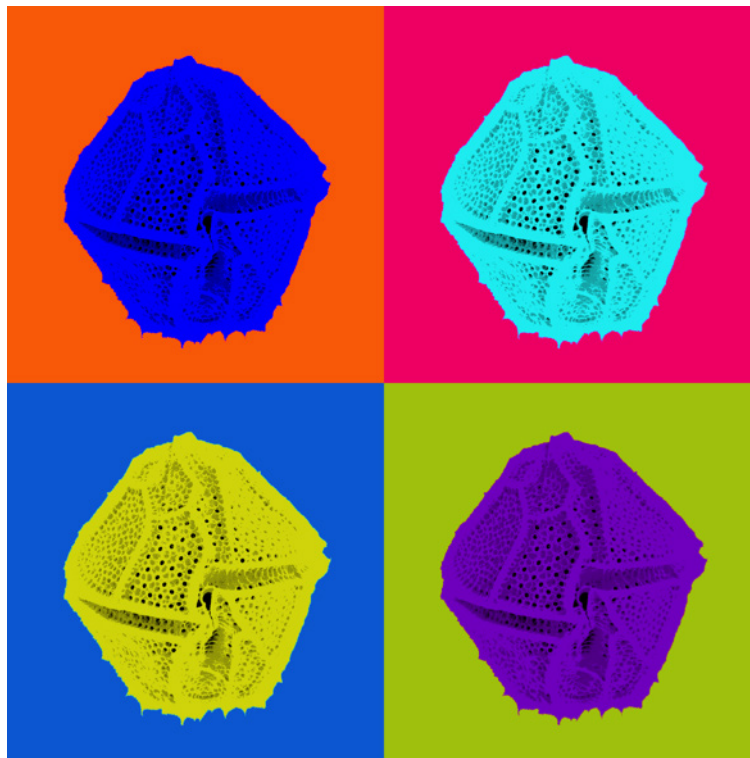


Società Botanica Italiana
Gruppo di Algologia
Riunione Scientifica Annuale

Trieste, 10-11 novembre 2017



Book of Abstracts

Società Botanica Italiana Gruppo di Algologia
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Sala Maggiore
Camera di Commercio Industria Artigianato e Agricoltura Venezia Giulia
Piazza della Borsa 14 - Trieste

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Organizzato da Istituto Nazionale di Oceanografia e di Geofisica Sperimentale - OGS, con la collaborazione di Università degli Studi di Udine, Università degli Studi di Trieste (Dipartimento di Scienze della Vita) e WWF Area Marina Protetta di Miramare. Con il Patrocinio della Regione Autonoma Friuli Venezia Giulia e del Comune di Trieste



RIUNIONE ANNUALE

Trieste, 10-11 Novembre 2017

Venerdì 10 Novembre

- 8.30 Registrazioni
9.00 Saluti di benvenuto

Microalghe I - Chairman: Adriana Zingone

- 9.15 **Monitoring of phytoplankton along Latium coast (Middle Tyrrhenian Sea) over the last fifteen years**
Bianco I., Viaggiu E. and Congestri R.
- 9.30 **Dynamics of the phytoplankton communities in the Mar Piccolo of Taranto, an Italian Long-Term Ecosystem Research (LTER-Italy) site**
Caroppo C., Rubino F.
- 9.45 **Harmful algal dynamics in the Gulf of Trieste during the last 30 years**
Cabrini M., Fornasaro D. and Kralj M.
- 10.00 **Phytoplankton diversity in Adriatic ports**
Mozetič P., Cangini M., Francé J., Bastianini M., Bernardi Aubry F., Bužančić M., Cabrini M., Cerino F., Čalić M., D'Adamo R., Drakulović D., Finotto S., Fornasaro D., Grilli F., Kraus R., Kužat N., Marić Pfannkuchen D., Ninčević Gladan Z., Pompei M., Rotter A., Servadei I., Skejić S.
- 10.15 **Survey on dinoflagellate cysts in recent marine sediments of the port of Trieste (Northern Adriatic Sea)**
Di Poi E., Beran A., Cabrini M.
- 10.30 **Microphytobenthic community structure in four Po Delta lagoons subjected to natural and anthropogenic disturbance**
Cibic T.
- 10.45 **Phytoplankton traits, functional groups and community organization**
Roselli L. and Litchman E.
- 11.00 **Application of taxonomic and morpho-functional properties of phytoplankton communities to water quality assessment for artificial lakes in the Mediterranean Ecoregion**
Vadrucci M. R., Barbone E., Ungaro N., Romano A. and Bucci R.
- 11.15 - 11.30 Coffee break

Macroalghe - Chairman: Annalisa Falace

- 11.30 **Two new species of the genus *Ceramium* Roth (Ceramiales, Rhodophyta) from the Venice Lagoon (Italy)**
Betto V.M., M. A. Wolf, A. Buosi, K. Sciuto, I. Moro, C.A. Maggs and Sfriso A.

- 11.45 Flora marina bentonica del Mediterraneo: Rhodophyta (*Rhodymeniophycidae* escluse)**
Cormaci M., Furnari G., Alongi G.
- 12.00 A journey of discoveries: New Zealand Kallymeniaceae**
D'Archino R.
- 12.15 Tracking the arrival and spread of non-indigenous macroalgae in New Zealand**
D'Archino R., Nelson W., Neill K., Sutherland J.
- 12.30 Citizens and scientists work together to monitor marine alien macrophytes**
Mannino A.M., Balistreri P., Deidun A.
- 12.45 Canopy-forming algae and biodiversity: the structure and large-scale spatial variability of macroalgal assemblages associated to *Cystoseira* beds**
Piazzì L., Pinna S., Ceccherelli G.
- 13.00 - 14.00 Pausa Pranzo**

Microalghe II - Chairman: Cecilia Totti

- 14.00 The ecology of the genus *Leptocylindrus* in a highly structured ecosystem, the northern Adriatic**
Pfannkuchen D. M., Kuzat N., Godrijan J., Smodlaka M., Baricevic A., Pustijanac E., Jahn R., Ivancic I., Pfannkuchen M.
- 14.15 The Ecology of One Cosmopolitan, One Newly Introduced and One Occasionally Ad-
vected Species from the Genus *Skeletonema* in a Highly Structured Ecosystem, the Nor-
thern Adriatic**
Smodlaka Tanković M., Pfannkuchen D.M., Godrijan J., Baričević A., Kužat N., Djakovac T., Pustijanac E., Jahn R., Pfannkuchen M.
- 14.30 Physiological adaptation within the Chaetoceraceae to the ecological challenges in the
northern Adriatic**
Kuzat N., Pfannkuchen D. M., Smodlaka M., Baricevic A., Pustijanac E., Gasparovic B., Novak T., Ivancic I., Pfannkuchen M.
- 14.45 *Ostreopsis ovata* Fukuyo 1981 along the eastern Adriatic coast**
Baričević A., Kužat N., Smodlaka Tanković M., Pfannkuchen D.M., Pfannkuchen M.
- 15.00 Ten years of research on *Ostreopsis cf. ovata* (Dinophyceae) in the Gulf of Trieste: an in-
terdisciplinary approach**
Honsell G., Ciminiello P., Dell'Aversano C., Pelin M., Penna A., Sosa S., Tubaro A.

Algologia applicata I - Chairman: Rossella Pistocchi

- 15.15 Hydrogel scaffolds from *Trichormus variabilis* (Nostocales, Cyanobacteria) exopolymers**
Bellini E., Ciocci M., Savio S., Antonaroli S., Seliktar D., Melino S., Congestri R.
- 15.30 Effect of CO₂ supply on the growth and nutrient uptake in microalgae cultures for indu-
ustrial applications**
Pezzolesi L., Guerrini F., Vanucci S., Pistocchi R.
- 15.45 BiOLEAP Biotechnological optimization of light use efficiency in algae photobioreactors**
Morosinotto T.

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

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

16.15 The use of Marine Resources for Restoration and Conservation of Artworks

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

16.30 - 16.45 Coffee break

Algologia applicata II - Chairman: Adriano Sfriso

16.45 Relazione ad invito - Mario R. Tredici

Food from Microalgae: Challenges and Opportunities

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

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

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

Sfriso A.A., Juhmani A.

17.45 Phytoplankton stable carbon isotopes as a tool to monitor CO₂ leakage at Carbon Capture and Storage sites

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

18.00 Plant essential oils as a safe and non-invasive technology for the remediation of biodeteriorated stone Cultural Heritages

Bruno L., Valle V., Gismondi A., Canuti L., Canini A.

18.15 - 19.30 Assemblea degli iscritti

20.30 Cena sociale

Sabato 11 Novembre

Fisiologia - Chairman: Giorgio Honsell

9.00 Gene expression changes in diatoms in response to external stimuli

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

9.15 Different features of the ultrastructure of autophagy in algae

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

9.30 Phosphorus and metal removal combined with lipid production by the green microalga *Desmodesmus* sp.: an integrated approach

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

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

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

- 10.00 The potential role of phytoplankton in traps of carnivorous bladderworts (*Utricularia*, *Lentibulariaceae*)**
Ellwood N.T.W., Congestri R. and Ceschin S.
- 10.15 Acidification and temperature effects on the gene expression of the coccolithophore *Pleurochrysis pseudoroscoffensis***
Spazzali F., Gerdol M., Cerino F., Antonioli M., Beran A., Pallavicini A. and Cabrini M.
- 10.30 Acclimation of *Caulerpa cylindracea* to ocean acidification**
Santin A., Moschin E., Lorenti M., Buia M.C. and Moro I.
- 10.45 - 11.00 Coffee break**

Microalghe III - Chairman: Marina Cabrini

- 11.00 Factors affecting growth and toxin production in *Prorocentrum hoffmannianum* (Dinophyceae) from Florida Keys**
Accoroni S., Ceci M., Dell'Aversano C., Tartaglione L., Romagnoli T., Totti C.
- 11.15 A multi-gene phylogeny to uncover species diversity in the planktonic diatom genus *Chaetoceros* Ehrenberg, 1844**
De Luca D., Sarno D., Kooistra WHCF
- 11.30 Speciation by time in diatoms: the case of *Pseudo-nitzschia allochroa* sp. nov., a cryptic species in the *P. delicatissima*-complex**
Percopo I., Sarno D., Ruggiero M.V., Rossi R., Piredda R. and Zingone A.
- 11.45 Towards the assessment of the diversity of the dinoflagellate genus *Tripes*: single cell approach**
Mordret S., Mollica T., Biffali E., Montresor M., Pannone R., Piredda R., Kooistra WHCF, Sarno D.
- 12.00 *In vitro* isolation of anti-*Alexandrium minutum* nanobodies from a pre-immune library**
Mazzege E., Beran A., Cabrini M., de Marco A.

Fanerogame marine - Chairman: Maria Cristina Buia

- 12.15 Assessment of *Posidonia oceanica* (L.) Delile (1813) meadows along the Italian coasts: an excursus of 25 years**
Micheli C.
- 12.30 Success of aquatic angiosperm transplantation in the northern Venice Lagoon**
Sfriso A., Buosi A., Facca C., Franzoi P., Scapin L., Zucchetta M., Bonometto A., Boscolo R., Rampazzo F., Oselladore F., Ponis E.
- 12.45 Preliminary study of primary production and nutrient uptake by two seagrasses (*Zostera marina* and *Zostera noltei*) in the Venice Lagoon**
Tomio Y., Buosi A., Wolf M.A. and Sfriso A.
- 13.00 Chiusura dei lavori**

Book of Abstracts

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

Stefano Accoroni¹, Martina Ceci¹, Carmen Dell'Aversano², Luciana Tartaglione², Tiziana Romagnoli¹, Cecilia Totti¹

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Prorocentrum hoffmannianum M.A.Faust is a benthic dinoflagellate that produces biotoxins which are causative of diarrhetic shellfish poisoning (DPS) in warm marine waters. The occurrence of this dinoflagellate was reported only in tropical areas (Belize, Florida, Mexico, Brazil, northern Australia and Hainan Island, China).

In this study, we investigated the effect temperature and nutrient depletion on *Prorocentrum hoffmannianum* isolated in from field samples (Florida Keys, Florida, USA) in 2014.

Monoclonal cultures were prepared by isolating single cells by the capillary pipette method. Cultures were maintained at a temperature of 21 ± 0.1 or 26 ± 0.1 °C and a light intensity of $90\text{--}100 \mu\text{mol m}^{-2} \text{s}^{-1}$ with light:dark cycle 12:12 h. For each temperature, *Prorocentrum hoffmannianum* were grown in f/4 medium (as control), N-free and P-free medium in triplicate. Subsamples (2 ml) were sampled every 2 days for 30 days. Cell counts were made following the Utermöhl sedimentation method, through epifluorescence microscopy.

Both temperature and nutrient condition significantly affected growth rates and maximum yield of *Prorocentrum hoffmannianum* showing the maximum values recorded in high temperature and replete medium. Production of okadaic acid was induced in all conditions (min 50 ± 46 pg/cell; max 643 ± 189 pg/cell), showing values up to 1 order of magnitude higher than that observed in other DSP species.

Toxin concentration (both on a per cell and a biovolume basis) resulted enhanced at 26 °C and in P depletion, corroborating the knowledge that toxin production is modulated by cell physiological conditions, which are affected by a wide spectrum of factors, including several environmental stressors such as nutrient availability.

Toxin fraction released in the growth medium was neglectable. No okadaic acid esters were detected in this strain of *Prorocentrum hoffmannianum*.

***Ostreopsis ovata* Fukuyo 1981 along the eastern Adriatic coast**

**Ana Baričević, Nataša Kužat, Mirta Smodlaka Tanković,
Daniela Marić Pfannkuchen, Martin Pfannkuchen**

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

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

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Cyanobacterial Extracellular Polymeric Substances (EPS) are highly complex and variable compounds exhibiting hydrophilic and anionic properties. EPS can comprise heteropolysaccharides, of six or more sugar units, proteins, nucleic acids and lipids. EPS highly retentive properties make them promising for producing hydrogel scaffolds for drug delivery and tissue engineering.

Released-EPS (R-EPS) were obtained by intensive indoor cultivation of a strain, VRUC 168, of the cyanobacterium *Trichormus variabilis* (Kützing ex Bornet & Flahault) Komárek & Anagnostidis, isolated from sediments of a Mediterranean coastal lagoon, in polyethylene bags (10L; BG11 medium; 25°C; L:D 12:12h; irradiance 80 $\mu\text{mol photons/m}^2/\text{s}$). At stationary phase, day 20, the culture medium was collected and R-EPS concentrated by centrifugation and rotary evaporation. R-EPS were then precipitated in cold ethanol and the pellet freeze-dried. This material was characterized by RP-HPLC, spectroscopic analyses, cytochemical staining, BCA and phenol-sulphuric acid assays.

R-EPS were used to produce hybrid hydrogels (EPS-Hys) for detoxification enzyme- and stem cells- carrier systems. To this end, a specific protocol for the synthesis of the EPS-hydrogel through photopolymerization was developed. Hydrogel stability, resistance to hydrolysis and mechanical properties were also assessed. Thus, hybrid hydrogels were tested for enzymatic encapsulation using thiosulfate:cyanide sulfur transferase (TST) and 3D scaffolding for human cardiac mesenchymal stem cells (cMSCs).

Mass cultures of *T. variabilis* reached productivity of $0.039 \pm 0.001 \text{ g(DW)L}^{-1}\text{d}^{-1}$ and the R-EPS yield was of 2 gDW. Characterization of this EPS fraction revealed the presence of carbohydrates with sulfated and carboxylic groups and 3.5% w/w of proteins. FT-IR spectrum showed peaks within 2900 and 3450 cm^{-1} , attributable to -CH and -OH group vibrations, and a peak around 1600 cm^{-1} attributable to -COOH group vibrations.

The fabricated hybrid hydrogels (EPS-Hys) resulted more stable and resistant to dehydration and spontaneous hydrolysis, at 37°C, than PEGDa-Hys and rheological analysis of hybrid hydrogels evidenced that cyanobacterial EPS improved hydrogel mechanical performances.

Enzymatic encapsulation test showed that TST activity was preserved in the hydrogel at 37°C. 3D scaffold application of the novel hybrid EPS-Hy showed effective adherence and growth of cMSCs cells, with 80% increase in cell viability after 3-day growth, suggesting a non-cytotoxic effect of the EPS-Hys on the stem cells. Results showed that *T. variabilis* S-EPS are a sustainable source of biomaterials adaptable to a large variety of biotechnological applications.

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

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The cosmopolitan genus *Ceramium* Roth (Ceramiaceae), with about 213 currently accepted species (and infraspecific) names, is one of the largest groups in the Rhodophyta. This taxon is characterized by cylindrical or slightly compressed thalli, whose axial cells can be incompletely to completely covered by cortical cells, with alternate to pseudo-dichotomous branching, and straight to inrolled apices. It typically occurs in eulittoral or shallow subtidal habitats and its worldwide distribution is often connected with naval traffics. In fact, *Ceramium* thalli are often carried by hull fouling.

The nomenclature and the taxonomy of this genus are still in a state of chaos. Taxonomic problems are, indeed, tied to a high degree of variations in the morphological characters classically used in species recognition: presence of cortical spines, numbers of periaxial cells, developmental patterns of the corticating filaments, branching pattern, and tetrasporangial features. At the same time, the identification of new species is often complicated by their small sizes and epiphytic life style, that make them cryptic.

Culture studies, suggesting a strong influence of the environment on morphology, and the use of molecular tools have questioned the validity of morphological features to discriminate different *Ceramium* species. In particular, as the inventory of *Ceramium* species in some areas, has been only based on morphological observations, more and more authors have started using molecular markers (e.g. *rbcL* gene and *rbcL-rbcS* intergenic spacer, SSU rDNA, ITS2) to determine species diversity.

Here we characterize different isolates, sampled in transitional waters of the Venice Lagoon (Italy), in order to better investigate presence and biodiversity of *Ceramium* in these areas. Through accurate morphological observations and phylogenetic analyses, based on the plastid ribulose-1,5-bisphosphate carboxylase/oxygenase gene (*rbcL*) as molecular marker, we identify two distinct *Ceramium* taxonomic entities, never reported before in this environment according to the available checklists. The validity of the two entities as recognizable species is also discussed.

Monitoring of phytoplankton along Latium coast (Middle Tyrrhenian Sea) over the last fifteen years

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Current knowledge of marine phytoplankton dynamics in Latium coastal waters is still scanty, with only few works, dating more than 10 years back, focussed on the community composition and spatio-temporal changes only in surface waters and within 500 m from coastline (Bianco *et al.* 2006; Congestri *et al.* 2004, 2006). The collaboration between the Regional Environmental Agency, ARPA Lazio and the Laboratory of Biology of Algae, University of Rome 'Tor Vergata', led to a first update of phytoplankton assemblages with over 250 *taxa* recorded, including toxic species, identified with light and electron microscopy.

The present work is a reappraisal of the Latium phytoplankton by a revision of the data collected from 2002 to 2016 (national monitoring programs: L.979/82, Water Frame Directive 2000/60/CE, Bathing Water Directive 2006/7/CE) in the light of new monitoring activities carried out in the Marine Strategy Programme, as quali-quantitative estimation of phytoplankton also in DCM (Deep Chlorophyll Maximum) and "offshore" waters (3, 6 and 12 NM). This ongoing programme started from July 2015, foresees bimonthly sampling along 4 transects, each including 3 sampling stations.

Data analyses of the whole dataset, more than one thousand samples, preliminarily showed:

- an increase in the number of *taxa* with over 350 recordings;
- the confirmed occurrence of potentially toxic dinoflagellates as *Alexandrium minutum*, *Dinophysis caudata*, *Dinophysis sacculus*, *Lingulodinium polyedrum*, *Phalacroma rotundatum*, *Ostreopsis ovata*, *Prorocentrum lima*, *Prorocentrum minimum* and several diatom species belonging to *Pseudo-nitzschia* genus already recorded before 2004. Moreover the ichthyotoxic raphidophycean *Chattonella subsalsa* and the harmful dinoflagellates *Dinophysis rapa*, *Gonyaulax spinifera*, *Karenia cf. brevis*, *K. cf. mikimotoi*, *K. cf. papillonacea* and *Protoceratium reticulatum*, were observed from 2005;
- the occasional presence of the non-indigenous diatom species *Pseudo-nitzschia multistriata* from 2001 as well as of the non indigenous benthic dinoflagellate *Ostreopsis ovata*, observed in water samples during the summer bloom events;
- a reduced numbers of *taxa* and lower abundances in offshore stations than in more coastal ones with a relevant contribution of the a-taxonomic group of nanoflagellates.

Bianco I., Congestri R., et al. 2006 -Biol. Mar. Med.13 (1): pp.947-950.

Congestri R., Bianco I., et al. 2006 - Biol. Mar. Med. 13 (1): pp.54-60.

Congestri R., Bianco I., Albertano P. 2004 - In Steidinger K.A., Landsberg J.H., Tomas C.R. & Vargo G.A. (Eds) – Proc. of the Xth Int. Conference on Harmful Algae. Florida Fish and Wildlife Conservation Commission and Intergovernmental Commission of UNESCO.

Plant essential oils as a safe and non-invasive technology for the remediation of biodeteriorated stone Cultural Heritages

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Biodeterioration of stone monuments is a well-known problem that endangers valuable archaeological sites worldwide. Environmental conditions and presence of natural or artificial light sustain the development of phototrophic biofilms on stone surfaces causing aesthetic and structural damages. These biofilms are complex communities mainly formed by cyanobacteria in association with bacteria, microalgae and fungi. Conventional methods used for the eradication of phototrophic biofilms, deteriorating valuable archaeological sites, consist in mechanical brushes coupled with the employment of biocides that are deleterious for the substrata, dangerous for the environment and harmful for human health¹. Recent researches highlighted the need of new eco-friendly solutions²⁻⁴. Different essential oils, not cytotoxic for human cells and widely employed for medical purposes⁵, proved to be effective against fungi and bacteria present on cultural heritage objects⁶.

In order to develop new non-invasive and safe technologies for the restoration and preservation of stone monuments, we decided to evaluate the potentiality of two plant essential oils to be used as green biocides with the final aim to design an easy-to-use protocol for the restoration of stone cultural heritages.

For this study, we used different concentrations of two plant essential oils distilled from *Lavandula angustifolia* Mill. and *Thymus vulgaris* L. against phototrophic biofilms made up by cyanobacteria isolated from Roman Catacombs. The effect of the essential oils was monitored by means the control of biofilm growth on agar and the evaluation of the photosynthetic activity with the portable fluorometer mini-PAM. The chemical profiles of the essential oils employed were investigated, in detail, via GC-MS. Moreover, the evaluation of the effect of the application on substrata was carried out on calcareous stones. The obtained results evidenced that the essential oils were effective on the reduction of the biofilm growth and the application did not alter the aspect of the substrata. In conclusion, the study showed the feasibility of the employment of essential oils as green and save new biocides for the restoration of Cultural Heritages.

Urzi et al. 2016. Science of Total Environment. 572 (1) 252–262

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Giovannini et al. 2016 Immunological Investigations, 45:1

Stupar et al. 2014 South African Journal of Botany 93, 118–1247

Harmful algal dynamics in the Gulf of Trieste during the last 30 years

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

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

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The Mar Piccolo of Taranto (Northern Ionian Sea), one of the most studied coastal areas in Italy, is a Long-Term Ecosystem Research site (LTER_EU_IT_095).

Phytoplankton data, both as active stages in the water column and cysts in the sediments, have been collected since 1991 (with gaps). Notwithstanding a reduction of nutrients caused by a reduction of wastewater inputs during the early 2000s, phytoplankton biomass (in terms of chlorophyll *a*) did not show significant variations over the years. However, other relevant changes occurred such as a reduction of the community size, due to a shift of dominance from diatoms to nanoflagellates, an increased duration of secondary blooms (beside the spring one), and the appearance of a diversified dinoflagellate community, comprised mixotrophic and heterotrophic species. An increase of the pico-sized planktonic component has also been recently detected. Moreover, wastewater diversion did not preserve the Mar Piccolo from harmful algal blooms and about 25 harmful species were monitored throughout the years.

These signals are in some ways mirrored in the sediments. In fact, the resting stages communities, during the last 20 years passed through many changes in their structure, even though calcareous dinocysts produced by Thoracosphaeraceae remained dominant. During the last 5 years, in particular, resting spores of some diatoms of the genus *Chaetoceros* were observed, together with the increase and new findings of some heterotrophic species, apparently a signal of the changes occurred in the Mar Piccolo. This change has been registered also with the disappearing of species, e.g. *Protoperidinium pentagonum* and *P. divaricatum*, whose cysts were regularly observed in surface sediments, even with low densities.

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

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

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

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Dopo una breve introduzione sui caratteri generali delle Rhodophyta (Alghe Rosse) e sulla loro classificazione, distribuzione geografica ed ecologia, viene riportata una chiave d'identificazione dei generi e dei *taxa* specifici e infraspecifici macroalgali di questo Phylum [ad esclusione delle Rhodymeniophycidae che saranno oggetto di altro contributo] presenti in Mediterraneo [Mar Nero escluso].

A seguito di una revisione tassonomico-nomenclaturale, 120 *taxa* a livello specifico e infraspecifico sono stati accettati e trattati. 44 generi, 15 famiglie, 12 ordini, 3 sottoclassi, 4 classi e 2 subphyla sono rappresentati. Inoltre 61 *taxa*, a livello specifico e infraspecifico, sono stati considerati *taxa excludenda* di cui 18 da confermare in Mediterraneo; 90 *taxa*, a livello specifico e infraspecifico, e 1 a livello di genere, sono considerati *taxa inquirenda*; 29 *taxa*, a livello specifico e infraspecifico, sono risultati nomi illegittimi; 33 *taxa*, a livello specifico e infraspecifico, e 1 a livello di famiglia, sono risultati invalidi; infine sono state individuate 17 combinazioni invalide, mentre vengono proposte 4 nuove combinazioni e un nome nuovo.

L'aggiornamento tassonomico segue i più recenti lavori basati sull'analisi molecolare. Di ogni *taxon* viene data una breve descrizione preceduta da alcuni riferimenti bibliografici riportanti sue illustrazioni e/o la sua distribuzione in Mediterraneo. Inoltre, la trattazione della maggior parte dei *taxa* è arricchita da note a supporto delle sinonimie indicate, o delle scelte tassonomiche seguite o delle motivazioni per cui il *taxon* è stato considerato *inquirendum* o *excludendum*. Il lavoro è completato da un glossario di 148 voci, da un indice di tutti i *taxa* citati nel testo e da 52 tavole.

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

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

A journey of discoveries: New Zealand Kallymeniaceae

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

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

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

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

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In coastal and temperate shallow waters, dinoflagellates can produce benthic resting stages to overcome unfavorable periods, and germinate in the water column once favorable environmental conditions are re-established. Studies on the composition, abundance and distribution of cysts in surface sediments of semi-enclosed bays such as ports can provide valuable information both to detect potential harmful and non-indigenous species and to foresee dinoflagellate dynamics inside the phytoplankton community. To this aim, a monitoring programme was carried out in the port of Trieste within the framework of the European IPA project BALMAS (Ballast Water Management System for Adriatic Sea Protection). In May 2014 and March 2015, 4 sites (Ts1-2-3-4) characterized by maritime traffic and anthropic pressures and a reference site (C1) located in the Marine Reserve of Miramare (Gulf of Trieste) were sampled. Surface sediments were collected by means of a box corer and samples consequently processed. The identification of the resting stages was based on the shape and morphology of cyst bodies (Matsuoka and Fukuyo, 2000) using the inverted microscope at 20x objective. Furthermore to verify the taxonomic identification some selected cysts were isolated and induced to germinate.

In this study, a total of 38 dinoflagellate taxa were recognized. Cyst assemblage resulted dominated by Gonyaulacales and calcareous Peridinales, mainly represented by the potentially toxic *Lingulodinium polyedrum* and by resting cysts of the genus *Alexandrium* in the size 20 μm . In May 2014 cysts varied from a minimum of 17 to a maximum of 232 cells g^{-1} of dry weight detected respectively at C1 and Ts4. In March 2015, cysts ranged from 21 to 60 cells g^{-1} of dry weight respectively at Ts2 and Ts4. The latter site Ts4 recorded the higher cyst deposits detected in the port. From the germination experiments carried out on the cysts collected during the second survey (March 2015), a new record for the northern Adriatic, the species *Alexandrium margalefii* was developed. Other potentially toxic morphotypes of the genus *Alexandrium* identified in both campaigns were already renowned in this area. Compared to previous few studies conducted in the Gulf of Trieste, the number of taxa and the most representative morphotypes were comparable to past studies whereas abundances were much lower than past monitoring. Our results remark the importance of these stages of dinoflagellate life cycle, and the necessity to intensify investigation especially in areas at high risk such as ports.

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

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Carnivorous aquatic plants of the genus *Utricularia* (Lentibulariaceae) are well known to capture small aquatic invertebrates, using structures called bladders. But information is coming to the fore suggesting that *Utricularia* could be re-classified as an omnivore as phytoplankton species can make up a major proportion of bladder contents. It is not fully understood if phytoplankton cells are preyed upon or if they are taken in merely as a consequence when invertebrate prey triggers the bladder. Past studies have indicated that both scenarios could be possible. Some studies now also suggest that a mutual-symbiotic relationship exists between *Utricularia* and the community in the within the bladders. The present study is to determine a potential role for phytoplankton in nutrient supply to *Utricularia*.

The identification and quantification phytoplankton species was carried out on the contents of bladders sampled from the carnivorous bladderwort *Utricularia australis*. Samples of three full *Utricularia* filaments were taken from 18 central Italian sites and the contents of 10 or 15 randomly chosen bladders were observed. These data were compared to the external phytoplankton community and water chemico-physical and nutrient variables. Every bladder was shown to contain some phytoplankton cells, with findings showing species spread across 6 divisions (Chlorophyta, Bacillariophyta, Pyrrophyta, Euglenophyta, Cryptophyta, Cyanophyta). Significant relationships of phytoplankton species assemblages within the bladders and those in the ambient water were found. Also in the majority of bladders there were a high proportion of live compared to dead cells. Many bladders had phytoplankton-only communities. It was not possible to say for certain if phytoplankton was preyed upon or not, or if there was a mutualistic-symbiotic relationship, but the presence of many living cells could suggests the latter option is possible. The findings here imply that the bladder phytoplankton community is a consequence of the ambient community given the relationships between them. The presence of phytoplankton-only contents in many bladders implies possible automatic-rather than prey-triggered bladder functioning.

Gene expression changes in diatoms in response to external stimuli

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Signal transduction mechanisms are still poorly defined in marine phytoplankton, including diatoms. Yet, understanding how cells sense and integrate environmental signals is fundamental to explain population dynamics, community interactions and ecosystem functioning. We use several approaches, mostly based on transcriptomics and loss of function strategies, to study molecular changes occurring in diatom cells upon reception of external stimuli, such as changes in turbulence, or chemical cues produced during sexual reproduction or grazing, using dedicated, custom-made instrumentation.

I will present data on diatom responses to turbulence. It is known that diatoms thrive in turbulent environments, however, despite several experimental and numerical studies, if and how diatoms may profit from turbulence is still an open question. One of the leading arguments is that turbulence favours nutrient uptake. To study this process, we designed and built the TURBOGEN, a prototypic instrument that can generate natural levels of microscale turbulence. We tested several diatom species using TURBOGEN, and demonstrated that diatoms actively respond to turbulence in non-limiting nutrient conditions by tuning their chain length. Differential expression analyses were conducted to study the transcriptional response of *Chaetoceros decipiens* to turbulence, one of the species displaying obvious morphological changes. *C. decipiens* responded to turbulence by activating energy storage pathways like fatty acid biosynthesis. In addition, in experiments lasting 12 days, we observed that in turbulence *C. decipiens* continued to take up phosphorus and carbon even when silicon was depleted. These findings indicate that turbulence affects diatoms in a more sophisticated fashion than what was accepted so far.

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

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Metal contamination is a serious environmental problem that affects life forms and changes the natural microbiota of aquatic ecosystems.

Chromium (Cr) is one of several heavy metals (HM) that causes serious environmental contamination in soil, sediments, and groundwater. Cr can exist in the environment as Cr(III) or Cr(VI) and particularly Cr(VI) form is extremely toxic, mutagenic, and carcinogenic.

Microalgae resistance and/or detoxification to heavy metals may occur through several mechanisms: binding to the cell wall; low plasma membrane permeability; active extrusion; biotransformation; compartmentalization of HM into vacuoles and other intracellular organelles; complexation with chelating agents, such as non-proteinaceous compounds (as malate, citrate, and polyphosphates) or metal-binding proteins, such as metallothioneins and phytochelatins. Phytochelatins (PCs) are heavy metals chelating cysteine rich polypeptides with the general structure $(\gamma\text{-Glu-Cys})_n\text{-Gly}$. Phytochelatins are produced by the enzyme Phytochelatin Synthase (PC-synthase) which uses glutathione (GSH) as its substrate. It is now well established that glutathione (GSH) and PCs are a central component of the plant defence system against various forms of stresses.

Several physiological studies in plants, indicated the role of PCs in the homeostasis and detoxification of toxic metals including Cr. However, synthesis of PCs has received little attention in algal cells and no data regarding Cr-induced PCs synthesis in microalgae exist.

In this work, we reported the first evidence of a PCs synthase gene from the microalga *Scenedesmus acutus*. Using degenerate primers, we amplified a partial cDNA fragment of nearly 1300 bp (*SaPCS*). The deduced protein shows a high identity homology (46%-74%) with other PCs synthase of algae and plants.

In order to define its role in Cr detoxification, we analysed by RT-PCR, the level of *SaPCS* transcription in two strains of *S. acutus* with different chromium sensitivity (Cr tolerant strain vs wild type) after 24h culture in standard and in Cr supplemented medium (1mg/l Cr (VI)). Since the pathway of Cr (VI) translocation is an active mechanism involving transporter of essential nutrients such as phosphate and sulphate, we also evaluated the role of PCs synthase in the two different *S. acutus* strains after sulphur starvation.

Our preliminary data indicate that in the Cr-tolerant strain *SaPCS* transcription is induced following Cr(VI) treatment both in S-sufficient and in S-replete condition whereas in the wild type the induction is significantly enhanced by sulphur starvation. These observations indicate that in *S. acutus* PC synthase is not only involved in Cr detoxification, but can also play a role in the balance the intracellular sulphur.

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

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The Euganean Thermal District is the oldest and largest thermal center in Europe and represents a reference in mud-based thermal treatments. The mature mud is the result of traditional methods, handed down over the centuries up to our days. During the mud maturation process, we can observe the formation of a biofilm, mainly consisting of cyanobacteria. They are suggested to produce active molecules conferring the therapeutic properties of the mud. Among them, *Phormidium* ETS-05, an endemic species firstly isolated from the Euganean District, is well known for the production of galactosyldiacylglycerols with anti-inflammatory effects (Bruno et al. 2005, doi: 10.1016/j.ejphar.2005.09.023), leading to the achievement of the European Patent on the active principles of the Euganean Thermal Muds in 2013 (EP1571203). Recently, another endemic species, *Cyanobacterium aponinum* ETS-03, has been studied for its ability to produce polysaccharides with immunostimulating effects (Gudmundsdottir et al. 2015, doi: 10.1016/j.imlet.2014.11.008; Gris et al. 2017, doi: 10.1007/s10811-017-1133-3). A raw protocol, called “Disciplinare per la Tutela del Marchio Collettivo di Origine del Fango del Bacino Termale Euganeo” has been written to formalize the traditional methods. Therefore, the scientific bases and a deep knowledge of all elements influencing the mature mud formation are still missing.

In the first part of this study, the maturation process of 2 thermal SPAs has been monitored every 15 days for 2 months, following the cyanobacteria population growth and composition as function of time and environmental parameters. In the second part, the presence and the total amount of the Patent target species, *Phormidium* ETS-05, in mature muds of 33 different thermal SPA, has been investigated as an indicator of the correct maturation process.

Results showed that, during maturation, the maintenance of temperature in a proper constant range is one of the crucial aspects to consider to have a proper final mature mud. The observations and the analysis of mature muds of different SPAs revealed that the temperature influences both the final concentration and the cyanobacteria population composition. The target species *Phormidium* ETS-05 was present in almost all the monitored SPAs, and represented always at least the 40% of the total cyanobacteria population.

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

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Since 2000s, benthic blooms of the harmful dinoflagellate *Ostreopsis cf. ovata* have been occurring with increasing frequency and extension along the Mediterranean coasts. Concomitantly to these events, episodes of human adverse effects associated mainly with cutaneous and inhalational exposure to marine aerosol and/or seawater have been recorded. Although high amounts of *Ostreopsis* toxins have been detected in edible marine organisms, no human cases of seafood poisoning ascribed to these compounds have been documented, so far. Mediterranean strains of *Ostreopsis cf. ovata* have been found to produce palytoxin (PLTX) analogues, with ovatoxin-a (OVTX-a) as the major toxin, in addition to other ovatoxins and low amounts of isobaric palytoxin.

In the Gulf of Trieste, *Ostreopsis cf. ovata* was first recorded in 2006, but the real beginning of an extensive research on this organism dates back to 2009 when a bloom occurred near the small beach of Canovella de' Zoppoli (Trieste). This improved the collaboration within a research team of biologists, chemists and toxicologists for an interdisciplinary study providing new insights on different features of this organism: from taxonomy to cell ultrastructure, genetic and molecular characterization, toxin profile, intracellular localization of toxins and other metabolites by immunochemistry and/or Raman spectroscopy. A short review of the results obtained is given and future perspectives of research are discussed.

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

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The northern Adriatic is a suitable ecosystem for observing phytoplankton under a variety of different ecological conditions. This shallow basin is characterized by a multitude of steep and quickly changing gradients (e.g. nutrients), both temporal and spatial. The current systems and the major freshwater input (River Po) generate strong gradients in nutrient concentrations with an expressed N/P imbalance and sustained phosphate limitation. The genus *Chaetoceros* is among the most diverse and species rich diatoms often dominating the phytoplankton community in the Adriatic Sea. There is a big number of species belonging to this genus with different physiology, seasonality and ecology.

We analyzed species from the genus *Chaetoceros*, with a special focus on their metabolic reaction towards phosphate limitation. Here we report data on species-specific growth rates under different nutrient regimes, phosphate uptake rates, alkaline phosphatase activity, localization and activation patterns and characteristics of alkaline phosphatase activity. Our results demonstrate a high interspecific variation in metabolic responses to phosphate limitation in sympatric congeneric species. Ecological characteristics and hence significance and function appears hence to be defined on the species level and appears far from homogeneous within genera. This results in a highly structured planktonic ecosystem that allows for a high level of sympatric congeneric species diversity.

Citizens and scientists work together to monitor marine alien macrophytes

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The spread of alien species is an ongoing phenomenon which is widely recognized as a major threat to biodiversity at different levels. The Mediterranean Sea is an important hotspot for marine alien species (ca. 1,000 such species recorded to date). The creation of early-warning systems is crucial for reducing the risk of invasive species introduction. Since intensive scientific monitoring programs could be very expensive, engaging citizens (e.g. tourists, fishermen, divers) through citizen science could be a useful tool for providing information and scientific data on the occurrence and spread of marine alien species. Citizen science is having an increasing success worldwide. The increase in the number of citizen science projects is possibly due to the wide availability of mobile technologies and internet access that enable an easy and cheap way to communicate and to interchange data.

The value of citizen science has been widely recognized. Despite this, in order to be used for scientific purposes and management decisions, the collected data need appropriate quality assurance measures such as validation and verification by taxonomic experts. We report on the experience of two citizen science projects: the Project “*Caulerpa cylindracea* – Egadi Islands” and the Project “Invasive Algae”, included within the “Seawatchers” platform. The first one, sponsored by the STEBICEF Department of the University of Palermo and by the Egadi Islands Marine Protected Area (MPA), aims at creating a database on the spread dynamics of *C. cylindracea* within the Egadi Islands MPA. The second one, coordinated by the Institute of Marine Sciences of Barcelona (CSIC, Spain), collects data on 10 marine invasive alien species. These projects highlight how important the contribution of citizen science campaigns is for collecting new data and information on non-native marine species. Moreover, in areas particularly vulnerable to biological invasions, such as Sicily, they represent an opportunity to promote the creation of early-warning systems, and an effective tool in the management of present and future introductions of alien species within the Mediterranean Sea.

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

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At the present, the identification of planktonic species in coastal water mostly relies on light microscopy observations, only in some cases through PCR analysis and for specific morphological analyses by electronic microscopy. The analyses are performed by highly trained personnel and require lab equipment and long time in order to get the results. The increasing danger represented by Harmful Algal Blooms (HABs) and the new legislation on ballast water management urge faster and possibly easy-to-perform diagnostic methods able to guarantee sensitivity and reliability. Immuno-reagents for microalgae capture would have the requisites of specificity necessary for developing semi-automatic sensitive biosensors to be operated on-site by generic personnel. This alternative is at the moment neglected because of the elevated costs for monoclonal antibody selection and production.

Here we demonstrate for the first time the feasibility to recover nanobodies (VHHs) selective for native surface epitopes of *Alexandrium minutum* by direct whole cell biopanning using a pre-immune phage display library. The recombinant nature of VHHs enabled their bacterial expression as eGFP fluorescent reagents. We demonstrated that these reagents are directly suitable for fluorescence microscopy and flow cytometry, but could be used also on solid surfaces such as ELISA plates. The isolated fluorescent nanobodies showed no cross-reactivity with other microalgae of the Collection of Sea Microorganisms (COSMI) available at INOGS such as *Scrippsiella lachrymosa* (COSMI n: 1049), *Prorocentrum minimum* (COSMI n. 1034), *Pleurochrysis cf. roscoffensis* (COSMI n. 5005), Cryptophycean *sp.* 2 (COSMI n. 3002), and *Phaeodactylum tricornutum* (COSMI n. 2007). They were able to bind selectively the target cells in both fixed and fresh samples with minimal processing, demonstrating the advantage of these reagents over conventional IgGs. The establishment of the protocol represents a proof-of-principle that can be applied to further species. It is possible to isolate monoclonal nanobodies in 1:3 of the time and at a cost roughly 1:10 of that necessary to select a monoclonal antibody by hybridoma technique. The production costs are between 10 and 100 times lower. Furthermore, nanobodies are easy to engineer and, therefore, optimal material for biotechnological applications (functionalization of solid surfaces, nanoparticles, sensor chips).

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

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During 25 years (1991-2016) of monitoring studies a time series of data of *Posidonia oceanica* (L.) Delile (1813) meadows growing along the Italian islands and in the northern-central Tyrrhenian coasts was estimated.

We have assessed the ecological status of the species integrating the knowledge acquired about genetic, biomass, density and morphological features of the populations in relation to different natural and anthropogenic impacts and various substrate composition (rocky, sand). *P. oceanica* meadows growing along the coastline are under continuous natural and anthropogenic pressure where the way meadow health correlates with its genetic and genotypic diversity (Micheli *et al.*, 2005; 2015).

During the years we have identified new diagnostic tools for effective monitoring of the conservation status of *P. oceanica* beds by applying innovative methodologies: a suite of indicator highlighted the seasonal variation of biomass, phenols and the main biotic parameters as well as pigment concentration of seagrass meadows (Rotini *et al.*, 2013). Knowledge of the ecological structure of meadows is considered a prerequisite for many multidisciplinary programs (Borfecchia *et al.*, 2013a,b) as well as the marine energy projects by which the renewable wave energy resource can be exploited in the Mediterranean (Micheli *et al.*, 2017; Borfecchia *et al.*, 2016). Assessment of *P. oceanica* meadows along the coasts allows us to evaluate the role of the species in sustaining the primary production and the trophic level in the Mediterranean.

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

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The dinoflagellate genus *Tripos* (previously included in *Ceratium*) represents a significant component of marine microplankton. The genus includes more than 120 morphologically described species and infraspecific taxa, such as forms and varieties, found from polar to tropical areas. The genus has been suggested as indicator of ocean warming because of its taxonomic richness, wide geographical distribution and geographical patterning of its taxa related to temperature. However, the great variability of shapes described even within a single strain, coupled with the lack of clear discriminating characters, makes challenging species circumscription based on morphological criteria.

Using a single-cell approach, 52 *Tripos* cells isolated from the Gulf of Naples were characterized from the molecular (28S and 18S) and morphological point of view. In most cases, sequences obtained from morphologically identical cells clustered in the same clade. The 28S phylogeny showed a better capacity to discriminate different species than 18S but also suggested the existence of cryptic diversity in the genus. As an example, cells morphologically identified as *T. trichoceros* clustered into two different clades.

Taxonomically validated sequences were added to the existing reference database DinoREF to improve the interpretation of metabarcoding data from a time series collected in the Gulf of Naples (LTER MC, 48 dates in 3 years).

I will present preliminary results and discuss problems and future possible approaches to study the diversity of this important but poorly known genus.

BiOLEAP Biotechnological optimization of light use efficiency in algae photobioreactors

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New renewable energy source are highly needed to compensate exhausting fossil fuels reserves and reduce greenhouse gases emissions. Some species of algae have an interesting potential as feedstock for the production of biodiesel thanks to their ability to accumulate large amount of lipids. Strong research efforts are however needed to fulfil this potential and address many issues involving optimization of cultivation systems, biomass harvesting and algae genetic improvement. This proposal aims to address one of these issues, the optimization of algae light use efficiency. Light, in fact, provides the energy supporting algae growth and must be exploited with the highest possible efficiency to achieve sufficient productivity and make their cultivation competitive.

Algae efficiency in converting solar radiation, however, depends on many environmental factors, including light intensity, temperature, nutrient and CO₂ availability. Optimizing microalgae productivity in such a complex environment hinges on our ability to describe, in a quantitative manner, the effect of these various parameters as well as their mutual interactions. Application of computational models that are capable of quantitative predictions can prove especially useful in identifying which parameters have the largest impact on productivity, thereby providing a mean for enhancing growth through design and operational changes. This information has now been employed to select *Nannochloropsis gaditana* strains with altered regulation of photosynthesis that showed improved productivity in lab scale photobioreactors.

Light influence on algae metabolism has also been investigated using a combination of genomic, transcriptomic and metabolomics analyses. These evidenced how light availability in *Nannochloropsis gaditana* modulates the carbon partitioning and TAG biosynthesis by affecting the transport of reduced carbon in and out of the chloroplast.

Phytoplankton diversity in Adriatic ports

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

Different features of the ultrastructure of autophagy in algae

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The concept of (eukaryotic) algae does not represent a monophyletic group, since some groups of algae derived from primary endosymbiosis and others from secondary endosymbiosis. For this reason the cell machinery does not work the same in every group. Concordantly, also autophagy showed quite variable features from the archaeplastida group (red algae, Glaucophyta, green algae and land plants) with respect, for instance to the heterokont algae. Some example are presented.

Some doubt arises also about the origin of autophagy, since this phenomenon is typical of eukaryotes and currently not much sequence similarity to autophagy-related eukaryotic genes has been found in prokaryotes. As a matter of fact autophagy consists in an activity of membranes and, by definition, prokaryotes should not have autonomous membranes with respect to the plasmamembrane. Nevertheless, cyanobacteria have a very developed membrane system, mainly devoted to photosynthesis, but also to the formation of gas vesicles. In cyanobacteria some open space, apparently surrounded by membranes, and containing granular material can be observed (Dvorak et al. 2015; Capelli et al. 2017), while there is evidence of protein recycling, particularly Rubisco.

While in green algae the ultrastructural features are relatively similar to autophagy in land plants as in *Dichtiosphaerium* sp., in the more distantly related *Heterosigma hakashiwo* (Raphidophyceae), autophagy assumes more extended features in the cytoplasm particularly in case of starvation (Papini et al. in press) and shows even evidence of nuclear blebbing, that is a typical feature of programmed cell death in animals.

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

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

Effect of CO₂ supply on the growth and nutrient uptake in microalgae cultures for industrial applications

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Atmospheric CO₂ concentration has been rising since the Industrial Revolution and is predicted to double by the end of this century. Microalgae play an important role in the carbon cycle of marine ecosystems and their responses to elevated atmospheric CO₂ are of considerable interest. The effects of CO₂ enrichment are variable and may depend on the availability of nutrients, since CO₂ supply leads to a higher growth rate and to an increased demand for nutrients.

CO₂ released by industrial activities contributes substantially to increase the atmospheric CO₂ level; thus it is becoming more and more important to find alternatives to control its release in the atmosphere. Flue gases produced by processes such as anaerobic digestion, for instance, could be potentially used for microalgae culturing, thus preventing their release and allowing the transformation of polluting gas fluxes into new and valuable microalgae products.

In this study physiological responses to CO₂ supply in two microalgal species that have potential industrial applications were investigated. In particular *Desmodesmus communis*, a green algae widely used for phycoremediation due to its high resistance to chemical and environmental stress, and the diatom *Phaeodactylum tricornerutum*, a PUFAs producer widely used in aquaculture, were studied using 1 L batch cultures or 70 L photobioreactors. Flue gases obtained by anaerobic digestion and enriched in CO₂ by the removal of the biogas fraction were also tested for microalgae culturing.

Results showed a marked increase of the growth and productivity in cultures with CO₂ addition respect to the ones grown with only air. Moreover, a different use of macronutrients by algal cells and changes in cellular composition were observed when CO₂ was supplied. Preliminary experiments performed using flue gases supplied to *P. tricornerutum* cultures reported no inhibition of the algal growth and promising results attesting their potential use to cultivate microalgae.

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

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

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

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

Phytoplankton stable carbon isotopes as a tool to monitor CO₂ leakage at Carbon Capture and Storage sites

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Carbon capture and storage technology (CCS) is expected to play a key role among strategies for the mitigation of climate change by reducing CO₂ emissions into atmosphere from fossil fuel combustion. Although a well-engineered storage site is not expected to leak, the risk of failure of CO₂ containment and the subsequent environmental impact is a major issue for the acceptance of this approach. This study aims to the evaluation of stable carbon isotope analysis as a tool for effective early warning of CO₂ migration from CCS, since different carbon sources have specific $\delta^{13}\text{C}$ (usually lower values in anthropogenic emissions than in natural CO₂).

Two culture experiments were conducted within 2L-photobioreactors under controlled conditions (light, temperature). In each experiment, the diatom *Thalassiosira rotula* was grown in two different media: one prepared with natural seawater and the other with artificial seawater, whose carbonate system was derived from anthropogenic CO₂. Both media were equally supplemented with nutrients (silicate, nitrate and phosphate), metals and vitamins. Daily variations of $\delta^{13}\text{C}$ in dissolved inorganic carbon (DIC) and in diatoms were analysed in order to study the phytoplankton response.

According to the experiments results, differences exist in microalgae isotope composition between natural and anthropogenic conditions. In natural medium, the diatoms $\delta^{13}\text{C}$ values did not show important deviations from the starting value ($-24.4 \pm 0.3\%$), whereas in the anthropogenic CO₂ condition, the uptake of ¹³C-depleted DIC ($-44.7 \pm 0.8\%$) resulted in the decreasing values of microalgae carbon isotope composition (until -44.4%). The rapid change in phytoplankton isotope composition confirms the method tested as reliable for identifying different CO₂ sources.

Phytoplankton traits, functional groups and community organization

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

Phosphorus and metal removal combined with lipid production by the green microalga *Desmodesmus* sp.: an integrated approach

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The quality of surface water bodies is still threaten by anthropogenic activities causing diffuse and/or point-source emissions of both organic and inorganic pollutants. Effluents rich in nutrients such as nitrogen (N) and phosphorus (P) can lead to eutrophication ^[1], while metals may be toxic for human and ecosystem health ^[2]. This work focused on the potential of *Desmodesmus* sp. for wastewater bioremediation and biodiesel production. This microalga was grown in two *Runs*, namely *Run 1* and *Run 2*, using a culture medium with a P content of 4.55 mg L⁻¹ to simulate a domestic or an industrial wastewater effluent. In *Run 2*, *Desmodesmus* sp. was exposed to a multi-metal solution of Cu (9.8 mg L⁻¹) and Ni (7.4 mg L⁻¹) and the biosorption ability of living cells was evaluated after 2 days of contact time. In both *Runs*, biomass production, P removal, adsorption capacity of freeze-dried biomass (30 minutes contact-tests, Cu and Ni mixed solution) and lipid yields were also evaluated. Results showed that P was completely removed in 21 days in *Run 1* (100%) and in 14 days in *Run2* (94%); furthermore, 94% of Cu and 85% of Ni were removed after 2 days of exposure in *Run 2*. Adsorption tests with non-living obtained in both *Runs* (freeze-dried biomass) showed that material from *Run 1* was able to adsorb 90% of Cu and 43% of Ni, while metal adsorption by non-living biomass from *Run 2*, previously exposed to metals for 2 days, was only 65% for Cu and 30% for Ni. This was likely due to saturation of free binding-site that occurred in living cells during the growth. Lipid analyses revealed that the presence of metals in *Run 2* decreased the lipid yield (from 17% in *Run 1* to 13% in *Run2*), but according to European and American standard, biodiesel quality from biomass obtained in *Run 2* was higher than that from *Run 1*. In a waste-to-value approach, residual biomass after lipid extraction from both *Runs* was employed for adsorption tests of 30 min and the data revealed that only the biomass from *Run 1* was still able to remove metals, even if with a low removal efficiency (10% and 14% of Cu and Ni, respectively). This study demonstrates the effectiveness of using this green microalga to integrate the removal of contaminants such as P, Cu and Ni with the use of the biomass obtained as feedstock for biofuel production, offering a new alternative solution to the problems of environmental pollution and energy shortage.

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The use of Marine Resources for Restoration and Conservation of Artworks

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

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Acclimation of *Caulerpa cylindracea* to ocean acidification

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Areas showing naturally high levels of CO₂, such as upwelling areas, rocky tidal pools, and volcanic underwater CO₂ vents have been recently used as natural laboratory where to study the effects of ocean acidification on benthic systems, in particular those where alien species are already settled (Hall-Spencer et al., 2008). Around the Castello Aragonese (Ischia Island, Gulf of Naples) shallow underwater vents naturally acidify the water, very close to pH values expected for 2100. In this area it has been possible to find patches of *C. cylindracea* Sonder both at ambient (8.1) and “acid” (7.8) conditions since the beginning of 21st century.

Through crossed transplant experiments *in situ* and by using an integrated approach we investigated short- and long- term effects of ocean acidification on *C. cylindracea*. Morphological and ultrastructural analyses were performed by using light and transmission electron microscopy; physiological analyses were performed by detecting chlorophyll and carotenoid contents and the *in situ* photosynthetic performancy (Diving-PAM fluorometer).

Short-term transplants from 8.1 to 7.8 and *viceversa* gave opposite results. Stress effects resulted in transplants from current to lower pH values: a decrease in the number of active chloroplasts, the appearance of dilatations between thylakoidal membranes, an increase in the amount of plastoglobules. A decrease in the photosynthetic efficiency also occurred, while carotenoids and non-photochemical yields were strongly increased. A completely opposite response was observed in transplants from lower to normal pH. However, no significant difference was evident when native plants at the two different pH conditions were compared, testifying a long-term acclimation at lower pH values.

Those preliminary results seem to confirm the high acclimation capacity of this species even in a new pH scenario, as already resulted according to variations in temperature and daylength (Raniello et al., 2006; Flagella et al., 2008).

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

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Mycosporine-like amino acids (MAAs) are a class of small, water-soluble molecules known for their capability of absorbing UV radiation and widespread across a variety of algae and cyanobacteria. Some red algae MAAs showed bioactivity with potential in drug discovery, but no evidence yet exist on MAA production, composition and potential application in cultured diatoms.

In this work, one brackish strain of *Staurosirella pinnata* (Ehrenberg) D.M. Williams & Round, VRUC 290, was mass cultivated in indoor polyethylene photobioreactors (10 L) to produce biomass for MAA extraction using methanol/water (20:80 v/v). Extracts were then characterized using LC-MS and subsequently analyzed for their anti-oxidant activity with 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and, finally, tested for their effect on melanoma cells (CHL-1) by flow cytometry.

Chromatographic profiles allowed identification of seven different mycosporines, two of which mycosporine-glycine and euhalothecine, present in relatively high amounts, 24.936 mg and 23.246 mg/g of dry weight extract, compared to concentrations recorded for other two diatom strains *Phaeodactylum tricornutum* and *Thalassiosira weissflogii* (SAG Culture Collection) cultivated in the same conditions in our laboratory. The radical scavenging activity of *S. pinnata* extract was assessed by DPPH assay showing high anti-oxidant activity, with an IC₅₀ of 15.0 +/- 0.1 µg.

Bioactivity of the extract was then tested on human melanoma CHL-1 cells and cytofluorimetric analyses (Fluorescence-Activated Cell Sorting) evidenced a strong cytotoxic effect of the extract at the concentration of 13.3 mg/ml (95% of increasing cell death) after 24h and 48h treatments. Finally, at the extract concentrations of 8 and 10 mg/ml an alteration of cell cycle was observed after 24 and 48h treatments. CHL-1 cells showed an increase in cells in G1 and G2/M phases while a decrease of cells in S phase was measured. These results suggest an anti-proliferative effect of the *S. pinnata* extract on the melanoma cell line.

Success of aquatic angiosperm transplantation in the northern Venice Lagoon

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

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

After three years from the beginning of the project ca. 44700 rhizomes have been transplanted in 35 sites placed in an area of ca. 36 km². Transplantations have been successful in 32 sites with a mean coverage of 60% and many areas have been completely colonized. However, results were very different among sites and were negatively affected by raining conditions and water temperature whose extreme values can severely hamper their spread. Despite the different and unpredictable climatic conditions now aquatic angiosperms have colonized all the bottoms close to salt marshes and the canal edges. They are self-sustaining and expand exponentially because they produce a high number of seeds that colonize all the suitable areas. If extreme climatic changes are not taking place and other human impacts will be not occur, in a few years the environmental conditions of the northern lagoon will be restored as before the anthropogenic impacts.

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

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Despite the high ecological and economical importance of transitional environments and their high productivity, they represent a sink for many pollutants including metals. The steady increase in the production and release of products, containing silver nanoparticles (AgNP), could lead to an increasing dispersion and diffusion of silver into transitional environments. In this study, *in vitro* experiments were conducted to examine the toxicity of AgNP using the marine macroalga *Ulva rigida* C. Agardh, the most common species in transitional environments. The toxicity of both AgNP and aqueous silver (AgNO₃) was estimated based on the implementation of both biochemical (lipid peroxidation) and physiological (primary production) biomarkers with conditions similar to the Venice Lagoon environment.

The primary production showed an adverse effect of AgNP starting from 0.1 ppm reaching complete inhibition at 5ppm. Conversely, AgNO₃ displayed a severe inhibition of primary production in *U. rigida* already at 0.01 ppm.

The lipid peroxidation, measured by malondialdehyde levels, showed that the lowest observed stress effect was at 1 ppm of AgNP. The oxidative stress generated by AgNP steadily increased up to a concentration of 30 ppm resulting in membrane disruption. In contrast, the oxidative stress generated by AgNO₃ was less significant, proving that AgNO₃ was very toxic (as measured by primary production) but did not produce stress at cellular level. The silver bioaccumulated by *U. rigida* from an AgNP solution of 1 ppm reached 8.5 mg Kg⁻¹fw and the bioaccumulation reached a saturation point at 11.3 mg Kg⁻¹fw from a 5ppm AgNP solution.

The concentration of silver in the water column don't exceed the ppt range, which is not toxic for *U. rigida*, but the reported concentrations for benthic marine organisms from the Venice Lagoon were in the ppm range. These concentrations of AgNP were found to be high enough when bioaccumulated to elicit a phytotoxic effect.

Keywords: *Ulva rigida*, Biomarkers, Silver Nanoparticles, Venice Lagoon.

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

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

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

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Coccolithophores are nanoplanktonic microalgae characterized by an exo-skeleton composed of minute calcified plates and play an important role in control of climate changes because they are involved in the sulfur cycle for the production of dimethylsulfide as well as in the carbon cycle for the photosynthesis and calcification processes. Recent studies on the ocean acidification due to the carbon dioxide increase in the atmosphere have provided evidence on a possible impact on coccolithophore calcification.

The aim of this study was: a) to investigate possible effects on gene expression of the coccolithophore *Pleurochrysis pseudoroscoffensis* due to changes in $p\text{CO}_2$ and temperature; b) to assemble de novo the genome and transcriptome of the coccolithophore.

In the framework of the project Acid.it (Costruzione di conoscenze e di strumenti a supporto della definizione di strategie di mitigazione ed adattamento agli effetti dell'acidificazione marina, con particolare riferimento ai mari italiani), culture experiments were performed in triplicate in 2.5 L-photobioreactors testing four $p\text{CO}_2$ /temperature conditions: control (21°C and 400 ppm CO_2), high temperature (25°C and 400 ppm CO_2), high $p\text{CO}_2$ (21°C and 700 ppm CO_2) and high temperature and $p\text{CO}_2$ (25°C and 700 ppm CO_2). Samples were collected at two different timing: after 11 and 20 days. The RNA from samples was isolated with a commercial kit, and the cDNA library were prepared for the sequencing with the Illumina Hiseq 3000/4000 technology. The reads obtained from the sequencing were trimmed and assembled with the Trinity software, the transcriptome is about 51000 contigs. The functional annotation was made with the software Trinotate, and for evaluate differentially expressed genes was used the program CLC Genomics Workbench.

Considering the gene expression, the only effect of CO_2 did not result in significant variations, whereas the only temperature had a more significant effect. However, these two factors seemed to show a combined effect, with a strong gene up-regulation and down-regulation at the end of the culture. The genes down regulated were related to protein transcription and translation, so to presume a complete stop of the protein synthesis. Among the upregulated genes, there were some involved in several metabolic pathways mainly related to nitrogen synthesis, but also genes involved in the coccolithogenesis and a carbonic anhydrase, suggesting a possible alteration of the production of the calcium carbonate skeleton.

Instead, for the genome we isolated the DNA and sent it to the sequencer center, where they prepare the library and sequenced them with the Illumina Hiseq 3000/4000 technology. The reads were then assembled with four different software (CLC G.W., Soap, Spades2 and Abyss). The better output, based on different parameters was the one with Abyss, and with another program (Jellyfish) we were able to estimate the genome size which is about 213Mb.

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

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Seagrass meadows are among the most productive ecosystems on Earth, that provide high-value ecosystem service and contribute to carbon sequestration. Nutrient and light availability are the primary physical factors controlling seagrass growth. The median carbon, nitrogen and phosphorus contents in seagrass leaves translate into a median atomic C:N:P ratio of 432:20:1. If we compare these values with the Redfield ratio for the production of organic matter by phytoplankton (C:N:P 106:16:1), we can deduce that seagrasses need about a quartet of the nitrogen and the phosphorus that are necessary for phytoplankton.

This is the first study *in situ* of seagrass primary production and nutrient uptake from the water column in the Venice Lagoon. We tested the Net Community Production (NCP) and the nutrient uptake (nitrogen and phosphorus) of *Zostera marina* and *Zostera noltei* through benthic incubation chambers. This work was carried out in summer (July and August 2017). The result showed that the NCP of *Zostera marina* decreased from July (mean 0.108 ± 0.02 mmoli/(m²*h*gr)) to August (mean 0.053 ± 0.08 mmoli/(m²*h*gr)), according to the growth rates of this specie. Moreover, the comparison of *Zostera marina* and *Zostera noltei* NCP, during the August sampling, highlighted that the species with the highest NCP was *Zostera noltei*, with a mean of 0.709 ± 0.35 mmoli/(m²*h*gr) against a mean of 0.108 ± 0.02 mmoli/(m²*h*gr) of *Zostera marina*. Indeed, in this month, the growth rates of *Z. noltei* increased while that of *Z. marina* decreased.

Finally, this study displayed that the nutrient uptake for both the species was higher for ammonium, with a mean of 17.1 nm/h*gr for *Z. marina* and a mean of 23.5 nm/h*gr for *Z. noltei* while the phosphorus and nitrate uptake were lower. In particular, the mean for both species of phosphorus was 5.2 nm/h*gr and of nitrate was 1.1 nm/h*gr.

Food from Microalgae: Challenges and Opportunities

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Providing adequate sources of food to the still growing world population is a global problem of difficult solution. Almost 800 million people suffer from hunger and more than 2 billion from micronutrient deficiencies (FAO, 2017).

Several planetary boundaries have been surpassed thanks to agriculture impacts on environment. Intensive agriculture is among the first causes of GHG emissions, loss of fertile soil, expansion of ocean dead zones, desertification and depletion of freshwater resources. Climate change is decreasing crop production in many areas. World's oceans have been overexploited and heavily polluted.

The looming food crisis, which encompasses an unprecedented rise of overweight and obesity, is not only a matter of calories, but also of lack of proteins and essential nutrients (vitamins, minerals and fatty acids).

Microalgae, and particularly those of marine origin, represent a sustainable alternative source of those essential amino acids and micronutrients that a healthy diet requires. They have several advantages over traditional crops: 1) can be cultivated on arid lands unsuitable for agriculture using saline or brackish waters; 2) do not require pesticides; 3) have less dependency on seasonal variations; 4) can accumulate high amounts of protein, oil or sugars, and 5) synthesize at the same time valuable molecules such as vitamins, pigments and polyunsaturated fatty acids. These properties have drawn significant interest in microalgae as source of biofuels and food in recent years.

However, several barriers still hinder the industrial use of microalgae as food and, especially, bioenergy sources. Many companies and institutionally supported projects have failed, particularly in the US and Europe, because based on the false assumption that algae can provide several times as much biomass per hectare than traditional crops. In reality, productivity of algae cultures is not much higher than that of C4 plants, and their industrial exploitation is limited by the need to sustainably cultivate selected strains at hundreds of hectares scale (Tredici, 2010).

Despite that large-scale commercial production of microalgae is mostly carried out in open ponds, many companies (and especially most of the start-ups in the algae biofuel sector) focus on photobioreactors (PBR). PBR provide a more controllable environment and limit the risk of contamination, but in general present a negative energy balance, and too high capital and operational costs when high quality biomass of selected strains is targeted. Some approaches and technological innovations developed at the University of Florence, which can help lifting these obstacles, will be discussed.

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

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

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