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In copertina: *Mastogloia sergiana* (diatomee) epifita su angiosperme marine dell'isola di Siladen (Bunaken Archipelago, Indonesia), foto di Chiara Pennesi

On the cover: *Mastogloia sergiana* (diatoms) epiphytic on seagrasses from Siladen Island (Bunaken Archipelago, Indonesia), photo by Chiara Pennesi

Diversità della flora marina colombiana: conoscenze attuali e prospettive future

B. Gavio

La Colombia è considerata uno dei 12 paesi “megadiversi” del mondo, con un’altissima diversità di ecosistemi e di specie presenti. È l’unico paese sudamericano a possedere un ambiente costiero sia sull’Oceano Atlantico (il Mar dei Caraibi) che nell’Oceano Pacifico, e i suoi ecosistemi marini e costieri sono parzialmente protetti grazie all’istituzione di due Riserve della Biosfera (*Seaflower* e Cienaga Grande de Santa Marta, nei Caraibi) e 16 Parchi Nazionali (11 sulla costa caraibica e cinque sul Pacifico).

Considerando la diversità di ecosistemi marini, anche la flora marina è considerata varia. Nonostante questo, e l’importanza delle macroalghe negli ecosistemi marini, lo studio della diversità algale nel passato è stato abbastanza limitato, con zone (il Parco Tayrona, le isole del Rosario) in cui gli studi sono stati abbastanza approfonditi e altre (la costa Pacifica e varie regioni della zona caraibica) molto meno studiate.

Negli ultimi anni, varie campagne di campionamento hanno portato a uno studio particolareggiato in varie regioni del paese; i risultati hanno rivelato una flora marina molto più diversa e varia di quello che è stato riportato storicamente. In particolare, si è osservato che sono molto abbondanti le specie di piccole dimensioni che comunemente venivano trascurate negli studi di biodiversità, rivelando anche nuovi registri per i Caraibi e l’Oceano Pacifico Orientale o la descrizione di nuove specie (p.e. *Crouania pumila*). Si è osservata una particolare abbondanza e diversità di cianobatteri associati alle barriere coralline. Non siamo in grado di dire se questa flora microbica abbia subito un’esplosione recente, dovuta alla degradazione delle barriere coralline, o se è sempre stata presente, considerando l’assenza di studi precedenti sulle alghe verdi-azzurre. Nonostante la conoscenza della diversità delle macroalghe in Colombia sia migliorata negli ultimi anni, rimane ancora molto da fare. Si sa ancora poco sul ricambio stagionale e i cambiamenti annuali o interannuali della flora mentre studi a livello molecolare sono ancora all’inizio. Considerando la grande diversità del paese, in futuro si dovrebbe stimolare anche l’interesse per la bioprospezione su queste specie, visto che molte alghe producono metaboliti secondari con attività antibiotica, antivirale e antitumorale.

AUTORE

Brigitte Gavio (bgavio@unal.edu.co), Departamento de Biología, Universidad Nacional de Colombia, Sede Bogotá, Carrera 30 Calle 45, Bogotá DC Colombia

Revision and cladistic analysis of the genus *Anorthoneis* (Bacillariophyceae), including the description of a new species

C. Pennesi, R. Majewska, F.A.S. Sterrenburg, C. Totti, T. Romagnoli, M. de Stefano

The genus *Anorthoneis* Grunow is a small benthic marine diatom genus belonging to the family Cocconeidaceae Kützinger, order Achnanthes Silva together with other monoraphid diatoms (*Cocconeis* Ehrenberg, *Amphicocconeis* De Stefano & Marino, *Psammococconeis* Garcia). It lives with the raphe valve attached to the substratum (e.g., rocks, sand grains, plants or animals) and the rapheless valve exposed to the surrounding environment. Generally, araphid valves are more silicified and carry more complex ornamentations than the corresponding raphid valves. Rapheless valves show a convex profile and a monolayered or bilayered ultrastructure, whereas the raphe valves are mainly concave and always monolayered. Finally, on both valves there are functionally elaborated girdle bands, which can be particularly complex on the raphe valve.

Here we present an ultrastructure-based complete revision of the diatom genus *Anorthoneis* using both light (LM) and electron microscopes (SEM). The original slides and type materials of all species belonging to the genus *Anorthoneis* have been analysed. New morphological features for the genus *Anorthoneis* on diatom frustules were revealed both in new and already described taxa providing detailed observations and SEM images (i.e., rota-like vela and ligulate structures). Moreover, the phylogenetic relationships based on morphological characters among all taxa were performed via parsimony method. The character states that describe variations of the internal and external central area tend to be homologous and to support an important first big clade which includes *A. dulcis* Hein, *A. tenuis* Hustedt, *A. vortex* Sterrenburg, *A. excentrica* (Donkin) Grunow, *A. hummii* Hustedt, *A. hyalina* Hustedt, *A. arthus-bertrandii* De Stefano & Pennesi sp. nov., *A. eurystoma* Cleve and *A. minima* Foged. In addition, a new species, *Anorthoneis arthus-bertrandii* De Stefano & Pennesi sp. nov., from Siladen Island, was discovered and described.

AUTORI

Chiara Pennesi (c.pennesi@univpm.it), Cecilia Totti, Tiziana Romagnoli, Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy

Roksana Majewska, School of Biological Sciences, Faculty of Natural Sciences North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

Frithjof A.S. Sterrenburg, National Natural History Museum "Naturalis", Leiden, Stationsweg 1581852 LN Heiloo, The Netherlands

Mario de Stefano, Dipartimento di Scienze e Tecnologie Ambientali Biologiche e Farmaceutiche, Seconda Università di Napoli, 81100 Caserta, Italy

Molecular underpinning of diatom response to sexual cues enabled by the genome sequence of *Pseudo-nitzschia multistriata*

S. Basu, S. Patil, D. Mapleson, M.T. Russo, L. Vitale, C. Fevola, F. Maumus, R. Casotti, T. Mock, M. Caccamo, M. Montresor, R. Sanges, M.I. Ferrante

Microalgae play a major role as primary producers in aquatic ecosystems, shaping diversity and functioning. Cell signalling regulates their interactions with the environment and other organisms, yet the molecular mechanisms underlying these processes are poorly defined. We investigated the cell response to cues released during sexual reproduction, a key phase of their life cycle, which impacts on population dynamics. We selected *Pseudo-nitzschia multistriata*, a marine planktonic diatom with a controllable life cycle, for genome sequencing. We define gene gains and losses, events of retrotransposition, and gene acquisitions via horizontal gene transfer, and identify a small number of conserved non-coding elements, likely involved in gene expression regulation. Through a transcriptomic study we show that sexual reproduction impacted the cell cycle progression, involved expression changes in 9% of genes and induced an asymmetric response of the opposite mating types. G protein-coupled receptors and cGMP appear to be implicated in the response. Overall, the response entails a modulation of cell cycle, meiosis-related and nutrient transporter genes, suggesting a fine control of nutrient uptake during sexual reproduction even under nutrient replete conditions. Finally, the genome sequence was exploited to characterize the gene set employed in the response to sexual cues, defining conservation and evolutionary rate of sex-related genes. Sexual stages are rare in natural phytoplankton samples and the identification of genes regulated during sexual reproduction provides markers for data-mining of metatranscriptomic datasets. Data on the genetic control of sexual reproduction in *P. multistriata* will improve our ability to understand the dynamics of toxic blooms.

AUTORI

Swaraj Basu, Shrikant Patil, Monia Teresa Russo, Laura Vitale, Cristina Fevola, Raffaella Casotti, Marina Montresor, Maria Immacolata Ferrante (mariella.ferrante@szn.it), Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale 1, 80121 Napoli, Italy

Daniel Mapleson, Mario Caccamo, 2The Genome Analysis Centre (TGAC), Norwich Research Park, Norwich, NR4 7UH, UK
Florian Maumus, URGI, INRA, Université Paris-Saclay, 78026 Versailles, France

Thomas Mock, School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK
Remo Sanges, Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Villa Comunale 1, 80121 Napoli, Italy

Biofilm diversity and structure in cooling towers industrial systems

L. Di Gregorio, V. Tandoi, S. Rossetti, R. Congestri, F. Di Pippo

Highly structured micro-consortia composed of microorganisms adhering to surfaces and immersed in a self-produced exopolymeric matrix, known as biofilms, profusely colonize cooling systems. These communities develop and adhere inside the heat exchangers and on the inner surfaces of cooling towers, promoting biofouling and causing serious equipment damages. While diversity, structure and function of aquatic and sub-aerial biofilms have been studied and the importance of bacterial biofilms in medical microbiology has been well established, little attention has been given to biofilm colonization in industrial settings. This study aims to assess biodiversity and structure of biofilms from different full scale cooling towers by applying CARD-FISH (Catalyzed Reporter Deposition Fluorescence *In Situ* Hybridization) combined with CLSM (Confocal Laser Scanning Microscopy) and NGS (Next Generation Sequencing). Biofilms, make up and cooling waters were seasonally sampled from the different sites with the scope to understand the possible effect of source communities and abiotic parameters on biofilm diversity and structure. In this on-going study, a complex architecture and high biodiversity were found in biofilm communities, where phototrophic (mainly Cyanobacteria and Diatoms) and heterotrophic (mainly Alpha- and Betaproteobacteria) microorganisms formed multilayered communities maintained by an exopolymeric matrix. The filamentous genera *Leptolyngbya*, *Microcoleus*, *Oscillatoria* (Oscillatoriales), *Nodularia*, *Calothrix* (Nostocales) along with a variety of coccal forms were the main Cyanobacteria found in the studied biofilm communities.

AUTORI

Luciana Di Gregorio, National Research Council, Water Research Institute, Area della Ricerca di Roma 1, Monterotondo stazione, Roma, Italy; University of Rome Tor Vergata, Department of Biology, via della Ricerca Scientifica 1, 00133 Roma, Italy

Valter Tandoi, Simona Rossetti, CNR-IRSA, National Research Council, Water Research Institute, Area della Ricerca di Roma 1, Monterotondo stazione, Roma, Italy

Roberta Congestri, University of Rome Tor Vergata, Department of Biology, via della Ricerca Scientifica 1, 00133 Roma, Italy

Francesca Di Pippo, CNR-IRSA, National Research Council, Water Research Institute, Area della Ricerca di Roma 1, Monterotondo stazione, Roma, Italy; CNR-IAMC, National Research Council, Institute for Coastal Marine Environment, Località Sa Mardini, Torregrande, 09170 Oristano, Italy

Characterization of a *Coccomyxa* (Chlorophyta, Trebouxiophyceae) strain associated to carnivorous plants of the genus *Drosera* L.

K. Sciuto, B. Baldan, I. Moro

Coccomyxa Schmidle is a genus of green microalgae that belongs to the class Trebouxiophyceae and, more precisely, to the *Elliptochloris*-clade (Darienko et al. 2010, Pröschold et al. 2011, Leliaert et al. 2012). Members of this taxon show a very simple morphology, with from irregular elliptical to globular cells (6-14 µm long, 3-6 µm wide), a parietal chloroplast without pyrenoid inside cells, and the absence of flagellated stages (Jaag 1933, Darienko et al. 2015). Moreover, a huge and often layered mucilaginous envelop distinguishes this genus by the related taxa *Choricystis* (Skuja) Fott and *Pseudococcomyxa* Korshikov (Fott 1976).

Coccomyxa has a worldwide distribution with representatives found in a broad range of terrestrial and aquatic ecosystems. Moreover, members of this taxon can be free-living and/or associated with other organisms, establishing symbiotic or parasitic relationships. The most frequent organisms forming associations with *Coccomyxa* are fungi and marine mussels (e.g., Lohtander et al. 2003, Zoller et al. 2003, Rodriguez et al. 2008, Syasina et al. 2012). Up to now the only documented association with higher plants is that reported between a *Coccomyxa* strain and *Ginkgo biloba* (Trémouillaux-Guiller et al. 2002, Trémouillaux-Guiller, Huss 2007).

Here we describe a green algal strain found in association with carnivorous plants of the genus *Drosera* L. The microorganism, isolated and maintained in culture, was subjected to a polyphasic characterization. A first molecular survey, based on the 18S rRNA and *rbcL* genes, led us to attribute the green alga to the genus *Coccomyxa*, as also supported by the subsequent light and electron (scanning and transmission) microscopy observations. In order to identify this algal strain at the species level, we carried out deeper molecular and phylogenetic analyses using the above reported molecular markers, as well as the ITS region. The analysis of the ITS2 secondary structure proved to be particularly useful and led us to hypothesize that the isolated microorganism represents a new species inside the genus *Coccomyxa*.

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AUTORI

Katia Sciuto (katia.sciuto@unipd.it), Barbara Baldan, Isabella Moro, Department of Biology, University of Padova, via U. Bassi 58/B, 35131 Padova, Italy

Marine canopies in a changing environment: projecting the distribution of *Cystoseira* brown algae under alternative climatic scenarios

R. Buonomo, R. Chefaoui, A.H. Engelen, L. Airoidi, E.A. Serrão

Canopy forming algae are key ecosystem engineer helping to sustain ecosystem services and function in rocky shore habitats. However, algal forests are globally threatened by cumulative anthropogenic impacts. Climate change is considered an important driver of future loss of these species. Evidence of accelerating climate change enhance the need of predicting the consequences for species distribution and range shifts in order to plan effective mitigation strategies. Species distribution modeling (SDM) approach allows describing current and future distribution and to predict possible effects of genetic erosion. We selected three representative intertidal brown algae species belonging to the genus *Cystoseira* and modelled the present and future climatic niche according to divergent emission scenarios. Additionally, we analyzed the genetic populations' structure as well as the microbial communities associated to the algae to examine the species diversity and the possible effects of distributional changes on genetic pools. The model predicted a northward range shift for the Atlantic species *C. tamariscifolia* and moderate to severe suitable habitat lost for *C. compressa* and *C. amentacea*, depending on the scenarios. The most susceptible species to projected climate change was predicted to be *C. amentacea*, with up to 94% loss of originally suitable area. High genetic structuring and diversity across areas was found for *C. tamariscifolia* and *C. compressa*, while *C. compressa* presented more admixed populations. Results also showed species-specific associated bacterial communities which vary according to the geographic distance among populations. According to our model, major consequences for the distribution range of the species were predicted already in the best-case future scenario. The area of predicted suitability loss corresponded to high genetic richness and distinct groups with specific associated microbial communities that could be lost in the near future.

AUTORI

Roberto Buonomo (roberto.buonomo2@unibo.it), CCMAR-CIMAR Centre of Marine Sciences Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal; Dipartimento di Biologia Evoluzionistica Sperimentale and Centro Interdipartimentale di Ricerca per le Scienze Ambientali, Università di Bologna, Via S. Alberto 163,1 48100 Ravenna, Italy

Rosa Chefaoui, Aschwin H. Engelen, Ester A. Serrão, CCMAR-CIMAR Centre of Marine Sciences Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

Laura Airoidi, Dipartimento di Biologia Evoluzionistica Sperimentale and Centro Interdipartimentale di Ricerca per le Scienze Ambientali, Università di Bologna, Via S. Alberto 163,1 48100 Ravenna, Italy

Prime osservazioni sullo status dei popolamenti a *Cystoseira* e della fauna associata nell'AMP "Isole Ciclopi"

G. Alongi, M. Catra, D. Serio, R. Leonardi, R. Sanfilippo, F. Sciuto, A. Viola, A. Rosso

Le comunità a *Cystoseira*, per l'alta persistenza e la buona resilienza, sono in grado di fornire la registrazione delle condizioni ambientali predominanti su un lungo periodo di tempo mentre sono relativamente poco sensibili alle fluttuazioni temporanee dell'ambiente come pure all'immissione sporadica e non massiva di inquinanti. Per tale motivo, lo studio di questi habitat costituisce un efficace strumento per la conoscenza dello stato di salute degli ambienti costieri. Negli ultimi decenni è stata evidenziata una regressione dei popolamenti a *Cystoseira* in ambienti ad elevata naturalità della fascia costiera siciliana in linea con quanto registrato in altre aree mediterranee. Risulta quindi indispensabile avere un quadro quanto più completo dell'attuale situazione ambientale anche per potere comprendere le cause che determinano un tale fenomeno, attraverso uno studio integrato di queste comunità.

In quest'ottica si inquadra il progetto FIR "Biodiversità e variazioni spazio-temporali di comunità a *Cystoseira* della Biocenosi delle Alghe Infralitorali nell'AMP Isole Ciclopi (Mar Ionio)", che si propone di monitorare alcune comunità a *Cystoseira* presenti nell'AMP "Isole Ciclopi" compresi gli epibionti animali a scheletro mineralizzato (e in particolare le componenti a briozoi, serpulidi, foraminiferi e ostracodi) per definirne composizione, struttura e distribuzione nelle diverse comunità anche in relazione all'alga substrato.

Di tale progetto vengono riportate alcune osservazioni relative ai primi campionamenti. Le comunità monitorate sono quelle presenti a -5m, -10m e -25m, profondità alle quali alla fine degli anni '90 erano stati segnalati popolamenti fitobentonici ben strutturati caratterizzati rispettivamente da *C. brachycarpa* J. Agardh *emend.* Giaccone, *C. sauvageana* Hamel e *C. spinosa* Sauvageau. Per ciascuna profondità e in tre diverse stazioni per un totale di 6 siti (2 per ciascuna comunità) sono stati condotti campionamenti in immersione con ARA, in aree fitosomologicamente omogenee; i prelievi sono stati effettuati con metodo "sorbona-grattaggio-sorbona" su superfici standard di 1600 cm².

Dalle prime osservazioni sembra evidente che nelle stazioni indagate non si riscontrano popolamenti strutturati ma piuttosto si registra un declino delle comunità a *Cystoseira*. La specie guida non presenta valori di ricoprimento specifico superiori al 50% e spesso viene del tutto sostituita da Sphacelariales, Dictyotaceae, Corallinaceae incrostanti e da un fitto feltro di specie filamentose. La componente animale è scarsa e include poche specie. I serpulidi comprendono in totale 20 specie: 16 serpulinae prevalentemente di piccola taglia, e 6 spirorbinae con popolazioni date essenzialmente da giovanili. I foraminiferi sono dati prevalentemente da specie delle famiglie Elphidiidae, Miliolidae e Cibicididae mentre gli ostracodi sono rappresentati fondamentalmente dal genere *Xestoleberis*. I briozoi sono presenti con 59 specie (14 ciclostomi, 3 ctenostomi e 42 cheilostomi) che formano essenzialmente colonie erette flessibili e incrostanti, tutte di piccola taglia ma comunemente fertili. Per quest'ultimo gruppo un confronto con dati dei primi anni '70 indicherebbe una riduzione della ricchezza specifica, presumibilmente correlabile alla mancanza delle alghe strutturanti.

AUTORI

Giuseppina Alongi (g.alongi@unict.it), Marcello Catra, Donatella Serio, Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Catania, Sezione di Biologia Vegetale, Via A. Longo 19, 95130 Catania, Italy

Riccardo Leonardi, Rossana Sanfilippo, Francesco Sciuto, Alfio Viola, Antonietta Rosso, Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Catania, Sezione di Scienze della Terra, Corso Italia 57, 95129 Catania, Italy

Declines of Mediterranean canopy-forming seaweeds: multiple drivers and potential management options

L. Airoidi, P. Mancuso , O. de Clerck

Along Mediterranean coasts, canopy-forming seaweeds are retracting particularly close to urban areas, and are replaced by turf-forming and ephemeral algae or barrens. We summarise results of project TETRIS (PRIN 2010-2011) aiming at developing adaptive management of multiple impacts in coastal systems, including canopy forests. We provide evidence that declines of canopy algae are dramatically extensive, largely underestimated and are driven by multiple local and global stressors. We suggest a potential influence of the microbial communities in modulating the responses to some of these stressors, and explore the future additional threats from climatic stressors. We finally demonstrate that the control of local stressors could work as a rapid and cost-effective strategy to limit at least some of the adverse effects, allowing more time to these ecosystem to adjust to climate change. We conclude discussing management actions to limit further losses of these valuable ecosystems.

AUTORI

Laura Airoidi (laura.airoidi@unibo.it), Dipartimento di Scienze Biologiche, Geologiche ed Ambientali, Università di Bologna, Italy

Paolo Mancuso, Dipartimento di Scienze Biologiche, Geologiche ed Ambientali, Università di Bologna, Italy; Department of Biology, Ghent University, Belgium

Olivier de Clerck, Department of Biology, Ghent University, Belgium

Si può prevedere il destino delle specie aliene introdotte? Il caso dell'ambiente di transizione Mar Piccolo di Taranto*

A. Petrocelli, G. Portacci, E. Cecere

Con circa 1000 specie rinvenute, appartenenti a tutti i taxa marini escluse le microalghe, il Mediterraneo è una delle zone geografiche più gravemente colpite dall'introduzione di specie aliene. E presto la situazione potrebbe diventare significativamente peggiore, a causa del recente allargamento del canale di Suez, che è considerato uno dei principali corridoi per il loro ingresso in questo bacino. Tuttavia, anche l'acquacoltura ha una responsabilità considerevole come vettore, soprattutto per quanto riguarda le macroalghe, introdotte principalmente nei sistemi di transizione. Il Mar Piccolo di Taranto, ad esempio, sede di una millenaria attività di molluschicoltura, rappresenta il terzo "hotspot" per il numero di specie di macroalghe aliene nel Mediterraneo. Pertanto, per poterne individuare tempestivamente di nuove, il bacino viene continuamente monitorato.

Una buona conoscenza delle caratteristiche ecologiche e del ciclo vitale delle specie introdotte (eco-fisiologia, modello riproduttivo, tasso di crescita, vulnerabilità alle malattie, alla predazione e alla competizione) permette di identificare i valori soglia delle variabili ambientali che possano favorirne la trasformazione da "specie aliene" in "specie aliene invasive". Pertanto, le suddette informazioni si possono rivelare utili per prevedere il destino di una specie potenzialmente invasiva a breve, medio o lungo termine, fornendo altresì le indicazioni necessarie per la scelta delle procedure di gestione adatte a minimizzarne l'impatto sugli ecosistemi e sulle attività umane. Negli ultimi 15 anni, nel Mar Piccolo di Taranto, sono state studiate le popolazioni di due alghe aliene potenzialmente invasive: l'alga bruna *Undaria pinnatifida* (Alariaceae, Laminariales) e l'alga rossa *Hypnea cornuta* (Cystocloniaceae, Gigartinales). Sono stati contemporaneamente considerati i valori estremi della temperatura dell'acqua di mare (settimana più fredda e settimana più calda), nonché la sua variazione annuale. Questi valori, in estate, sono risultati frequentemente superiori a quelli limite per lo sviluppo dei gametofiti di *U. pinnatifida*, specie temperato-fredda, inducendone probabilmente un'alta mortalità e il fallimento della riproduzione, che ha portato al conseguente crollo del popolamento. Tant'è che lo studio della dinamica della popolazione di questa specie ha mostrato un andamento di tipo "boom and bust", che è risultato in un'apparente estinzione. Viceversa, proprio i valori estivi di temperatura, frequentemente superiori ai 30° C, in sinergia con le strategie di riproduzione vegetativa (propaguli, frammenti di tallo, tetraspore) messe in atto da *H. cornuta*, specie tropicale, potrebbero averne favorito l'aumento considerevole e costante di biomassa sin dalla sua introduzione, tanto che essa è diventata la specie dominante in estate nel Secondo Seno.

Pertanto, noto l'andamento delle variabili ambientali in una determinata zona, in questo caso della temperatura, e la regione fitogeografica di appartenenza della specie introdotta, è possibile formulare una prima ipotesi circa il suo potenziale invasivo in quella zona. Ciò conferma, quindi, che il concetto di invasività di una specie aliena non è assoluto ma relativo all'area in cui viene introdotta.

AUTORI

Antonella Petrocelli (antonella.petrocelli@iamc.cnr.it), Giuseppe Portacci, Ester Cecere, Istituto per l'Ambiente Marino Costiero (IAMC)-CNR, UOS Taranto, via Roma 3, 74123 Taranto, Italy

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Ecological structure and mapping of *Posidonia oceanica* meadows in the island of Pantelleria (South Tyrrhenian): a selected site to detect sea wave energy

C. Micheli, F. Borfecchia, L. De Cecco, A. Belmonte, G. Bracco, G. Mattiazzo, M.V. Struglia, G. Sannino

During the summer 2015, we have investigated the ecological structure of *Posidonia oceanica* (L) Delile meadows located along the northern coast of Pantelleria Island, a zone interested to a possible impacts of sea wave renewable energy production. This small Island is situated at 110 km southwest of Sicilia island, Italy (36°47'27"N 11°59'38"E).

In this work we describe, for the first time, the ecological scenario of the *P. oceanica* ecosystem and its change after the impact arising from the installation of offshore devices for exploitation of the Inertial Sea Wave Energy Converter (ISWEC). This innovative prototype has been developed by the Polytechnic of Turin: it is characterized by high conversion efficiency, resulting from its adaptability to different wave conditions, and by a limited environmental impact due to its mooring innovative method which does not make use of fixed anchors to the seabed. In order to allow a suitable sea energy harvesting, ISWEC has been installed at 30 meters bathymetry depth, 800 m offshore of coastal zone: a strategic site near the important *P. oceanica* meadows. Genetic, morphological and physiological data of the collected *P. oceanica* plants were analyzed. Then, data were processed and integrated for providing a suitable point calibration to the subsequent spectral clustering methods on purpose implemented for monitoring and mapping the meadows in the coastal zone of interest using satellite remote sensing techniques.

The preliminary results showed that the environmental parameters are linked to energy production (such as the sea currents) and the distribution patterns of these different sea energy contributions can be considered useful indicators for the identification of possible natural and anthropogenic impacts on the ecosystem.

The exploitation of marine renewable energy can play a key role in providing electrical power to Italian small islands contributing to climate change mitigation. Such research represents an innovative solution for supporting alternative green energy production and, at the same time, for monitoring activities for coastal environments, including water quality and vulnerable marine ecosystems.

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AUTORI

Carla Micheli (carla.micheli@enea.it), Alessandro Belmonte, ENEA, Agenzia nazionale per le nuove tecnologie, l'energia e lo sviluppo economico sostenibile. Dipartimento di Tecnologie Energetiche, DTE-BBC-BBE. Lab. Biomasse e Biotecnologie per l'Energia. Centro Ricerche Casaccia, Via Anguillarese 301, 00123 Roma, Italy

Flavio Borfecchia, Luigi De Cecco, ENEA, Dipartimento di Sostenibilità Dei Sistemi Produttivi e Territoriali SSPT-PROTER-OAC. Centro Ricerche Casaccia, Via Anguillarese 301, 00123 Roma, Italy

Giovanni Bracco, Giuliana Mattiazzo, Politecnico di Torino, Dipartimento di Ingegneria meccanica, Torino, Italy

M. Vittoria Struglia, Gianmaria Sannino, ENEA, Dipartimento di Sostenibilità Dei Sistemi Produttivi e Territoriali, SSPT-MET-CLIM. Centro Ricerche Casaccia, Via Anguillarese 301, 00123 Roma, Italy

High resolution melting: an innovative approach for molecular characterization of *Pseudo-nitzschia* spp. (Bacillariophyceae)

S. Casabianca, L. Pugliese, F. Perini, F. Andreoni, A. Penna

The planktonic pennate diatom *Pseudo-nitzschia* (Bacillariophyceae) is a cosmopolitan genus commonly found in the polar, temperate, subtropical and tropical regions. Since the genus *Pseudo-nitzschia* includes a large number of species, their accurate taxonomical identification is needed, as they can be associated to DA production. By now, species identification or description is often performed by integrating different methodological approaches based on scanning and transmission electron microscopy, and molecular analyses. As microscopy based methods don't always provide the necessary resolution required for the identification of various *Pseudo-nitzschia* species, different molecular methods, such as qPCR, microarray and dot blot hybridization systems have been applied for phytoplankton diagnostic species identification. Moreover, among the molecular techniques used to analyse small genetic mutations, as single nucleotide polymorphisms (SNPs), we have the post PCR high resolution melting (HRM) curve analysis. This analysis can be an alternative technique to rapidly and accurately identify microalgal species and/or genotype cultured strains. Different melting profiles are obtained from the transition of double-strand (dsDNA) to single-strand DNA (ssDNA) as a result of a gradual temperature increase after PCR amplification. To date, only one study applied HRM to rapidly discriminate *Karenia mikimotoi* strains from distinct geographic localities. The HRM analysis is faster, and less expensive than alternative approaches, such as microarray or microsatellites. In the present study, we investigated the genetic diversity of several *Pseudo-nitzschia* monoclonal cultures obtained from environmental samples collected over one year in the NW Adriatic Sea using HRM based assay. The developed and optimized method was able to distinguish three different *Pseudo-nitzschia* variants corresponding to three different species or complex, namely *P. pungens*, *P. calliantha* and *P. delicatissima*/*P. cf. arenysensis* complex. The method was subsequently applied for the analysis of unknown strains of *Pseudo-nitzschia* spp. collected in the NW Adriatic Sea during the period of survey. Among the 29 *Pseudo-nitzschia* spp., 7 strains were identified as *P. pungens*, 12 strains were identified as *P. calliantha* and 10 strains were identified as *P. delicatissima*/*P. cf. arenysensis*. Finally, representative strains of *Pseudo-nitzschia* spp. identified by HRM assay were analysed by LSU and ITS rDNA sequence alignment and phylogenetic analyses. The species-specific taxonomical assignment was confirmed. In conclusion, the post PCR HRM assay developed in this study appears to be a promising tool for simultaneous detection and discrimination of the *Pseudo-nitzschia* spp. The assay offers several advantages: it is specific, reproducible and rapid when applied to several simultaneously processed cultured samples. The HRM assay depends on available reference genotypes, and it analyses monoclonal cultures of the species under investigation. In the future, the sampling of potentially numerous harmful species in the Mediterranean Sea could make use of the application range of the HRM assay.

AUTORI

Silvia Casabianca (silvia.casabianca@uniurb.it), Laura Pugliese, Federico Perini, Francesca Andreoni, Antonella Penna, Dipartimento di Scienze Biomolecolari, Università di Urbino, Viale Trieste 296, 61121 Pesaro, Italy

The SXT gene and paralytic shellfish poisoning toxins as markers for the monitoring of HAB dinoflagellate blooms

F. Perini, C. Dell'Aversano, S. Capellacci, L. Tartaglione, M.G. Giacobbe, S. Casabianca, S. Fraga, P. Ciminiello, M. Scardi, A. Penna

Paralytic shellfish poisoning (PSP) is a serious human illness caused by the ingestion of seafood contaminated with paralytic shellfish toxins, composed of saxitoxin and its derivatives (STXs). These toxins are also produced by marine dinoflagellate *Alexandrium* spp. STXs can affect the human neuro-vegetative system leading to muscular paralysis and death. These toxins are a family of neurotoxins produced by some freshwater prokaryotic cyanobacteria and marine eukaryotic dinoflagellates. The first two genes that start the biochemical synthesis of STX, also those best characterized, are the *sxtA* (coding for a polyketide synthase) and *sxtG* (coding for an amidinotransferase). STXs mainly affect the marine trophic web accumulating in many organisms. The accumulation of these compounds in seafood has a major economic impact on aquaculture industries. Due to the increased risks posed to human health by PSP toxins worldwide, including also the Mediterranean Sea, it is crucial to investigate the potential correlation between STX-production and *sxt* gene content in environmental samples during toxic blooms. This study aimed to illustrate a potential scenario of STX producing harmful *Alexandrium minutum* and to investigate STX-related risk in the Mediterranean Sea. Field samples were collected in Syracuse Bay (Ionian Sea) on May 2014 during a bloom event. Field samples were processed for quantitative molecular qPCR and LC-HRMS analyses. Firstly, the *sxtA* gene content, as well as toxin profile and content, were investigated in the Mediterranean *A. minutum* populations. The median *sxtA1* gene copy number/cell was 2.2 and no difference in the *sxtA1* gene copy number was found among *A. minutum* populations. A qPCR assay was applied to quantify the *sxtA1* gene copy number in *A. minutum* strains in relation to STX production and rapidly quantify the abundance of *sxtA1* gene in field samples. In field samples, a positive correlation was found between cell densities determined by microscopy and *sxtA1* gene copies by qPCR in surface water ($n=29$, Pearson's $r=0.978$ $p<<0.0001$). The range of *sxtA1* gene content was $2.55 \times 10^8 \pm 3.08 \times 10^7$ - $1.38 \times 10^5 \pm 1.80 \times 10^3$ copies/L. Further, the *sxtA1* gene content was correlated with toxin presence in environmental samples to provide an indication of PSP risk during a bloom. The amount of *sxtA1* gene was in the range of 1.38×10^5 - 2.55×10^8 copies/L and the STX concentrations ranged from 41-201 nmol/L. This scenario illustrates the potential risk of real PSP occurrences, even though the toxin amount on a per cell basis was lower than in other areas worldwide, but in high biomass blooming events, the potential risk of toxin accumulation in shellfish is likely. Efficient monitoring strategies by combined molecular and chemical methodologies could play a fundamental role in preventing and managing health and economic risks related to STX-producing *Alexandrium* spp. blooms in coastal and marine farm areas.

AUTORI

Federico Perini (federico.perini@uniurb.it), Samuela Capellacci, Silvia Casabianca, Antonella Penna, Dipartimento di Scienze Biomolecolari, Università di Urbino, viale Trieste 296, 61121 Pesaro, Italy

Carmela Dell'Aversano, Luciana Tartaglione, Patrizia Ciminiello, Dipartimento di Sostanze Naturali, Università Federico II, via D. Montesano 49, 80131 Napoli, Italy

Maria Grazia Giacobbe, CNR IAMC, spianata S. Raineri 86, 98122 Messina, Italy

Santiago Fraga, Instituto Espanol de Oceanografia (IEO), Subida Radio Faro 50, Vigo, Spain

Michele Scardi, Dipartimento di Biologia, Università Tor Vergata, via della Ricerca Scientifica 1, 00133 Roma, Italy

Il DNA metabarcoding nello studio della distribuzione spaziale e temporale delle microalghe marine tossiche

M.P. Tomasino, R. Piredda, P. Sánchez, B. Duarte, B. Fosso, I. Caçador, L. Amaral-Zettler, G. Pesole, D. Sarno, A. Zingone

Il DNA metabarcoding, cioè l'identificazione contemporanea di specie attraverso marcatori molecolari ottenuti da DNA ambientale, è uno strumento efficace per valutare la biodiversità dei microrganismi in campioni naturali, permettendo di rilevare la presenza di molte specie in modo relativamente rapido e preciso. In particolar modo nelle microalghe, in molti casi difficili da fissare e spesso impossibili da identificare, questo è l'unico approccio che permette di coprire tutto lo spettro della diversità. Inoltre, in virtù dello sviluppo di tecnologie di sequenziamento 'High throughput' (HTS), è oggi possibile ottenere da un singolo campione un numero elevatissimo di sequenze, incluse quelle di specie molto rare.

In questo studio abbiamo testato la fattibilità e l'efficacia di questo approccio nello studio della distribuzione di microalghe tossiche (Harmful microalgae, HMA). Il primo passaggio è stato la costruzione di un database di riferimento (18S-HMA db), utilizzando il marcatore ribosomiale 18S rDNA e verificando le sequenze disponibili per 95 delle 113 specie di HMA note, nonché per le loro specie congeneri non tossiche. Da questo è stato estratto il solo frammento V4, che è quello più utilizzato negli studi di metabarcoding, e ne è stato verificato il potere discriminante, cioè la capacità di distinguere in maniera non ambigua le specie tossiche.

Il database finale, costituito da 642 sequenze di riferimento (V4-18S HMA), è stato quindi utilizzato per analizzare: i) il dataset dell'Ocean Sampling Day (OSD), costituito da un data set di sequenze V4 ottenute attraverso un campionamento sincrono su scala globale (154 siti) condotto il 21 giugno 2014. In questo dataset abbiamo rilevato la presenza di 49 diverse specie di HMA in 147 siti OSD, e abbiamo identificato le specie più frequenti e i siti hot-spot per la diversità delle HMA. ii) Il data set raccolto alla stazione a lungo termine MareChiara (LTER-MC, Golfo di Napoli, Mar Mediterraneo), ottenuto da 48 campioni raccolti in tre anni (2011-2013). In questo set di dati sono state rilevate 26 HMA, tre delle quali mai registrate prima nella zona. Per le specie più abbondanti, la distribuzione stagionale rifletteva quella rilevata utilizzando la microscopia ottica, ma ne approfondiva l'identificazione allargandola, ad esempio, alle specie criptiche del genere *Pseudo-nitzschia*. Quindi, nonostante alcune limitazioni, l'HTS-DNA metabarcoding risulta essere un approccio adeguato per la individuazione di microalghe dannose anche rare in programmi di monitoraggio, nonché per la valutazione della loro biogeografia.

AUTORI

Maria Paola Tomasino, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italia; CNR, via Amendola 165/A, 70126 Bari, Italia

Roberta Piredda, Diana Sarno, Adriana Zingone (zingone@szn.it), Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italia

Bruno Fosso, Graziano Pesole, Istituto di Biomembrane e Bioenergetica, CNR, via Amendola 165/A, 70126 Bari, Italia

P. Sánchez, Institut de Ciències del Mar, CSIC-Barcellona, Spagna

Bernardo Duarte, Isabel Caçador, MARE - Centre of Marine and Environmental Sciences, Facoltà di Scienza dell'Università di Lisbona, Portogallo

Linda Amaral-Zettler, The Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, Woods Hole, Massachusetts 02543, USA

The “epidemic” bloom of a toxic and ecologically relevant marine diatom

M.V. Ruggiero, D. D'Alelio, G. Procaccini, M. Montresor

Epidemic population expansions were postulated by John Maynard Smith in his influential paper entitled “How clonal are bacteria?” (*PNAS*, 1993). According to that definition, bacteria evolve following three main and non-mutually exclusive models, depending on the reciprocal frequency of genetic divergence (*via* mutation) and recombination (*via* horizontal gene transfer or *via* bacterial sex). At low recombination rate (due to geographical, ecological and/or genomic barriers), clonal divergence drives diversification processes. Conversely, when sex barriers are unrestricted, genetic recombination limits the emergence of clonal structures and the diversification processes display a tangled web of genetic relations among panmictic genetic types. A third way is given by “epidemic populations”: therein, a background level of frequent recombination is disrupted by occasional clonal expansion of a few genotypes, which dominate over the others. Epidemic boosts are well known for prokaryotes but population genetics studies revealed that parasitic protozoa can also have an epidemic genetic structure (*e.g.*, MacLeod *et al.*, *PNAS* 2000) and this suggests that Maynard Smith's model is also valid for unicellular eukaryotes.

We studied over several years the population genetics of the potentially toxic marine planktonic diatom *Pseudo-nitzschia multistriata* at the Long Term Ecological Research station MareChiara (LTER-MC) in the Gulf of Naples. The genetic fingerprint of natural populations succeeding along time revealed different populations alternating over the years. Two main blooms were recorded during summer 2013: one in August-September and a minor one in October. Genotypic diversity was high during the first bloom ($G/N = 97.95$), but considerably dropped at the beginning of the second bloom ($G/N = 24.61$), which was due to a few strains, one of them largely dominating the population in the Gulf of Naples. Comparable studies carried out on various planktonic marine and freshwater diatoms always showed populations characterized by high genotypic diversity. The present study is the first report of an epidemic dynamics (*i.e.*, a sudden collapse in genetic diversity related to an extreme clonal dominance) for a free-living protist and paves the way to further functional studies on the microevolutionary processes driving the structure and dynamics of diatom populations and their blooms.

AUTORI

Maria Valeria Ruggiero, Domenico D'Alelio (dalelio@szn.it), Gabriele Procaccini, Marina Montresor, Stazione Zoologica Anton Dohrn Napoli, Villa Comunale, 80121 Napoli, Italy

Port Baseline Surveys during the BALMAS project: indigenous, alien and harmful microalgal species

M. Bastianini, F. Bernardi Aubry, S. Finotto, A. Penna, F. Perini, M. Cabrini, D. Fornasaro, M. Cangini, S. Pigozzi

Ballast water transferred by vessels has been widely recognized as a prominent vector of harmful aquatic organisms and pathogens (HAOP) species, which are, according to the United Nations, one of the four greatest pressures on the world's oceans and seas, causing global environmental changes and also posing a threat to human health, property and resources. In the Adriatic Sea, a strategic common cross-border approach was recognised to be crucial because of the shared, specific, vulnerable, economically important, semi-enclosed environment, in which control over HAOP as well as international shipping cannot be limited by political borders.

The main BALMAS (Ballast Water Management System for Adriatic Sea Protection) objective is to establish a common cross-border system, which will link all researchers, experts and responsible national authorities from Adriatic countries in order to avoid unwanted risks to the environment from the transfer of HAOP. This will be achieved through control and management of ships' ballast waters and sediments.

This work will report about the results regarding microalgal aspects of the project.

The total list of phytoplankton taxa found during PBS in the Adriatic ports of Trieste, Venezia, Ancona and Bari comprises more than 300 entries. Some of them are reported as new records for the Adriatic Sea. *Skeletonema tropicum*, an alien species in marine habitats along the Italian coasts, was identified. 9 potentially toxic diatoms and 33 dinoflagellates, included in the IOC-UNESCO Taxonomic Reference List of Harmful Microalgae, have been identified to the species level.

Molecular methods allowed the identification of dinoflagellate cysts belonging to *Alexandrium pacificum*, formerly classified as *A. catenella* Group IV. *A. pacificum*, a PSP producing organism, is a potentially non-indigenous species for the Adriatic Sea, mostly considered as invasive along the Spanish, French and Italian coasts of the Mediterranean Sea. The new detection of resting stages in harbors sediments suggests that this harmful alga is spreading in the Northern Adriatic Sea.

AUTORI

Mauro Bastianini (mauro.bastianini@ismar.cnr.it), Fabrizio Bernardi Aubry, Stefania Finotto, Istituto di Scienze Marine – Consiglio Nazionale delle Ricerche, Castello 2737/f arsenale Tesa 104, 30122 Venezia, Italy

Antonella Penna, Federico Perini, Dipartimento di Scienze Biomolecolari, Università di Urbino, Viale Trieste 261, 61121 Pesaro, Italy

Marina Cabrini, Daniela Fornasaro, OGS (Istituto Nazionale di Oceanografia e di Geofisica Sperimentale), via A. Piccard 54, 34151 Trieste, Italy

Monica Cangini, Silvia Pigozzi, Fondazione Centro Ricerche Marine Cesenatico, viale A. Vespucci 2, 47042 Cesenatico (FC), Italy

Phytoplankton in the ships' ballast waters sampled in three Adriatic ports

M. Cabrini, F. Cerino, E. Di Poi, D. Fornasaro

Ships' ballast waters represent one of the most serious threats of the world to human health and marine resources. Indeed, many organisms can survive at ballasting operations and long transfers. To establish a common cross border system among all researchers, experts and responsible national authorities in the Adriatic countries, a strategic IPA project BALMAS (Ballast Water Management System for Adriatic Sea Protection), was promoted. From June to September 2015, thirty ballast tanks of ships incoming in the Port of Trieste, Venice, and Ancona were sampled. Vessels were selected to analyse ballast waters from different donor ports in the Mediterranean Sea, in order to check the presence of living phytoplankton, NIS and harmful microalgae. For the viable cell analysis, water samples were stained with Fluorescein Diacetate (FDA) as a selective viability indicator and processed using an epifluorescence microscope. For phytoplankton quantitative and qualitative analyses, 500 ml water samples were fixed with Lugol (1% final concentration). Cell counts were carried out according to the Utermöhl's method (Zingone et al. 2010).

All the ballast samples from Trieste showed viable cells exceeding the phytoplankton threshold of 10 cells mL⁻¹, as DEFINED by IMO regarding ballast water quality standards. On the contrary, viable cells below the threshold were found in 20% of the ballast samples of Venice and in 30% of the samples of Ancona. Phytoplankton community in Trieste resulted dominated by small flagellates, whereas in Venice and Ancona, the community was mainly represented by small diatoms. Taxonomic analyses showed the presence of some potentially harmful species such as *Pseudo-nitzschia multistriata*, *Pseudo-nitzschia* spp., *Dinophysis caudata*, *D. sacculus*, *Noctiluca scintillans*, *Prorocentrum cordatum* in the ballast tanks of several ships incoming in Trieste, Venice, and Ancona. Among the potentially harmful species, *Pseudo-nitzschia multistriata*, which is also considered a NIS for the Mediterranean area, was only found in a ballast tank sampled in Venice.

These results confirm that inside the ballast tanks microalgae may survive and be potentially transferred via ships in the port areas, with a risk for human health and possible ecological and economic impacts for coastal waters.

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AUTORI

Marina Cabrini (mcabrini@inogs.it), Federica Cerino, Elena Di Poi and Daniela Fornasaro, OGS- Istituto Nazionale di Oceanografia e di Geofisica Sperimentale, Via Auguste Piccard, 54, 34151 Trieste, Italy

Image analysis methods to monitor toxic cyanobacteria in freshwater ecosystems

E. Gandola, E. Viaggiu, G. Morabito, R. Congestri

Human nutrient loading and climate change (warming, altered rainfall) synergistically enhance cyanobacterial blooms in aquatic ecosystems. Bloom-forming cyanobacterial taxa can be harmful from environmental, organismal, and human health perspectives by outcompeting beneficial phytoplankton, depleting oxygen upon bloom senescence, and producing a variety of toxic secondary metabolites (e.g., cyanotoxins). 40 different cyanobacterial genera, out of the ca 2000 described, are able to produce neuro and hepato-toxins that can reach critical concentration during bloom events (CyanoHABs) with ultimate water contamination limiting the use and sustainability of freshwater resources (drinking water reservoirs, water systems for aquaculture and recreation). Accordingly, the World Health Organization and regional authorities developed strategies aimed at controlling and mitigating CyanoHABs, with recent implementation of Alert Level Frameworks (ALFs) and Water Safety Plans (WSPs), all based on the estimation of cyanobacterial cell concentration in the water systems (Lucentini et al. 2017). It is known that cyanobacteria exhibit inconspicuous morphological traits (coccal, solitary or colonial, and filamentous forms) making the counting procedure difficult, time consuming and biased by the operator subjectivity.

In this scenario, we developed ACQUA, a fully automated image analysis algorithm able to discriminate 5 of the most widespread toxic filamentous genera in natural samples using bright field microscopy (Gandola et al. 2016). ACQUA has the capacity to precisely evaluating filament morphometry and to estimate cell abundance, cell/mL, as an output. Furthermore, we are currently focussing on the development of MAIA (Microcystis Automated Image Analyser), a fully automated algorithm designed for analysing images of the coccal, colonial genus *Microcystis*, whose toxic species commonly occur as dense, tridimensional aggregates in natural samples. MAIA is designed to extract depth profiles from 2D images and to reconstruct 3D information with ultimate cell abundance estimation for each colony in the field of view.

The application of ACQUA to Lake Vico (Italy) water samples led us to collect a large morphometric dataset that was correlated with toxin profiles showing that filament elongation in *Planktothrix rubescens* anticipated toxin retrieval in water, prospecting potential for early warning of water contamination.

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AUTORI

Emanuele Gandola (gandola@mat.uniroma2.it), University of Rome 'Tor Vergata', Department of Biology, Laboratory of Biology of Algae, via della Ricerca Scientifica, 00133 Rome, Italy; University of Rome 'Tor Vergata', Department of Mathematics, via della Ricerca Scientifica, 00133 Rome, Italy

Emanuela Viaggiu, University of Rome 'Tor Vergata', Department of Biology, Laboratory of Biology of Algae, via della Ricerca Scientifica, 00133 Rome, Italy; AlgaRes S.r.l., University Spinoff of Rome 'Tor Vergata', c/o Science Park, via della Ricerca Scientifica, 00133 Rome, Italy

Roberta Congestri, University of Rome 'Tor Vergata', Department of Biology, Laboratory of Biology of Algae, via della Ricerca Scientifica, 00133 Rome, Italy; AlgaRes S.r.l., University Spinoff of Rome 'Tor Vergata', c/o Science Park, via della Ricerca Scientifica, 00133 Rome, Italy

Giuseppe Morabito, CNR - Institute of Ecosystem Study, Largo Tonolli 50, 28922 Verbania Pallanza, Italy

New observations on the benthic algal flora of the area of Ancona

F. Rindi, B. Gavio, T. Romagnoli, A. Falace, P. Díaz-Tapia

Ancona is located at the northern edge of the only long stretch of rocky shore on the Adriatic coast of central Italy. In general, the benthic algal flora of this area has been understudied in comparison with many other Mediterranean regions. Detailed investigations were mainly concentrated in two periods: the years 1940-1945, in which Irma Pierpaoli made extensive collections mainly in the area of the harbour of Ancona; and the years 1960-1976, when Solazzi and collaborators published several reports on the benthic vegetation of the Riviera del Conero. We are currently reassessing the macroalgal flora of this area (harbour of Ancona and Riviera del Conero) by examination of numerous new collections made from several sites and at different times of the year. Collections made in the last 4 years indicate that not less than 135 species are present. An obvious difference from previous studies is the presence of at least six introduced species: *Antithamnion hubbsii*, *Codium fragile*, *Grateloupia turuturu*, *Neosiphonia harveyi*, *Polysiphonia morrowii* and *Sargassum muticum*. *N. harveyi* is nowadays one of the most common red seaweeds in the area of Ancona and its identity was confirmed by molecular data. The collections allowed to record some species so far unreported in the area; at the same time, we have not been able yet to recollect other species previously recorded. Several species are strictly associated with one or a few sites, often showing strong differences in seasonal occurrence. In the near future, we are planning to integrate the morphological identifications with large-scale DNA barcoding data for the most common species.

AUTORI

Fabio Rindi (f.rindi@univpm.it), Tiziana Romagnoli, Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Via Breccie Bianche, 60131 Ancona, Italy

Brigitte Gavio, Departamento de Biología, Universidad Nacional de Colombia, Carrera 30, Calle 45, Bogotá, Colombia

Annalisa Falace, Dipartimento di Scienze della Vita, Università di Trieste, Via L. Giorgieri 10, 34127 Trieste, Italy

Pilar Díaz-Tapia, BioCost Research Group, Universidade da Coruña, Campus da Zapateira, 15071 A Coruña, Spain

Macroalgal response to the lagoon recovery and aquatic plant transplantation in the Venice Lagoon

A. Sfriso, A. Buosi, A.A. Sfriso

The northern region of the Venice Lagoon is showing a rapid ecological recovery. The decrease of the trophic level, especially phosphorus concentration, both in water column and surface sediments, and the reduction of anthropogenic impacts such as the harvesting of the Manila clam *Ruditapes philippinarum* (Adams & Reeve) prevent the growth of opportunistic species such as Ulvaceae, Cladophoraceae, Gracilariaceae and Solieriaceae. These taxa are common in the most eutrophicated areas of the lagoon such as the historical center of Venice but in the northern lagoon they are almost disappeared.

To colonize the bottoms of this area an important contribution came from the project LIFE 12 NAT/IT/000331 with the transplantation of aquatic plants in 35 areas (17 areas in 2014 and 18 areas in 2015) and in the corridors that connect the different areas. Plants have taken root in 32 areas out of 35 and spread along the edges of salt marshes colonizing even the shallows of the most protected areas. Their presence favored also the bottom colonization by many sensitive macroalgae which, year after year, are increasing the range of expansion. The abundance of Rhodophyceae has increased by 101% whereas the abundance of Chlorophyceae only by 26%. At the end of 2014 the mean number of macroalgal taxa per station recorded in spring and autumn was 16.6. In 2015, the number of taxa was 20.8.

On average, the number of sensitive taxa per station increased from 1.53 (2014) to 2.53 (2015). The first records of 2016 show a further increase. Many taxa never recorded in the last 30 years or present only in border areas such as *Polysiphonia spinosa* (C. Agardh) J. Agardh, *Palisada patentiramea* (Montagne) Cassano *et al.*, *Laurencia obtusa* (Hudson) J. V. Lamouroux and the small calcareous species, are spreading everywhere and contribute to the overall environmental recovery.

AUTORI

Adriano Sfriso (sfrisoa@unive.it), Alessandro Buosi, Department of Environmental Sciences, Informatics & Statistics, Via Torino 155, 30172 Mestre-Venice, Italy

Andrea A. Sfriso, Department of Molecular Sciences and Nanotechnologies, Via Torino 155, 30172 Mestre-Venice, Italy

Inventory of the distribution and abundance of alien macroalgae in the Venice lagoon

A. Sfriso, A. Buosi, A.A. Sfriso

Recently, a revision of the non indigenous macroalgae in the lagoon of Venice (Sfriso, Marchini, 2014; Marchini et al. 2015) confirmed the presence of 28 taxa of extra-Mediterranean origin with the exclusion of many cryptic species or species whose origin must be confirmed. Some NIS have colonized the whole lagoon and have a significant biomass, others are rare or are located in particular areas of the lagoon. Out of them, 9 taxa are present with a fresh biomass in the order of hundreds or thousands of tonnes; 7 taxa show a biomass ranging from 25 to 500 kg; 10 taxa, mostly species of small or very small size, have a biomass <1 kg. Two taxa which are difficult to determine have an unknown biomass.

The species that shows the highest biomass is *Gracilaria vermiculophylla* (Ohmi) Papenfuss (about 12500 tonnes of fresh weight) even if that species colonises only confined areas (ca. 25 km²). *Agardhiella subulata* (C. Agardh) Kraft & M.J. Wynne colonises 216 km² at least with a slightly lower biomass: ca. 10800 tonnes. Other species such as *Hypnea flexicaulis* Y. Yamagishi & M. Masuda, *Scytosiphon dotyi* M. J. Wynne, *Solieria filiformis* (Kützinger) Gabrielson range from 3600 to 5400 tonnes. *Sargassum muticum* (Yendo) Fensholt and *Undaria pinnatifida* (Harvey) Suringar which grow mainly in the hard substrata of Chioggia and the historical centre of Venice with people concern, show a biomass of 5900 and 125 tonnes, respectively. All the other taxa have a biomass almost negligible or difficult to measure because are very small or rare. The species most abundant are free-floating taxa which grow mainly in the soft bottoms of the lagoon. The species attached to hard substrata, even though are abundant as *S. muticum* or *U. pinnatifida* and can present a fresh biomass up to 10 kg m⁻², show a limited biomass because the reduced presence of an appropriate substratum. On the whole the total NIS biomass is <45000 tonnes, about half of the total macroalgal biomass estimated by Sfriso, Facca (2007) for the whole soft substrata of the lagoon (ca. 432 km²).

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AUTORI

Adriano Sfriso (sfrisoad@unive.it), Alessandro Buosi, Department of Environmental Sciences, Informatics & Statistics, Via Torino 155, 30172 Mestre-Venice, Italy
Andrea A. Sfriso, Department of Molecular Sciences and Nanotechnologies, Via Torino 155, 30172 Mestre-Venice, Italy

Adding molecular data to Venice Lagoon macroalgae

S. Armeli Minicante, T. Melton, J. Lopez-Bautista

Marine biodiversity is in rapid and continuous change. For this reason it is important to continue to monitor the biodiversity of an area even with the aid of techniques for a rapid species identification. Venice Lagoon is a peculiar environment, with an high biodiversity and a significant human influence. At present, few studies of molecular systematics concern the macrophytes of Venice Lagoon.

Using the plastid marker *tufA*, new molecular data have been obtained for Ulvaceae samples from Venice Lagoon adding information to the list of green algae of the Italian coasts.

AUTORI

Simona Armeli Minicante (simona.armeli@ve.ismar.cnr.it), CNR-ISMAR Istituto di Scienze Marine, Arsenale 104, Castello 2737F, 30122 Venezia, Italy

Trey Melton, Juan Lopez-Bautista, University of Alabama, Department of Biological Sciences, 500 Hackberry Lane, Tuscaloosa, AL (USA)

Aggiornamento tassonomico dell'ordine Gelidiales Kylin 1923 (Rhodophyta)

A. Bottalico

Nel 1956 Kylin in "Die Gattungen der Rhodophyceen" fornì un quadro sinottico di tutti i generi di alghe rosse conosciuti a quel tempo, suddivisi in ordini e famiglie. In particolare, l'ordine Gelidiales, eretto dallo stesso autore nel 1923, comprendeva l'unica famiglia Gelidiaceae Kützing con 11 generi, compreso *Gelidiella* Feldmann & Hamel. Sin dalle sue origini, l'ordine Gelidiales ha subito numerosi rimaneggiamenti grazie a studi morfologici e all'avvento delle indagini molecolari cominciate negli anni '90 del secolo scorso. Il numero di generi appartenenti alle Gelidiaceae è diminuito a 4, inclusi due generi nuovi *Capreolia* Guiry & Womersley e *Gelidiophycus* G.H. Boo, J.K. Park & S.M. Boo, e sono state descritte nuove specie.

Per il genere *Gelidiella*, nel 1961 Fan stabilì la famiglia Gelidiellaceae, basata sull'assenza di rizine e di riproduzione sessuale. Tuttavia, per lungo tempo la validità di entrambi i caratteri diagnostici, e quindi della famiglia, fu messa in discussione da numerosi ricercatori. Nel 2004 Santelices descrisse il nuovo genere *Parviphycus* al quale trasferì le specie di *Gelidiella* che presentavano i seguenti caratteri: divisione subdistica delle cellule subapicali, assiali e periaassiali disposte in una fila mediana visibile in tutto il tallo, tetrasporangi originati dalle cellule periaassiali e regolarmente disposti in piani paralleli trasversali. I caratteri del nuovo genere si riferiscono alle specie di *Gelidiella* di piccolissima taglia (fino a poco più di 1 cm) che formano fitti tappeti a livello intertidale e sulla volta delle grotte semisommerse, come la specie tipo *Parviphycus adnatus* (Dawson) Santelices. Da allora, il numero di specie in *Parviphycus* è salito a 10, 7 specie trasferite da *Gelidiella* e 3 nuove specie descritte lungo le coste pugliesi: *P. felicinii* Perrone & Delle Foglie, *P. albertanoae* A. Bottalico, G.H. Boo, C. Russo, S.M. Boo & C. Perrone e *P. bompardii* A. Bottalico, C. Russo, G. Furnari & C. Perrone.

In seguito alle indagini sulle caratteristiche dei sori tetrasporangiali in *Gelidiella* e del sistema prostrato nelle Gelidiales, iniziate nel 1994, Perrone *et al.* nel 2006 emendarono e ristabilirono la famiglia Gelidiellaceae e separarono i generi *Pterocladia* J. Agardh e *Pterocladiella* Santelices & Hommersand nella nuova famiglia Pterocladaceae Felicini & Perrone. Recenti analisi molecolari e filogenetiche hanno dimostrato la monofilia delle Gelidiellaceae e Pterocladaceae, supportando pienamente il loro status di famiglia nell'ambito delle Gelidiales. Nel 2007 Tronchin e Freshwater descrissero il nuovo genere *Aphanta* e lo ascrissero temporaneamente alle Pterocladaceae. Nel corso del 2016, Boo *et al.* in uno studio filogenetico basato su 5 diversi marcatori molecolari, tra cui *CesA* utilizzato per la prima volta nelle Gelidiales, hanno individuato un nuovo clade corrispondente alla famiglia Orthogonacladiaceae G.H. Boo, L. Le Gall, K.A. Miller & S.M. Boo con il nuovo genere *Orthogonacladia* G.H. Boo & L. Le Gall e alla quale è stato trasferito il genere *Aphanta*.

In un altro lavoro che combinava 3 diversi marcatori (*cox1*, *rbcl* e *psA*) è stato descritto un nuovo scenario in cui la famiglia Gelidiellaceae risulta suddivisa in 5 generi, 2 già conosciuti e 3 nuovi. Raccolte più estensive ed analisi allargate a più marcatori potranno ulteriormente chiarire le relazioni filogenetiche, i pattern di speciazione e la biogeografia dei membri di questa complessa famiglia.

AUTORE

Antonella Bottalico (antonella.bottalico@uniba.it), Dipartimento di Biologia, Università degli Studi di Bari "A. Moro", Via E. Orabona 4, 70125 Bari, Italy

Phycological jokes...and more. III Contribution

G. Furnari

Following an almost well-established tradition, a new series of original jokes are presented aiming at inducing listeners to smile. As in the previous contributions they are mainly based on algal names but also on different topics.

Because jokes are most probably little intelligible to people not speaking Italian, the author begs foreign colleagues' pardon.

AUTORE

Giovanni Furnari (furnari.giovanni41@gmail.com), Dipartimento di Scienze Biologiche, Geologiche e Ambientali dell'Università di Catania, Sezione Biologia Vegetale, via A. Longo 19, 95125 Catania, Italy

Growth responses of photosynthetic microorganisms to Red Dwarf star light

D. Simionato, R. Claudi, A.C. Pozzer, A. Segalla, M.S. Erculiani, B. Salasnich, D. Billi, L. Coccola, L. Poletto, T. Morosinotto, N. La Rocca

The recent discovery of a number of rocky exoplanets orbiting in the habitable zone of their Red Dwarf stars is offering new challenges to answer the question whether life could be possible in other worlds beyond our solar system. In this frame a scientific collaboration between astrophysics and biologists recently started aiming to perform laboratory simulations replicating the light irradiation on the surface of an Earth-like planet orbiting around the mean habitable zone of a Red Dwarf star (M type star) and to analyze the capability of photosynthetic microorganisms to exploit this light to grow and evolve oxygen. Red Dwarf stars represent perhaps the 76 % of our Milky Way galaxy's stars, have some 10 to 50 % of the Sun's mass and are dimmer and redder, strongly radiating at invisible infrared wavelengths. A light simulator of the different stellar spectra in the wavelength range (365-940 nm), also able to reproduce the spectra of Red Dwarf stars, have been developed by researcher of the Astronomical Observatory in Padova. Biological experiments were thus carried out starting with selected species of cyanobacteria recently demonstrated to perform a far-red light photoacclimation (FaRLiP) when exposed to light centered at 720 nm. This ability was discovered to be dependent on the presence and expression of a peculiar gene cluster leading to the reorganized of the photosynthetic apparatus and to the synthesis of different chlorophyll forms (chl d and f) (Gan, Bryant 2015). Growth and photosynthetic performances of these cyanobacteria irradiated with simulated Red Dwarf star light were compared with that of the same organisms exposed to far-red (720 nm) or solar lights. The experiment was then extended to other photosynthetic organisms also including green microalgae. Preliminary results of this pioneering experiment will be presented.

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AUTORI

Diana Simionato, Anna Caterina Pozzer, Anna Segalla, Tomas Morosinotto, Nicoletta La Rocca (nicoletta.larocca@unipd.it), Dipartimento di Biologia, Università di Padova, via U. Bassi 58/B, 35121, Padova, Italy

Riccardo Claudi, Marco Sergio Erculiani, Bernardo Salasnich, Osservatorio Astronomico, vicolo dell'Osservatorio 5, 35122 Padova, Italy

Daniela Billi, Dipartimento di Biologia, Università di Tor Vergata, via Ricerca Scientifica, 00133 Roma, Italy

Lorenzo Coccola, Luca Poletto, Luxor, Istituto di Fotonica e Nanotecnologie CNR, via Trasea 7, 35131 Padova, Italy

Exploiting microalgae as green factory for the production of high value compounds

D. Simionato, M. Salvalaio, T. Morosinotto, N. La Rocca

Microalgae in the last years are receiving increasing attention for their possible biotechnological utilization. In fact, they could be exploited for the production of molecules which can be introduced in the industry at multiple levels. Among the diverse applications, fields of interest are aquaculture, pharmaceuticals, nutraceuticals and cosmetics. These fields share the utilization of molecules such as omega-3 or carotenoids which could be added to human and animal nutrition with already recognized benefits.

In this work we focused the attention on two microalgae belonging to different phyla. The first is the marine *Nannochloropsis gaditana*, an Eustigmatophyceae which is already utilized in aquaculture and which is actually under evaluation of the European Commission for its introduction in the human nutraceuticals as source of eicosapentaenoic acid (EPA), a lipid belonging to omega-3 group. The second microalga is the freshwater *Chlorella zofingiensis*, which could be used in alternative to *Haematococcus pluvialis* for the production of carotenoids. Both species have been exposed to different growth conditions modulating illumination regime, nutrient concentration or carbon dioxide availability in order to evaluate which stimuli are responsible for a major accumulation of omega-3 or carotenoids in these microalgae.

In the case of *N. gaditana*, the EPA content was assessed to be the 5% on a dry weight basis with a daily productivity of 4 mg EPA liter⁻¹ day⁻¹. The rate of accumulation of this compound has been found to be dependent from a combination of stimuli such as, in particular, light intensity and CO₂. The results obtained are very interesting since from literature the declared productivities are between 5 and 11 mg EPA liter⁻¹ day⁻¹.

In the case of *C. zofingiensis* the interest relies, above all, on the production of secondary carotenoids, in particular astaxanthin. This ketocarotenoid possess a strong antioxidant activity and it already has a profitable market all over the world. Actually, it is synthetically produced or naturally produced from *H. pluvialis*. The natural astaxanthin is more stable respect with the synthetic one and, in addition, *C. zofingiensis* presents a lot of advantages respect with *H. pluvialis*. For these reasons, it could be considered a valid alternative for the production of astaxanthin. In this work, it was observed that *C. zofingiensis* responds to high light and nitrogen deprivation increasing the production of secondary carotenoids in the expense of primary ones. In particular, when these two stresses are applied to the cultures, the rate of ketocarotenoids production is even speed up. From an industrial point of view, it was found that *C. zofingiensis* can accumulate a concentration of astaxanthin up to 5 mg/g dry weight which is a result in line and in some cases even higher with those found in literature.

AUTORI

Diana Simionato (diana.simionato@unipd.it), Maddalena Salvalaio, Tomas Morosinotto, Nicoletta La Rocca, PARLAB, Dipartimento di Biologia, Università degli Studi di Padova, via U. Bassi 58/B, 35121 Padova, Italy

Cultivation of photosynthetic microorganisms in continuous steady state reactor towards a large scale industrial production

E. Sforza, E. Barbera, C.E. de Farias Silva, B. Gris, N. La Rocca, A. Bertucco

The large scale production of photosynthetic microorganisms for industrial and biofuel applications has received enormous attention in the last decade, due to their fast growth rate, higher photosynthetic efficiency and productivity, and high carbohydrates or lipid content. Microalgae and cyanobacteria are the organisms commonly investigated, even though they may differ in terms of macromolecules and pigments composition, light utilization, growth rates and biomass productivity. When considering the possible exploitation of photosynthetic organisms for biofuel production, a large scale approach is the only way to understand the technical and economic feasibility of the whole process. Thus, moving from lab to industrial scale, the impact of variables involved in the cultivation step increases. Even not considering the technical challenges of downstream process, that are currently under deep investigation, some critical aspects concerning the cultivation are not overcome yet. The main key issues still needing an efficient improvement are related to the photosynthetic efficiency, the CO₂ and nutrient supply, and the management of cultivation conditions to stimulate the accumulation of target product of interest. As data of large scale production are still limited, a deeper comprehension of the variables and mechanisms involved in the cultivation step is needed: based on lab measures, the dependence of growth parameters on key variables can be obtained, and can be then applied in reliable models needed to develop industrial process by simulation. Of course the experimental approach, even at lab scale, should be carefully considered and set: the common batch method to evaluate growth capabilities is strongly limited by a number of factors, such as the acclimation of preinoculum and a changing biomass concentration over time, which makes it more difficult to explain the results. On the opposite, in a steady-state continuous system, biomass production is constant and well acclimated to the environmental conditions. Working in a lab scale continuous system is essential even from a biological point of view, because, once a steady state is reached, all the transient acclimation phenomena are achieved, allowing a more reliable measurement of physiological parameters. Thus, if the crucial point is to understand the feasibility of large scale production of microalgae and cyanobacteria, continuous cultivation should be recognized as the best method to deeply understand microalgal acclimation and physiology.

AUTORI

Eleonora Sforza (eleonora.sforza@unipd.it), Elena Barbera, Carlos Eduardo de Farias Silva, Alberto Bertucco, Dipartimento di Ingegneria Industriale, Università di Padova, via F. Marzolo 9, 35131 Padova, Italy

Barbara Gris, Nicoletta La Rocca, Dipartimento di Biologia, Università di Padova, via U. Bassi 58/B, 35121 Padova, Italy

First report of polyunsaturated aldehydes production by three benthic diatoms

S. Pichierri, L. Pezzolesi, C. Samori, C. Totti, R. Pistocchi

The production of secondary metabolites have been reported in many phytoplankton organisms including diatoms that have been shown to produce and release different types of fatty acid derived as polyunsaturated aldehydes (PUAs) able to affect the reproduction of several invertebrates and phytoplankton growth. Until now, the interest towards these biologically compounds focused on planktonic diatoms, especially on their relationship with zooplankton species. This study investigated the possible production of PUAs in the three benthic diatoms commonly occurring in the benthic flora of temperate regions: *Tabularia affinis*, *Proschkinia complanatoidea* and *Navicula* sp. The results highlighted the production of PUAs by all three benthic diatoms tested only during the late stationary and decline phases of growth. No traces of PUAs were found until day 28, confirming the release of these compounds into the medium only after the cell destruction. The detected amounts showed of low values in the micromolar range and appeared comparable with those of planktonic species able to affect the growth of other phytoplankton species. The PUAs percentage respect to the total aldehydes (saturated and unsaturated) ranged from 62 to 94% with different values among the diatoms tested: almost the totality of PUAs produced by *Navicula* sp. (89%) consisted of the low or medium-chained compounds hexanal and octanal (i.e. C6 and C8, respectively), while in *T. affinis* and *P. complanatoidea* the low medium-chained represented the 47 and 44% respectively, and longer chained compounds (between C9 and C13) increased their contribute. Moreover, the long chained aldehydes (C9-C13) produced by the three species tested could have a higher biological activity, respect to the short chained ones produced by the planktonic diatoms, as already reported in literature.

AUTORI

Salvatore Pichierri (s.pichierri@pm.univpm.it), Cecilia Totti, Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Breccie Bianche, 60131 Ancona, Italy

Laura Pezzolesi, Rossella Pistocchi, Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Bologna, via Sant'Alberto 163, 48123 Ravenna, Italy

Chiara Samori, Dipartimento di Chimica "G. Ciamician", Università di Bologna, via Selmi 2, 40126 Bologna, Italy

***Desmodesmus communis* for bioremediation of herbicide contaminated drinkable waters**

L. Pezzolesi, P. Blair Vasquez, P. Galletti, C. Samorì, E. Tagliavini

In the last 10 years, various sources of drinkable waters of several communities in the Caribbean lowlands in Costa Rica have been found to be polluted with bromacil, a common uracil-herbicide largely used in pineapple plantations (69% of worldwide production of pineapple comes from Costa Rica). The concentrations detected in the environment range between 2-2.5 µg/L being, therefore, more than 20-times higher than the current regulation (the maximum permissible level of a single herbicide in drinkable water is 0.1 µg/L).

In the present study, the microalga *Desmodesmus communis* (Chlorophyceae) has been investigated as a possible tool for a sustainable bioremediation of bromacil, meeting the need of bioremediation approaches with low environmental impact. Microalgae have been commonly used for the bioremediation of inorganic pollutants. As for organic pollutants, even though it is reknown that algae have the ability of uptaking and removing organic pollutants via both biosorption and/or metabolization, their use for bioremediation hasn't been extensively explored. Specifically, *D. communis* has been here proposed for its high resistance to chemical and environmental stress, due to the presence of algaenans in the cell wall.

The effect of bromacil on the growth and on the photosynthetic efficiency of *D. communis* was firstly determined in order to understand the maximum levels of bromacil which the algae can withstand. For both responses, the EC50 values obtained (19-27 µg/L) where above the concentrations present in drinkable water in Costa Rica. Phycoremediation experiments were performed in batch or semi-continuous configurations, both with light and under the dark. Under a batch mode with light exposure the best results were achieved: *D. communis*, in fact, was able to decrease the concentration of bromacil in the medium from 4 µg/L to 0.4 µg/L, therefore at levels close to the law limits. The algal biomass collected after the phycoremediation process was characterized in order to understand the fate of the herbicide and its effect on cellular metabolism. No differences between the treated and the control algal cells were found in terms of morphology, and of lipids and carbohydrates content; however, the treated algae had a higher content of proteins than the control, suggesting that sub-lethal concentrations of bromacil could induce a stress to *D. communis* cells.

In conclusion, *D. communis* results a highly resistant microalga whose growth is not affected by the concentration of bromacil detected in the environment. *D. communis* could be proposed as a successful technology to be used for phycoremediation of bromacil from contaminated waters.

AUTORI

Laura Pezzolesi (laura.pezzolesi@unibo.it), Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Bologna, via Sant'Alberto 163, 48123 Ravenna, Italy

Paola Blair Vasquez, Paola Galletti, Chiara Samorì, Emilio Tagliavini, Dipartimento di Chimica "G. Ciamician", Università di Bologna, via Selmi 2, 40126 Bologna, Italy

Epigenetic mechanisms are involved in Cr(VI) tolerance and in response to S deprivation in *Scenedesmus acutus*

M. Ferrari, A. Torelli, A. Sardella, M. Marieschi, C. Zanni, R. Cozza

Tolerance, avoidance, and resistance are three major strategies followed by plants to counter the recurring biotic and abiotic stresses (Dhar et al. 2014). These mechanisms involve genes associated with several interconnected pathways which lead them towards better stress tolerance. Long-term strategies include transgenerational changes involving development of heritable gene expression changes. Epigenetics, referred to heritable changes in gene expression without mutating DNA sequences, play a role in the induction of transgenerational stress resistance upon chemical exposure. Gene expression driven by developmental and stress cues often depends on nucleosome histone post-translational modifications and sometimes on DNA methylation. Levels and patterns of cytosine DNA methylation, a heritable epigenetic marker with a conserved role in gene silencing, appear to vary drastically among different organisms. Recently discovered epigenetic features in various environmentally relevant species are reviewed in Vandegheuchte, Janssen (2014). Very few data about the epigenetic features exist in algae (Feng et al. 2010, Zemach et al. 2010, Veluchamy et al. 2014).

To gain additional information on the involvement of epigenetic mechanisms in heavy metal tolerance, we analyzed the global DNA methylation in two strains of the freshwater green alga *Scenedesmus acutus* with different chromium sensitivity. In this alga a transient increase in Cr(VI) tolerance (Gorbi et al. 2007) was observed following a 3-day sulfur starvation both in the wild type and in a Cr-tolerant strain and was lost after 2-day recovery in standard medium. The analysis of methylation was thus conducted after Cr(VI) exposure following 3-day culture in standard or S-deprived medium. Significant differences both in the level and in the pattern of the labeling of the DNA cytosine-rich zones were observed between the two strains. Moreover S-starvation induced a significant hypomethylation in both strain. These data suggest that an epigenetic mechanism could be at the basis of the Cr tolerance in *S. acutus*, as supported by previous data reporting that the acquired tolerance was inherited and maintained through the progeny.

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AUTORI

Anna Torelli (anna.torelli@unipr.it), Alessio Sardella, Matteo Marieschi, Corrado Zanni, University of Parma, Department of Life Sciences, University of Parma, Parco Area delle Scienze 11/A, 43124 Parma, Italy
Michele Ferrari, Radiana Cozza, University of Calabria, Department of Biology, Ecology and Earth Science, Via P. Bucci, 87036 Arcavacata di Rende, Cosenza, Italy

Is a different sulfate assimilation responsible for the different chromium tolerance of two strains of the green unicellular alga *Scenedesmus acutus*? -APS reductase

A. Sardella, M. Marieschi, C. Zanni, A. Torelli

In the freshwater green alga *Scenedesmus acutus*, sulfur starvation induces a transient increase of Cr(VI) tolerance, both in the wild type and in a Cr-tolerant strain, associated to an increased capacity of sulfur uptake and assimilation leading to an increase in cysteine synthesis during the recovery in standard medium (Gorbi et al. 2007). Despite being chromium tolerance not related to the induction of phytochelatin, the tolerance to this metal seems strictly connected to sulfur metabolism (Pereira et al. 2008, Schiavon et al. 2008). It is well known that sulfur starvation induces a huge variation in gene expression in *Chlamydomonas reinhardtii* (Zhang et al. 2004) as well as in other algae and in land plants (Bochenek et al. 2013). Among the genes over-expressed there are plasma membrane high affinity sulfate transporters and many enzymes of the sulfate assimilation pathway. The key regulatory enzyme of the pathway is APS reductase (APR) which control the 90% of sulfate flux toward the production of cysteine (Davidian, Kopriva 2010). In order to verify if differences in APS reductase expression is involved in the observed transient increase in Cr-tolerance, as well in the differential sensitivity shown by the two *S. acutus* strains, we tried to clone fragments of APS reductase gene. Primers designed on the sequence of *C. reinhardtii* allowed the partial cloning of the APR gene homologous. Our analyses, conducted on algae pre-cultured for 3 days in standard and S-deprived medium before Cr exposure, show that the enzyme activity and transcription are both affected by nutrient deprivation and metal exposure. No differences were however observed between the wild type and the Cr tolerant strains exposed to similar conditions, thus indicating that the two strains different chromium sensitivity does not rely on a different regulation of the enzyme.

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AUTORE

Alessio Sardella (alessio.sardella@student.unife.it), Matteo Marieschi, Corrado Zanni, Anna Torelli, Dipartimento di BioScienze, Università di Parma, Parco delle Scienze 11/A, 43124 Parma, Italy

Let us introduce you to the new benthic toxic *Ostreopsis* species from the eastern Mediterranean Sea: *Ostreopsis fattorussoi* sp. nov.

S. Accoroni, T. Romagnoli, A. Penna, S. Capellacci, P. Ciminiello, C. Dell'Aversano, L. Tartaglione, M. Abboud–Abi Saab, V. Giussani, V. Asnaghi, M. Chiantore, C. Totti

The genus *Ostreopsis* belongs to the family of Ostreopsidaceae (Gonyaucales, Dinophyceae), which includes two genera of benthic dinoflagellates (i.e., *Ostreopsis* and *Coolia*). The type species, *Ostreopsis siamensis* Schmidt was first described in the Gulf of Siam (Thailand) in 1900. In the following years, several other species have been described by other authors: *O. lenticularis* Fukuyo, *O. ovata* Fukuyo, *O. heptagona* Norris, Bomber & Balech, *O. mascarenensis* Quod, *O. labens* Faust & Morton, *O. marina* Faust, *O. belizeana* Faust and *O. caribbeana* Faust.

In the Mediterranean Sea, two genotypes corresponding to the morphotypes *O. cf. ovata* and *O. cf. siamensis* have been recorded to date. In addition to these two species, recently a new genotype corresponding to a new species of *Ostreopsis* has been found in both the Atlantic Ocean (Canary Islands) and Mediterranean Sea (Greece Cyprus Island and Lebanon) and named *O. fattorussoi* Accoroni, Romagnoli & Totti. This new benthic toxic dinoflagellate is described from eastern Mediterranean Sea Lebanon and Cyprus coasts, supported by morphological and molecular data. The plate formula, Po, 3', 7'', 6c, 7s, 5''', 2''''', is typical for the *Ostreopsis* genus. It differs from all other *Ostreopsis* species in that i) the curved suture between plates 1' and 3' makes them approximately hexagonal, ii) the 1' plate lies in the left-half of the epitheca and is obliquely orientated leading to a characteristic shape of plate 6''. The round thecal pores are bigger than the other two Mediterranean species (*O. cf. ovata* and *O. cf. siamensis*). *O. fattorussoi* is among the smallest species of the genus (DV: 60.07±5.63 µm, AP: 25.66±2.97 µm, W: 39.81±5.05 µm) along with *O. ovata*. Phylogenetic analyses based on the LSU and ITS rDNA shows that *O. fattorussoi* belongs to the Atlantic/Mediterranean *Ostreopsis* spp. clade separated from the other *Ostreopsis* species. This benthic dinoflagellate has been detected along the Lebanon coast throughout the year 2015 (with temperatures ranging from 18 to 31.5 °C), with bloom occurring in June and August and a significant correlation was highlighted between *Ostreopsis* growth rates and phosphate concentrations. *O. fattorussoi* is a toxic species producing OVTX-a and structural isomers OVTX-d and -e, so far found only in *O. cf. ovata*, and three exclusive palytoxin-like compounds (OVTX-i, OVTX-j1, OVTX-j2, and OVTX-k). All the data collected on this new species about its toxicity so far, however, would suggest a lower risk to human health and marine fauna to that of *O. cf. ovata*.

AUTORI

Stefano Accoroni (s.accoroni@univpm.it), Tiziana Romagnoli, Cecilia Totti, Dipartimento di Scienze del Mare, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy

Antonella Penna, Samuela Capellacci, Dipartimento di Scienze Biomolecolari, Università di Urbino, Viale Trieste 296, 61100 Pesaro, Italy

Patrizia Ciminiello, Carmela Dell'Aversano, Luciana Tartaglione, Dipartimento di Farmacia, Università degli Studi di Napoli "Federico II", Via D. Montesano 49, 80131 Napoli, Italy

Marie Abboud–Abi Saab, National Council for Scientific Research /National Center for Marine Sciences, P.O. Box 534, Batroun, Lebanon

Valentina Giussani, Valentina Asnaghi, Mariachiara Chiantore, Dipartimento di Scienze della Terra, dell'Ambiente e della Vita, Università degli Studi di Genova, Corso Europa 26, 16132 Genova, Italy

Phosphatase activities of an epiphyte community during a bloom of *Ostreopsis cf. ovata* in the northern Adriatic Sea

N.T.W. Ellwood, C. Totti, E. Razza, R. Congestri, A. Campanelli, M. Marini, S. Accoroni

In many coastal environments there is an increasing amount of organic nutrients deriving from anthropogenic and natural activities. It is also coming to light that many phototrophs are able to make use of organic nutrients, mainly through indirect studies of surface enzyme activities of target organisms or through direct studies of growth in media containing organic only nutrients. Yet many studies on coastal eutrophication have focused mainly on inorganic nutrient fractions. As *Ostreopsis cf. ovata* blooms are increasing in number and intensity in many coastal zones it was decided to follow a bloom through its entire cycle in the Conero Riviera (N Adriatic Sea) during summer-autumn 2015, monitoring the diverse ambient nutrient fractions and the phosphomonoesterase (PMEase) and phosphodiesterase (PDEase) activities of the epiphytic community involved.

Analyses of ambient nutrient fractions revealed very high N:P values suggesting that primary productivity was P limited (TIN:FRP = 359 ± 113 ; FON:FOP = 203 ± 71.3 ; TN:TP = 178 ± 50). In addition, the dissolved organic P was on average 85% of the total dissolved P, so there was little P about and what there was, was mostly organic. So high phosphatase activities were expected and indeed a rapid increase in PMEase and PDEase activities was observed from the epiphytic microalgal community and this coincided with the onset of an increase in growth of *Ostreopsis*. Chromogenic and fluorogenic staining of samples showed that activity was closely associated with the cells (cell surface, cytoplasm and intercellular space), and the EPS of *Ostreopsis*. The EPS of various organisms has been shown to accumulate a wide range of dissolved and particulate organic compounds. Therefore, the presence of elevated PMEase and PDEase activities close to the cells of *Ostreopsis* could provide plentiful P for growth and in the same instance minimise any loss to the surrounding waters. Having large quantities of broadly specific phosphatases within and closely associated with its cells would allow *Ostreopsis* to rapidly take advantage of any high concentration nutrient pulses and EPS-accumulated organics.

AUTORI

Neil Thomas William Ellwood (neilthomaswilliam.ellwood@uniroma3.it), Dipartimento di Scienze, Università Roma Tre, Viale G. Marconi 446, 00146 Roma, Italy

Cecilia Totti, Emanuela Razza, Stefano Accoroni, Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy

Roberta Congestri, Department of Biology University of Rome "Tor Vergata", Via Cracovia 1, 00133 Rome, Italy

Alessandra Campanelli, Mauro Marini, Istituto di Scienze Marine (Consiglio Nazionale delle Ricerche), Largo Fiera della Pesca, 60125 Ancona, Italy

Epiphytic dinoflagellates associated to marine phanerogams in the Colombian Caribbean along different climatic periods

J.E. Mancera-Pineda, B. Gavio, N. Arbelaez, A. Ruiz

The epidemiological reports for 18 Caribbean territories show that the ciguatera incidence level has increased over the last 31 years from 34/100 000 to 45/100 000 average annual incidence; however, its impact on tourism, public health, and marine ecosystems is still poorly understood. Ciguatera fish poisoning is a seafood-borne illness caused by the consumption of fish that have accumulated lipid-soluble ciguatoxins, produced by dinoflagellates of the genera *Gambierdiscus*, *Ostreopsis*, *Coolia* and *Prorocentrum*. To determine the occurrence of epiphytic toxic dinoflagellates in the coastal waters of Caribbean Colombia, we analyzed seagrass beds (*Thalassia testudinum*) in three marine reserved regions: San Andrés island (Seaflower International Biosphere Reservation), Barú island (Corales del Rosario National Natural Park), and Chengue Bay (Tayrona National Park). Samples were taken in different climatic periods from 2007 till 2015. Three quadrants were haphazardly placed in sample site and all the material was extracted, each substrate species (seagrass and macroalgae) was placed in a separate bag with 250 mL of a solution composed of 50% filtered seawater and 50% Transeau (6 parts of water, 3 parts of 90% ethanol, 1 part of 100% formalin). The bags were agitated vigorously to guarantee that all epiphytic microalgae left the substrate. In the laboratory the water of each bag was filtered through a 50 µm net; the filtered water was let standing for several days, after which we collected all the deposit and enough supernatant to fill a 10 mL bottle. We used SEM micrographs to identify species and Sedgewick-Rafter chambers under a Zeiss Primo Star optical microscope to count cells. We have found 21 species belonging to seven genera of epiphytic dinoflagellates. Maximum cell densities of dinoflagellates were higher in Chengue Bay (20109 cells/g wet weight), followed by San Andrés Island (2793 cells/g wet weight), and Barú Island (1032 cells/g wet weight). These densities are low if compared with other Caribbean sites, however, some of these genera as *Prorocentrum*, *Ostreopsis* and *Gambierdiscus*, have been associated with toxins causing the Diarrhetic Shellfish Poisoning and ciguatera. *Prorocentrum lima*, a cosmopolitan species distributed in tropical and temperate seas, characterized in different regions as toxin producers (okadaic acid and its analogs), is the most abundant and frequent of the 21 taxa observed in the epiphytic dinoflagellate community on *T. testudinum*. Cell densities ranged from 232 to 15867 cells/g wet weight, with an average of 3081 cells/g wet weight and maximum values in August and September. These results suggest that *P. lima* population is likely to benefit during periods of low rain, low salinity, high temperature and low inorganic nutrients.

AUTORI

J. Ernesto Mancera-Pineda (jemancerap@unal.edu.co), Brigitte Gavio, Anderson Ruiz, Departamento de Biología, Facultad de Ciencias, Universidad Nacional de Colombia, Ciudad Universitaria, Bogotá, Colombia
Natalia Arbelaez, Centro de Estudios en Ciencias del Mar, CECIMAR, Universidad Nacional de Colombia, sede Caribe, Santa Marta, Colombia; Instituto de Investigaciones Marinas y Costeras, INVEMAR, Santa Marta, Colombia

An efficient and selective phycoerithrin extraction method from macroalgae

A.A. Sfriso, M. Gallo, F. Baldi

The red seaweeds are fast colonizers of shallow waters in the Venice Lagoon and dwell on the bottom of turbid waters where other seaweed production is limited. Their growing abilities in these environments are related to the high content of phycobiliproteins that allows to convey more efficiently a broader part of the light spectrum energy to the photosynthetic system. Research in the last years focalized the attention on purification protocols for these pigments which are powerful antioxidants and extremely fluorescent non toxic proteins with an increasing role in biomedical and biotechnological applications. Little attention has been given to an effective quantification of these fluorophores in macroalgae. A fast and selective method for total phycoerithrin extraction and determination has been set up for a routine investigation in algal samples. A pestle mashing technique with a freeze-thawing cycle and the use of EDTA 1mM (not buffered) with an extraction time of 24 hours was the best choice to extract only the phycoerithrin biliprotein pigment with a 95-98% yield, as confirmed by solid fluorescence. This is currently the most selective and efficient method for phycoerithrin extraction. The role of pH and buffer concentrations on the different biliproteins extraction has been confirmed.

AUTORE

Andrea A. Sfriso (asfriso@hotmail.it), Michele Gallo, Franco Baldi, Dipartimento di Scienze Molecolari e Nanosistemi, Università Ca' Foscari Venezia, Via Torino 155, 30172 Mestre-Venice, Italy

Key points for cellulase conversion of cellulose to glucose in *Chaetomorpha linum* from the Orbetello lagoon

A. Papini, U. Bardi, B. Di Vico, C. Gonnelli, L. Lazzara, C. Nuccio, C. Tani, P. Di Falco, R. Ballini

The filamentous macroalga *Chaetomorpha linum* (O.F.Müller) Kützing (Cladophoraceae) increased its abundance in the Orbetello lagoon during the last ten years. For this reason it is currently necessary to collect tons of the alga to reduce its impact on the lagoon environment. The presence of *C. linum* in coastal ecosystems is very common in Europe and the abundance of the biomass produced by this alga already suggested the use of it for industrial applications such as biofuel production (Bastianoni et al. 2008). Since the main part of the dry mass obtained from *Chaetomorpha* dessication is composed of cell walls cellulose, we tested a process of extraction of glucose from cellulose with the use of the enzyme cellulase. This enzyme is not able to convert the whole cellulose biomass, since it cannot cleave cellobiose (Drissen et al. 2007). We produced a model showing that this enzyme is theoretically capable to convert to glucose about one third of the biomass, while the rest would remain in solution as cellobiose. One of the main concern with cellulose conversion is the pretreatment preliminarily to the following enzymatic digestion (Schulz-Jensen et al. 2013). We tested various pretreatment processes such as: autoclaving at high temperature and pressure to partially demolish the biomass and filtering (or not) after the autoclave step. We showed that autoclave step increased the successive reactions, but not by filtering, since filtering would reduce the total glucose yield. A microscopy analysis showed that entire fragments of the alga thallus were still present in the last solution, hence suggesting that the preliminary step for algae grinding should be improved in order to gain better access to the internal cellulose wall by the lytic enzyme (only cellulase in our investigation).

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AUTORI

Alessio Papini (alpapini@unifi.it), Cristina Gonnelli, Luigi Lazzara, Caterina Nuccio, Corrado Tani, Pietro Di Falco, R. Ballini, Dipartimento di Biologia, Università di Firenze, Via Micheli 1-3, 50121 Firenze, Italy
Ugo Bardi, B. Di Vico, Dipartimento di Chimica, Università di Firenze, Via della Lastruccia, 3-13, 50019 Sesto Fiorentino, Firenze, Italy

Freshwater coralline *Pneophyllum cetinaensis*: osmotic adaptability across salinity gradients

S. Kaleb, A. Žuljević, F. Vita, A. Falace

The first coralline alga from a freshwater environment has been recently found on the Cetina River (Croatia), to 300 m above the sea level (75 km from the river mouth) and has been described as *P. cetinaensis* sp. nov. The marine-freshwater transition most probably occurred during the last glaciation. The brackish-water ancestor was probably an opportunistic wide tolerant taxa, preadapted to osmotic stress and rapid changes in water salinity and temperature.

To test *P. cetinaensis* osmotic adaptability and its recover capability from salinity stress two trials were set up: i) *P. cetinaensis* samples were maintained for 32 days at three salinity conditions: < 0.5, 12 and 35 PSU; ii) after 16 days at < 0.5, 12 and 35 PSU, the water was replaced with Cetina river freshwater and algae were cultivated in the recover condition for 16 days. Spore germination and development in different salinity conditions was also assessed.

While at 35 PSU the thalli did not manage to survive nor to recover, algae exposed to 12 PSU showed evidences of stress, but were vital throughout the trial. Moreover, they recovered from hyperosmotic stress displaying ultrastructural changes. Spore adhesion and germination disc development occurred only in the freshwater condition.

AUTORI

Sara Kaleb, F. Vita, Annalisa Falace (falace@univ.trieste.it), Department of Life Science, University of Trieste, Via L. Giorgieri, 10, 34127 Trieste, Italy

Ante Žuljević, Institute of Oceanography and Fisheries, 21000 Split, Croatia
