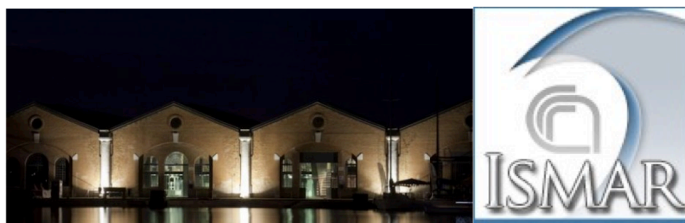




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The synergic effects of hydrodynamics, temperature, and the N:P ratio on bloom dynamics of the toxic benthic dinoflagellate *Ostreopsis cf. ovata*

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Intense blooms of the toxic benthic dinoflagellate *Ostreopsis cf. ovata* have been a recurrent phenomenon along several Mediterranean coasts during summer in the last few years. Blooms are associated with both noxious effects on human health and mortality of benthic marine organisms, due to the production of palytoxin-like compounds. We investigated the temporal trend of *O. cf. ovata* bloom focusing on the role of environmental factors on the bloom dynamics using an interannual dataset (2007-2012) from the Conero Riviera (N Adriatic Sea). Generally, the temporal trend of blooms showed the first cell appearance at the end of July/early August, the maximum abundances in late-summer at end of September/early October reaching up to 10^6 cells g^{-1} fw on macrophyte samples, and the decline of the blooms at end October/early November. The synergic effects of hydrodynamics, temperature, and the N:P ratio of water column nutrients seems to drive the bloom dynamics of *O. cf. ovata*. Hydrodynamism play a major role as calm conditions appeared to be a prerequisite for blooms. When suitable hydrodynamic conditions exist, *O. cf. ovata* blooms appear to be triggered by a combination of optimal temperature and available nutrients. Temperature above 25 °C would allow the germination of cysts leading to the bloom onset, and N:P ratio around Redfield value is a necessary condition to allow cell proliferation supporting high growth rates. Once the bloom has started it may be maintained at temperature values even below 20 °C and at N:P ratios that are in excess of the Redfield ratio (likely attributable to *O. cf. ovata* uptake), suggesting other physiological mechanisms important to maintain the bloom, including allelopathic and mixotrophic interactions and metabolic dissipatory strategies. Bloom decline occurs when temperatures dropped below 18 °C. The net effect of the synergy between local hydrodynamic conditions, temperature, and N and P availability may explain why in the N Adriatic Sea there is a delay between the maximum temperature values and the peak *O. cf. ovata* bloom differently from what observed in other Mediterranean regions.

Inhibitory effect of polyunsaturated aldehydes (PUAs) on the growth of toxic benthic dinoflagellate *Ostreopsis cf. ovata*

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During the last decades, massive blooms of the toxic benthic dinoflagellate *Ostreopsis cf. ovata* have occurred in different coastal areas of the Mediterranean Sea typically during the late summer period. *O. cf. ovata* blooms have been related to human intoxication and to mass mortalities of benthic invertebrates due to the production of palytoxin-like compounds, including isobaric palytoxin, ovatoxins and mascarenotoxins.

In the last decades, several microalgae have been shown to release a wide range of secondary metabolites that mediate interactions between individuals of different species. A variety of diatoms genera produce and release different types of fatty acid derived long-chained polyunsaturated aldehydes (PUAs). These compounds have been related to multiple functions such as adverse effect on the reproduction of various invertebrates, intra- or interspecific signals, and have effects on a variety of marine organisms as well, including co-occurring microalgae. Moreover, several studies have highlighted changes on growth, cell membrane permeability, flow cytometric properties and cell morphology due the exposition of microalgae to PUAs.

The relationships between *O. cf. ovata* and co-occurring microalgae has been investigated previously (Pichierri et al., 2014), showing that some benthic diatoms exerted a negative effect on the growth rate of *O. cf. ovata*. The aim of this study is to investigate the effects of three toxic algal-derived PUAs, namely the 2E,4E-decadienal, 2E,4E-octadienal and the 2E,4E heptadienal, on the growth and cell morphology of *O. cf. ovata*.

O. cf. ovata was cultured under controlled laboratory conditions at different PUAs concentrations (from 0.1 to 36 $\mu\text{mol l}^{-1}$) and using two different exposition times (24 and 72 h).

Our results showed a clear decrease of *O. cf. ovata* growth when cultures were exposed to the various PUAs compared with the control. After 72 h of exposition at the maximum concentration tested, the growth decrease respect to the control was 92.4%, 81.5% and 48.6% for 2E,4E-decadienal, 2E,4E-octadienal and 2E,4E heptadienal, respectively. Although a concentration-dependent decrease of the growth rate was observed for all three aldehyde compounds, the effect was stronger with longer-chained molecules than with shorter-chained ones.

Moreover, at the highest concentrations of 2E,4E-decadienal (from 6 to 36 $\mu\text{mol l}^{-1}$), aberrant forms of *O. cf. ovata* cells were observed, showing a marked decrease of cell dimensions, a contraction of cytoplasm and the formation of abnormal vesicle-like structures.

These data pointed out for the first time the negative effect of PUAs, which are compounds produced by some diatoms species, on the growth and morphology of *O. cf. ovata* cells and confirm the possible allelopathic role of these molecules in the relationships among phytoplankton species. Further studies are needed to better clarify the interactions between diatoms and *O. cf. ovata*, especially on bloom-forming dynamics.

Some aspects on bacterial community associated with *Ostreopsis* cf. *ovata* in batch cultures

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Extensive blooms of the toxic epiphytic/benthic dinoflagellate *Ostreopsis* cf. *ovata* are being reported with increased frequency and areal distribution in temperate coastal regions including the Mediterranean Sea. *O.* cf. *ovata* outbreaks are of human and environmental health concern due to the production of isobaric palytoxin and a wide range of ovatoxins. In the last years, among the ecological factors that trigger or regulate the algal bloom dynamics, bacteria-microalgae interactions have received increasingly attention.

This study investigated the dynamics and phylogenetic structure of the bacterial community co-occurring with *Ostreopsis* cf. *ovata* in batch cultures during different algal growth phases. Cultures of a *O.* cf. *ovata* strain isolated from the NW Adriatic Sea were maintained under controlled conditions for a period of 42 days. *O.* cf. *ovata* and bacterial cell abundances were monitored along with the removal of major nutrients from the medium. Phylogenetic composition of bacterial community was assessed by next generation sequencing of bacterial 16S rDNA hypervariable regions.

Bacterial community showed a biphasic growth curve with the first exponential step occurring in parallel with the algal exponential phase and the second one in concomitance with the algal mid-stationary phase. Ion torrent data revealed the presence of 12 bacterial phyla, 17 classes and 150 genera all along the experiment. A self-sufficient consortium for vitamin synthesis composed by only few genera belonging to *Alphaproteobacteria* (>60% of relative abundance) and *Sphingobacteria* (2-34%) dominated the community. Our results allow to postulate on specific mutualistic and antagonistic interactions between *O.* cf. *ovata* and the associated microbial community in batch cultures, which will ultimately affect algal cellular physiology and potentially toxin dynamics. Moreover, the data pave the way for further investigations on relationships between bacteria-*O.* cf. *ovata* interactions and vitamins availability in the environment.

The use of organic phosphorus by *Ostreopsis cf. ovata*: a mixotrophic strategy to account for its success?

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Many coastal environments are suffering increasing levels of organic nutrients deriving from anthropogenic and microbial activities and this will affect the natural communities of these environments. In addition, the importance of phosphorus as a nutrient that is limiting primary productivity in these areas is also coming to light. Likewise, in recent years, incidences involving *Ostreopsis ovata* blooms in temperate coastal zones have been reported with increasing frequency, intensity and distribution. So it was decided to investigate a potential link between these actualities. Studies of *O. ovata* 'success' have covered many aspects of its ecology, however, to date there has been no information given about its possible use of organic substrates as nutrient source. Yet there are many reports in the literature on diverse algal groups that can maintain 'normal' activity by making use of organic substrates. In this preliminary study, the phosphatase assay was used as convenient and rapid method to assess possible organic nutrition. Phosphatases are a group of enzymes that hydrolyse simple organic compounds and release inorganic phosphate. Evidence has shown that the bulk of enzyme activity is associated with the cell wall or its immediate surroundings, leading to the release of inorganic phosphate external to the cytoplasmic membrane and the subsequent uptake of much of this into the cell.

Here we report on the phosphatase activities of *O. ovata* cultured in media containing inorganic or organic phosphate and natural samples. Primarily the results show that *O. ovata* could utilise organic P compounds as growth continued unimpeded in organic P only media and the occurrence of high surface phosphatase activity associated with the cells. The results also highlight what seems to be a high constitutive phosphatase activity. This signifies that even when experiencing high ambient nutrient levels, that rates of activity can continue to supply 'luxury' levels of P to the cells. It is likely that *O. ovata* would thrive under high nutrient conditions and have a competitive edge over other groups of organisms that may have product repressible phosphatases.

A first analysis of harmful and non indigenous microalgae in ships' ballast waters in the port of Trieste (NE Adriatic Sea)

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The introduction of Non Indigenous Species (NIS) and Harmful Aquatic Organisms and Pathogens (HAOP) through ships' ballast water is recognized by United Nations as one of the four most serious threats of the world to human health, property and resources. In the world 3-5 billion tons of ballast waters are annually discharged from the ships (IMO, 2000-2014), with the consequence that 7.000 species among microorganisms, plants and animals can be transferred from a sea to another. In the frame of the BALMAS (Ballast Water Management System for Adriatic Sea Protection) project, the port baseline survey (PBS) and the monitoring of the ships' ballast waters incoming in the port of Trieste were carried out. The PBS was performed from 2014 to 2015 to seasonally analyse the phytoplankton communities. Sampling was carried out in 6 stations located in the port of Trieste and in the reference site (C1) located inside the Marine Reserve of Miramare. 10 ship' ballast tanks, coming in the Port of Trieste, were sampled in June 2015 to monitor the presence of NIS and harmful microalgae. For the viable cell analysis, water samples were stained with Fluorescein Diacetate (FDA) as a selective viability indicator and processed using an epifluorescence microscope. For phytoplankton quantitative and qualitative analyses, 500ml-water samples were fixed with Lugol (1% final concentration). Cell counts were carried out according to the Utermöhl's method (Zingone et al., 2010).

Phytoplankton community showed approximately the same species composition in the 6 port stations and in the reference site. Only one NIS was recorded, the diatom *Pseudo-nitzschia multistriata*. The harmful species were identified among dinoflagellates (*Alexandrium pseudogonyaulax*, *Dinophysis caudata*, *D. fortii*, *D. sacculus*, *D. tripos*, *Lingulodinium polyedrum*, *Phalacroma mitra*, *P. rotundatum*, *Prorocentrum cordatum*, *Protoceratium reticulatum*) and diatoms (*Pseudo-nitzschia multistriata*, *P. cf. galaxiae* and *Pseudo-nitzschia* spp.). In all ballast water samples, viable cells exceeded the phytoplankton limits of 10 cells mL⁻¹ as defined by IMO regarding ballast water quality standards. Finally, potentially toxic species were found (*Pseudo-nitzschia* spp., *Dinophysis sacculus*, *Noctiluca scintillans*, *Prorocentrum cordatum*).

Results showed the possibility of survival of phytoplankton organisms inside the ballast tanks and their potential transfer *via* ships in the port areas, with possible ecological, economic and human-health effects in the coastal marine environments.

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Fitoplancton metodiche di analisi quali-quantitativa. In: Metodologie di studio del plancton marino. Socal, G. Buttino, I. Cabrini, M. Mangoni, O. Penna, A., Totti, C. (eds.), Manuali e Linee Guida ISPRA SIBM Roma, 213-237.

Impact of driving factors on macrophyte dominance in the Italian transitional environments

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Macrophyte metrics and community composition in lagoons and ponds (Grado-Marano lagoon, Venice lagoon, Po Delta lagoons, Valli di Comacchio Ponds, Pialassa della Baiona Pond, lagoon of Lesina, Lagoon of Orbetello), representing more than 75% of the Italian transitional water surface, show a strong relationship with the ecological status of these environments.

Anthropogenic impacts, affecting the availability of nutrients in the water column and surface sediments, the increase of water turbidity and phytoplankton (as chlorophyll-*a*) concentration, act as main drivers, changing macrophyte assemblages and the conditions governing their presence/absence.

Macrophyte behavior is similar in all the examined environments, in spite of the different basin morphology and species richness and composition. That trend almost follows the same pattern both in choked and restricted basins. It does not always depend on the number of taxa but mainly on their ecological value.

The quick response to environmental changes makes the study of macrophyte assemblages as the most suitable and rapid method to assess the environmental quality and to forecast its short term trend.

Macrophytes and ecological status in the northern basin of the lagoon of Venice.

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The northernmost part of the lagoon of Venice in the '70s-'90s underwent strong changes mainly due to nutrient inputs and clam harvesting. Those anthropogenic impacts led to the almost complete disappearance of aquatic angiosperms and sensitive macroalgae, also reducing biomass and coverage of opportunistic species like Ulvaceae, Cladophoraceae and Gracilariaceae (Sfriso & Facca 2007). In the last decade environmental conditions changed again: nutrients, especially phosphorus, decreased significantly and clam harvesting declined for the reduction of clam stocks, triggering the colonization of sensitive species and allowing the spreading of aquatic angiosperms, re-introduced with the support of Regional and European projects. In order to determine the species diffusion, 17 stations have been monitored in spring and autumn 2014, before/during angiosperm transplantation, and in spring and autumn 2015 after their first-year dissemination. The results show a significant spreading of sensitive taxa and angiosperms that increased in great part of the basin, especially in Valle Lanzoni, Palude Maggiore and Palude della Centrega. In contrast, in Palude della Rosa and Valle Ca' Zane, affected by the outflows of the Dese and Silone rivers, because of the high rainfall recorded in 2014, ca. twice higher than in the previous decade, an abnormal growth of Ulvaceae was recorded. However, in general the ecological conditions of the studied areas have improved confirming the positive trend recorded after 2011 when great part of Valle Lanzoni, Palude Maggiore and Palude della Centrega were almost deprived of vegetation, except for *Vaucheria submarina*, the only species able to grow in the presence of high water turbidity and sedimentation rates.

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What is *Lithophyllum incrustans*? Resolving taxonomic misconceptions with the use of DNA sequence data from type specimens

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Lithophyllum incrustans is the type species of *Lithophyllum*, one of the most widespread and ecologically important genera of coralline algae. Originally described from Sicily, this species is mostly reported as intertidal and shallow subtidal crust on Atlantic and Mediterranean shores of Europe. Using DNA sequence data (*rbcL*, COI and *psbA* genes) from historical herbarium specimens and new collections from many sites around Europe, we clarified the taxonomic relationships between *L. incrustans* and some closely related species. Partial *rbcL* sequences obtained from the type specimens of *L. incrustans*, *L. hibernicum* and *L. bathyporum* allowed to link unambiguously these names with molecular clades. Based on these results, we conclude that in Atlantic Europe *L. incrustans* is a subtidal species occurring both as crust and rhodolith, whereas crustose forms of *Lithophyllum* found in the intertidal and shallow subtidal belong mostly to *L. hibernicum*. New DNA sequence data obtained from samples from Italy suggest that a similar situation exists in the Mediterranean. So far, molecular confirmation of the presence of *L. incrustans* has been possible only for the Gulf of Trieste; deep subtidal samples (15-20 m depth) collected in this area belonged to the molecular lineage corresponding to the genuine *L. incrustans*. Conversely, samples of *Lithophyllum* collected in the intertidal and shallow subtidal zone at many sites in Italy were not representative of *L. incrustans*, and belonged to many different lineages. These results indicate that the assemblage of encrusting coralline algae living at this level of the shore is much more diverse than currently appreciated; detailed studies combining DNA sequence data and traditional morphological data will be necessary to fully elucidate its diversity.

New records of Charophytes occurring in some Apulian transitional water systems

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Charophytes or stoneworts (Streptophyta, Charophyceae) are a large and morphologically complex group of brackish and freshwater algae, greatly important for both their ecological and evolutionary significance. Stoneworts play different roles in aquatic ecosystems, having a strong positive effect on water quality and transparency. They are very susceptible to human induced eutrophication, mechanical disturbance as well as pollution. Therefore, charophytes represent good indicators of undisturbed aquatic ecosystems, but they are rapidly declining all over Europe. Several species, characterizing some habitats of community interest (Habitat Directive 92/43/EEC), are currently recognized as species of high conservation value and are included in many European Red Lists.

Despite the ecological importance of stoneworts, data on their occurrence and abundance, as well as on their distribution in Italy, are scarce and fragmentary and an Italian Red List is consequently still lacking.

The present study aims at giving a contribution to the knowledge of charophyte species occurring in some Apulian transitional water systems. Four taxa (two of the genus *Chara* and two of the genus *Lamprothamnium*) were found in three out of the eight investigated coastal lagoons. One taxon was identified at generic level exclusively and requires further investigation. All the species found belong to the IUCN threatened categories throughout Europe. A comparison with literature data, when available, is also discussed in order to identify changes eventually occurred over time.

Effect of nano-Ag particles on the Adriatic Sea green macroalga *Ulva laetevirens* Areschoug, 1854 (preliminary results)

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Silver nanoparticles (ENP) are one of the most widely used engineered nanomaterial in a broad variety of applications, such as anti-microbial coatings, textiles, cosmetics, pharmaceuticals, electronics, plastics and food. A recent survey by Piccinno et al. (2012) highlighted 55 (5.5-550) and 5.5 (0.6-55) t y⁻¹ as the median (25/75 percentile) of production/utilization quantities of n-Ag, becoming a key parameter in environmental risk assessment and monitoring. As reported by Gottschalk et al. (2013), the estimate of n-Ag release that could reach wastewater treatment plant (WWTP) is in the range of 0.05-0.2 ug l⁻¹ and 1-8 mg kg⁻¹ as biosolids.

Therefore, n-Ag could be entered in the aquatic compartment, as the northern Adriatic Sea, which is strongly influenced by river runoff. Recent literature highlighted that n-Ag may be directly and/or indirectly toxic in the marine organisms, e.g. causing oxidative stress or damaging cell membranes but results are still contradictory (Macken et al., 2012). Upon entering marine ecosystems, complexation and speciation of silver has to be taken into account when assessing silver ecotoxicity. The aim of this preliminary research is to compare toxicity of n-Ag with AgNO₃ in the marine macroalga *Ulva laetevirens* Areschoug, 1854, using the concentration of chlorophyll-*a* as biological marker, in order to highlight the role of ENP physico-chemical parameters with respect to free Ag⁺.

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Macken A, Byrne Hj, Thomas Kv. (2012) - Effects of salinity on the toxicity of ionic silver and Ag-PVP nanoparticles to *Tisbe battagliai* and *Ceramecium tenuicorne*. *Ecotox. Environ. Safe.*, 86: 101-110.

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Seasonal variations of native agar from three different Gracilariaceae

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The seaweeds collected by companies or local governments in Italy were considered as waste by law till the emanation of the decrees 152/2006 and 2/2009. This abundant resource is still not exploited and can become an issue during summer. In the year 2014 an hypo-anoxic event occurred in the Venice Lagoon (Bastianini et al., 2013) and repeated itself in the summer 2015 due to fast algal bloom and degradation. These events led to perform an investigation in two stations Tresse (TR) and San Giuliano (SG) on the sides of the bridge watershed that connect Venice with Mainland, where there are high nutrient loads and algal growth. Three Gracilariaceae were selected for their abundance, fast growth and potential biotechnological and commercial value: *Gracilaria gracilis* (Stackhouse) M. Steentoft et al. (1995), *Gracilariopsis longissima* (S.G. Gmelin) M. Steentoft et al. (1995) and *Gracilaria vermiculophylla* (Ohmi) Papenfuss (1967). Monthly samples were collected of the investigated species to measure quantity, quality and variations of useful compounds. The main biochemical components of these species are sulphated polysaccharides, commonly regarded as agar. Even though the agar extracted from other red algae as *Gelidium* has an higher quality, because of the not-sulphated polysaccharide that has an higher gel strength, some pretreatments (with diluted caustic soda or acetic acid) have been developed for the gel desulphurization in *Gracilaria*, species that are now exploited worldwide for agar production.

Measures of Native Agar, the simplest gel obtained by boiling extraction without chemical pretreatments were performed on the red seaweeds monthly. These measures represent the maximum quantity of extractable agar. The algae were collected, dried, ground and the agar was extracted at 100°C for 2 hrs with bi-distilled water. The native agar was measured gravimetrically on aluminium capsules after desiccation at 105° C.

The seaweeds presented different agar production patterns in the stations globally ranging from 22% to 62% of the algal dry weight: at TR both *G. longissima* and *G. vermiculophylla* reached a mean value of 54%, lower at SG with 42% and a higher seasonal variation. *G. gracilis* produced the lowest values with a mean of 49% at TR and 45% at SG. The agar content at TR was more constant ranging in all species, present in all the campaigns, from 36% to 64%. At SG the production was more instable (22%-62%). Moreover *G. gracilis* completely disappeared in June and July while *G. longissima* e *G. vermiculophylla* only displayed a decrease in polysaccharide production. *G. longissima* and *G. vermiculophylla* are good candidates for agar production in the Venice Lagoon and can ensure a “stable” production of agar with maxima during spring and mid-summer.

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Surveys on the systematics of *Pleurastrum* and *Chlorococcum* (Chlorophyta) genera and finding of a new polar taxon

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The genus *Pleurastrum* (Chlorophyta) Chodat is found in soil, freshwater habitats, and lichen symbiont and it includes species that can undergo different growth forms (coccoid, sarcinoid, and filamentous) depending on field and culture conditions (Sluiman & Gartner 1990). Because of its high polymorphism, the taxonomic history of this genus is confusing, with authors questioning its monophyly and several taxonomic revisions during years (Friedl 1996). Currently, only two species are confirmed under this genus: the type species *Pleurastrum insigne* and *P. photoheterotrophicum*. Many more species are recognized under the genus *Chlorococcum* (Chlorophyta) Meneghini, whose type species is *Chlorococcum infusionum*. This group of coccoid green microalgae is ubiquitous, since it is found in soil, freshwater, and marine environments, and it is reported from such diverse habitats like hot springs and Antarctica. The simple morphology and the lacking of unambiguous diagnostic characters make the identification of members of this genus very difficult (Brown & McLean 1969).

This research work was started in order to characterize two coccoid green algal strains isolated from Terra Nova Bay, Victoria Land (Antarctica). Morphological, ultrastructural and molecular analyses were carried out on the two isolates, as well as on *Pleurastrum* and *Chlorococcum* strains got from International culture collections for comparison. The molecular analyses were based on the *rbcL* and *tufA* genes and on the ITS region, with a focus on the ITS2 structure.

Our results allow us to attribute the two isolated strains, together with two of the comparison strains, to a new polar taxon and to further circumscribe the genera *Pleurastrum* and *Chlorococcum*, with the synonymization of different species. Moreover the validity of the genus *Tetracystis* created by Brown & Bold in 1964 by the re-assigning of species previously belonging to the genus *Chlorococcum* will be debated.

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Photoautotrophic biodiversity of the Giant cave (Trieste, Italy)

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Caves, and more in general the hypogean environment, can be considered extreme habitats for their life-limiting conditions, of which light intensity is one of the most important factors. While at the cave threshold cyanobacteria must compete for sunlight with other organisms (e.g. microalgae, bryophytes, ferns), in the deepest recesses these prokaryotes are usually the unique phototrophs. In show caves, in particular, cyanobacteria represent the major part of the microflora growing in proximity of artificial lighting, also called *Lampenflora*, where they are both at the base of several food chains and a possible cause of damage to the cultural heritage (Lamprinou et al. 2014).

However, due to the particular conditions found in this environment, and the consequent natural selection pressures, some unusual taxa of cyanobacteria, apparently not reported from other environments, have been isolated from caves (Lamprinou et al. 2012a, Whitton 2012). There are many studies on the hypogean flora worldwide (Lamprinou et al. 2011, 2012a, 2012b), but caves of some areas have not been investigated yet or studies were just based on morphological observations. Here we report a first survey on the photoautotrophic biodiversity of the Giant cave (Trieste, Italy). This is a Karst show cave, which in 1995 was put in the Guinness Book of Records as the world's largest tourist cave and which has been open to public for over 100 years. The artificial lightening and, most likely, also the flow of people have favored the proliferation of *Lampenflora*, largely represented by cyanobacteria, during the years. A deep characterization of the Giant Cave's cyanobacteria has not been carried out up to now.

Here we give an overview on the different isolated photoxygenic strains (several cyanobacteria and one diatom) and in particular we focus on two cyanobacteria that were studied more in detail using a polyphasic approach. The characterization of these two microorganisms was carried out through morphological, ultrastructural, biochemical, molecular and phylogenetic analyses. These last were based on the 16S rDNA gene and the 16S-23S ITS spacer.

Lamprinou et al. 2011 *Int J Syst Evol Microbiol* 61:2907-2915

Lamprinou et al. 2012a *Int J Speleol* 41:267-272

Lamprinou et al. 2012b *Int J Syst Evol Microbiol* 62:2870-2877

Lamprinou et al. 2014 *Int J Speleol* 43:335-342

Whitton 2012 *The ecology of cyanobacteria: their diversity in space and time, II.*

Diatoms and other epibionts associated with olive ridley (*Lepidochelys olivacea*) sea turtles from the Pacific coast of Costa Rica

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Although the sea turtles have long been familiar and even iconic to marine biologists, many aspects of their ecology remain unaddressed. The present study is the first of the epizoic diatom community covering the olive ridley turtle's (*Lepidochelys olivacea*) carapace and the first describing diatoms living on sea turtles in general, with the primary objective of providing detailed information on turtle epibiotic associations. Samples of turtle carapace including the associated diatom biofilm and epizoic macro-fauna were collected from Ostional beach (9° 59' 23.7'' N 85° 41' 52.6'' W), Costa Rica, during the *arribada* event in October 2013. A complex diatom community was present in every sample. In total, 11 macro-faunal and 21 diatom taxa were recorded. Amongst diatoms, the most numerous were erect (*Achnanthes* spp., *Tripterion* spp.) and motile (*Haslea* sp., *Navicula* spp., *Nitzschia* spp., *Proschkinia* sp.) forms, followed by adnate *Amphora* spp., while the most common macro-faunal species was *Stomatolepas elegans* (Cirripedia). Diatom densities ranged from 8179 ± 750 to 27685 ± 4885 cells mm⁻². Epizoic microalgae were either partly immersed or entirely encapsulated within an exopolymeric coat. The relatively low diatom species number, stable species composition and low inter-sample dissimilarities (14.4% on average) may indicate a mutualistic relationship between the epibiont and the basibiont. Dispersal of sea turtle diatoms is probably highly restricted and similar studies will help to understand both diatom diversity, evolution, biogeography and sea turtle ecology and foraging strategies.

First report of *Alexandrium margalefii* (Balech 1994) in the northern Adriatic Sea

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Some of the cysts analysed microscopically from sediment sampling during campaign PBS II (31 March 2015) in the frame of the Project BALMAS (Ballast Water Management System for Adriatic Sea Protection) were determined as potentially belonging to *Alexandrium margalefii* (Balech, 1994).

These cysts have in common the spherical shape of 40-45 µm in diameter with granular content and a yellow-orange accumulation body. Their appearance is very similar to that described for the western Mediterranean by Bravo et al. (2006) but differs from that described by Hallegraeff et al. (1991) regarding the size (28-34 µm). Hallegraeff et al. (1991) mentioned the mucoid characteristic of *A. margalefii* cysts, which was also observed for the cysts found in BALMAS. To confirm the diagnosis, cysts were isolated and incubated for further studies. One of the cysts germinated and vegetative cells were reisolated and cultured. The strain was examined using calcofluor staining. The typical conformation of plate 1' and 6'' found in the vegetative cells confirmed the diagnosis.

A. margalefii had never been reported in Northern Adriatic Sea before, either from plankton or sediment samples. The presence of cysts of this species in low abundances has been detected in sediments of all the 5 sites analysed: sites TS 1-2-3-4, located within the port of Trieste and in control site C1 inside the Marine Reserve Park of Miramare.

Accurate identification of *Alexandrium* species is extremely important as this genus includes toxic and troublesome species that affect public health and therefore have economic impact on aquaculture activities. The recognition of the cysts stages particularly of toxic microalgae represent a early warning for future bloom events.

Bravo I., Garces E., Diogene J., Fraga S., Sampedro N., Figueroa R. I. 2006 Resting cysts of the toxigenic dinoflagellate genus *Alexandrium* in recent sediments from the western Mediterranean coast, including the first description of cysts of *A. kutnerae* and *A. peruvianum*. Eur. J. Phycol., 41 (3): 293-302.

Hallegraeff G.M., Bolch C. J., Blackburn S.I., Oshima Y. 1991 Species of the toxigenic dinoflagellate genus *Alexandrium* in Southeastern Australian waters. Bot. Mar., 34: 575-587.

Microphytobenthic community response to high CO₂ concentration and temperature in the Aeolian Islands (Tyrrhenian Sea, Italy)

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Aeolian Islands are a volcanic archipelago in the Tyrrhenian Sea (Sicily). Scientific interest toward this area has increased after a great gas emission occurred in November 2002. We conducted four samplings during three years (2012–2014) at the Panarea and Basiluzzo Islands, in order to get an insight of the ecosystem changes and to understand how the benthic community has adapted to higher CO₂ concentrations and higher temperature.

Near the Basiluzzo Island, we sampled sediments at two stations (B1, B3) influenced by gas emissions, and one reference station without emissions (B2). Close to the Panarea Island we also sampled at two very close points characterised by different CO₂ concentration and sea bottom temperature (CB-HOT, CB-COLD). We aimed to investigate potential changes in the microphytobenthic community abundance and composition and relate them to the presence/absence of the hydrothermal vent.

At the Basiluzzo sampling site, the highest microphytobenthic densities were consistently recorded at St. B1, with its absolute maximum in October 2012 (20045 ± 2344 cells cm⁻²). Differences in the microalgal densities between St. B2 and St. B3 were not so marked. Even greater differences were observed between St. CB-COLD and St. CB HOT where at the latter microphytobenthic abundance in June 2012 reached 243362 ± 14384 cells cm⁻² vs 2510 ± 30 cells cm⁻², respectively.

In contrast, the high abundances were coupled with low community richness and diversity, particularly at St. CB-HOT where the diversity value (H') reached on average only 1.02 ± 0.21 compared to 2.88 ± 0.25 at St. CB-COLD.

Focusing on diatom living forms, the motile ones were predominant at all stations reaching the highest relative abundance at St. CB-HOT (91.45%). The greatest differences among the Basiluzzo stations were detected within the tychopelagic (*Bacillaria paxilifera*, *Ceratoneis closterium* and *Paralia sulcata*) and planktonic (mainly *Fragilaria* sp.) living forms. The tychopelagic forms were more abundant at the reference St. B2 with 13.92% compared to St. B1 and B3 with 2.01% and 5.27%, respectively. On the other hand the planktonic forms were more represented at St. B1 (37.76%) and less abundant at the reference station (7.41%). The values at St. B3 were again in-between the two (16.28%).

The microphytobenthic abundance and species composition were remarkably different among stations indicating a significant influence of the CO₂ concentration and temperature on this phototrophic community.

Phylogeny and Species Diversity of the Planktonic Diatom Family Chaetocerotaceae inferred from ribosomal DNA sequences and morphology

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The centric diatom family Chaetocerotaceae is distributed worldwide and constitutes a prominent component of the plankton. The hallmark of this family forms the siliceous hollow tubes, called setae, protruding from the valve ends following cell division. The family includes two extant genera: *Bacteriastrum* and *Chaetoceros*, the latter is highly diverse. Species diversity is still poorly resolved. Morphological information of natural populations, along with molecular sequence data of cultured strains, provides the basis for the species characterization. In the present study we focus on the phylogeny of the Chaetocerotaceae. Species-level diversity was assessed using a culture-based approach for taxonomic identification (280 strains), followed by molecular characterisation of the nuclear encoded SSU (18S) and partial LSU (28S) ribosomal DNA sequences. We inferred ML and Bayesian phylogenies of the Chaetocerotacean species, as well as morphological characters and their states. Phylogenies were used to circumscribe clades. We recovered ca. 73 of *Chaetoceros* and 7 species of *Bacteriastrum*. With the phylogeny now densely packed with species, surprisingly, only a few states of morphological characters showed homoplasy, whereas the remainder is shared derived or shared ancestral. Using the rDNA phylogeny of this family, we updated the descriptions of the species in the *C. socialis* complex, the *C. curvisetus* complex and the *C. lorenzianus* complex. We provide descriptions of several species new to science, found in the Gulf of Naples, Italy.

Protist diversity in surface sediments at the LTER station MareChiara in the Gulf of Naples: a NGS approach coupled with SDC estimates for diatom resting stages

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Surface sediments host a diverse community of protists including benthic residents and resting stages of planktonic species. Resting stages of dinoflagellates can be identified and quantified by examining sediment samples, while detecting resting stages of diatoms relies on indirect approaches such as the Serial Dilution Culture (SDC) method.

We present results of a pilot study carried out on surface sediments at the Long Term Ecological Research station MareChiara in the Gulf of Naples. Our aims were to: i) test the most appropriate method to extract DNA from sediments; ii) assess protist diversity in sediment samples (with focus on resting stages produced by planktonic species) through an NGS approach; iii) compare the results obtained by NGS for diatoms with estimated of most probable numbers of diatom resting stages obtained through the SDC method.

We sequenced both the V4 and the V9 regions of the SSU rDNA with Illumina and obtained a comparable number (about 2,000) of unique haplotypes. Taxonomic assignment was done using an in-house modified version of the R2 database. The benthic protist community was dominated by taxa belonging to Stramenopiles, followed by Alveolata and Rhizaria. A good match in terms of taxa composition and dominance was obtained when comparing NGS data for Bacillariophyta with the results of the SDC method. Our results show that NGS sequencing, coupled with the more ‘classical’ methods, represents a valuable approach to the study of benthic communities, both active and resting.

Biodiversity in the planktonic diatom family Chaetocerotaceae

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Chaetocerotaceae is one of the most diverse marine planktonic diatom families. Its two genera, *Chaetoceros* and *Bacteriastrium* currently include over ca 230 and 15 taxonomically accepted species, respectively. We set out to assess its diversity at the LTER station MareChiara in the Gulf of Naples (GoN), Mediterranean Sea, at Roscoff, along the French Atlantic coast, and at Las Cruces in an upwelling zone on the central Chilean coast. Colony and spore morphology, frustule ultrastructure as well as nuclear LSU and SSU rDNA sequences have been gathered from ca 240 monoclonal strains. Results showed 55 genetically distinct species, many of these being new to science. Phylogenies inferred from the aligned sequences revealed several major clades of morphologically similar species. SSU sequences in one of these clades contained up to 6 introns of ca. 140 base pairs each. In addition, many of the internal standard sequencing primers showed mismatches in critical positions. The sequences of the V4 region in the SSU rDNA of all these species were used as taxonomic barcodes (references) and blasted against an environmental V4-Next Generation Sequencing dataset gathered from 40 plankton samples taken over three years at the LTER station in the GoN. Results provide an unprecedented picture of the diversity and seasonal cycle in this species-rich family. This approach provides insights in the occurrence and distribution of species along the seasons and it permits in-silico discovery of species new to science.

Coupling digital photography and image processing for detecting the growth on different lithotypes of *Pleurocapsa* sp. and *Leptolyngbya* sp. isolated from Pompei frescoes: a lab study

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In the framework of a study on the algal flora attacking the frescoes of the archeological site of Pompei, two strains of Cyanobacteria were isolated and attributed to the genera *Oculatella* and *Pleurocapsa*, on the basis of molecular analyses. The strains were used in a laboratory study for monitoring their ability to colonize different lithotypes, namely tufa, mortar, porphyry, brick and calcarenite, and glass. Batch cultures of *Oculatella* and *Pleurocapsa* were grown in Bold's Basal Media (BBM) in a climatic chamber under 60 PAR continuous illumination and at 22 ± 2 °C. Three blocks (about 2x1x1.5 cm) of each lithotype were immersed in the flasks when culture reached 1.0 OD and kept in the above described conditions for twelve weeks. Then, the algal populations settled on the blocks were kept in air, closed in sealed chambers under different moistures or light wavelengths. The biofilms growth was macroscopically monitored by measuring the surface coverage and the amount increase of green colour over 12 weeks. The surface of each specimen exposed to colonisation was photographed with a Nikon 5100 digital camera and Digital image analysis was performed on each photographs with Image J platform. The selected substrates showed the following bioreceptivity for the growth of both Cyanobacteria: tufa, brick, porphyry, calcarenite, mortar and glass. Relative humidity (RH) strongly affects the growth of algal biofilm on air. A values of RH close to 100% was required to maintain cyanobacterial colonization. Both strains were able to growth at all wavelegths ranging from 470 and 680 nm but with different kinetics. Moreover, we have observed a correlation between effects of wavelenghts and lithotype on biofilm growth.

Genetic structure of population of *Cyanidiophytina* (Rhodophyta)

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Cyanidiophyceae are one of the most intriguing groups of microorganisms challenging extreme environmental conditions of volcanic and post-volcanic areas, typically characterized by strong acidity, mostly generated by high amounts of sulfur, and high temperatures. The interest in this group of microalgae has prompted researchers to explore a large number of geothermal sites dispersed in different continents, and in most cases the pH of the sampling points was less than 5, thus confirming the strong acidophily of Cyanidiophyceae; *vice versa*, the temperature does not seem to be a limiting factor for these organisms, having been found in a wide temperature range, from 19 ° C to 55 ° C in Phlegrean Fields. The specific ecological and physiological requirements by Cyanidiophyceae should reduce their dispersal ability; however, this sets paradoxically against their global distribution; infact, the data so far collected suggest that thermoacidic environments throughout the world are inhabited by mixed populations of Cyanidiophyceae (Iceland, Italy, USA, New Zealand, Japan, Taiwan) with a level of genetic polymorphism higher than expected. Here we analyze the level of haplotype diversity for *Galdieria sulphuraria*, *G. maxima* and *Cyanidioschyzon merolae*, three thermoacidophilic species globally distributed; the detected haplotypes were employed to understand if they derived from a single or independent evolutionary lineages through haplotype networking and nested clade analysis. The analysis of intra and interpopulation genetic diversity, carried out by DNAsp, have shown that italian population of *G. sulphuraria* possess the highest haplotype and nucleotide diversity level, followed by New Zealand, USA and Iceland; *G. sulphuraria* showed the widest dispersal areal, explainable with a high dispersal ability followed by extinction events, likely due to the evolution of the geography of the Earth. *G. partita* and *G. daedala* clustered separately, the former with Taiwan haplotypes, the latter with Turkey haplotypes, thus suggesting that they would represent two distinct evolutionary lineage, additional to *G. sulphuraria*. Networking analysis have also revealed the coexistence of two different populations of *G. maxima* in Turkey, one correlated to western populations and the other to the eastern populations of *G. maxima*; these data let us speculate that Turkey would be the origin of *G. maxima*, this hypothesis being supported by the fact that Turkey traces back its origin to Gondwana and during its long evolutionary history, the population of *G. maxima* would have splitted in two sub-populations. *C. merolae* populations dispersed throughout the world (USA, Italy, Turkey and Indonesia), instead, seems to be derived from the same evolutionary lineage: different haplotypes of the same single network differs few mutational steps each other. The hypothesis is that the RubisCo in *C. merolae* is not subject to high level of mutatiol rates because this microalgae lives in that acidothermal zones closer to water sounces, where the environment is more stable. The alternative hypothesis could be that this species has a moderate ability to move colonizing acidothermal regions throughout the world.

A complete outfitting of meiotic genes in the extremophilic *Galdieria* and *Cyanidioschyzon* (Cyanidiophytina, Rhodophyta)

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Meiosis came into view early in the phylogenetic history of eukaryotes and the constantly discovery of genes encoding proteins for meiosis in putatively asexual protists allows new insights on the evolution of sexual reproduction.

Here we have investigated the presence of meiotic genes in *Galdieria* and *Cyanidioschyzon* (Cyanidiophytina), two unicellular asexual red algae usually challenging the harshness of the thermoacidic environments; these extremophilic algae have appeared on the earth scenario very early in the long phylogenetic history of photosynthetic protists, just after the evolution of eukaryogenesis.

Based on the meiosis detection toolkit, comprising meiotic genes widely present in eukaryotes and exclusively functioning in meiosis, our survey interested 9 core meiotic genes and 11 additional genes that are required in meiosis. Phylogeny was estimated for Cyanidiophytina through a Bayesian inference and a RAxML likelihood analysis was performed on several members among animals, fungi and plants and related photosynthetic protists as well as *Chondrus*, a well-known sexual alga belonging to Rodophyta.

Surprisingly, 8 of 9 core genes were found to be present in *Galdieria* and 7 of 9 in *Cyanidioschyzon*. Our results clearly showed that these primitive eukaryotic algae possess almost the complete set of genes that are known to have an exclusive role in meiosis with the only exception of Hop1, that is anyway often missing in many sexual organisms, despite its important role in synaptonemal complex assemblage. It is also notable that the repair protein Rad52 is present in both *Galdieria* and *Cyanidioschyzon*; as far as we know, there is no evidence of the presence of this protein in plants and green algae lineages. Interestingly, *Chondrus* seems not require 6 of the inventoried genes to undergo meiosis, which means that probably other different proteins are required in meiotic machinery of red algae.

Phycological jokes...and more. II Contribution.

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Aiming at inducing listeners to smile, in this second contribution a new series of original jokes are presented. As in the first contribution they are mainly based on algal names but also on different topics.

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

Microscopy applied to the study of the biodeterioration of fossil bones in the Pleistocenic site of La Polledrara di Cecanibbio (Rome, Italy)

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La Polledrara di Cecanibbio is one among the richest palaeontological sites of elephant fossil remains in Europe, discovered in 1984. The archaeological investigation finished in 2014 unearthing a portion of paleo-surface corresponding to an ancient river bed that incised the sediments of the Monti Sabatini Volcanic Complex during the Upper and Middle Pleistocene. The river deposited the bone remains of large mammals, particularly of elephants (*Palaeoloxodon antiquus*), after their transport during the high flow stage. Thanks to the exceptionality of the finds, their excellent preservation and exclusive taphonomic contest, the “musealization” of the site was conducted at the beginning of 2000 and it is currently under way¹.

The capillarity of tuff sediments arouse a serious problem of humidity that, coupled with the presence of light, favours a huge development of phototrophic biofilms on the fossils exposed *in situ*. This causes an aesthetic damage and represents a serious threat for the special heritage exposed at the site. Among the afflicted zones, two areas have been selected for the investigation of the biodeterioration process. The sampling was conducted using adhesive tape strips in order to collect the samples in a non-invasive way for the substratum and at the same time non-destructive for the biofilms².

The adhesive tape strips were observed directly at the Light and Confocal Laser Scanning Microscopes (CLSM) showing that most of the biofilms were mainly composed of cyanobacteria sometimes associated with microalgae, fungi and mosses. In particular, the cyanobacterium *Leptolyngbya* sp. resulted as the main component of the deteriorating biofilms. The interaction between the phototrophic community and the bone substratum was also investigated in order to evaluate the possible damage induced by the biofilm growth to the fossil remains. Cross and thin sections of colonized elephant bones were observed at the Scanning Electron Microscope, Petrographic microscope and CLSM. The images showed some filaments of *Leptolyngbya* sp. penetrating inside the superficial cortical portion of the bone for more than 50 µm up to reach, in some cases, the osteons suggesting an endolithic activity into the phosphatic substratum causing mechanical damages. The research represents the first report of *Leptolyngbya* sp. on a peculiar non-carbonate substratum, probably with an endolithic activity. More studies are needed to better understand the deterioration mechanism along with specific preservative actions to protect the inestimable cultural and scientific value of the fossil remains exposed at the site.

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[2] Urzi, C., De Leo, F. 2001. Journal of Microbiological Methods 44, 1–11

Produzione di Spirulina “High Quality” in un impianto pilota a conduzione annuale

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I cianobatteri del genere *Arthrospira* (*A. platensis* e *A. maxima*), comunemente denominati “Spirulina” in seguito alla caratteristica forma elicoidale, sono organismi fotoautotrofi coltivati da anni in vari paesi del mondo. L’elevato tenore proteico (50-70% del peso secco) ed il contenuto significativo di antiossidanti, acidi grassi polinsaturi, vitamine e minerali rendono Spirulina un vero e proprio superalimento ed aprono gli orizzonti ad innumerevoli impieghi nel settore farmaceutico. Tuttavia i sistemi di coltivazione all’aperto ad oggi utilizzati sono facilmente soggetti a contaminazioni chimiche e biologiche che minano la qualità e la sicurezza del prodotto.

Ceppi di *Arthrospira* sp. sono stati isolati da campioni naturali provenienti dal Marocco; in seguito a screening su colture di laboratorio un solo ceppo è stato selezionato per lo scaling-up ed identificato geneticamente come *Arthrospira platensis*. Previa ottimizzazione dei terreni di coltura, la crescita algale, la produttività, la composizione della biomassa e gli aspetti sensibili nella coltivazione sono stati valutati nel corso di oltre un anno di sperimentazione in diversi sistemi all’interno di un impianto pilota costituito da una serra ad elevata automazione.

Tassi di crescita positivi sono stati registrati con temperature del mezzo di coltura comprese tra i 4 e i 39 °C. La produttività massima in fotobioreattori anulari su litro di coltura, senza insufflazione di anidride carbonica, era di circa tre volte maggiore rispetto a quella osservata in raceways (0.22 e 0.08 g l⁻¹ giorno⁻¹, rispettivamente; $p < 0.01$, ANOVA), tuttavia i primi sono risultati di più difficile gestione. Correlazioni significative sono emerse tra produttività e durata del fotoperiodo ($r = 1$; $p < 0.05$, correlazione lineare di Pearson). Carenze di azoto nitrico nel terreno di coltura si riflettevano in primo luogo sul contenuto cellulare di ficocianina, clorofilla, proteine e carotenoidi totali nell’ordine. Studi sul riciclo e reintegro del terreno di coltura in raceways hanno messo in luce la fattibilità del processo senza ripercussioni sulla qualità del prodotto, in particolare dal punto di vista microbiologico. Grazie ad opportune procedure di filtrazione dell’acqua, sanificazione dei sistemi e monitoraggio delle colture, nel corso della sperimentazione non si sono mai verificate contaminazioni da parte di altri ceppi algali.

La sperimentazione ha consentito la messa a punto di protocolli e tecnologie ad hoc per la coltivazione di Spirulina, oltre alla stesura di un disciplinare di produzione per una Spirulina di elevata qualità e purezza, destinata al mercato nutraceutico e farmaceutico di alta fascia.

Optimizing biomass and high value compound production in *Cyanobacterium aponinum* PCC 10605

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Cyanobacterium aponinum PCC 10605, is a thermotolerant cyanobacterium species firstly isolated from Euganean Thermal Springs muds and later found in other thermal districts and in the Oman desert.

Recent research highlighted the interest on this strain due to its possible application for biotechnological purposes. It has been in fact considered for biofuel production and the U.S. Department of Energy financed its genome sequencing.

Moreover *C. aponinum* PCC 10605 was recently demonstrated to synthesize and release exopolysaccharides (RPS) with immunomodulating properties, which may contribute to the beneficial effects in psoriasis patients (Gudmundsdottir et al., 2015).

In this work we evaluated the effects of different environmental parameters such as temperature, light intensity and carbon source availability on growth rate, biomass accumulation and high value compound production, focusing our attention on RPS but also on zeaxanthin and phycocyanin synthesis levels.

With a first set of experiments we determined the temperature and the type of carbon source (5% CO₂ or 5,5 g/L sodium bicarbonate) giving the organism optimal growth rate. With this purpose the cyanobacterium strain was cultivated in tubular reactors under constant light (100 μmol photons m⁻² s⁻¹), by changing the other operating parameters.

C. aponinum PCC 10605 resulted to have the higher growth rate ($\mu \approx 0,6$ 1/d) at 35°C with sodium bicarbonate as inorganic carbon source, reaching a biomass productivity of about 0,75 g L⁻¹ day⁻¹ of dry weight, but being also able to similarly exploit CO₂.

In the second set of experiments, we tested the influence of light on growth and biomass production by using a multicultivator system (PSI, MC1000): a small photobioreactor with eight culture slots of 80 ml, each illuminated by a set of led lights individually adjustable at different light intensities and all culture tubes immersed in a water bath with controlled temperatures.

Maintaining the temperature at 35°C and sodium bicarbonate as carbon source, we tested 8 light intensities ranging between 15 to 650 μmol photons m⁻² s⁻¹. For all the last growing conditions we analyzed RPS, zeaxanthin and phycocyanin levels, finding the optimal irradiation inducing their higher productivity. The RPS monosaccharide composition was also determined.

Gudmundsdottir et al., 2015 Immunology Letters 163:157-62

Generation and selection of *Nannochloropsis gaditana* with altered photosynthetic apparatus for more efficient biodiesel production

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Microalgae have the potential to be one of the most promising feedstock for next generation biofuels thanks to a high productivity per area and the possibility of growing in unproductive land without competing with food crops. In this work the attention is focused on *Nannochloropsis gaditana* as a candidate for biodiesel production, due to its ability to accumulate large amounts of lipids, especially during nutrient starvation.

This marine microalga is photoautotroph and depends on sunlight for energy to support its biomass accumulation. One of the major problems associated with growing algae in any large scale photobioreactor or pond is that cultures have high optical densities, resulting in strongly inhomogeneous light distributions. This condition reduces photosynthetic efficiency and, as a consequence, overall productivity.

In this work we generated by random insertional mutagenesis *N.gaditana* mutant strains and we optimized a screening that exploits growth curves, pigments quantification and fluorescence measurements *in vivo* to detect the strains with an altered photosynthetic apparatus. Among these we started with a deeper characterization of the ones having a phenotype potentially beneficial under the specific artificial conditions of a photobioreactor. For example we identified strains with a reduced chlorophyll content, potentially interesting to increase the light penetration in industrial culture conditions and improving biomass accumulation. Some of these strains exhibit improved photosynthetic activity and, in one case, this trait corresponded to improved biomass productivity in lab-scale cultures. At the same time, we are working to characterize at the molecular level these strains to identify the genes responsible of the phenotype, contributing to a better understanding of composition and regulation of photosynthetic apparatus in this alga.

Mass cultivation of an isolate of *Staurosirella pinnata* for photonics application coupled to lipid production

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Diatoms have been identified as a versatile biomass feedstock upon which future biorefineries can be established. Although diatoms fulfill the major prerequisites of a sustainable fatty acid and other value added product feedstock, there are still technical challenges holding back the large scale production in an economic and environmentally-sound process.

In this work we mass cultivated a native isolate of the colonial, araphid diatom *Staurosirella pinnata* (Ehrenberg) D.M. Williams & Round, from biofilms of a Mediterranean coastal lagoon, for lipid production coupled to the use of frustule biosilica, a by-product of the lipid conversion process, as dye traps and scatterers in random lasers.

The diatom strain was selected for its ease of growth and harvesting in 5 liter indoor reactors. No nutrient deprivation was applied to preserve pore pattern integrity during laboratory culture and manipulation. Total lipids were quantified at the end of the growth experiment after chloroform/methanol extraction of the biomass. Biosilica culture content was then evaluated (mg product/mg freeze dried biomass) after hot acid cleaning of frustules. The dye-binding analysis of frustules was performed using 10 µg/ml Rhodamine B solution and 10 mg/ml frustule suspension at different frustules/Rhodamine B w/w ratios by Fluorescence Spectroscopy and Epifluorescence, Confocal Microscopy. Fourier Transform Infrared Spectroscopy, in the range 4000-400 cm⁻¹, was also used for chemical analysis of frustules. Random laser experiments were conducted on 10% and 20% of polyacrylamide gels.

Our past research evidenced multiple scattering and localization of light of fossil (commercial diatomite) and extant multispecies frustules material, obtaining first experimental evidence of random lasing effect from Rhodamine B in polymethylmethacrylate (Lamastra et al. 2014).

Here we went beyond focussing on biosilica obtained from one diatom species intensively cultivated, providing large amount of homogeneous and predictable nanostructured material to employ for random lasing as well as dye trapping purposes. This integration process demonstrated application potential, contributing to the abatement of diatom mass culture costs and is prospect to inspire novel developments in photonics, materials science and nanotechnology.

This work is conducted in the frame of the project 'Phantom – PHotonics Application in diaTOM frustules' (Uncovering Excellence 2014, University of Rome 'Tor Vergata' Grant to PP, LB, RC). RC acknowledges ESI408 Cost Action EUALGAE.

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Nonliving macrophytes used as adsorbents for the removal of metals and semimetals ions from wastewater solution

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During the last decades, the use of biosorbents as nonliving macrophytes beached on the coasts has become interesting due to high adsorption capacities and to the low costs of the sorbents. In this period, the ability of seaweeds in removing heavy metals has been used both for pharmaceutical applications (i.e., detoxification of heavy metals in the human body due to a very efficient adsorption of the contaminants), and in industrial wastewater treatment. Different algae have been used for these purposes, and among them, the brown algae have proven to be the most effective and promising substrates for the removal of metallic cations.

This work was aimed to assess the performance of different nonliving macrophytes in arsenic(V), vanadium(III) and molybdenum(V) sorption.

Tests on the arsenic biosorption were carried out on brown algae (*Cystoseira* sp., *Dictyopteris* sp., *Eisenia* sp.), green algae (*Caulerpa* sp., *Ulva* sp.), red algae (*Ceramium* sp., *Gracilaria* sp., *Porphyra* sp.) and a marine angiosperm (*Zostera marina*). The different nonliving macrophytes were pre-treated with different waters (i.e., deionized, acid and basic waters), and the biosorption was tested at equilibrium pH (range 1–8), and under high (10 mg l^{-1}) and low ($100 \text{ }\mu\text{g l}^{-1}$) arsenic concentration. All the investigated species exhibited significant arsenic(V) adsorption. Indeed, they showed a good performance at pH= 6, with the highest observed adsorption value ($1.3\pm 0.1 \text{ mg g}^{-1}$) for the red alga *Ceramium* and the seagrass *Zostera*. Such value was comparable with those of activated carbon and other low-cost adsorbents reported in the literature under similar experimental conditions. Furthermore, red algae known to be bad cationic metal sorbents, showed very good anionic sorption performance.

Vanadium and molybdenum biosorption was investigated using biomass of *Posidonia oceanica* previously treated with deionized water acid. The combined effects on biosorption performance of the equilibrium pH (range 1–12) and metal concentrations were investigated in an ideal single metal system and in real-like multicomponent systems. The results suggested that the biomass of *P. oceanica* can be used as an efficient biosorbent for the removal of vanadium(III) and molybdenum(V) from aqueous solutions offering an eco-compatible solution for the reuse of the waste material consisting of leaves accumulated on the beach due to both human activities and sea storms.

Microalgae and water pollution: a study on heavy metal uptake, phosphorus removal and biomass reuse

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Widespread applications of microalgal-based systems in wastewater treatment have been proposed since 50s. Wastewater treatment facilities take advantage of microalgae ability to reduce excess nutrients, such as dissolved nitrogen and phosphorus, and heavy metals attaining regulatory concentrations in the effluent (EU Water Framework Directive 2000/60/EC) as long as valuable biomass growth to harvest and process for bioproduct market. Anthropogenic waste production can significantly increase nutrient and heavy metal water load posing hazard to the ecosystem and human health. Cyanobacteria and green microalgae can remove metals from the surrounding environment either for an active process, absorption, or as passive adsorption of cations on the cell surface of both living and non-living cells. Phosphorus (P) assimilation is an active process and some microalgae can efficiently store this key element for their growth and metabolism.

Part of this study focused on the uptake of Copper (Cu) and Nickel (Ni) by *Chlorella vulgaris* CCAP 211/12, *Nostoc* sp. VRUC167, isolated from a dystrophic coastal lagoon, *Desmodesmus* sp. VRUC281 and *Phormidium* sp. VRUC169, both from the secondary sedimentation tank of a municipal wastewater treatment plant, in batch cultures. Strains grown in BG11 and BBM media were exposed to metal ion species at the concentration of 5, 15 and 30 mg L⁻¹ for 12 days, then the biomass was harvested by centrifuging and metal concentrations measured by ICP-OES analysis, both in the culture media and in the cells after ultrasound assisted acid extraction.

Secondly, we investigated the ability of *Desmodesmus* sp. VRUC281 and *Phormidium* sp. VRUC169 to grow attached to two artificial materials: polystyrene foam and polycarbonate. Polystyrene (EPS) was selected as growth substratum for a first P-removal experiment. EPS slides were submerged in a 6 L water carrier, where cells, suspended in BG11 medium circulated for 28 days thanks to an air pump. At the end of the experiments, biofilms forming on the slides were easily scraped off and no-centrifuging was needed to de-water cells P concentrations were then measured in the medium by means of molybdenum-blue colorimetry.

Our results encourage the research in this field for an efficient and less expensive water clean up by integrating phosphorus and heavy metal removal using biofilms of native strains in mono- or mixed culture. Heavy metal adsorption tests are foreseen using the inactivated biomass (non-living cells) obtained at the end of the previous experiments, to further exploit algal potential for noxious pollutants.

Seaweed mapping in the Mar Piccolo of Taranto (Ionian Sea, Southern Italy)

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The Mar Piccolo of Taranto is a coastal semi-enclosed basin with lagoon features, divided in two smaller inlets by two promontories of land. Its benthic vegetation has been deeply studied since the end of the eighties, and in these years, continuous changes in phytobenthos composition and distribution occurred, mainly linked to anthropogenic activities.

In the framework of the Ritmare project, several monitoring activities were carried out in the Mar Piccolo of Taranto. In particular, the macroalgal vegetation was mapped by innovative remote sensing techniques, as a result of the collaboration among the laboratories of ENEA Research Centre of Casaccia (Roma), IAMC-CNR Taranto (1) and OGS Trieste (2).

Two macroalgae, *Hypnea cornuta* (Kützing) J. Agardh and *Caulerpa prolifera* (Forsskål) J.V. Lamouroux, were investigated at different sampling stations: *C. prolifera* was found in three sites of the basin, one in the first inlet and two in the second inlet, whilst *H. cornuta* was observed only at two stations of the second inlet.

Different parameters were used to build maps of the two algal species based mainly on their taxonomy, peculiarities in the growth period, coverage (%) and biomass (g).

Maps of their distribution were obtained by means of atmospherically corrected Landsat 8 OLI multispectral data acquired in June 2013 and suitably calibrated using in situ point measurements at the sampling stations.

The thematic maps of the seaweed distribution in the Mar Piccolo of Taranto are in agreement with the data collected *in situ*. The obtained results are very promising for tracking macroalgal species in water bodies when subjected to both anthropogenic pressure and climate changes effects. Moreover, the abilities and the strategies of algae to use nutrients could offer an attractive solution for a possible application of their biomass in the bio-energy production. In these ecosystems, macroalgae could be used as natural laboratory to evaluate the potential employment of their biomass for energy use. Therefore, this methodology could be a useful tool for the sustainable management of these environments.

Research carried out within the framework of the Flagship Project RITMARE

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L'istituzione dell'*Algarium Veneticum* presso la Biblioteca Storica di Studi Adriatici (ISMAR-CNR Venezia)

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La Biblioteca Storica di Studi Adriatici si compone di quattro fondi principali provenienti dagli istituti confluiti nell'attuale Istituto di Scienze Marine nel 2003, dalla sede veneziana del Regio Comitato Talassografico Italiano (1910), poi Istituto di Biologia del Mare (1968), dall'ingente archivio dell'Istituto di Studi Adriatici, attivo dal 1931 alla prima metà degli anni 1970, dall'Osservatorio per la Pesca Marittima (che alla fine dell'ultima Guerra Mondiale raccoglieva l'eredità dell'Istituto di Biologia Marina di Rovigno) ed infine dall'Istituto per la Dinamica delle Grandi Masse (CNR-ISDGM, 1969-2002). A questi si aggiungono fotografie, manoscritti, cartografie, taccuini ed un prezioso archivio di corrispondenza, di verbali dei diversi Istituti e di tavoli internazionali (Società Geografica Internazionale, Commissione Internazionale per il Mare Mediterraneo, ecc.) svoltesi dal 1909 al 2002, che documentano gli intensi scambi culturali tra i paesi rivieraschi e, attraverso questi, tra Occidente e Oriente. Il repertorio scientifico e culturale sui Balcani è probabilmente unico in Italia, mentre il preziosissimo patrimonio scientifico incentrato sull'Adriatico e sulla Laguna documenta il passaggio tra l'approccio naturalistico-descrittivo delle scienze ambientali a quello sperimentale di impianto galileiano, con l'avvio di misure quantitative di crescente precisione ed accuratezza.

Spicca in questo contesto un inedito erbario algologico raccolto da Michelangelo Minio tra il 1942 e il 1950. In seguito al ritrovamento nel 2013 di questa raccolta di interesse storico e scientifico, si è ritenuto opportuno procedere all'istituzione del primo erbario censito per l'Istituto di Scienze Marine (ISMAR-CNR).

Il nuovo erbario, istituito presso la sede ISMAR CNR di Venezia, è stato registrato dal New York Botanical Garden con il nome di *Algarium Veneticum* e con Index Herbariorum <ISMAR> (<http://sciweb.nybg.org/science2/IndexHerbariorum.asp>).

Attualmente l'erbario comprende la collezione algologica storica di Vatova, intitolata “*Distribuzione e polimorfismo di Gracilaria confervoides nella laguna di Venezia*” e include 19 carpette contenenti più di mille esemplari di *Gracilaria* raccolti tra il 1942 e il 1950. Nella collezione è presente anche una sezione di miscellanea che include esemplari di differenti taxa algali, quali Ulvales, Ceramiales e Bangiales.

La collezione è stata digitalizzata mediante l'ausilio di uno scanner planetario digitale Bookeye® 3. I metadati associati saranno disponibili sulle piattaforme “Atlante della Laguna” (www.atlantedellalaguna.it) e “CIGNo” (<http://cigno.ve.ismar.cnr.it/>) e sul sito della Biblioteca Storica di Studi Adriatici (<http://bsa.ve.ismar.cnr.it/>). Inoltre sarà effettuata una revisione tassonomica delle specie algali presenti nella collezione algologica storica, utilizzando le tecniche di DNA *barcoding* specifiche per i campioni antichi.

L'ampliamento dell'*Algarium Veneticum* con l'inclusione di raccolte algologiche moderne, provenienti dalla Laguna di Venezia e dal Mare Adriatico, permetterà infine di approfondire gli studi floristici e monitorare i cambiamenti vegetazionali nell'area alla luce dei crescenti impatti antropici sull'ecosistema lagunare e, in particolare, della diffusione crescente di specie “aliene” di varia origine.

Coastal transformation and marine habitat loss in the Gulf of Naples

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Coastal areas are undergoing continuous transformation to sustain the increasing residential, commercial, and tourist activities. However, the proliferation of artificial infrastructures (i.e. marinas and breakwaters) may contribute to destroy and fragment marine natural habitats, to alter the connectivity among populations and, as a consequence, the natural seascape. In particular, the upper sub tidal rocky fringe, where the coastal transformations are more conspicuous, represents the natural habitat of dominant engineering algae, such as *Cystoseira* and *Sargassum*; some of them, because of their sensitivity to environmental variability, are considered biological elements of water quality. The recent attempt to measure historical changes in macro algal diversity in the Gulf of Naples has highlighted a dramatic decrease of *Cystoseira* species in the upper sub littoral zones and at the same time the lack of information on their up to date extension. To verify the influence of coastal development on the decline of *Cystoseira* species in the Gulf, the percentage of natural/artificial coastal length has been estimated with Google Earth orto-photos. The results testified a correspondence: the higher was the *Cystoseira* spp. loss, the greater was the development of artificial urban infrastructures. To follow the temporal dynamics of these shallow systems, a complete re-monitoring of the fucoid distribution along the Neapolitan coast has been planned. Up to now, the occurrence of these species has been mapped and digitalized in a qGIS database (scale 1:2,500) for the shallowest fringe of the island of Ischia. An ongoing homogenization of diversity at a medium scale has been recorded: *Cystoseira compressa* (the less sensitive species) is the widespread species; fragmented stands of *Cystoseira amentacea* (the most sensitive) still exist. Their distribution is limited to natural habitats slope more gently while the highest diversity has been reached in rocky pools. Occasional settlements were recorded on artificial structures when natural marine habitat was preserved in the surrounding area and the distance among the algal patches could not be a limiting factor in the spreading of the species. These preliminary data seem to suggest that a stronger collaboration between engineers and ecologists could favour a better coastal management in order to mitigate the impacts of artificial infrastructures on natural marine ecosystems and avoid the loss of biodiversity.

Red algal diversity in Tunisia revealed using DNA barcoding

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Tunisia, located at the hinge between eastern and western Mediterranean, extends over 1670 km of coastline that constitute a transition zone and habitat diversity between the two basins of the Mediterranean. The coasts are characterized by alternating rocky and soft bottom, promoting marine and coastal biodiversity particularly interesting. However, despite some efforts to better study the biodiversity, data are insufficient due to the heterogeneity of the work. Also, species inventory and their identification pose many problems, particularly concerning the phyla reputedly difficult, such as those of algae. The Tunisia Seaweed Project aims to extensively survey the marine algal flora in Tunisia and generate morphological and molecular data for all three major groups of macroalgae present. DNA barcode data were produced for the red algal samples from the *cox1* and *rbcL* genes of the mitochondrion and plastid, respectively. Phylogenetic trees were built from the molecular data produced thus far in order to assess the algal diversity. This approach is a fast-and-friendly tool for the prompt identification of macroalgal taxa even for non-trained taxonomists and resulted in two new report in Tunisia, *Grateloupia filicina* and the alien species *Hypnea cornuta*. Furthermore, it highlighted the presence of other Mediterranean taxa whose status should be better investigated (e.g. *Halymenia floresii*, *Palisada* sp., *Peyssonnelia* sp.). Additional molecular studies as well as careful morphological re-examination of preserved samples will be undertaken to clarify the relationships between these and other genera within the red algae in Tunisia and assign proper taxonomic names where necessary.

Evolution of photoprotection mechanisms upon land colonization: evidences of PSBS dependent NPQ in late Streptophyta algae

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Light is the energy source for photosynthetic organisms but, if absorbed in excess, it can drive to the formation of reactive oxygen species and photoinhibition. One major mechanism to avoid oxidative damage in plants and algae is the dissipation of excess excitation energy as heat, a process called Non Photochemical Quenching (NPQ). Eukaryotic algae and plants, however, rely on two different proteins for NPQ activation, the former mainly depending on LHCSR, whereas in the latter the major role is played by a distinct protein, PSBS. In the moss *Physcomitrella patens*, which diverged from vascular plants early after land colonization, both these proteins were found to be present and active in inducing NPQ, suggesting that during plants evolution both mechanisms co-existed. In order to investigate in more detail NPQ adaptation towards land colonization, we analyzed Streptophyte algae, the latest organisms to diverge from the land plants ancestors. Among them we found evidences of a PSBS dependent NPQ in species belonging to Charales, Coleochetales and Zygnematales, the latest groups to diverge from land plants ancestors. On the contrary earlier diverging algae, as Mesostigmatales and Klebsormidiales, likely rely on LHCSR for their NPQ activation. Presented evidence thus suggests that PSBS dependent NPQ, although possibly present in some Chlorophyta, was stably acquired before late Streptophyte algae diverged from land plants ancestors.

Evaluation of the effects of different light intensities and carbon sources on growth of two species of cyanobacteria.

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Cyanobacteria are prokaryotic photosynthetic organisms receiving growing attentions in the last years because of their high solar energy conversion efficiency, fast growth and valuable compounds production. However, these organisms are highly susceptible to changes in environmental parameters such as temperature, light intensity, pH and carbon source availability.

In this work, two different cyanobacteria species, *Synechococcus* sp. PCC 7002 and model organism *Synechocystis* sp. PCC 6803, were studied to evaluate the effects of these operating parameters on growth, biomass accumulation and biological-interest compounds production.

In order to optimize growth conditions, one of the most important parameter to consider is the carbon source. These cyanobacteria strains were cultivated in tubular reactors at the temperature of 28°C, under control light (100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) with 5% CO₂ and sodium bicarbonate, from 5.5 to 88 g/L. As a result, *Synechocystis* was able to better exploit CO₂, while *Synechococcus* is more susceptible to changes of pH when cultivated in CO₂, so experiments were performed using sodium bicarbonate, maintaining the pH value in the range between 8 and 9. In these conditions, *Synechococcus* was able to growth reaching significant biomass concentration and carbohydrate content.

For *Synechocystis* sp. PCC 6803, also the influence of light on growth was evaluated. In a multicultivator system, different light intensity was tested, from 75 to 375 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The specific growth rates obtained from these experiments showed photosaturation effects at high light intensities, but no photoinhibition phenomena were detected.

These preliminary results suggest the high potential of these two cyanobacteria strains for high-scale industrial production.

Evaluation of the oxidative stress induced by Cr(VI) in *Scenedesmus acutus* in relation to sulfur availability

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Many reports indicate that Cr(VI) tolerance is strictly connected to sulfur metabolism (Pereira et al., 2008; Schiavon et al., 2008). In the freshwater green alga *Scenedesmus acutus* sulfur starvation induces a transient increase of Cr(VI) tolerance both in the wild type and in a Cr-tolerant strain (Gorbi et al 2007). This transient increase is lost after 2-day recovery in standard medium. In a previous work we observed a significant increase in FeSOD levels after S-starvation. In order to verify if the enhancement of FeSOD levels leads to a better detoxification of the oxidative stress and could be responsible of the transient tolerance increase to Cr(VI), we analyzed the thiobarbituric acid-reactive substances (TBARS), as an index of lipid peroxidation. After a 3 day pre-culture in standard (+S) or sulfur deprived (-S) medium, algae (S-sufficient and S-replete respectively) were resuspended in standard medium and exposed to 1mg Cr(VI)/l.

In S-sufficient conditions lipid peroxidation reached a peak after 24h from medium renewal and decreased thereafter restoring the t0 basal levels after 96h culture. No differences were observed between the two strains. As a consequence of S deprivation, S-replete cells had an initial TBARS value significantly higher than the S-sufficient ones, but were able to maintain significantly lower level of lipid peroxidation during the following 4 day culture. This observation confirms that the higher level of FeSOD previously observed in S-replete cells has a role in preventing oxidative stress. In our experimental conditions, lipid peroxidation seems however mainly affected by the culture refreshing (nutrients renewal, restoration of algal cell density) rather than to chromium exposure, since similar TBARS values were observed in the controls and in the Cr(VI) treated samples. A pre-eminent role of the nutrition deprivation in inducing oxidative stress is also suggested by the observation that FeSOD levels were significantly higher after 3 day pre-culture in S-deprived medium than after 4 days of 1mg Cr(VI)/l exposure.

The transcript analysis indicates that the response to the stress is mainly due to the chloroplastic FeSOD rather than to the mitochondrial MnSOD enzyme suggesting that chloroplast is the main cell compartment involved in the response to nutritional stress. FeSOD induction during the pre-culture in S-deprived medium seems thus involved in the reduction of the oxidative stress and could contribute to the transient increase in Cr(VI) tolerance induced by S-starvation, warranting protection to the cells while they prepare specific defenses to cope with the additional stress due to metal exposure. The prompter response of the Cr-tolerant strain in the activation of antioxidant enzymes could be involved in the different Cr(VI) sensitivity of the two strains.

Gorbi et al., 2007, *Aquat. Toxicol.* 84:457-464

Pereira et al., 2008, *Toxicol Sci* 106(2): 400-412

Schiavon et al., 2008, *J. Environ. Qual.* 37: 1536-1545

Responses of *Chlamydomonas reinhardtii* to sulfur starvation and anoxia

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Temporary anoxia is not uncommon in many freshwater and in some marine environments. Anoxia alters cell metabolism especially with respect to allocation of reducing power to reductive metabolic pathways. Consequently this modifies C allocation and elemental stoichiometry. These responses are controlled by regulatory processes that change the expressed proteome. In order to study these processes we grew the model organism *Chlamydomonas reinhardtii* cc-125 under a factorial set of oxic, anoxic, S replete and S deplete growth condition. Cell elemental and organic composition, growth, cell size, and the expression of S and anoxia related genes were measured, and H₂ production were measured. The results show an overall prevalence of S deprivation responses over those determined by anoxia, possibly because of the hierarchical higher position of reductant management. The results will be discussed with respect to energy management, elemental resource use efficiency and C allocation.

Index of authors

A

Abdelkafi Slim 34
Accoroni Stefano 1, 2, 4
Alboresi Alessandro 27
Algeri Matteo 25
Antonaroli Simonetta 28
Antonioli Marta 5
Armeli Miniante Simona 32

B

Baldi Franco 11
Bellan Alessandra 27
Beolchini Francesca 29
Beran Alfred 15
Bertazzini Michele 37
Bertucco Alberto 26, 36
Bolaños Federico 14
Borfecchia Flavio 31
Bottalico Antonella 9
Brunelli Andrea 10
Bruno Laura 24, 28, 30
Buia Maria Cristina 33
Buosi Alessandro 6, 7, 10

C

Cabrini Marina 5, 15
Carbone Dora Allegra 20
Cavedon Giorgio 26
Cecere Ester 31
Ceregato Alessandro 32
Cerino Federica 5
Chaves Gerardo 14
Chiarore Antonia 33
Cibic Tamara 16, 31
Ciniglia Claudia 20, 21, 22
Cioppa Diana 21, 22
Congestri Roberta 4, 28, 30
Costa Giulia 30

D

De Angelis Roberta 28
De Cecco Luigi 31
De Natale Antonino 20
De Stefano Mario 14, 28
De Vittor Cinzia 16
Del Mondo Angelo 22
Del Negro Paola 16
Di Poi Elena 5, 15

E

Ellwood Neil 4
Emiliani Guido 25
Eren Ayla 21

F

Facca Chiara 6
Falace Annalisa 8
Falsetti Alessia 30
Forlani Giuseppe 37
Fornasaro Daniela 5
Furnari Giovanni 23

G

Gabrielson Paul W. 8
Gallo Michele 11
Gaonkar Chetan C. 17, 19
Genovese Giuseppa 34
Gerotto Caterina 35
Giordano Mario 38
Glibert Patricia M. 1
Grech Daniele 33
Gris Barbara 26, 36
Guerrini Franca 2, 25
Guidi Flavio 3, 25

H

Hernandez-Kantun Jazmin J 8

I

Iovinella Manuela 21, 22

K

Kooistra Wiebe H.C.F 17, 18, 19

L

La Rocca Nicoletta 26, 36
Lamastra Francesca Romana 28
Lange Carina 17, 18, 19
Le Gall Line 8
Lenucci Marcello 26

M

Majewska Roksana 14
Manghisi Antonio 34
Mann David 17, 19
Marano Federica 24
Marchiselli Simone 25
Marcomini Antonio 10

Marieschi Matteo 37
Marini Mauro 1
Mazzotti Matilde 25
Melino Sonia 28
Meneghesso Andrea 27
Micheli Carla 31
Miladi Ramzi 34
Montresor Marina 17, 18, 19
Morabito Marina 34
Moro Isabella 12, 13
Morosinotto Tomas 26, 27, 35
Moschin Emanuela 13
Mulas Martina 33

N

Nanni Francesca 28

P

Patti Francesco Paolo 33
Peña Viviana 8
Pennesi Chiara 16, 29
Perin Giorgio 27
Pessina Andrea 38
Pezzolesi Laura 2, 8
Petrocelli Antonella 31
Pichierri Salvatore 1, 2
Pignatelli Vito 31
Pinna Adriano 25
Pinto Gabriele 20, 21 22
Piredda Roberta 18, 19
Pistocchi Rossella 2, 3, 25
Pollio Antonino 20, 21, 22
Proposito Paolo 28

R

Rindi Fabio 8
Rogelja Manja 16
Romagnoli Tiziana
Romano Elena 24
Rubino Fernando 31
Rugnini Lorenza 30

S

Santoro Mario 14
Sardella Alessio 37
Sarno Diana 17, 18, 19
Sciuto Katia 12, 13
Sforza Eleonora 26, 36
Sfriso Adriano 6, 7, 10

Sfriso Andrea A **6, 7, 11**
Sigovini Marco **32**
Silva Carlos. Eduardo de Farias **36**

T

Tomasino Maria Paola **18**
Torelli Anna **37**
Totti Cecilia **1, 2, 4, 29**
Trincardi Fabio **32**

V

Vanucci Silvana **3**

W

Wolf Marion A **12**

Z

Zanni Corrado **37**
Zingone Adriana **18, 19**