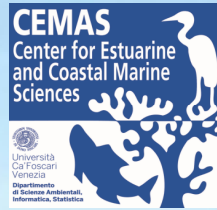




Università  
Ca' Foscari  
Venezia



*Società Botanica Italiana*

*Gruppo di Algologia  
Riunione Scientifica Annuale*

**Auditorium Santa Margherita**

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**Col Patrocinio di**





**Società Botanica Italiana**  
**Gruppo di Algologia**  
**Riunione Scientifica Annuale**

**Venice, October 18<sup>th</sup> -19<sup>th</sup>, 2013**

<b>Friday</b>	<b>October 18<sup>th</sup></b>
<b>08.30</b>	Participant registration
<b>09.00</b>	Meeting Opening and Welcome Greetings
<b>09.10</b>	Invited talk <b>AlgaeBase: the evolution of a web resource.</b> <i>Michael Guiry, Wendy Guiry</i>
<b>09.45 – 11.00</b>	<b>Microalgae: Chlorophyceae</b> Chairman: Neil T.W. ELLWOOD
<b>09.45</b>	News from Antarctica: another group of microalgal strains divorce the old genus <i>Scenedesmus</i> (Scenedesmaceae, Chlorophyta). <i>Katia Sciuto, Carlo Andreoli, Elie Verleyen, Isabella Moro, Nicoletta La Rocca</i>
<b>10.00</b>	Ketocarotenoid production by a new Antarctic Scenedesmaceae strain. <i>Tomas Morosinotto, Isabella Moro, Anna Segalla, Nicoletta La Rocca</i>
<b>10.15</b>	Biomass production and nutrient removal of <i>Scenedesmus</i> sp. under diverse culture conditions. <i>Lorenza Rugnini, Neil T.W. Ellwood, Laura Bruno</i>
<b>10.30</b>	Risposte morfologiche di ceppi di <i>Scenedesmus acutus</i> a diverse concentrazioni di cromo <i>Davide Cozza, Nunzia Maradei, Anna Torelli, Ida Perrotta, Corrado Zanni, Matteo Marieschi, Radiana Cozza</i>
<b>10.45</b>	<i>Marco Favaro</i> - Comune di Venezia, Osservatorio della laguna e del Territorio
<b>11.00 – 11.30</b>	Coffee break

<b>11.30 – 13.00</b>	<b>Microalgae: diatoms</b> Chairman: Giorgio SOCAL
<b>11.30</b>	Rejuvenating aquatic bloom-forming protists: Genetic and microscopic observations of environmentally triggered auxosporulation in the centric marine diatom <i>Skeletonema marinoi</i> . <i>Anna Godhe, Anke Kremp, Marina Montresor</i>
<b>11.45</b>	Coupling biomaterials and silica nano-structures in diatoms. <i>Alessandra Antonucci, Damiano Salvatori, Francesca Lamastra, Ilaria Cacciotti, Francesca Nanni, Sonia Melino, Mario De Stefano, Roberta Congestri</i>
<b>12.00</b>	Identificazione molecolare "Whole-Cell FISH" di diatomee tossiche del genere <i>Pseudo-nitzschia</i> : applicazione su campioni ambientali. <i>Luisa Ruffolo, Davide Cozza, Diana Sarno, Isabella Percopo, Radiana Cozza</i>
<b>12.15</b>	A revision of diatom genus <i>Diploneis</i> Ehrenberg ex Cleve, with the description of two new species <i>Chiara Pennesi, Michel Poulin, Christopher Lobban, Andrea Caputo, Tiziana Romagnoli, Cecilia Totti</i>
<b>12.30</b>	Study of Diatoms and stomatocysts of algae Chrysophyceae in the sediments of Lake Colbricon Inferiore (East Trentino) <i>Roberta Bari, Michele Zannoni, Andrea Lami, Simona Musazzi, Renata Trevisan</i>
<b>12.45</b>	Two new cryptic species of the genus <i>Pseudo-nitzschia</i> H. Peragallo in H. & M. Peragallo, <i>P. autumnalis</i> sp. nov. and <i>P. arctica</i> sp. nov. <i>Diana Sarno, Sergio Balzano, Pricillia Gourvil, Anna Italiano, Nina Lundhölml, Isabella Percopo, Maria Valeria Ruggiero, Raffaele Siano, Anna Tammilehto, Daniel Vaultot, Adriana Zingone</i>
<b>13.00 – 14.00</b>	Lunch
<b>14.00 – 15.45</b>	<b>Macroalgae diversity and distribution</b> Chairman: Ester CECERE
<b>14.00</b>	Biodiversity and spatial variability of macroalgal assemblages on biogenic reefs in the northern Adriatic Sea. <i>Annalisa Falace, Daniele Curiel, Sara Kaleb, Chiara Miotti, Enric Ballesteros</i>
<b>14.15</b>	Macrophyte Biodiversity in four stations along the Northern Adriatic Sea. <i>Alessandro Buosi, Chiara Facca, Adriano Sfriso</i>
<b>14.30</b>	Macrophytes and Ecological Status in Transitional Systems: turbid waters and trophic conditions. The Veneto and Emilia-Romagna lagoons as study case. <i>Adriano Sfriso, Chiara Facca, Alessandro Buosi</i>
<b>14.45</b>	Historical changes in fucoid seaweed richness in the Gulf of Naples. <i>Maria Cristina Buia, Antonia Chiarore, Martina Mulas</i>
<b>15.00</b>	Identification of Ulvaceae (Chlorophyta) along the Italian coasts by means of the DNA barcoding tool. <i>Simona Armeli Minicante, Antonio Manghisi, Giuseppa Genovese, Marina Morabito</i>

<b>15.15</b>	Updating of the <i>Grateloupia turuturu</i> (Rhodophyta, Halymeniales) population status in the TWS Mar Piccolo of Taranto. <i>Ester Cecere, Roberto Carlucci, Giuseppe Portacci, Antonella Petrocelli</i>
<b>15.30 – 16.15</b>	<b>Cyanobacteria</b> Chairman: Roberta CONGESTRI
<b>15.45</b>	A new filamentous cyanobacterium from the Euganean Thermal District (Padova, Italy). <i>Maria Alessia Fuiano, Nicoletta Rascio, Carlo Andreoli, Roberto De Philippis, Nicoletta La Rocca, Isabella Moro</i>
<b>16.00</b>	Characterization of three biofilm-forming cyanobacteria and their potential in biotechnological applications. <i>Alessandra Gismondi, Francesca Di Pippo, Simonetta Antonaroli, Roberta Congestri, Laura Bruno</i>
<b>16.15 – 16.45</b>	Coffee break
<b>16.45 – 18.00</b>	<b>Macroalgae taxonomy</b> Chairman: Donatella SERIO
<b>16.45</b>	<i>Ulva ardreana</i> : a new species replacing the invalid " <i>Ulva bifrons</i> ". <i>Mario Cormaci, Giovanni Furnari, Giuseppina Alongi</i>
<b>17.00</b>	A census of J.P.L. Dangeard's invalid taxa with proposals to resolve the nomenclatural problems of some of them <i>Michael J. Wynne, Giovanni Furnari</i>
<b>17.15</b>	A species of <i>Parviphycus</i> (Gelidiales, Rhodophyta) new to the Mediterranean Sea. <i>Antonella Bottalico, GaHun Boo, Christian Russo, SungMin Boo, Cesira Perrone</i>
<b>17.30</b>	Thermal pollution and settlement of new tropical alien species: the case of <i>Grateloupia yinggehaiensis</i> (Rhodophyta) in the Venice Lagoon. <i>Marion Adelheid Wolf, Adriano Sfriso, Isabella Moro</i>
<b>17.45</b>	On the occurrence of <i>Uronema marinum</i> (Chaetophorales, Chlorophyta) in the north-western lagoons of the northern Adriatic Sea, Mediterranean Sea (Italy) <i>Adriano Sfriso, Alessandro Buosi, Andrea A. Sfriso</i>
<b>18.00 – 19.00</b>	<b>Member meeting</b>
<b>20.30</b>	Social dinner

<b>Saturday October 19<sup>th</sup></b>	
<b>09.00 - 10.45</b>	<b>Microalgae: Harmful algae events and Long Term Research</b> Chairman: Cecilia TOTTI
<b>09.00</b>	The dinoflagellate <i>O. cf. ovata</i> : a small timely and cost-effective biological factory. <i>Laura Pezzolesi, Silvana Vanucci, Franca Guerrini, Patrizia Ciminiello, Carmela Dell'Aversano, Rossella Pistocchi</i>
<b>09.15</b>	Interannual variability of <i>Ostreopsis cf. ovata</i> blooms along the Conero Riviera (2006-2012) <i>Stefano Accoroni, Salvatore Pichierri, Tiziana Romagnoli, Mauro Marini, Antonella Penna, Cecilia Totti</i>
<b>09.30</b>	Relationships of the harmful benthic dinoflagellate <i>Ostreopsis cf. ovata</i> with microphytobenthos community. <i>Salvatore Pichierri, Stefano Accoroni, Tiziana Romagnoli, Chiara Pennesi, Cecilia Totti</i>
<b>09.45</b>	Ecology of the dinoflagellate <i>Ostreopsis cf. ovata</i> : interannual variability in the Gulf of Naples and future scenarios. <i>Davide Di Cioccio, Maria Cristina Buia, Vincenzo Saggiomo, Adriana Zingone</i>
<b>10.00</b>	Distribution and multiannual trends of potentially toxic microalgae in shellfish farm areas along the Sardinian coast. <i>Anna Maria Bazzoni, Tiziana Caddeo, Silvia Pulina, Cecilia T. Satta, Bachisio M. Padedda, Nicola Sechi, Antonella Lugliè</i>
<b>10.15</b>	The Redentore fish die-off in the Lagoon of Venice: an integrated view. <i>Mauro Bastianini, Fabrizio Bernardi Aubry, Francesco Acri, Federica Braga, Chiara Facca, Adriano Sfriso, Stefania Finotto</i>
<b>10.30</b>	The LTER-MC phytoplankton time series: from research to marine observatory <i>Adriana Zingone, Isabella Percopo, Diana Sarno</i>
<b>10.45 – 11.15</b>	<b>Microalgae: Coccolithophyceae</b> Chairman: Adriana ZINGONE
<b>10.45</b>	First report of <i>Pleurochrysis cf. pseudoroscoffensis</i> in the Gulf of Trieste <i>Marina Cabrini, Federica Cerino, Alfred Beran, Edvino Cociancich, Cinzia Comici, Cinzia Fabbro, Daniela Fornasaro, Annalisa Franzo, Gianmarco Ingrosso, Martina Kralj, Marina Lipizer</i>
<b>11.00</b>	Coccolithophore diversity and distribution in a coastal site of the Gulf of Trieste <i>Federica Cerino, Elisa Malinverno, Daniela Fornasaro, Marina Cabrini</i>
<b>11.15 – 11.45</b>	Coffee break

<b>11.45 – 12.15</b>	<b>Microalgae: Eustigmatophyceae</b> Chairman: Rossella PISTOCCHI
<b>11.45</b>	Biotechnological optimization of light use efficiency in <i>Nannochloropsis</i> cultures for biodiesel production. <i>Giorgio Perin, Stefania Basso, Diana Simionato, Andrea Meneghesso, Caterina Gerotto, Tomas Morosinotto</i>
<b>12.00</b>	Response to different illumination in <i>Nannochloropsis gaditana</i> , a algal candidate for biofuels production. <i>Andrea Meneghesso, Diana Simionato, Tomas Morosinotto</i>
<b>12.15 – 13.15</b>	<b>Macrophytes</b> Chairman: Adriano SFRISO
<b>12.15</b>	Biomass trend of <i>Posidonia oceanica</i> (L) Delile recorded along the central Tyrrhenian sea. <i>Valentina Gnisci, Selvaggia Cognetti, Alessandro Belmonte, Carla Micheli, Flavio Borfecchia, Luigi De Cecco, Sandro Martini, Viviana Piermattei, Marco Marcelli</i>
<b>12.30</b>	Molecular response of <i>Sargassum vulgare</i> to decreased pH in natural acidified waters. <i>Amit Kumar, Francesco Paolo Patti, Anna Palumbo, Maria Cristina Buia</i>
<b>12.45</b>	Genetic variability of <i>Sargassum vulgare</i> along Ischia island. <i>Antonia Chiarore, Francesco Paolo Patti, Maria Cristina Buia</i>
<b>13.00</b>	Sulfated polysaccharides from macroalgae: isolation and preliminary evaluation of antiprotozoal activity. <i>Simona Armeli Minicante, Silvia Michelet, Fabrizio Vitale, Federica Bruno, Germano Castelli, Adriano Sfriso, Marina Morabito, Giuseppa Genovese</i>
<b>13.15</b>	Closing greetings

## AlgaeBase: the evolution of a web resource

**Michael Guiry, Wendy Guiry**

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AlgaeBase has been under construction since 1996, initially as a database of the seaweeds of the north-eastern Atlantic for private use as an *aide-mémoire*. It was extended to the Mediterranean as part of an EU project. Initially programmed in FileMaker, the database was put on line in 1998, but it quickly became apparent that the internet demand for such information outstripped the capabilities of this software. In 2004, it was completely reprogrammed in MySQL, and named AlgaeBase. Although all Irish public funding abruptly ceased in 2008, we have managed to keep the data on-line. In 2012, sponsorship from Ocean Harvest, an Irish company, was received and this will allow us to continue for the present.

AlgaeBase was intended to be an information source on the taxonomy and distribution of all algae, but it quickly became apparent that taxonomy is inseparable from nomenclature and we have been greatly assisted in this by having on-line access to the cards of Paul Silva's *Index Nominum Algarum*. In recent years, we have put a lot of effort into adding PDFs of publications. In this we have been somewhat thwarted by the legal aspects of copyright; technically, we cannot offer PDFs of works before 1923. Nevertheless, we have managed to make available over 5,000 PDFs, many of rare and beautiful items found only in a handful of major libraries worldwide.

Another issue is that of the legal requirements of the *International Code of Nomenclature for Algae, Fungi, and Plants*. Most problematical of all is the provision in Art. 13 for later-starting-point dates for the families *Nostocaceae*, *Desmidiaceae*, and *Oedogoniaceae* (each in the original sense of defined publications). Many authors have seen fit to ignore these provisions. This is not the only article of the Code ignored by authors: many names published are invalid for reasons that some clearly regard as insignificant, or in ignorance of the requirements. In recent years, high-impact on-line journals in particular often completely ignore the necessities of the *Code*. Some of the conventions of taxonomy are also disregarded by authors; for example, the requirement for considering a genus taxonomically in terms of its type species. The considerable extent of this problem is evident in the number of genera that still have no type species designated, although some authors seem to have no issues with describing a new entity without even mentioning its type. Some recent taxonomic proposals are also invalid as the entity proposed included the type of another name.

The proverb "*Homo proponit, sed Deus disponit*" can be applied to taxonomy in that one may be free to make any taxonomic proposals one wishes (provided somebody will publish them validly), but the final arbiter of acceptance is usually some authoritative source that surpasses all others. Essentially, like all the sciences, taxonomy is a web of hypotheses, the acceptance of which is based on the quality of the arguments presented. Unfortunately, AlgaeBase has, for some groups, become such an authoritative source for nomenclatural and taxonomic matters, which it was never intended to be.

## News from Antarctica: another group of microalgal strains divorce the old genus *Scenedesmus* (Scenedesmaceae, Chlorophyta)

**Katia Sciuto<sup>1</sup>, Carlo Andreoli<sup>1</sup>, Elie Verleyen<sup>2</sup>, Isabella Moro<sup>1</sup>, Nicoletta La Rocca<sup>1</sup>**

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Microalgal world is getting more and more consideration for the numerous biotechnological applications in which microalgae can be employed. In spite of this, some authors underline how the biodiversity of these organisms is largely underestimated (Pulz & Gross 2004, Stengel et al. 2011). One of the reason is the high phenotypic plasticity and the consequent existence of several cryptic species, which cannot be detected based only on the classical morphological method.

The genus *Scenedesmus* Meyen (Scenedesmaceae, Chlorophyta) falls within this context, as exhaustively summarized by Krienitz & Bock (2012) who defined it “a nightmare for field ecologists who wish to determine a taxon in a fixed sample under the inverted microscope”. Indeed, the introduction of molecular and phylogenetic techniques in systematics studies revealed several phylogenetic lineages inside *Scenedesmus* and led up to the separation and description of new distinct genera: e.g. *Acutodesmus* (Hegewald) Tsarenko; *Comasiella* Hegewald et al.; *Desmodesmus* (Chodat) An et al.; *Hylodesmus* Eliáš et al.; *Pectinodesmus* Hegewald et al.

The classically used marker for molecular studies on Scenedesmaceae was the 18S rDNA. Although useful for a first phylogenetic identification, the resolution power of this locus to discriminate among species is limited (An et al. 1999, Hegewald & Wolf 2003). A promising molecular marker to solve systematic relationships, not only inside the genus *Scenedesmus*, resulted to be the internal transcribed spacer 2 (ITS2) and studies show how including ITS2 secondary structures improves accuracy and robustness of phylogenetic reconstructions (Keller et al. 2010, Markert et al. 2012). Finally, the *rbcL* and *tufA* genes have been recently proposed as DNA barcoding markers (Hall et al. 2010), as well as they were used to infer phylogenetic relationships inside green microalgal genera (Fučíková et al. 2011, 2013).

Here we focus on a coccoid microalga isolated from the Gondwana Lake, Victoria Land, Antarctica, which, from a preliminary phylogenetic analysis based on the 18S rDNA gene, formed a clade with other Antarctic microalgal isolates (De Wever et al. 2009). Other phylogenetic analyses, using the ITS2 region and the *rbcL* and *tufA* genes, were subsequently performed, including also sequences obtained for 24 Scenedesmacean strains got from international culture collections.

The obtained results led us to ascribe the Antarctic strains, as well as one of the strains got from culture collections for comparison, to a new genus of the family Scenedesmaceae. A polyphasic approach, including morphological and ultrastructural observations, pigment composition analyses, and data on the growth range parameters, was also carried out on the Gondwana strain, which represents the holotype of the type species of the new genus.



## **Ketocarotenoid production by a new Antarctic Scenedesmaceae strain.**

**Tomas Morosinotto, Isabella Moro, Anna Segalla, Nicoletta La Rocca**

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Microscopic algae, have a high ecological importance as they can inhabit all major ecosystems, from cold, Arctic, and Antarctic regions, through extremely alkaline or saline habitats and arid soils. To cope with such competitive environments, these organisms have developed adaptive strategies that result in a significant level of structural-chemical diversity. For this reason extremophilic microalgae are considered a major natural source for a vast array of high-valuable compounds including pigments, fatty acids, polysaccharides, amino acids, minerals, vitamins and a variety of secondary metabolites with potential nutraceutical and pharmaceutical use. Future success in meeting the challenging demands of these products by industries mainly depends on selecting the right algae with relevant properties for specific culture conditions and products.

Within a project on the screening for high-value compound production from all the extremophilic microalgal strains isolated by the research group of Prof. C. Andreoli, we selected an Antarctic isolate for its ability to synthesize antioxidant ketocarotenoids. The synthesis of these compounds, identified by mass spectrometry as cantaxanthin, astaxanthin and its esters and ketolutein, the latter usually not accumulated in microalgae, was also accompanied by the production of large amounts of lipids. This process is part of a photoprotective mechanism activated by the microalga to avoid the risk of photooxidative damage by excess of light. Indeed, the strain, native of the snow fields of the Gondwana Lake (Antarctica), is exposed to low temperature and irradiance most of the year, but can experience higher photodamaging light intensities in summer during snow and ice melting. Our interest in this isolate derived also from the fact that it resulted to belong to Scenedesmaceae, a family receiving increasing attentions in biotechnology due to the rapid growth rate, the ability of oil accumulation and the use in wastewater and waste treatment of some of their species. Considering the commercial high-value of ketocarotenoids we have hypothesized a possible use of this microalga as biotechnological resource.

In the presented study we have tested various algal growth conditions, including different culture media, temperatures (from 4 to 25°C) and light intensities (from 10 to 100  $\mu\text{E}$ ), in order to obtain a trade-off between massive growth and high content of the desired metabolites. We also investigated the effects of static, mixed and atmospheric or 5%  $\text{CO}_2$  air filled culture maintenance. In all the considered conditions we analyzed morphology, ultrastructure, lipid accumulations (nile red staining), chlorophyll and carotenoid content, carotenoid profile (HPLC) and photosynthetic activity (PAM). Unexpectedly, considering its origin, the microalga showed a faster growth rate at higher temperatures. The best biomass production (1,7 g(DW)L<sup>-1</sup>) was obtained at 22°C in stationary phase of mixed cultures at 35  $\mu\text{E}$  while the best ketocarotenoid/biomass ratios was achieved at the same temperature but at the light intensity of 100  $\mu\text{E}$ . Not more positive results were gained with  $\text{CO}_2$  enriched medium.

## **Biomass production and nutrient removal in *Scenedesmus* sp. under diverse culture conditions**

**Lorenza Rugnini<sup>1</sup>, Neil Thomas William Ellwood<sup>2</sup>, Laura Bruno<sup>1</sup>**

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Algal-based biotechnology has been investigated for wastewater treatment over the past few decades. Human activities has greatly increased the input of nitrogen and phosphorus into bodies of water. This input induces eutrophication and causes deterioration in natural water quality. Microalgae remove N and P from water mainly by uptake into algal cells. The advantages of using algae for that purpose include: the low cost of the operation, the possibility of recycling assimilated nutrients into algae biomass as a fertilizer, and the discharge of oxygenated effluent into the water body.

The nutrient removal performance of the green microalga *Scenedesmus* sp. at different phosphorus concentrations, was investigated along with the uptake rate. Biomass production of 112 and 110 mg DW L<sup>-1</sup> was obtained in *Scenedesmus* sp. grown for 240 hours in artificial wastewater at P concentrations of 2.70 and 2.55 mg L<sup>-1</sup>, respectively. In artificial wastewater enriched with 17.38 mg P L<sup>-1</sup>, the microalga achieved a final biomass production of 64 mg DW L<sup>-1</sup>. The strain of *Scenedesmus* sp. showed the ability to remove on average 97% of P in batch cultures (50 mL volume) when the initial concentration was between 0.28 and 2.55 mg P L<sup>-1</sup>. At higher P concentrations (10.52-17.38 mg L<sup>-1</sup>), the removal efficiency of *Scenedesmus* sp. (less than 30%) was compared to that of the cyanobacterium *Phormidium autumnale*, known to have rapid P uptake and growth under elevated nutrient concentrations. In batch culture at 10.52 mg P L<sup>-1</sup>, *P. autumnale* showed a biomass production (404 mg DW L<sup>-1</sup>) and a P removal (53%) much greater than *Scenedesmus* sp. (78 mg DW L<sup>-1</sup>; 37% P-removal). However, a pilot-experiment in a tubular photobioreactor (100L) was conducted and over 95% P removal was achieved by *Scenedesmus* sp. at 16 mg P L<sup>-1</sup>, after 120 hours and 97% by the end of the experiment (240 hours). Under all tested conditions and culture systems, the uptake rates were evaluated. The highest values of uptake rates were reached within 72 h from the onset and then a drastic decrease was observed when the stationary phase of growth was reached.

Based on these results the green microalga *Scenedesmus* sp. showed an excellent phosphorus removal ability.

Integrated tertiary algal system could be used for wastewater treatment and bioremediation to polish waters by the removal of nutrients and especially phosphorus from industrial, municipal and agricultural wastes. Since the economic production of algal biomass requires significant inputs of nutrients, it would be possible to offset water treatment costs by utilising algae in tertiary processes and harvesting excessive biomass for the production of biofuels or valuable secondary metabolites.

## **Risposte morfologiche di ceppi di *Scenedesmus acutus* a diverse concentrazioni di cromo**

**Davide Cozza<sup>1</sup>, Nunzia Maradei<sup>1</sup>, Anna Torelli<sup>2</sup>, Ida Perrotta<sup>1</sup>, Corrado Zanni<sup>2</sup>, Matteo Marieschi<sup>2</sup>, Radiana Cozza<sup>1</sup>**

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La comprensione dei meccanismi di come e perché le diverse specie di organismi siano differentemente sensibili e/o tolleranti alla presenza dei metalli pesanti è di grande interesse anche per i risvolti applicativi di queste ricerche nel settore del biomonitoraggio e del biorimedio. Per quanto riguarda le microalghe, le ricerche eco-tossicologiche sono in momento di forte espansione e, sebbene sia riconosciuto che le microalghe presentino meccanismi costitutivi di rimozione di metalli analoghi a quanto avviene nelle piante, i processi molecolari e cinetici che riguardano i siti di legame dei metalli alla superficie delle microalghe, l'eventuale ingresso e la successiva detossificazione, sono ancora poco conosciuti. Test di tossicità condotti su una popolazione di *Scenedesmus acutus* (Chlorophyceae) con concentrazioni sub-letali di cromo esavalente (CrVI) hanno permesso di isolare un ceppo tollerante al cromo [1]. Tale ceppo cromo-tollerante (Cr-t) costituisce un ottimo modello per studiare i meccanismi di detossificazione e tolleranza (ed eventualmente dell'accumulo) del metallo in questa microalga. In questo studio riportiamo le risposte morfologico-ultrastrutturali del ceppo Cr-t, confrontato con il ceppo non tollerante (wilde type, Wt) dopo test di tossicità con CrVI alle concentrazioni rispettivamente di 1 mgCrVI/l e 2 mgCrVI/l, per 72 ore. Preliminarmente ai test di tossicità, l'analisi ultrastrutturale dei due ceppi (Wt e Cr-t) posti in coltura senza aggiunta di CrVI (coltura di controllo) evidenzia differenze a carico della parete, che nel ceppo Wt risulta essere più spessa e stratificata. Il test di tossicità con 1 mg Cr(VI)/l per 72h, induce variazioni nell'ultrastruttura soprattutto nel ceppo Wt, che presenta parziale distacco del plasmalemma, iniziali lesioni a livello dei mitocondri e del cloroplasto e aumento dei granuli di amido. In entrambi i ceppi, ma ancora soprattutto nel Wt, si rilevano numerose macchie elettrondense a livello dei vacuoli, indicative del legame tra gruppi fosfato e tracce di Cr [2]. Il trattamento con 2 mg Cr(VI)/l, induce alcune alterazioni anche nel ceppo Cr-t ed accentua il grado di degenerazione a livello intracellulare del Wt; in entrambi i ceppi si evidenzia un citoplasma vescicolato e, relativamente al ceppo Cr-t, la parete sviluppa numerosi prolungamenti. Sono in corso analisi per determinare la quantità e la localizzazione intracellulare del metallo. Complessivamente, le osservazioni ultrastrutturali indicano che la concentrazione 1 mg Cr(VI)/l altera la struttura del ceppo Wt ma non produce alterazioni significative sul ceppo Cr-t; di contro, la concentrazione 2 mg Cr(VI)/l altera in modo significativo l'ultrastruttura di entrambi i ceppi. Questi dati indicano che, già a tempi relativamente brevi di esposizione (72h), *Scenedesmus acutus* attua, in parallelo a risposte molecolari che meritano di essere indagate, chiare risposte morfologiche che, soprattutto nel ceppo Cr-t, sono volte alla tolleranza e/o detossificazione del metallo.

[1] MG. Corradi, G. Gorbi, A. Ricci, A. Torelli, M. Bassi (1995) *Ecotoxicol. Environ. Saf.* **32**: 12-18

[2] K. Nishikawa, Y. Yamakoshi, I. Uemura, N. Tominaga (2003) *FEMS Microbiol. Ecol.* **44**: 253-259



**Rejuvenating aquatic bloom-forming protists: Genetic and microscopic observations of environmentally triggered auxosporulation in the centric marine diatom *Skeletonema marinoi***

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*Skeletonema marinoi* is an important primary producer in temperate coastal water. It forms benthic resting stages that preserve very well in anoxic sediment and can survive at least hundred years. This allows simultaneous studies of populations from discrete time intervals that can serve as unique material for comparative temporal studies. *Skeletonema* is easy to isolate and maintain in culture, and the survival of monoclonal cultures after single cell isolation is almost 100%. This makes *Skeletonema* a suitable model organism for studying protist population genetics, dispersal ecology and microevolution. However, progress is stalled due to the limited knowledge of its life cycle. We have investigated environmental factors as potential triggers of auxosporulation. To describe the progression of the enlargement process and identify the sexual or asexual origin of the involved stages, induced cultures of Baltic *S. marinoi* were monitored and studied in light, confocal and electron microscopy. We obtained evidence for the production of flagellate gametes and auxospores surrounded by thin scales. Pedigree analyses using microsatellite markers confirmed that environmentally-induced auxospores are formed sexually by both homothallic and heterothallic reproduction.

## Coupling biomaterials and silica nano-structures in Diatoms

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Diatoms have significant biogeochemical and ecological roles and thanks to their unique morphologies impress and inspire scientists from different research fields. A new interdisciplinary area, diatom nanotechnology, has recently developed and lies on the interaction between biology, biochemistry, biotechnology, physics, chemistry, material science and engineering.

In the frame of the ongoing national project “*Photonic and Micromechanic Properties of Diatoms*” - FIRB 2008, we have been surveying frustule capacity of manipulating light in a number of centric and pennate diatoms kept in culture or collected in the field. The interaction of the complex, quasi-ordered patterns of diatom pores with light has been studied using a fundamental biology approach and, more recently, in view of nanotechnology application..

All the data we present have been obtained by collaborating with the Materials Science laboratories of the University of Rome “Tor Vergata” and led us to anticipate some promising perspectives in this field.

Due to the well known frustule optical behavior as a photonic crystal, we have set up an experiment to test siliceous powder, from diatomite earth and acid cleaned frustule from diatom cultures, as a Random Laser tool: a system in which light is multiply scattered, owing to randomness, and amplified by stimulated emission.

A parallel activity concerned the optimization of a method to extract frustule-associated peptides from cultured material, especially in strains producing high amount of exopolymeric substances, that ultimately hamper collection of peptides from diatom biosilica structures. These peptides, known as silaffins, possess interesting silica-binding properties and, among others, scaffolding potential for tissue regeneration.

This investigation was made possible by the use of high-performance equipment such as Confocal Laser Scanning Microscope (CLSM) with Spectral Analysis, Scanning Electron Microscope – Energy Dispersive X-ray Analyser (SEM-EDX), Picosecond Pulsed Laser Source and relied on the production of large amount of diatom biomass in intensive culture of at least eight different strains.

## Identificazione molecolare “Whole-Cell FISH” di diatomee tossiche del genere *Pseudo-nitzschia*: applicazione su campioni ambientali

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Il genere di diatomee planctoniche *Pseudo-nitzschia* annovera numerose specie potenziali produttrici di acido domoico, una neurotossina responsabile di una sindrome neurologica nell'uomo nota come ASP (Amnesic Shellfish Poisoning) e di episodi di mortalità massiva della fauna marina. La tossicità può variare tra specie morfologicamente identiche o molto simili e geneticamente diverse (criptiche o pseudocriptiche). Pertanto l'identificazione specifica è indispensabile soprattutto sul piano del monitoraggio precoce di eventuali fioriture. Tecniche molecolari che impiegano sequenze dirette contro l'rRNA ribosomiale costituiscono un valido strumento per identificare e rilevare specie potenzialmente tossiche.

Nel seguente lavoro, applicando la metodologia WC-FISH (Whole Cell Fluorescent In Situ Hybridization) che prevede l'uso di sonde a DNA (oligoprobes) fluorescenti complementari a regioni target dell'rRNA 28S (LSU), è stato effettuato il riconoscimento delle 4 specie criptiche appartenenti al *P. delicatissima* complex presenti in diversi periodi dell'anno nel Golfo di Napoli, i.e. *P. arenysensis*, *P. autumnalis* sp. nov., *P. delicatissima* e *P. dolorosa*. Le sonde sono state impiegate sia su colture unialgali (per la calibrazione metodologica e la valutazione delle specificità attraverso i cross-test) che su campioni ambientali (field-test) raccolti nel Golfo di Napoli in corrispondenza di fioriture di *Pseudo-nitzschia*. I dati di abbondanza delle diverse specie sono stati confrontati con quelli di abbondanza del *P. delicatissima* complex ottenuti con il metodo classico di conteggio al microscopio ottico (Utermöhl).

Gli esperimenti condotti sulle colture hanno confermato la specificità delle sonde dirette verso i quattro cladi testati. I field-test hanno evidenziato una correlazione tra l'efficienza quantitativa della WC-FISH e il numero di specie di *Pseudo-nitzschia* presenti nei campioni. Infatti nel campione estivo, in cui la WC-FISH rileva la presenza della sola *P. delicatissima*, l'abbondanza stimata ( $2.96 \times 10^4$  cell. l<sup>-1</sup>) rappresenta l'89% dell'abbondanza del *P. delicatissima* complex stimata con il metodo Utermöhl. Diversamente, nei campioni autunnali e in quelli di inizio primavera, quando la WC-FISH rileva, in modo variabile, tutti i 4 cladi, la somma delle abbondanze delle singole specie arriva a coprire un valore che va dal 9% al 23% rispetto a quella stimata con il metodo classico. In effetti, esperimenti condotti su campioni artificiali composti da un numero crescente di specie (da 1 a 4) hanno evidenziato che l'efficienza delle sonde specifiche diminuisce all'aumentare del numero di specie presenti. È perciò ipotizzabile che le sonde, avendo sequenze molto simili, competano per i siti target delle diverse specie del complex, legandosi ad essi debolmente in maniera aspecifica e venendo rimosse nella successiva fase di post-ibridazione, con conseguente perdita di segnale. A sostegno di questa ipotesi è stato verificato, sempre in campioni artificiali, che ad incrementi scalari della concentrazione della sonda specifica corrisponda un aumento della percentuale di detection. Questi risultati evidenziano che il metodo WC-FISH risulta poco attendibile in termini quantitativi, ma idoneo a rilevare *in situ*, velocemente e con costi contenuti, la presenza di specie non distinguibili con i tradizionali metodi di osservazione.

## A revision of diatom genus *Diploneis* Ehrenberg ex Cleve, with the description of two new species

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Benthic diatoms represent an important component of aquatic ecosystems, both for their contribution to primary production and their role in the food chain. Approximately 60% of the known diatom genera are represented by benthic species. The genus *Diploneis* together with other biraphid (i.e., *Mastogloia*, *Amphora*) and monoraphid (i.e., *Cocconeis*, *Achnanthes*) genera is highly diverse and quantitatively significant in benthic communities. The systematic of benthic diatoms is still partly unexplored and most of the species are described only by means of historical observations at light microscope. Consequently, in most cases, a revision of their classification and phylogeny is highly recommended.

The genus *Diploneis* is a large group that at present includes 504 taxa, of which 80 have been flagged as taxonomically accepted. The genus includes mainly benthic marine species, with a few freshwater species and until now, it has been reported only as epipelagic. Hustedt (1933) listed 99 species and divided them into seven sections based both on the ecological origin and the morphological features of the valves.

On the basis of an extensive sampling carried out from 2005 to 2011 in different tropical and subtropical areas, we present and describe 12 *Diploneis* species, including two new ones: *D. cerebrum* sp. nov. and *D. crispanti* sp. nov. belonging to the Hustedt's (year) groups V and IV, respectively. Samples were collected from epiphytic diatom communities (seaweeds and seagrasses) in Guam Island (western Pacific Ocean, USA), Siladen Island (Celebes Sea, Indonesia), Sharm el-Sheikh (Red Sea, Egypt) and from epipelagic diatoms (soft sediments) from Florida (Indian River lagoon, USA). Moreover, this study provides novel information on the frustule ultrastructure, detecting two morphological variants of the raphe structure never described until now in this genus. In particular, in *D. cerebrum* the raphe shows externally two straight branches with two central 'flaps', while *D. crispanti* shows a structure called 'rolled flaps' on the raphe ends. Furthermore, the internal valve of *D. cerebrum* has small globular papillae in the internal junctions of the boundary walls of the chambered areolae. Concerning the ecological distribution of *Diploneis*, this genus, previously described in literature as epipelagic, was very abundant also in epiphytic communities. This result may shed light upon the ecology of this genus, which is poorly known.



## Study of Diatoms and stomatocysts of algae Chrysophyceae in the sediments of Lake Colbricon Inferiore (East Trentino)

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Lo studio riguarda l'identificazione ed il conteggio delle Bacillariophyceae e delle stomatocisti delle alghe Chrysophyceae in una carota di sedimento del Lago Colbricon Inferiore (Trentino Orientale) situato ad una quota di 1910 m s.l.m, localizzato sulla catena del Lagorai all'interno del Parco Naturale di Paneveggio – Pale di S. Martino tra le cime del Colbricon e del Cavallazza. Una volta prelevata, la carota è stata analizzata, datata e suddivisa in 79 campioni i quali sono stati trattati per ottenere solamente le valve di Diatomee e le cisti di Chrysophyceae. Un primo lavoro ha riguardato la determinazione e conteggio delle diatomee, queste alghe unicellulari sono ritenute ottimi indicatori biologici che permettono di valutare la vulnerabilità di un ecosistema. La fase di osservazione si è svolta con l'utilizzo di un microscopio ottico con obiettivo ad alto ingrandimento per immersione ad olio (100X), per la determinazione si sono consultate diverse chiavi dicotomiche, mentre per il conteggio si sono seguiti precisi protocolli. Dai dati così ottenuti, utilizzando il programma Craticula 2, si è ottenuto il profilo stratigrafico delle specie. L'analisi delle associazioni di diatomee e il loro profilo stratigrafico hanno messo in luce una loro linearità nel tempo, in particolare si è rilevata una dominanza dei generi *Fragilaria* e *Cyclotella*, con un diverso rapporto tra i due che ha portato quindi a differenziare la carota in tre zone. Il secondo lavoro ha riguardato la determinazione delle stomatocisti delle Chrysophyceae. Molte delle specie di queste microalghe danno origine a statocisti: cisti sessuali durante l'accoppiamento e cisti di resistenza. Nel momento in cui si ripristinano le condizioni ambientali favorevoli esse sono in grado di ritornare allo stato vegetativo o possono essere incorporate nel sedimento diventando delle componenti importanti dei records sedimentari di laghi, fiumi e stagni. Ciascuna statospora presenta caratteristiche morfologiche specifiche: una parete primaria sottile non ornamentata, la successiva deposizione di ulteriori strati di silice da cui si possono formare una parete secondaria, una terziaria anche una quaternaria, un poro, un collare e vari tipi di ornamentazione della superficie esterna quando sono presenti. Le stomatocisti sono state osservate attentamente e conteggiate attraverso l'utilizzo del Microscopio Ottico ad Immersione al 100x; in seguito sono state identificate seguendo come guide: l' "Atlas of Chrysophycean Cysts" Volume I (1995) di K. E. Duff, B. A. Zeeb & J. P. Smol; l' "Atlas of Chrysophycean Cysts" Volume II (2001) di A. N. Wilkinson, B. A. Zeeb & J. P. Smol; l'atlante "Chrysophycean cysts from the Pyrenees" (2001) di Sergi Pla; l' "Atlas of Chrysophycean Cysts of Lake Baikal" (2006) di A. D. Firsova & YE. V. Likhoshway. Le statocisti riconosciute e fotografate sono state confrontate con quelle classificate da questi autori in base alla loro morfologia e alle dimensioni, considerando che ciascuno di essi ha utilizzato una propria numerazione per determinarle.

Si è riscontrato che le cisti prive di ornamentazione o con una ornamentazione semplice sono le più numerose; esse possono rappresentare dimensioni ed ornamentazioni differenti o più o meno sviluppate in base ai vari livelli di maturità da loro raggiunti. Nella numerazione effettuata da Duff, Zeeb, Smol e Wilkinson alcune stomatocisti da loro classificate vengono considerate da Pla come diverse forme di crescita della stessa statociste e non come singole cisti ciascuna propriamente numerata. Le statospore sono spesso associate ai frustoli delle alghe Diatomee, infatti il rapporto C/D (Lotter et al., 1997) viene sfruttato come un importante indicatore nelle ricostruzioni paleoecologiche e paleoambientali.

**Two new cryptic species of the genus *Pseudo-nitzschia* H. Peragallo, *P. autumnalis* sp. nov. and *P. arctica* sp. nov.**

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*Pseudo-nitzschia* H. Peragallo is a world-wide distributed genus of planktonic raphid diatoms which includes several species that produce domoic acid, the neurotoxin responsible of amnesic shellfish poisoning (ASP) in sea birds, marine mammals and humans. After the first ASP event in 1987, caused by *Pseudo-nitzschia multiseriata* (Hasle) Hasle, various studies have focused on this genus, providing new information on several aspects of its ecology, toxicity, distribution and physiology. The increased interest has led to the discovery of a large number of new species, which include numerous examples of cryptic and pseudo-cryptic species. Particularly two large groups of taxa, the *P. delicatissima* and *P. pseudodelicatissima* species-complex, include each several species which are distinct based on ultrastructural and/or molecular features.

Here we report the finding of two new species which are described based on morphological, molecular (ITS1-5.8S-ITS2, LSU and rbcL) and toxicological data. The two species, tentatively named *P. arctica* (*P. pseudodelicatissima* complex) and *P. autumnalis* (*P. delicatissima* complex), were isolated from the Arctic Sea and the Gulf of Naples, respectively.

The recognition of *P. arctica* and *P. autumnalis* as new species is based on clear molecular differences in all the three phylogenetic markers. However each species is morphologically identical to at least one already known species. *Pseudo-nitzschia arctica* is identical to *P. fryxelliana*, recently described from a far distant area, the Eastern Pacific Ocean (WA, USA), from which it markedly differs from the molecular point of view. On the other hand, *P. arctica* is very closely related to two other polar species *P. granii* (Arctic) and *P. subcurvata* (Antarctic), which however are very different in ultrastructure (e.g. they both lack a central larger interspace) and even in shape. *Pseudo-nitzschia autumnalis* is morphologically identical to other two species that bloom in the Gulf of Naples, *P. delicatissima* and *P. arenysensis*, and, although distinct from the molecular point of view, it is closely related to the two latter species. Interestingly, while the two known species bloom in spring, *P. autumnalis* is only found from August, with a peak in September.

The finding of the two new species poses interesting questions on speciation patterns and evolution in this widespread genus. In the case of *P. arctica*, its phylogenetic relatedness with morphologically distinct species is puzzling, and casts doubts on taxonomic characters so far considered important for separating groups of species. As for *P. autumnalis*, a case of speciation due to isolation by time is proposed.

## **Biodiversity and spatial variability of macroalgal assemblages on biogenic reefs in the northern Adriatic Sea**

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The aim of this study was to increase the knowledge of species composition and structure of macroalgal assemblages growing on rocky outcrops of the northern Adriatic, investigating their spatial variability. These habitats, recently assimilated to the coralligenous, can be considered “island of biodiversity” that rise from muddy seabeds.

Macroalgae were sampled in spring through early summer by scraping quadrats of 2500 cm<sup>2</sup> on the upper horizontal surfaces of 37 rocky outcrops. A total of 173 macroalgal taxa were collected, of which 124 Rhodophyta, 25 Ochrophyta and 24 Chlorophyta. The more abundant taxa were coralline algae (25 taxa) and Peyssonneliaceae (7 taxa), some of which are important coralligenous bioconstructors. The algal assemblages growing on Adriatic outcrops can be considered to have a high species richness as they include a high percentage of the flora of the region.

Despite the great biodiversity a high variability both in the number of species and coverage was detected. The morphology of the outcrops, the distance from the coast and the depth were identified as the main factors accounting for this variability. Both the mean total algal coverage (14.8 %) and encrusting layer coverage (8.0 %) were low when compared to typical Mediterranean coralligenous habitats. Offshore outcrops subject to low turbidity and eutrophication levels had several characteristic Mediterranean coralligenous taxa.

## Macrophyte biodiversity in four areas along the Northern Adriatic Sea.

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Abundance and species composition of benthic macrophytes were investigated in four areas along the coasts of the Northern Adriatic Sea between May 2012 and May 2013. From West to East they are: Lido-Malamocco, Porto Santa Margherita, Sistiana e Punta Salvore (Savudrija, Croatia).

Macrophytes were collected randomly in each station by SCUBA divers at depths ranging from 0 to 8 meters, fixed with 4% formaldehyde and determined in laboratory. In order to obtain the greatest number of species, macrophytes were sampled within a ray of 100 m. In Spring and Autumn, macrophytes were also sampled by a frame of 40×40 cm (1600 cm<sup>2</sup>) according to [1] and the per cent coverage of each species was determined in laboratory by the resuspension method [2]. On the whole, 158 specific and intraspecific taxa (42 Chlorophyta, 22 Ochrophyta and 94 Rhodophyta) were recorded.

Species richness and abundance varied significantly among the stations, in particular Salvore and Lido-Malamocco displayed the highest species richness but macrophyte assemblages were very different. Lido exhibited a high biodiversity, but many species were thionitrophilic and opportunistic whereas Salvore was colonised by highly sensitive taxa and the seagrass *Cymodocea nodosa*. The flora recorded at Sistiana was quite different and the species richness and coverage were lower than at Salvore and at Lido-Malamocco, while Porto Santa Margherita showed only 15 species.

The highest coverage at Salvore was mainly represented by *Cymodocea nodosa*, *Cystoseira compressa*, *C. spinosa* and *C. crinita* while at Sistiana the coverage was partitioned among *Ulva laetevirens*, *Dictyota dichotoma*, *Corallina elongata* and *Halymenia floresii*. Gracilariaceae, Ulvaceae and *Hypnea musciformis* were the most abundant taxa at Lido-Malamocco. At Porto Santa Margherita, where the species richness and coverage was the lowest, the coverage was shared by only 3 species: *Ulva laetevirens*, *Caulacanthus ustulatus* and *Polysiphonia denudata*.

The sampling of some environmental parameters shows that marine vegetation along the northern Adriatic Sea appears to be relatively diversified mainly depending on the hydrodynamics and light and nutrient availability.

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**Macrophytes and Ecological Status in Transitional Systems:  
Turbid Waters and Trophic Conditions.  
The Veneto and Emilia-Romagna lagoons as study case.**

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Since 2009 the transitional areas of the Veneto and Emilia-Romagna Regions in the Northern Adriatic Sea (lagoon of Venice, lagoons and ponds of Po Delta, Pialassa della Baiona pond) have been sampled in spring and autumn to assess the ecological status by the biological element “Macrophytes”, according to the requirements of the European Water Framework Directive (2000/60/EC). During sampling (118 sites in Venice lagoon; 21 sites in Po Delta and 4 sites in Pialassa della Baiona) the most common parameters of the water column and surface sediments have also been collected and processed to determine their relationship with the dominant macrophyte taxa (Ulvales, Gracilariaceae/Solieriaceae, Cladophorales, *Vaucheria submarina*, *Cymodocea nodosa*, *Zostera marina*, *Nanozostera noltii*, *Ruppia cirrhosa*, all other taxa), their biomass and cover and the presence of sensitive taxa. Results highlighted that the worst environmental conditions of these environments were characterized by the absence of macrophytes. Increasing with the ecological status we found the Gracilariaceae/Solieriaceae group and *Vaucheria submarina* whereas Ulvales and Cladophorales characterized environmental conditions a little higher and similar to those associated with the macroalgal biomass and cover. Vice versa, the best ecological conditions were associated with the presence of *Cymodocea*, *Zostera*, sensitive macroalgae and the number of taxa. *Nanozostera* and mostly *Ruppia*, as expected, showed an intermediate ecological status with *Ruppia* generally associated with lower ecological conditions.

The environmental parameters explaining the worst environmental conditions were the concentrations of nutrients in surface sediments, the presence of fine sediments and their porosity and moisture. The following were the concentrations of nutrients in the water column and phytoplankton. The highest conditions were related to the sediment density, the state of oxygenation and the water column transparency.

The index MaQI well reflected this macrophyte and parameter distribution confirming that the worst ecological conditions of transitional areas were associated with the absence of macrophytes and the presence of turbid waters. In this latter case, the dominant taxa were the Xanthophyceae *Vaucheria submarina*, able to grow also inside surface sediments, and terete to broadly flattened macroalgae, such as the group Gracilariaceae/Solieriaceae which, due to their cylindrical habit and the presence of accessory pigments, can grow in less enlightened environments affected by high sediment resuspension and settlement.

## Historical changes in furoid seaweed richness in the Gulf of Naples

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An attempt to measure historical changes in macroalgal diversity has been set up for the Gulf of Naples (South Italy), subject in the last century to intense anthropic pressures, ranging from dense urban settlements to industrial areas located on the coast, and from intense maritime traffic to land runoff. In order to assess the changes in the macroalgal diversity and to quantify the species loss since 1860, a research project started in may 2013 to assess the actual occurrence of *Cystoseira* and *Sargassum* spp., canopy-forming species particularly sensitive to anthropogenic pressure.

From the literature, a total of 19 Fucales have been inventoried, both in upper and lower sublittoral zones. Historical sites along the upper sublittoral zones of the gulf were revisited firstly. Preliminary results confirm a decrease in the diversity of the upper sublittoral furoids due to the loss of seven *Cystoseira* spp. This decrease is more evident in the inner parts of the gulf, where the industrial and urban settlement pressures were the highest. In the Pozzuoli and Naples Bays (the most studied areas) a high number of species was lost in their shallowest fringe: on a total of 13 species, only 6 are still present. All of them occur in the Bay of Pozzuoli but are limited to the west coast of the bay, on the opposite side of the ex-Italsider location. Up to now, *S. acinaroum* is still present in the Gulf of Pozzuoli only, even if a floating specimen has been collected on the Maronti beach, on the south side of the Ischia island. A more dramatic situation resulted for the Bay of Naples: the urban development has deeply changed the coastal line of the whole east side of the bay, where no furoid were recorded. Only scattered findings were recorded on the westernmost point of the bay. Another area where a loss of Fucales has been reported is the north side of the Ischia island: seven species disappeared on this coast characterized by higher population density and marittim traffic. Wherease, on the opposite side of the island, fringes of Fucales, not previously reported, occur in some shallower exposed sites. It is worth noting that, on the south side of this phloegrean island, *C. barbatula*, a species found by Sonder in the Gulf, described by Kutzing in 1860 and since then never been recorded again, has been collected and identified during this campaign. Along the coasts of the Peninsula Sorrentina and Capri island, past records were scattered and mainly focused on deep banks. Our current shallow records for the Capri area represent the first occurrences of Fucales for this island, generally poor studied.

Those preliminary results, even if limited to the upper sublittoral zones, demonstrate that there is a lack of furoid seaweeds in the gulf. However, data on the only presence of Fucales do not give evidence on the status of these habitats, both shallow and deep, and further studies are needed.

## Identification of Ulvaceae (Chlorophyta) along the Italian coasts by means of the DNA barcoding tool

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Species of the genus *Ulva* (Chlorophyta) are among the most common coastal seaweeds worldwide; they have proved as useful model organisms, as indicators of eutrophication, as sources of food and pharmaceutical compounds, as well as biomass for energy production. However, in foliose Ulvaceae simple morphology and anatomy, rampant convergence, remarkable degrees of phenotypic plasticity in response to environmental factors tend to confound attempts at identification and make cryptic introductions difficult to detect.

Aware of the problematic status of the morphologically defined species within the *Ulva* complex, researchers have extensively used molecular methods to clarify the taxonomy of this group. The plastid marker *tufA* has been tested and proposed as a viable marker for species identification among Ulvaceae.

During a census of macroalgal diversity into Venice and Cape Peloro lagoons, the DNA barcodes revealed cryptic diversity within the genus *Ulva*, including non-indigenous taxa of presumed indo-pacific origin.

DNA barcoding demonstrated as a quick and efficient tool for the accurate identification of troublesome species, which is the base for accurate environmental management.





## Updating of the *Grateloupia turuturu* (Rhodophyta, Halymeniales) population status in the TWS Mar Piccolo of Taranto

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The Mar Piccolo of Taranto is the third hot spot for the introduction of alien seaweeds in the Mediterranean Sea after the Lagoon of Thau (France) and the Lagoon of Venice. Since 1986, 13 alien seaweeds were recorded in this basin, showing different behaviours. As an example, *Undaria pinnatifida* (Harvey) Suringar disappeared 10 years later its introduction, even though its population was initially considered as well established. *Hypnea cornuta* (Kützinger) J. Agardh, still present, made two established populations with different increasing densities during 13 years. *Codium fragile* (Suringar) Hariot subsp. *fragile* appeared and disappeared repeatedly during 11 years of observations, never making stable populations.

*Grateloupia turuturu* Yamada was firstly observed in the Mar Piccolo during February 2007. Both gametophytic and tetrasporophytic generations were observed. Thalli of different dimensions, both erect and crustose, were found on natural and artificial substrata, with a marked preference for plastic nets commonly used in mussel farming.

Throughout six years, from October 2008 to July 2013, the number and the blade lengths (in mm) of upright thalli were monthly measured.

Erect thalli resulted to be present with different periodicity: from October 2008 to June 2009, from August 2009 to June 2010, from December 2010 to April 2011, during January and February 2012 and from January to March 2013.

In these periods, the statistical processing of the blade lengths did not show any significant temporal trend, even though they highly fluctuated. On the contrary, if other biometric indicators were used, such as the median blade lengths, the 3<sup>th</sup> quartile lengths and the 95<sup>th</sup> percentile lengths, considering the two macro-seasons summer-autumn and winter-spring, significant differences ( $p < 0.05$ ) in the thalli lengths between the two periods were observed. This indicates that *G. turuturu* shows a different growth pattern between warm and cold period in the Mar Piccolo of Taranto, contrarily to what happens in other localities.

Concerning the mean density values, highly significant differences ( $p < 0.001$ ) in the abundance of *G. turuturu* population between warm and cold period were also observed. However, even though fluctuations in the abundance indices occurred, no significant temporal trend throughout the six year investigated period (2008-2013) was evidenced.

The observed shortening of the presence of the erect thalli indicates a regression of the *G. turuturu* population, which might be destined to completely disappear, as already occurred for *U. pinnatifida*.

The present study confirms, as already hypothesized, that the high summer temperatures of the Mar Piccolo do not allow the persistence of upright blades but only the oversummering of crustose thalli. Probably, this is because *Grateloupia turuturu* is a cold temperate species, native from the Japanese waters of the Pacific Ocean like *U. pinnatifida*.

A continuous monitoring is advisable to know the fate of this alien species and observations are still ongoing.

Research carried out within the framework of the Flagship Project RITMARE

## **A new filamentous cyanobacterium from the Euganean Thermal District (Padova, Italy).**

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The Euganean hills, lying in the south-western area of Padova, are internationally known for the hyperthermal springs, which are exploited, both for water and mud properties, by the numerous SPAs in Abano, Montegrotto, Battaglia, and Galzignano towns.

Studies showed that mud therapeutic properties do not depend only on heat, but are chiefly due to active biological compounds produced by the colonizing microbial communities.

On the mud surfaces, in fact, large populations of microorganisms develop, forming blue-green mats, mainly composed of filamentous and coccoid cyanoprokariotes, many of which are notoriously thermotolerant.

In the past some authors reported, among the filamentous cyanobacteria living in the Euganean District, the presence of a strain that, for the spiralled shape and morphological features, was ascribed to the genus *Spirulina* Turpin ex Gomont.

Recently, during a series of samplings on mats covering the thermal muds, we found a filamentous cyanobacterium, which, at the beginning, could be assigned to the genus *Spirulina*, and we succeeded in isolating and maintaining this strain (named ETS-11) in culture.

In order to establish the taxonomic position of ETS-11 we carried out a polyphasic investigation by morphological, ultrastructural, biochemical and molecular surveys.

The phylogenetic analyses, based on the 16S rDNA gene, showed a clear distinction of strain ETS-11 from the clades including species ascribed to *Spirulina* and *Arthrospira* genera. It grouped, instead, with other cyanobacterial strains, all living in thermal environments.

These preliminary results suggest that ETS-11, together with the other thermal strains, are members of a probable new genus, for which we propose the name *Thermospirulina*.

## Characterization of three biofilm-forming cyanobacteria and their potential in biotechnological applications

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Aquatic biofilms are complex-surface attached communities of both phototrophic (mainly cyanobacteria and microalgae) and heterotrophic microorganisms. There is an increasing interest in understanding how these communities operate, not only for their ecological importance but also for the potential in biotechnological applications (Abed et al. 2009). In fact, phototrophic biofilms offer the possibility to couple wastewater treatment with intensive production of biomass, that is ultimately suitable for the extraction of added value compounds. A number of microalgae and most recently benthic cyanobacteria (Bruno et al. 2012), have been for instance recognized as a feedstock for biofuels, being their capacity of converting carbon dioxide into carbon-rich lipids competitive and more sustainable with respect to agricultural oleaginous crops (Mata et al. 2010). In addition, much attention has recently been paid to algal and cyanobacterial exopolysaccharides by the food, pharmaceutical and biomedical industries.

In this study, three biofilm-forming cyanobacteria, isolated from the dystrophic (hyper-eutrophic) coastal lagoon of Cabras (Oristano, Sardinia), were isolated and characterized by a polyphasic approach, involving microscopy analysis, autoecological investigation and gene sequencing. The isolates were grown separately in batch systems and in a semi-continuous flow incubator, specifically designed for biofilm culture. The biomass obtained was then used for the extraction, quantification and determination of exopolysaccharides (capsular and released fractions) and lipids.

The strains were identified as *Anabaena augstumalis*, *Calothrix* sp. and *Nostoc* sp. and their phylogenetic position investigated. The tested cultures produced variable amount of exopolysaccharides with different ratios of hydrophobic and hydrophilic moieties depending not only on the strain and the polysaccharide fraction (i.e., whether capsular or released), but also on the growth system used. The variety in monosaccharide units composition was up to 11 different monomers with the highest percentage in Glucose (50-60%) for all the strains studied. The lipid fractions obtained had fatty acid composition suitable for high-quality biodiesel, with high proportions of saturated and monounsaturated fatty acids. Incubator experiments also allowed for analysing the nutrient removal ability of the different strains that formed thick biofilm in the incubator system, in particular the nitrogen and phosphorus stripping.

The use of microalgal communities able to grow attached to the substrata is particularly promising for biotechnological application, making the harvesting process simpler and less expensive compared to planktonic algae mostly employed for these purposes.

## ***Ulva ardreana*: a new species replacing the invalid “*Ulva bifrons*”**

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*Ulva bifrons* Ardré was described by Ardré on the basis of some specimens collected at Sezimbra (Portugal), but in the protologue no holotype was designated by the Author. Therefore, according to art. 40.1 of the International Code of Nomenclature for Algae, Fungi, and Plants (Melbourne Code), “*Ulva bifrons*” is an invalid name. Up to date this failure has seemed to have gone unnoticed, so that the binomial “*Ulva bifrons*” has been currently used. The invalid “*Ulva bifrons*” is replaced with the new species: *Ulva ardreana* Cormaci, G. Furnari *et* Alongi. The specific epithet honours Madame F. Ardré, who first found this species. For the designation of the holotype, we first tried to find Ardré’s specimens in PC Herbarium, but no specimens of *Ulva bifrons* were there found. Thus, we looked for specimens collected along Portuguese coast held in PO Herbarium (Herbário, Instituto de Botânica, Univesidade do Porto, Porto, Portugal) and collected as near as to the type locality of “*Ulva bifrons*”. Among these specimens we chose the collection from Douro Litoral, Foz do Douro (Portugal), held in the Herbarium sheet PO 3480.

*U. ardreana* consists of thalli blade-like, up to 2.5 cm high, with denticulate edges and sometime perforated by scattered holes, with denticulate edge too. The two blade sides differ greatly each other both in surface view and in transverse section. In superficial view, one side shows closely arranged polygonal cells, while the other one shows cells with rounded corners, often in pair, loosely arranged and separated by thickened and often stratified cell walls.

In transverse section the dimorphism between the two layers, adherent each other, is evident. The one consists of squarish to slightly vertically elongated cells, the other of vertically elongated cells palisade like arranged. At the edge of the blade, only a wedge-shaped cell separates the two layers. This remarkable dimorphism between the two sides of the blades and in particular the typical difference in both size and shape of the cells of the two layers, distinguishes *Ulva ardreana* from the other blade-like *Ulva* species. In each cell, a single parietal hood-like chloroplast occurs, with 1-2(-3-4) pyrenoids.

*Ulva ardreana* is a rather rare species found in the midlittoral zone epiphytic on different seaweeds like *Cryptonemia seminervis* (C. Agardh) J. Agardh, *Gelidium spinosum* (S.G.Gmelin) P.C. Silva and *Pterosiphonia complanata* (Clemente) Falkenberg as well on *Caulacanthus ustulatus* (Mertens *ex* Turner) Kützinger. Up to data *U. ardreana* (as *U bifrons*) has been reported from Portugal; Atlantic coast of Morocco, Spain and France; Mediterranean Sea; Pakistan.

**A census of J.P.L. Dangeard's invalid taxa with proposals to resolve the nomenclatural problems of some of them**

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Attention is called to the failure of P.J.L. Dangeard to designate Types for essentially all of his new algal taxa proposed after 1957. Designation of Types on or after 1 Jan., 1958 is a requirement of Art. 40.1 of the International Code of Nomenclature for Algae, Fungi, and Plants (Melbourne Code) for valid publication of new taxa of the rank of genus or below. So, numerous names of both genera (about 15) and taxa at specific and infraspecific level (about 60) proposed by Dangeard, are invalid. Some of Dangeard's invalid species were later transferred to other genera or became the purported types of new genera, but all of these subsequent names were also invalid, as were any proposed new species assigned to Dangeard's invalid generic names. The names *Neostromatella* M.J. Wynne, G. Furnari *et* R. Nielsen gen. nov. and *N. monostromatica* M.J. Wynne, G. Furnari *et* R. Nielsen sp. nov. are proposed, replacing the invalid names *Stromatella* Kornmann & Sahling and *Stromatella monostromatica* (P.J.L. Dangeard) Kornmann *et* Sahling, respectively, based on *Ulvella monostromatica* P.J.L. Dangeard nom. inval. The names *Neodangemannia* M.J. Wynne, G. Furnari, A. Kryvenda *et* T. Friedl gen. nov. and *N. microcystis* M.J. Wynne, G. Furnari, A. Friedl *et* O'Kelly are proposed, replacing the invalid names *Dangemannia* T. Friedl *et* O'Kelly and *D. microcystis* (P.J.L. Dangeard) T. Friedl *et* O'Kelly, respectively, based on *Ulvella microcystis* P.J.L. Dangeard nom. inval. The name *Umbraulva dangeardii* M.J. Wynne *et* G. Furnari sp. nov. [= *Ulva olivascens* J.P.L. Dangeard nom. inval. and *Umbraulva olivascens* (P.J.L. Dangeard) G. Furnari nom. inval.] is proposed too. These are "first steps" in resolving the many nomenclatural problems left by Dangeard's failure to designate type specimens.

## A species of *Parviphycus* (Gelidiales, Rhodophyta) new to the Mediterranean Sea

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This study aims at describing a new species of *Parviphycus* (Gelidiellaceae) from the Low Adriatic Sea, supported by both morphological observations and molecular analyses of *rbcL* and *cox1* genes. Species discrimination within the family Gelidiellaceae is notoriously difficult using meaningful diacritical characters for the morphology because plants are the smallest in the Gelidiales and morphologically too variable. The red algal genus *Parviphycus* was established by Santelices to accommodate some species of *Gelidiella* Feldmann & Hamel showing distichous pattern of apical division, axial and periaxial cells forming a distinctive transverse row evident throughout the thallus and tetrasporangia regularly arranged in parallel transverse rows, often in chevrons. Seven species are currently recognized in the genus and three taxa have been recorded from the Mediterranean Sea: *P. antipae* (Celan) Santelices, *P. pannosus* (Feldmann) G. Furnari and *P. felicinii* Perrone & Delle Foglie, recently described from the southeastern Italy.

The new species was collected on the southeastern Apulian coast, at Mola di Bari. Plants were mostly erect and assembled in dense tufts; they bear branched compressed axes, with isodiametric outermost cortical cells irregularly arranged in surface view and apical swollen tetrasporangial sori with tetrasporangia in transverse tiers. From a morphological point of view, the new species is easily discernable from *P. antipae*, but hardly from *P. felicinii* and/or *P. pannosus* when young and in the vegetative status. The characteristics of the outermost cortical cells, branching and definitive size, however, are actually crucial in separating the species. Furthermore, the new taxon was successfully characterized on molecular bases. The *rbcL* tree showed that it was clearly separated from other *Parviphycus* species and formed a clade with *P. felicinii* and *P. pannosus*. As additional significant evidence based on molecular approach, the topology of *cox1* tree was similar to that of *rbcL*.

## Thermal pollution and settlement of new tropical alien species: the case of *Grateloupia yinggehaiensis* (Rhodophyta) in the Venice Lagoon

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To this day the Venice Lagoon has become increasingly affected by the introduction of allochthonous macroalgae mainly coming from the Indo-Pacific area. In fact, in consequence to the recent climate changes and temperature increase, such species could simply find numerous habitats suitable for their growth. One local process that contribute to water temperature changes is thermal pollution. A common cause of thermal pollution is the use of water as a coolant by power plants and industrial manufacturers. Water used as a coolant is returned to the natural environment at a higher temperature, and can affect ecosystem composition.

This is the case of the industrial area of Porto Marghera hosting the Fusina thermoelectric power plant. Annual reports of water quality show that temperatures recorded in different monitoring stations near the power plant are at least 5°C higher than those of other areas, with peaks of over 33 °C. This microclimate has enabled the spread of a new alien macroalgal species: *Grateloupia yinggehaiensis* (Rhodophyta) native of the tropical area of the Hainan province (China).

This species was identified and characterized in 2012 by H.W. Wang and R.X. Luan and is one of the entities of the systematically debated group named '*Grateloupia filicina*' complex.

Originally described from the Gulf of Trieste, Adriatic Sea, *G. filicina* has, subsequently, been considered to have a cosmopolitan distribution. In fact any *Grateloupia* specimens with a similar thallus morphology has been identified as this species. Slightly deviant morphologies led to the description of several varieties and forms from most of the world's oceans. Only recently, De Clerck et al. (2005) through a *rbcL*-based molecular phylogeny indicated the presence of extensive cryptic diversity in the *G. filicina* group. In fact, samples accredited to the same name, but from disjunct geographic areas, often belong to different genealogical lineages.

During a monitoring survey on macroalgal biodiversity in the Venice Lagoon in 2008 we found near the industrial area of Porto Marghera some samples of a new species belonging to the genus *Grateloupia*, never recorded before in this area.

Morphological observations showed green-dark red, foliose thalli attached to solid bedrock by a discoid holdfast with margin of the blades often generating more or less irregular proliferations. A stable population was discovered in the same sampling site in 2013. Molecular analyses were carried out on a series of samples both collected in 2008 and in 2013 using the plastid *rbcL* marker to obtain a correct identification of the specimens. The phylogenetic reconstruction showed that the sequences of the Venetian *Grateloupia* clustered both with two sequences of *G. yinggehaiensis* from China and with other two sequences of the '*G. filicina*' group from Madagascar and Sri Lanka. This is the first finding of the alien macroalga *G. yinggehaiensis* in the Mediterranean Sea.

It is highly probable that this species has been introduced from the Indian Ocean via shellfish transfers. In fact the Hainan province not only exports groupers, spanish mackerels, and tuna but has over 400 hatcheries for the production of scallops, shrimps, and *Tilapia*.

## On the occurrence of *Uronema marinum* (Chaetophorales, Chlorophyta) in the north-western lagoons of the northern Adriatic Sea, Mediterranean Sea (Italy)

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We report on the morphology and reproductive features of the green alien macroalgal species *Uronema marinum* Womersley, a small species of Australian origin, in the north-western Adriatic lagoons in the Mediterranean Sea. It was in summer 2012 that *U. marinum* was identified for the first time in the lagoon of Venice, in the lagoons and ponds of the Po Delta and in Pialassa della Baiona in Emilia-Romagna. Although *Uronema* filaments have already been observed from some years, until now they were not noticed because of their small size and similarity with young stages of other filamentous green algae such as *Ulothrix implexa* (Kützing) Kützing. They differ in determinate growth, curved morphology, basal holdfast, the elongated shape of the cells, a larger diameter towards the apex and their reproductive stages.

In the European Atlantic Coasts, from France to Norway, another species is reported: *Uronema curvatum*, now *Okellya curvata* (Printz) Leliaert & Rueness [1], that has a similar shape, but lacks pyrenoids.

That species, observed throughout the year, is known from shallow subtidal coasts of the south-western Australia, Micronesia and Hawaii and was probably imported in the lagoons of the Adriatic Sea with the seed of the Manila clam *Tapes philippinarum* (Adams & Reeve) introduced for economic purposes.

Its presence was also correlated to some water column and surface sediment parameters and the presence of the most frequent macroalgae which colonize these environments including some NIS (48 sites sampled both in late spring and autumn). *Uronema marinum* showed a very high correlation ( $p < 0.001$ ) with *Gracilaria vermiculophylla* (Ohmi) Papenfuss and significant correlations ( $p < 0.05$ ) with *Agardhiella subulata* (C.Agardh) Kraft et Wynne, *Gracilariopsis longissima* (S.G. Gmelin) M. Steentoft, L.M. Irvine & W.F. Farnham and the concentration of reactive phosphorus. Both *G. vermiculophylla* and *A. subulata* come from the Pacific Ocean [2]. *Uronema marinum*, has the same origins and may have been introduced attached on one or both those species. *Gracilaria vermiculophylla* and *A. subulata* were also significantly correlated with each other ( $p < 0.001$ ).

*Uronema marinum* is currently widespread in the whole lagoon surfaces, but it is particularly abundant in stagnant and confined areas rich in nutrients where Gracilariaceae and Solieriaceae prevail on Ulvaceae due to the high water turbidity.

[1] F. Leliaert, J. Rueness, C. Boedeker, C.A. Maggs, E. Cocquyt, *et al.* (2009) *Eur. J. Phycol.* **44**(4): 487-496.

[2] A. Zenetos, S. Gofas, C. Morri, A. Rosso, D. Violanti, *et al.* (2012) *Med. Mar. Sci.* **13**(2): 328-352.



## The dinoflagellate *O. cf. ovata*: a small timely and cost-effective biological factory

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*Ostreopsis* species are important components of subtropical and tropical marine environment, as well as of temperate areas. In the last years *O. cf. ovata* blooms are among the most intense of the entire Mediterranean coasts, leading to ecological and human health problems, that are associated to the toxins (palytoxin-like compounds) produced by these algal cells. In general, growth dynamics and toxicity of dinoflagellates reflect the physiological status of the organism. So far, there are only few studies concerning the physiological responses of dinoflagellates on different nutrient resources, nutrient uptake, and toxin production dynamics, and they are mainly focused on N-producing toxins algae (e.g. *Alexandrium* spp.). In the present study the cellular production of the main biochemical compounds which are likely involved in the growth and toxicity dynamics of *O. cf. ovata* was investigated from the exponential to the late stationary phase using batch cultures of an Adriatic strain. Cultures were established at two different nutrient levels, corresponding to a five-fold diluted f/2 ("standard culture medium") and to a N,P-limited condition which is comparable to the nutrient concentrations found during the algal blooms in the Adriatic coastal area. Removal of major nutrients from the medium was monitored along with the cell concentration, the biovolume, the cellular elemental composition (in terms of Carbon, Nitrogen, Phosphorus); nutrient uptakes, as well as toxin production rates, were calculated in the different growth periods. Dissolved nutrients resulted totally depleted while cells entered the stationary growth phase and significant differences were observed between the two nutrient conditions. In particular, the content of carbon (C), nitrogen (N), and phosphorus (P) resulted affected by the nutrient availability, as also observed for the toxin production dynamics and their cellular content. The palytoxins amount within the culture was found to increase during the growth, as well as the total and extracellular released polysaccharides and the lipids content, while proteins were mainly produced by cells during the exponential phase. The trend of growth and production of the main cellular compounds in *O. cf. ovata* leads us to hypothesize that the fast growth of this dinoflagellate, associated with the rapid use of environmental resources (nutrients, and phosphates in particular), may be an ecological/adaptive strategy which could favor this organism in the competition with other species.

## Interannual variability of *Ostreopsis* cf. *ovata* blooms along the Conero Riviera (2006-2012)

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*Ostreopsis* cf. *ovata* is a toxic benthic dinoflagellate distributed in both tropical and temperate areas that, in recent years, occurred in the Mediterranean region with increasing frequency. Since 2006, blooms of *O. cf. ovata* occur along the rocky costs of the northern Adriatic Sea. During blooms, toxin analysis revealed a high total toxin content (up to 75 pg cell<sup>-1</sup>), including putative palytoxin and ovatoxins [1]. *Ostreopsis* blooms have been associated with the death of both benthic invertebrates (limpets, sea urchins and mussels) and several macroalgae, and with noxious effects on human health. Toxins were also found in natural mussel tissues which showed clear signal of suffering on their general health status [2].

In this study, we investigated the interannual variability of *O. cf. ovata* blooms in the Conero Riviera (N Adriatic Sea), in terms of temporal trend and bloom intensity, and the potential role of environmental factors (temperature, hydrodynamism and nutrient concentration) on the bloom dynamics. Sampling was carried out in 2006, 2007, 2009, 2010, 2011 and 2012 on several benthic substrata (macroalgae, pebbles, mussels) and in the water column. Cell abundances were expressed as cells g<sup>-1</sup> fw / dw, cells cm<sup>-2</sup>.

Annual maxima on macrophyte samples were observed in late-summer, with abundances of the order of magnitude of 10<sup>6</sup> cells g<sup>-1</sup> fw (corresponding to 10<sup>7</sup> cells g<sup>-1</sup> dw and 10<sup>4</sup> cells cm<sup>-2</sup>). The highest abundance (1.9x10<sup>6</sup> cells g<sup>-1</sup> fw) was recorded in 2012 on *Hypnea musciformis* which, due to thallus morphology, has proven to be a very suitable substratum for *Ostreopsis* development. The temporal trend of *O. cf. ovata* blooms was similar in each investigated year, with the first cell appearance occurring at the end of July/early August, the peak in late-summer (end of September/early October) and the bloom decline at the end October/early November, often in concomitance with prolonged conditions of stormy sea. Exceptionally, in 2011 bloom peak was observed earlier than other years, i.e. at the end of August. Hydrodynamism seems to play a major role in regulating the bloom dynamics of *O. cf. ovata*, especially in terms of maximum abundances reached. The role of temperature is not clear, as blooms mostly developed when temperatures were decreasing, differently from what observed in other Mediterranean areas. However, a triggering role of temperature may be hypothesized for cyst germination, as in experimental conditions we observed that *O. cf. ovata* cyst germination occurs only at T<sub>≥</sub>25 °C. Although a relationship with nutrient concentration (DIN, and phosphate) was not highlighted, N:P ratio values near the Redfield value seem to enhance bloom development, once the temperature threshold values of 25 °C is reached. The early bloom in 2011 could be explained considering that optimal N:P ratio values were recorded earlier than in others years. This suggests that bloom dynamics is affected by several factors which can act synergically.

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[2] S. Gorbi, G.C. Avio, M. Benedetti, C. Totti, S. Accoroni, *et al.* (2013) *Fish. Shellfish. Immun.* **35**: 941-50.

## Relationships of the harmful benthic dinoflagellate *Ostreopsis* cf. *ovata* with microphytobenthos community

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The occurrence of benthic dinoflagellates belonging to the genus *Ostreopsis* have been recorded in both tropical and temperate regions. During the last decade, massive *O. cf. ovata* blooms have occurred in different coastal areas of Mediterranean Sea including the northern Adriatic Sea where have been observed since 2006, typically during the late summer period. This study was carried out during from March to October 2009 in Portonovo (Conero Riviera, N Adriatic Sea), with the aim of investigate the relationships between the *O. cf. ovata* bloom and the entire microphytobenthic community. The site is characterized by a rocky bottom, exposed to wave action and affected by a moderate human impact. Macrophytes (*Dictyota dichotoma*, *Ulva rigida*) and hard substrata (pebbles) were sampled for microphytobenthos identification and counting. Cell abundances were expressed as cells g<sup>-1</sup> fw/dw and cells cm<sup>-2</sup> for seaweeds, cells cm<sup>-2</sup> for hard substrata. Biomass (µgC cm<sup>-2</sup>) was calculated on a biovolume basis.

The results show that microphytobenthic communities were dominated by diatoms, followed by cyanobacteria. Dinoflagellates represented a minor component, in terms of abundance, even during the *Ostreopsis* bloom, while they were important contributors to biomass. Microphytobenthos exhibited a clear seasonal behaviour: diatoms showed their annual maximum in spring (315,776 ± 45,209 cells cm<sup>-2</sup>, 22 ± 7 µgC cm<sup>-2</sup>), while cyanobacteria (filamentous forms attributed to Oscillatoriales) and dinoflagellates in summer (1,041,513 ± 55,190 cells cm<sup>-2</sup>, 5 ± 3 µgC cm<sup>-2</sup> and 11,534 ± 13,486 cells cm<sup>-2</sup>, 44 ± 7 µgC cm<sup>-2</sup>, respectively). During the summer bloom of *O. cf. ovata*, which represented the 99.75% of total dinoflagellates, this group reached its annual maximum representing the 7% of total microphytobenthos abundance and the 88% of total biomass.

Benthic diatom community structure showed a dominance of motile species over the entire study period (mainly *Navicula* spp., *Nitzschia* spp.), followed by erect (*Licmophora* cf. *abbreviata*, *Tabularia* spp.), adnate (*Cocconeis scutellum*), planktonic (*Cylindrotheca closterium*) and tube-dwelling diatoms. All these growth forms showed their maximum in spring while decreased in summer. Considering only the period of *O. cf. ovata* proliferation, motile diatoms showed significantly higher percent abundances in correspondence to bloom peak, suggesting that their life strategy is the most successful in presence of a conspicuous benthic mat, due to the ability of these biraphid taxa to move, which may make them superior competitors for nutrients and light. Moreover, significantly lower values of Shannon diversity index were observed during the *O. cf. ovata* bloom than in the rest of the year, suggesting a negative effect due to both the shadowing of the conspicuous mucous mat and the presence of *O. cf. ovata*, which could exert allelopathic effect toward other microalgae.

**Ecology of the dinoflagellate *Ostreopsis cf. ovata*:  
interannual variability in the Gulf of Naples and future scenarios**

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The phenology and ecology of the benthic toxic dinoflagellate *Ostreopsis cf. ovata* were investigated in the Gulf of Naples (Tyrrhenian Sea) to assess distribution patterns and evaluate factors affecting the development of the blooms. Samplings were carried out during summer/early autumn from 2007 to 2013 in two sites in Naples (Gaiola and Rocce Verdi). In addition, from 2011 six stations in two different hydrodynamic conditions (exposed and sheltered) were sampled in the Ischia Island (Castello Aragonese) along a pH gradient (6.8 - 8.1) produced by natural CO<sub>2</sub> vents, in order to determine future scenarios in the predicted ocean acidification conditions. *O. cf. ovata* abundance peaks in Naples stations showed a marked seasonality over the years, with a bimodal distribution: a first, more intense bloom in July (avg.  $1.73 \cdot 10^5$  cells g<sup>-1</sup> fw) and a second, minor and less constant bloom in late September (avg.  $1.39 \cdot 10^5$  cells g<sup>-1</sup> fw). Seawater temperatures, salinity and nutrient availability do not seem to play a major role in bloom timing and intensity; rather, the effect of light exposure and hydrodynamic conditions likely contribute to the dynamics of *O. cf. ovata* blooms. Results from Castello Aragonese stations showed maximum values in July 2011 at all the exposed stations (up to  $1.15 \cdot 10^6$  cells g<sup>-1</sup> fw) with significant differences from sheltered stations. The acidification gradient did not show significant effects on the distribution pattern, indicating that *O. cf. ovata* is tolerant to a wide range of pH values. Therefore, ocean acidification is not likely to represent a hindrance to the spreading and intensification of its blooms. Differences observed between sheltered and exposed sites rather highlight the role of hydrographic factors, either directly or through their effects on the biota in the two sites. Changes in these factors under the pressure of climate change could be major drivers for long term trends of *O. cf. ovata* blooms.

## **Distribution and multiannual trends of potentially toxic microalgae in shellfish farm areas along the Sardinian coast**

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Italy is the third largest European producer of edible molluscs bivalves, after Spain and France. In Sardinia, which is one of the major producer amongst Italian regions, mussel farming occupies a prominent position, with relevant economic and social interests. The mussel farms are mainly located in coastal and lagoon areas of the provinces of Oristano, Cagliari, Olbia-Tempio and Ogliastra. The management of these areas is strictly dependent on the water quality, guaranteed by a constant monitoring program, required by European legislation (2004/853/EC). It includes the warning detection of potentially harmful algal species (HAS) which can affect human health through the consumption of seafood products. Anyway, these programs are not finalized in the understanding of the patterns of the blooms and of the causes of the arising of the harmful events. In this study, we analyse multiannual data on the geographical distribution and abundance of potentially HAS in 18 Sardinian mussel farming sites. The data mainly derive from Regional monitoring activities. Mann-Kendall test was performed to detect significant multiannual trends in the abundances of the main harmful species.

The results showed the constant detection of HAS (in particular *Pseudonitzschia* spp., present at all the considered areas during all periods of the year) and the detection of a high numbers of harmful Dinophyceae, mainly belonging to the genera *Dinophysis*. Moreover, the detection of *Alexandrium catenella* e *A. minutum* in mussel farming in Olbia and Oristano areas has occurred, respectively, since 2002 and 2006, causing eight cases of PSP positivities events, always in coincidence of modest abundances of the involved species.

Statistical analysis showed the temporal increasing trend of *Pseudonitzschia* spp., *Dinophysis acuminata*, *Prorocentrum lima* and *P. mexicanum* in some study areas, while *A. catenella*, *D. fortii*, *Dinophysis* sp. and *Prorocentrum* sp. decreased significantly during the considered period in different areas. Other species (e.g. *A. minutum*, *Alexandrium* sp. and *D. sacculus*) increased in some areas and decreased in others during the analysed period.

The results highlight as the systematic controls operated in shellfish farming areas can contribute to the general topic on the expansion of toxic algae. Specifically, it can help in identifying on the species which are showing trends in increasing or decreasing in a specific geographical area, helping to find most suitable management practices.

## **The Redentore fish die-off in the Lagoon of Venice: an integrated view.**

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During the 2013 Redentore week-end in the central area of the Lagoon and the centre of Venice a huge die-off involved fishes molluscs and crustaceans of the order of 5 tons of collected organisms in the meantime strong sulphur dioxide emissions pervaded the centre town and the surroundings. The first environmental analysis showed an extensive hypoxic area where macro and microalgal populations were thriving in nutrient rich, warm and low salinity waters. Some algal species already linked with fish die-off were detected, among these *Peridinium quinquecorne* and *Prorocentrum rhathymum*. From the seaweeds side huge biomass of *Ulva*, *Gracilariopsis*, *Gracilaria* and *Agardhiella* developed adding an over sustainable biomass to the ecosystem of the lagoon. The network of sensors of local authorities followed the extension of the event in real time allowing researcher to spot the most critical zones and scheduling appropriate discrete samplings. Satellite images showed that the blooms were intense also in the whole northern Adriatic basin so the usually tidal “refreshing” mechanism didn’t work anymore. From the climatic point of view spring and summer during 2013 have been characterized by large amounts of nutrient rich freshwaters coming from over snowed mountains .

## **The LTER-MC phytoplankton time series: from research to marine observatory**

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Over the last years researchers in the field of marine phytoplankton have been called to provide information and answers on a series of issues which are relevant to societal needs. These include human health, the safe and sustained use of marine resources and the quality of the marine environment. One of the most recent initiatives is the Marine Strategy Framework Directive and the need to define and reach the Good Environmental Status (GES). Long term series of data are a powerful tool to assess the status of the marine environment and monitor its changes under the impact of climate variations and anthropogenic impacts. In the Gulf of Naples, the Long Term Ecological Research station MareChiara (LTER-MC) is sampled since 1984. Phytoplankton data have been collected at this station with the main aim to conduct research on phytoplankton variations over multiple time scales in relation to environmental parameters. Within the National flagship project RITMARE, an effort is presently made to widen the aims of this long term project as to include a more relevant role in relation with societal needs. To this end, a series of activity are ongoing which include a website devoted to the station, a *quasi* real-time delivery of the phytoplankton data collected weekly and the publication of the phytoplankton dataset following good data management practices and quality control procedures. We present here the first results of these activities and discuss related problems and the perspectives of this initiative.

## First report of *Pleurochrysis cf. pseudoroscoffensis* in the Gulf of Trieste

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During the summer 2012, a bloom of a coccolithophore was reported in the inland channel Est-Ovest located in the western coast of the Gulf of Trieste. By the scanning electron microscopy analysis, the coccolithophore was identified as *Pleurochrysis cf. pseudoroscoffensis*, a microalgae never reported in this area. To study this bloom, a weekly sampling programme was carried out from 24 July to 10 September in four stations along the channel. Physico-chemical (temperature, salinity, dissolved oxygen, nutrient concentrations, chlorophyll and pheopigment concentrations, pH, and total particulate carbon and nitrogen) and biological parameters (diversity and abundance of phytoplankton community) were analyzed. Phytoplankton abundance were always above  $10^6$  cells  $l^{-1}$ . At the beginning of the monitoring the community was dominated by *Pleurochrysis cf. pseudoroscoffensis* (80% of the total phytoplankton), while at the end of the sampling period diatoms and phytoflagellates dominated. In the late summer 2013 a similar bloom of *Pleurochrysis cf. pseudoroscoffensis* was again observed in the same area. The absence of currents in that area and the scarcity of rains during the summer probably supported the development of microalgal blooms that caused uneasiness at the yacht basin located in the channel. A periodic programme of environmental and biological monitoring should be required to better manage the anomalous events through tools of mitigation such as systems able to improve the water circulation, to reduce the algal concentrations and to better the ecosystem conditions.



## Coccolithophore diversity and distribution in a coastal site of the Gulf of Trieste

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Coccolithophores are nanoplanktonic microalgae characterized by an exo-skeleton composed of minute calcified plates. They are found in all oceans, from equatorial to sub-polar areas, and under favorable conditions can proliferate in dense and extensive blooms. They are among the main marine primary producers, representing about 15% of the total phytoplankton biomass. They also play an important role in control of climate changes, playing a vital role in the sulfur cycle for the production of dimethylsulfide as well as in the carbon cycle due to the photosynthesis and calcification processes. Recent studies on ocean acidification due to the carbon dioxide increase in the atmosphere have provided evidence on a possible impact on coccolithophore calcification. In the framework of the project MEDSEA (Mediterranean Sea Acidification in a changing climate, EU-FP7, 2011-in progress), it was proposed to deepen the knowledge on the coccolithophore associations at a coastal site in the Gulf of Trieste, for which a long-term time series of data on plankton is available (C1-LTER 1986-present).

Main aims of this study were to assess the contribution of coccolithophores to the phytoplankton community, also in relationship to the available time series for the site, to identify the seasonal distribution of different species from August 2011 to July 2012, and to compare the results of the coccolithophore analyses obtained by different methods (phase contrast and polarized light microscopy and scanning electron microscopy).

Coccolithophores showed a high interannual variability and a typical seasonal pattern, with maxima in the autumn-winter and minima in summer. During the analyzed period, the coccolithophore community was characterized by species adapted to a variety of environmental conditions: in autumn-winter, *Emiliania huxleyi* dominated, followed by *Acanthoica quattrosopina*, *Syracosphaera pulchra* and some minor species (only present in autumn, e.g. *Ophiaster hydroideus*, *Calciopappus caudatus*, *Michaelsarsia adriaticus*); in spring the association was dominated by holococcolithophores and *E. huxleyi*; in summer, *E. huxleyi*, small species belonging to *Syracosphaera* genus, *Rhabdosphaera clavigera* and holococcolithophores were present. The comparison between the total coccolithophore abundances obtained at phase contrast microscope with the Utermöhl method and those obtained at polarized light microscope, revealed good correspondence in winter and autumn, less in spring. The polarized light microscope counting indeed allowed to recognize very small and/or less calcified species that would otherwise be lost with the Utermöhl method, improving the assessment of the coccolithophore biodiversity.

## **Biotechnological optimization of light use efficiency in *Nannochloropsis* cultures for biodiesel production.**

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New renewable energy sources are highly needed to compensate exhausting fossil fuels and reducing greenhouse gases emissions. Microalgae have reemerged as potential next-generation feedstock for biofuels and they are considered very promising on the long term, since they have a potentially high productivity per area and they can be grown on marginal land without competing with food crops. Our work is focused on the seawater microalgae of the *Nannochloropsis* genus, which combine a fast growth rate with a strong accumulation of lipids.

Solar radiation provides all energy supporting algae growth and lipids production: for this reason the available radiation must be exploited with the highest possible efficiency to optimize productivity and make their cultivation on a large scale competitive. Investigation of the molecular bases affecting light use efficiency is thus seminal to elucidate the connection between light and lipid production and cultures productivity.

To this aim we first characterized the functional properties and the biochemical composition of the photosynthetic apparatus of *N. gaditana*. This work allowed evidencing a conserved organization of photosystems in different organisms with antenna proteins of Photosystem I tightly associated with the reaction center and while in Photosystem II antennas are loosely bound. Such a flexible association in the latter is correlated with the requirement of a regulatory response which requires a dynamic association with the reaction center.

Light use efficiency is influenced not only by the composition of the photosynthetic apparatus but also from several regulatory responses which can be differently activated. For this reason we analyzed the *Nannochloropsis* genome and identified genes potentially involved in regulatory mechanisms of photosynthesis. We selected few of these genes as targets for genetic manipulation in *Nannochloropsis* exploiting the ability of this organism to perform high-efficiency homologous recombination. Once strains with altered regulation of photosynthesis are available they will allow investigating their influence in cultures light use efficiency, identifying strategies for an increase of *Nannochloropsis* productivity.

## **Response to different illumination in *Nannochloropsis gaditana*, a algal candidate for biofuels production.**

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Solar light provides energy to support all metabolism of photosynthetic organisms but if absorbed in excess illumination may easily drive the production of reactive oxygen species and damage of the photosynthetic apparatus. Photosynthetic organisms evolved the ability of responding to variations in light intensity and they maximize the light harvesting efficiency to support photosynthesis when solar radiation is limiting while dissipating any energy in excess. Changes in light intensity may occur with different timescales spanning from seconds, when clouds or the overlaying canopy interfere with the incident sunlight, up to weeks, as with seasonal changes. For this reason plants evolved multiple protection mechanisms, with different activation timescales, enabling response to the short- and long-term variations in environmental cues.

In this work the responses of seawater microalgae *Nannochloropsis gaditana* to different light intensities have been analyzed both assessing fast protection mechanisms such as the Non Photochemical Quenching (NPQ) and xanthophyll cycle as well as the long term acclimation to different light intensities. Differences and similarities with respect to response observed in other algal species can provide information on how these responses adapted during algae evolution.

This work will also provide valuable information for the optimization of light use efficiency in algae large scale cultivation systems, an interesting perspective for species such as *Nannochloropsis gaditana*.

## **Biomass trend of *Posidonia oceanica* (L) Delile recorded along the central Tyrrhenian sea**

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Vegetative growth events of *Posidonia oceanica* (L) Delile were observed along the Tyrrhenian coasts, during the monitoring activities carried out in late spring 2013. Twenty different sites were investigated with lepidochronological, biometric and RAPD genetic analyses in the shoreline upper and below Civitavecchia Port (from S. Severa to Marina di Tarquinia)

Estimation of primary production was carried out using lepidochronological data of orthotropic rhizomes collected at random by SCUBA diving at the 20 sites. Meadow density (number of shoots per square meter) was estimated *in situ* using a 40X40 cm quadrat. In all the measurement taken for each site, meadow density showed lower values than those found in previous monitoring studies. In particular in the vicinity of a power plant of Tor Valdaliga, the values of density and cover of *P. oceanica* meadows, were significantly higher than those relative to the other stations situated along the coast.

Leaf biomass trend (g/leaf/shoot/m<sup>2</sup> DW) showed a very variable set of data, probably due to the different anthropogenic impacts to which the plants are subjected. The results showed that the development of *P. oceanica* growth could be influenced by the typology of different substrates.

The species *P. oceanica* can maintain its ecological status by vegetative reproduction and facilitative interaction of clone integration with fixed mutations.

This coastal zone studied represents one of the most rapidly changing environments, mainly caused by anthropogenic activities of industry, urbanization and tourism, as well as natural process of hydro-dynamism and coastal erosion.

## Molecular response of *Sargassum vulgare* to decreased pH in natural acidified waters

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Fucoid algae are important primary producers and key species structuring the biodiversity in rocky intertidal zone. Increasing global temperature and changing water chemistry due to acidification emerged as new stress in the marine ecosystem. The ability of these species to cope up harsh shallower coastal environment determines the success of particular species in given eco-region. Though we have reasonable understanding of physiological process to the different stress, such as photosynthetic efficiency, molecular basis of responses and tolerance is largely unknown. In the present study, using the acidified area around Castello Aragonese (Ischia) as a natural laboratory, we are trying to understand the tolerance capability of *Sargassum vulgare* to changes in water pH conditions, thanks to a gradient of CO<sub>2</sub> gas emissions.

Populations from acidified and normal waters (San Pancrazio, Ischia Island) were genetically compared. Based on the previous genomic information on several brown algal species, a set of genes responsible for photosynthesis, energy metabolism and stress responses were selected for gene expression studies. Unambiguous primers were designed for those genes and PCR was carried out. The amplified products were sequenced and analyzed to check for correct amplification. Using the sequences generated in this study, species specific pair of oligos were designed and used in real time qPCR.

Preliminary results suggest that photosynthetic genes shows adaptation of *Sargassum vulgare* at molecular level in acidified waters. To get a detailed understanding on tolerance mechanism, a study related to nitrosative stress responses as well as role of secondary messenger (Nitric oxide) in regulation of important genes, is undergoing. In the future, the study will be carried out to determine the genetic connectivity of *Sargassum vulgare* at different spatial scale, from local to basin level. Combining genetic, biochemical and molecular informations, we expect to understand the adaptation and tolerance of *Sargassum vulgare* and scale up the information to further fucoid seaweeds, as *Cystoseira* spp.

## Genetic variability of *Sargassum vulgare* along Ischia island

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*Sargassum vulgare* (C. Agardh, 1820) is a brown algae characteristic of infralittoral rocky shore from surface to about 40 meters depth, it is a cosmopolitan species distributed in the Mediterranean sea except of northern Adriatic and Gulf of Lion. In this study we analysed the distribution of haplotypes of some *Sargassum vulgare* populations along the coasts of Ischia island. Three sampling zones has been chosen: Castello Aragonese, Lacco Ameno and S. Pancrazio. Castello Aragonese is characterized by a natural CO<sub>2</sub> emissions that lowers the pH of surrounding water creating an acidic environment whereas other two zones are characterized by normal pH. We used three different molecular markers for analysing genetic variability: PsbA, a plastidial gene, ITS a nuclear gene and Cox3 a mitochondrial gene. PsbA gene was chosen because it codify for the D1 protein that is very important for photosynthesis, moreover it is largely used for specific characterization (highly conserved and little variable gene). ITS 1 e 2 (*Internal Transcribed Spacers*) are non functional region of ribosomal RNA placed among structural rRNA regions. These genes are largely used for molecular phylogeny studies because of the presence of repeated copies and the high mutation rates on these fragments. Finally Cox3 gene is widely used in fine genetic studies at interspecific and intrapopulation levels. Techniques involved in this studies are DNA extraction, PCR amplification of target gene and their sequencing. Finally the sequences were analysed using bioinformatic softwares for understanding the phylogenetic relationship among individuals of three zones by construction of networks and cladograms. Results indicate that instead of geographical boundaries, *Sargassum vulgare* populations off Ischia island show genetic flux of informations. Nevertheless Castello Aragonese populations outdistance from others because of the higher genetic mutations and it establish detached haplotypes characteristic of acidic site.

## **Sulfated polysaccharides from macroalgae: isolation and preliminary evaluation of antiprotozoal activity**

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Marine algae are a great sources of natural products that play an invaluable role in the drug discovery process. Many reports have been published about isolated compounds from algae with biological activity, demonstrating their ability to produce metabolites different from those found in terrestrial species, with high complexity and unlimited diversity of pharmacological and/or biological properties. Among the natural compounds, the sulphated polysaccharides produced by green, brown and red seaweeds are numerous and with various structures. They are widely used in food and cosmetic industries, but also acknowledged as endowed with a rather low toxicity and numerous biological activities, including antiviral, anticoagulant, anti-tumoral, antimetastatic and anti-inflammatory effects, worthwhile for clinical uses.

In this study, sulphated polysaccharides were extracted from different species of macroalgae: *Chaetomorpha linum* (O.F. Müller) Kützing, *Gracilaria bursa-pastoris* (S.G. Gmelin) P.C. Silva, *Gracilaria viridis* Sfriso, Wolf, Sciuto, M. Morabito, Andreoli et Moro, *Agardhiella subulata* (C. Agardh) Kraft et M.J. Wynne, *Hypnea cornuta* (Kützing) J. Agardh, *Sargassum muticum* (Yendo) Fensholt and *Undaria pinnatifida* (Harvey) Suringar. The samples were collected in Venice Lagoon and in Lake Ganzirri (Italy) and the polysaccharide extracts were tested against the protozoan *L. infantum* (MHOM/IT/80/IPT1), the prevalent agent of Leishmaniasis in the Mediterranean, endemic in Italy.

Leishmaniasis is a disease with a worldwide distribution affecting both humans and animals. There is increasing awareness that drug treatment can be complicated by variation in the sensitivity of *Leishmania* species to drugs, variation in pharmacokinetics, and variation in drug-host immune response interaction.

Preliminary results showed that polysaccharide extracts had remarkable antileishmanial activity revealing the studied species as a great source of natural antiprotozoal products.





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