (reservoirs) of the Apulia Region (Southern Italy). Lake ecological status was evaluated according to the requirements of the 2000/60/EC Water Framework Directive. Phytoplankton taxonomic composition, biovolume and chlorophyll a data were used in an application of the Italian methods (Italian Phytoplankton Assessment Method or New Italian Method) for water quality assessment of artificial lakes. Even though all the reservoirs were classified as being in good ecological status, the phytoplankton communities of the six reservoirs were different and characterized by highly site-specific components and by distinctive dominant taxa. The influences of environmental variables on the phytoplankton communities were analyzed. Results of Constrained Coordinate Analysis (CCA) indicated strong influences of the trophic gradient and water depth on the phytoplankton assemblages, but significant factors are also the surrounding landscape and the origin and the history of the lakes. A functional traits-based analysis was carried out to support the water quality assessment tools. Twenty-one morpho-functional groups were identified. Most of them were common in all six lakes, so functional properties clearly recur among different phytoplankton species.

AUTORI

Maria Rosaria Vadrucci (m.vadrucci@arpa.puglia.it), Antonella Romano, Roberto Bucci, Arpa Puglia–Dipartimento Provinciale di Lecce, Via Miglietta 2, 73100 Lecce
Enrico Barbone, Nicola Ungaro, Arpa Puglia –Direzione Scientifica, Corso Trieste 27, 70126 Bari
Autore di riferimento: Maria Rosaria Vadrucci