RIUNIONE SCIENTIFICA
GRUPPO DI LAVORO PER L'ALGOLOGIA
Società Botanica Italiana

Bari, 15-16 Novembre 2019

A cura di:

Antonella Bottalico, Cesira Perrone, Anna Lisco, Annamaria Mincuzzi

Comitato organizzatore:

Antonella Bottalico

Stampa: Tipolito Vitetum s.n.c. - Bitetto, novembre 2019

PROGRAMMA

VENERDI' 15 NOVEMBRE

08:30 ***Registrazione***

09:00 ***Saluti istituzionali***

09:30 **Recent progress on the genus *Pterocladiella* (Rhodophyta): taxonomy, species delimitation and biogeography**
Boo G.H.

10:15 ***Coffee break***

MACROALGHE – Moderatore: A. Bottalico

10:45 Benthic marine flora of the Mediterranean Sea: Rhodophyta – Rhodymeniophycidae I. Acrosymphytales, Bonnemaisoniales, Gelidiales, Gigartinales, Gracilariales
Cormaci M., Furnari G., Alongi G.

11:00 Preliminary results on the DNA barcoding identification of the littoral macroalgal flora of Tiskerth islet, Bejaia (Algeria)
Fellah F., Djenidi R., Manghisi A., Spagnuolo D., Genovese G., Morabito M.

11:15 First results of the macroalgal diversity in Terra Nova Bay (Ross Sea, Antarctica) using DNA barcoding
Milan M., Sciuto K., Cecchetto M., Fontaneto D., Schiaparelli S., Moro I.

11:30 Updating macroalgal biodiversity in the Venice lagoon (North Adriatic Sea): revision of complex genera and new introductions
Wolf M.A., Buosi A., Juhmani A-S.F., Sciuto K., Moro I., Sfriso A.

11:45 Circumscription of *Lithophyllum racemus* from the western Mediterranean Sea reveals the cryptic species *Lithophyllum pseudoracemus* sp. nov.
Caragnano A., Rodondi G., Basso D., Peña V., Le Gall L., Rindi F.

12:00 Macrophyte diversity in two polyhaline coastal lakes of Apulia (Italy)
Lisco A., Ungaro N., Boo G.H., Bottalico A.

12:15 Will the restoration of canopy-forming brown algae keep up the pace with climate changes?
Falace A., De La Fuente G., Savonitto G., Peplis M., Chiantore M.C.

12:30 Microcalcareous seaweeds a sentinel of trophic changes and CO_2 trapping in transitional water systems
Sfriso A., Buosi A., Tomio Y., Wolf M.A., Juhmani A-S.F., Mistri M., Munari C., Sfriso A.A.

12:45 Preliminary study for the mass cultivation of *Porphyra dioica* J. Brodie et L.M. Irvine
Spagnuolo D., Manghisi A., Morabito M., Cremades J., Genovese G.

13:00 ***Pausa pranzo***

15:00 **ApuliaKundi – "SpirulinaK" feeding in a sustainable way**
Chiapperini D., Settanni R.

MICROALGHE 1 – Moderatore: R. Pistocchi

15:15 Marine phytoplankton database of Latium coastal waters (Middle Tyrrhenian Sea – Italy): checklist and Self Organizing Maps analyses of data collected from 2002 to 2017
Bianco I., Russo T., Viaggiu E., Sarno D., Caroppo C., Congestri R.

15:30 Occurrences of phytoplankton bloom along coastal marine areas of Apulia Region (Italy)
Vadrucci M.R., Roselli L., Ciciriello P., Di Festa T., D'Arpa S., Florio M., Maci F., Ranieri S., Spinelli M., Pastorelli A.

15:45 Phytoflagellates blooms below the Antarctic land fast ice: Changes in functioning of a coastal Antarctic ecosystem?
Escalera L., Bolinesi F., Mangoni O., Saggiomo V., Saggiomo M.

16:00 Arctic phytoplankton diversity and distribution from the TARA Oceans Polar Circle campaign
Scalco E., Bowler C., de Vargas C., Karp-Boss L., Iudicone D., Sarno D., Zingone A.

16:15 Distribution and trends of harmful algal blooms (HABs) in the Mediterranean Sea
Zingone A., Escalera L., Aligizaki K., Fernández-Tejedor M., Ismael A., Montresor M., Mozetic P., Tas S., Totti C.

16:30 Distribution and potential toxicity of benthic harmful dinoflagellates in waters of Florida Bay and the Florida Keys
Accoroni S., Totti C., Romagnoli T., Giulietti S., Glibert P.M.

16:45 First occurrence of *Margalefidinium* cf. *polykrikoides* blooms in Ionian Sea, Italy
Roselli L., Vadrucci M.R., Belmonte M., Ciciriello P., Rubino F., Ungaro N., Caroppo C.

17:00 ***Coffee break***

MICROALGHE 2 – Moderatore: A. Zingone

17:30 High Throughput Sequencing reveals marked seasonality for dinoflagellates and a distinctive winter assemblage at LTER-MC (Gulf of Naples, Mediterranean Sea)
Mordret S., Piredda R., Montresor M., Zingone A., Kooistra W.H.C.F., Sarno D.

17:45 Microalgae: a sustainable way for cattle wastewater recycling
Rugnini L., Guzzon A., Savio S., Migliore L., Thaller M.C., Congestri R., Bruno L.

18:00 Removal of total and extra-cellular cyanotoxins from *Microcystis aeruginosa* using chlorinated compounds
Simonazzi M., Pezzolesi L., Guerrini F., Calfapietra A., Vanucci S., Pistocchi R.

18:15 Recovery of rare earths and metals from spent fluorescent lamps by *Galdieria sulphuraria* (Rhodophyta)
Iovinella M., Palmieri M., Race M., Davis S., Ciniglia C.

18:30 Amenità ficologiche (Phycological jokes)
Furnari G.

19:00 ***Assemblea dei soci***

20:30 ***Cena sociale***

SABATO 16 NOVEMBRE

09:00 **The results of the ARPA monitoring on the qualitative and quantitative consistency of marine phytoplankton**
Ungaro N.

MICROALGHE 1 – Moderatore: C. Totti

09:30 First survey of epibenthic diatom communities on marine phanerogams and invertebrates from Omani coasts
De Stefano M., Perricone V., Roviello V., Dobretsov S.

09:45 Preliminary data on the bioactivity of *Phaeodactylum tricornutum* Bohlin lipidic extract on Brown Adipocyte Tissue cells
Savio S., Turchi R., Guidobaldi G., Arnaìz E., Lettieri-Barbato D., Aquilano K., Congestri R.

10:00 Biodiversity of diatom community on corals and seagrasses from Saudi Arabian coasts of the Arabian Gulf: a taxonomical, ecological and environmental approach
Rabaoui L., Moritz J.H., Qurban M.A., Perricone V., Roviello V., De Stefano M.

10:15 Morphological and molecular characterization of *Pseudo-nitzschia* spp. in the NW Adriatic Sea
Giulietti S., Accoroni S., Romagnoli T., Bacchiocchi S., Totti C.

10:30 ***Coffee break***

MACROFITE – Moderatore: G. Alongi

11:00 The response of *Fucus virsoides* (Fucales, Ochrophyta) to a glyphosate-based herbicide exposure: ecophysiology and metabolomics approach
Falace A., Kaleb S., Felline S., Del Coco L., Fraschetti S., Fanizzi F.P.

11:15 Natural recolonization of *Posidonia oceanica* ten years after the laying of a submarine gas pipeline
Cotugno M., Lorenti M., Scipione M.B., Buia M.C.

11:30 Seaweeds: the sticky fly paper for microplastics in transitional water systems
Sfriso A.A., Munari C., Mistri M.

11:45 Bioactive compounds from *Ulva australis* Areschoug collected in the North Adriatic Sea
Trentin R., Custódio L., Rodrigues M.J., Moschin E., Sciuto K., da Silva J.P., Moro I.

MICROALGHE 2 – Moderatore: R. Congestri

12:00 Killing them gently; control of phototrophic biofilms growing on stone monuments using plant products
Ellwood N.T.W., Rugnini L., Sprocati A.R., Migliore G., Tasso F., Alisi C., Bruno L.

12:15 Molecular mechanisms regulating sexual reproduction and mating type determination in the diatom *Pseudo-nitzschia multistriata*
Russo M.T., Annunziata R., Borgonuovo C., Manfellotto F., Marotta P., Sanges R., Montresor M., Ferrante M.I.

12:30 Chromium tolerance in *Scenedesmus acutus* M. mediated by sulphur uptake
Ferrari M., Marieschi M., Cozza R., Torelli A.

12:45 ***Chiusura dei lavori***

RIASSUNTI

Recent progress on the genus *Pterocladiella* (Rhodophyta): taxonomy, species delimitation, and biogeography

G.H. Boo

The agar-producing red algal genus *Pterocladiella* comprises 24 species from temperate and tropical seas. Many species are economically important sources of food, agar and agarose. However, our taxonomic knowledge of the genus remains largely incomplete as some regions have seldom been investigated. Molecular taxonomic studies on the genus from Madagascar, Philippines, and Pacific America revealed several new species. To understand the species diversity and contemporary global distribution, five markers (mitochondrial *cox*1, *cob* and plastid *psa*A, *psb*A, *rbc*L) have been used from samples collected at global scale. Species delimitation approaches were investigated with coalescent analyses based on mitochondrial sequences. Results revealed that *Pterocladiella* comprised about 45 molecular species, nearly doubling in the number of the previously described species. Morphological studies are needed to publish new species. Results of biogeographical analyses, inferring the origin and divergence center of the genus are also introduced.

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Benthic marine flora of the Mediterranean Sea: Rhodophyta - *Rhodymeniophycidae* I. Acrosymphytales, Bonnemaisoniales, Gelidiales, Gigartinales, Gracilariales.

M. Cormaci, G. Furnari, G. Alongi

Among the benthic marine algae, the Phylum of Rhodophyta contains the greatest number of species (more than 7000) (Guiry, Guiry 2019). For this reason, we planned to treat that Phylum in more than one contribution. In the first one (Cormaci et al. 2017) we treated Compsopogonophyceae, Rhodellophyceae, Stylonematophyceae, Bangiophyceae and subclasses of Florideophyceae excluding Rhodymeniophycidae.
Rhodymeniophycidae G.W. Saunders *et* Hommersand, including at present 15 Orders and 68 Families with 5.214 *taxa* at specific and infraspecific level (Guiry, Guiry 2019), and represented in the Mediterranean Sea by 12 Orders and 41 Families with a number of species of about 10% of the above reported total number, will be treated in more than one contribution. In this first contribution the following 5 Orders are treated: Acrosymphytales, Bonnemaisoniales, Gelidiales, Gigartinales, Gracilariales, including 25 Families. The present contribution follows the layout of the previous three ones (Cormaci et al. 2012a,b; 2014; 2017). They can be freely downloaded from the site of the Bollettino dell'Accademia Gioenia di Catania: www.gioenia.unict.it/bollettino_on_line.
As concerns information on general characters of Rhodophyta, different proposals of classification, their world distribution and the importance of some of them in characterization of interesting Mediterranean submerged *habitat*, see Cormaci et al. (2017). The identification key reported in this contribution is based on some "Groups" established in the previous contribution on Rhodophyta (Cormaci et al. 2017). The present key, as the previous one, is based in part on empirical features (important from a practical point of view) and in part on taxonomic characters.
As in the previous contributions, a morpho-anatomical description of *taxa* treated is given. Moreover, for the major part of species both an essential iconography and main references to publications in which additional illustrations are present, are given too. As well, for species not illustrated in this contribution, references to specific recently published papers in which the *taxon* is illustrated, are given.
Moreover, the treatment of most *taxa* is enriched with bilingual notes (Italian and English), a glossary, an index of all *taxa* quoted in the text and an *errata corrige* to the previous contribution on Rhodophyta by Cormaci et al. (2017).

Letteratura citata

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Preliminary results on the DNA barcoding identification of the littoral macroalgal flora of Tiskerth islet, Bejaia (Algeria)

F. Fellah, R. Djenidi, A. Manghisi, D. Spagnuolo, G. Genovese, M. Morabito

Algeria with its large Mediterranean facade, more than 1200 km, is a country deeply influenced by the sea, which implies the existence of thousands of marine species, notably algae, which have a very important biomass. The first studies on algal flora in Algeria date back to the end of the 18th century (e.g. Desfontaines 1798-1799; Montagne 1846), to which in 20th century were added the notable works of J. Feldmann (1931 and following), the inventory of Perret-Boudouresque and Séridi (1989), and, recently, the pubblications of Ould Ahmed et al. (1995), Seridi et al. (2007) and Ould Ahmed et al. (2013). However, these algological works are essentially based on the morphological and anatomical identifications, which have been challenging due to the plasticity of characters. Marine macroalgae can be notoriously difficult to identify owing to their relatively simple morphology and anatomy, convergence, rampant phenotypic plasticity, and alternation of heteromorphic generations (Saunders 2005). Algal taxonomists have overcome such difficulties using molecular assisted alpha taxonomy (MAAT), a technique that uses differences in gene sequence data to delineate genetic species groups that are subsequently analysed morphologically and assigned to existing species or established as novel taxa (see Manghisi et al. 2019 and references therein). In this regard, our long-term project focuses on the compilation of a DNA barcode-assisted floristic list of macroalgae collected along the Algerian coast. The first collections were carried out on submerged rocks at a depth of 0-3 m in Tiskerth (36.8153 N 4.9778 E), a small islet in the region of Boulimat, Bejaia, Northeastern Algeria, on September 2018. Collected algae were sorted and morphologically identified following Perret-Boudouresque and Séridi (1989). DNA barcoding identification resolved 13 genetic species, highlighting cryptic diversity relative to taxa reported from the Mediterranean versus from other geographical regions with taxonomic and nomenclature updates provided where possible. To ensure an effective monitoring of biodiversity change over time we propose the use of DNA barcode-assisted floristic lists, which are more convenient and accurate than lists devised from traditional approaches, because associated barcode sequences are easily trackable and each specimen is unequivocally linked to a permanent genetic label regardless of any subsequent taxonomic or nomenclature variation. DNA barcoding surveys contribute to new records to DNA libraries of life, increasing our biodiversity knowledge, which is linked to geographical information and is also freely available to the scientific community. Furthermore, in the context of environmental management, the added value of DNA barcode-assisted identifications is that they give objective and verifiable data, an essential strength if we consider that floristic lists are extensively used by ecologists and environmental agencies as the basis for monitoring studies. To the best of our knowledge, this is the first study of the Algerian macroalgal flora using DNA barcoding methods.

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AUTORI

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First results of the macroalgal diversity in Terra Nova Bay (Ross Sea, Antarctica) using DNA barcoding

M. Milan, K. Sciuto, M. Cecchetto, D. Fontaneto, S. Schiaparelli, I. Moro

During the XXXIV Italian Expedition (Austral summer 2018-2019) of the Italian National Antarctic Research Programme (PNRA), several biological samples were collected in Terra Nova Bay (Ross Sea, Antarctica) in the framework of the "TNB-CODE", (Terra Nova Bay barCODing and mEtabarcoding of Antarctic organisms from marine and limno-terrestrial environments). This research program aims at producing sound checklists of all major groups occurring in the area by using a molecular approach. In this contribution we focus on the coastal marine macroalgae. Specimens were stored as exsiccata on herbarium sheets or as dried specimens on boulders in the case of encrusting Corallinales. A DNA barcoding approach was applied to these samples in order to speed taxa classification and clarify taxonomic positions. For a first screening of the macroalgal samples, the plastidial 23S rRNA marker was amplified by using universal primers designed specifically for photosynthetic organisms and sequenced. Once determined the higher taxonomic ranks of the specimens, for given samples further molecular markers were used in order to more accurately identify the macroalgae at the genus and species levels, choosing from time to time the more suitable loci for the purpose (e.g., *rbc*L, *cox*1, *tuf*A, *psb*A). Morphological observations were also carried out, as well as an accurate comparison of our specimen distribution with the data available in literature.
Here we present the first obtained results and discuss the further possible developments of this research.

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Updating macroalgal biodiversity in the Venice lagoon (North Adriatic Sea): revision of complex genera and new introductions

M.A. Wolf, A. Buosi, A-S.F. Juhmani, K. Sciuto, I. Moro, A. Sfriso

The North Adriatic Sea and, in particular the Venice Lagoon, is a very interesting area for systematic and ecological investigations on macroalgae, because of the high natural biodiversity of the Mediterranean Sea and the extensive anthropogenic impacts (e.g., shipping, aquaculture activities, environmental pollution) that favour the introduction and settlement of allochthonous species. Since 2016 to 2019 our research group carried out several studies on different macroalgal groups in order to understand the biodiversity and distribution of these taxa in the Venice Lagoon. In particular, we focused on species and genera that are poorly known and/or whose taxonomic status is controversial. We report the first finding of *Calonitophyllum* Aregood, a monospecific genus of the family Delesseriaceae for which *C. medium* (Hoty) Aregood represents the only species known so far. This genus is present in North and South America and was never found in the Mediterranean Sea. It is possible that specimens of *C. medium* have previously been misidentified with *Radicilingua thysanorhizans* (Holmes) Papenfuss, a species widely distributed in the Mediterranean. The phylogenetic analyses, based on the plastid *rbc*L marker, have shown that the Adriatic samples are genetically distinct from the American ones and probably represent a new species of the genus never described before. The second genus that we took into consideration was *Chaetomorpha* Kutzing (Cladophorales, Chlorophyta). In this case morphology is very simple, and only few characters are available to discriminate species, e.g.: attached or unattached growth, filament diameter, shape and size of the basal cells, length/diameter ratio of cells, presence of constrictions between cells. Phylogenetic analyses, based on the partial nuclear small subunit (SSU) rDNA, coupled with morphological data have allowed the rediscovery of a *Chaetomorpha* species described from the Venice Lagoon (Italy) in the early 20th century and then forgotten: *Chaetomorpha stricta* Schiffner. Another study was focused on the genus *Centroceras* Kützing, a small red algal taxon characterized by simple filamentous thalli with erect axes arising from a prostrate system. Until 2009 the type species of this genus, *C. clavulatum* (C. Agardh) Montagne, was considered a cosmopolitan taxon and the only species reported in the Mediterranean Sea. In this study we have found out that the species actually present in the Adriatic Sea is *C. gasparrinii* (Meneghini) Kützing and we excluded *C. clavulatum* from the algal flora of this region. The North Adriatic samples of *C. gasparrinii* differed morphologically from those of other regions for the number of periaxial cells, suggesting the presence of different subspecies. Therefore, we have proposed the new subspecies *C. gasparrinii* subsp. *minor*. Wolf et al. to encompass the Adriatic specimens of this species. The last taxon examined was the genus *Ceramium* Roth. Through phylogenetic analyses, using the plastid *rbc*L marker, we identified six distinct taxonomic entities, of which four represented taxa not currently recognized in the Venice Lagoon or the wider Mediterranean Sea. One of these was the poorly known species *Ceramium connivens* Zanardini, which is non-spiny and fully corticated when mature, with short naked internodes developing in older axes. Two entities with erect partially corticated thalli without spines corresponded to the new species *Ceramium rothianum* Wolf *et al.* and to the forgotten species *Ceramium nodosum* (Kützing) A.W. Griffiths & Harvey. The last entity, characterized by prostrate axes giving rise to narrow erect incompletely corticated, non-spiny axes, has been identified with the rare, poorly known species *Ceramium incospicuum* Zanardini.

In addition to the above studies on complex or poorly known genera, we investigated the presence of non-indigenous species (NIS). Identification of these alien taxa on the basis of morphological characters is difficult due to their simple morphology, phenotypic plasticity and the occurrence of morphologically overlapping species. Through molecular analyses based on the DNA barcoding method we were able to recognize *Acanthosiphonia echinata* (Harvey) A.M. Savoie & G.W. Saunders (Rhodomelaceae, Ceramiales)*, Aglaothamnion halliae* (Collins) Aponte, D.L. Ballantine & J.N. Norris (Callithamniaceae, Ceramiales), *Melanothamnus japonicus* (Harvey) Díaz-Tapia & Maggs (Rhodomelaceae, Ceramiales) and *Polysiphonia schneideri* Stuercke & Freshwater (Rhodomelaceae, Ceramiales)*,* all new introductions for the North Adriatic Sea. The presence of closely related cryptic species in the Venice Lagoon, such as *Aglaothamnion tenuissimum* (Bonnemaison) Feldmann-Mazoyer, *Melanothamnus harveyi* (Bailey) Díaz-Tapia & Maggs and *Polysiphonia denudata* (Dillwyn) Greville ex Harvey (now *Carradoriella denudata* (Dillwyn) A.M. Savoie & G.W. Saunders), and the lack of diagnostic characters can lead to the underestimation of the biodiversity of these genera.

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Circumscription of *Lithophyllum racemus* from the western Mediterranean Sea reveals the cryptic species *Lithophyllum pseudoracemus* sp. nov.

A. Caragnano, G. Rodondi, D. Basso, V. Peña, L. Le Gall, F. Rindi

The taxonomy and systematics of coralline algae (Corallinophycideae, Rhodophyta) in the Mediterranean Sea are still based mainly on morpho-anatomical data. Although traditional coralline taxonomy has been integrated with DNA sequencing for almost 20 years, only in the last few years investigations of molecular-assisted alpha taxonomy have been applied to Mediterranean corallines (Wolf et al. 2015, 2016; Pezzolesi et al. 2017; Peña et al. 2018; Pezzolesi et al. 2019). Molecular phylogenetic and taxonomic investigations have revealed a substantial amount of cryptic diversity that has led to gross underestimation of species numbers in individual geographical regions (Hernandez-Kantun et al. 2016).
The genus *Lithophyllum* is an important carbonate producer and builder of three-dimensional frameworks, such as coralligenous concretions in the Mediterranean (Ballesteros 2006; Pezzolesi et al. 2017) and reefs in the tropics (Abbott et al. 2012; Caragnano et al. 2016). Pezzolesi et al. (2019) in a large-scale molecular phylogenetic study of one of the main coralligenous bioconstructor, the *Lithophyllum stictiforme*, based on samples obtained from a wide geographical range in the western and central Mediterranean, found at least 13 cryptic species.
Morpho-anatomical observation integrated with molecular analysis (*psb*A, *rbc*L, COI, *cox*2-3) of samples of *Lithophyllum racemus* from the North-Western Mediterranean (including the type material) reveals the occurrence of *Lithophyllum pseudoracemus*., a new species recovered as sister taxon to *L. racemus*. The morpho-anatomical discrimination of the two species is based on the presence/absence of thricocytes, the size of the epithallial cells, the height of the sporangial conceptacle chamber and the pore canal length. The two species are widespread in the western Mediterranean and there is not a geographic separation of their distributions.

Letteratura citata

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Macrophyte diversity in two polyhaline coastal lakes of Apulia (Italy)

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Transitional waters, which include coastal lagoons, saline lakes, river estuaries and deltas, are highly dynamic and heterogeneous systems in which macrophytes (i.e. macroalgae and angiosperms) are important primary producers. Moreover, macrophyte assemblages are a Biological Quality Element (BQE) proposed by the European Water Framework Directive (WFD, 2000/60/EEC) to assess the ecological status of these aquatic systems and knowledge of their diversity is a key point for conservation and sustainable management purposes.
Lesina and Varano are the biggest brackish lakes of southern Italy, located in the northern side of the Gargano Promontory (Adriatic coasts) and placed in the Gargano National Park Territory. Lesina displays a narrow shape, elongated from east to west direction, and it communicates with the Adriatic Sea through two artificial channels: Schiapparo at the eastern side and Acquarotta at the western side. The lake exhibits a permanent E-W salinity gradient due to the fact that freshwater inputs occur at the eastern end (Manini et al. 2005). Varano is surrounded by steep hills at south, east and west, whereas the north side faces the sea and it is characterized by sandy dunes. Two artificial channels (Capojale and Varano) at the east and west sides provide a connection to the Adriatic Sea.
These basins, classified as polyhaline coastal lagoons, are annually monitored for the BQE "macrophyte" by the Department of Biology, University of Bari, on behalf of the Apulian Environmental Agency (ARPA Puglia). Inventory of the submerged macroflora in these lagoons was completed during the monitoring program carried out from 2011 to 2018. A total of 45 and 69 macrophytes were collected in Lesina and Varano, respectively. The identification of some critical *taxa* in the order Gracilariales was also ascertained by molecular analyses. No Ochrophyta-Phaeophyceae were found in Lesina in all the examined period. In the latter lagoon the finding of two characean macroalgae is noteworthy, while in Varano lake the foxtail stonewort *Lamprothamnion papulosum* (Wallroth) J. Groves, previously reported in dense populations in the central part of the basin (Corbetta 1970), was never found. The chorological spectra show for both lagoons a high occurrence of Cosmopolitan and Atlantic elements. Throughout the study period the ecological status was evaluated as "Good", according to MaQI classification (Sfriso *et al.*, 2009), both for Varano lake and for two out of the three water bodies in which Lesina lagoon has been divided (Regione Puglia 2009), whereas that one located in the western part, mainly affected by human impacts, showed a “Scarce-Sufficient” ecological status. Results of the present study have provided an up-to-date checklist, also increased in terms of number of *taxa*, with respect to the scarce and fragmentary available literature, as reported by Cecere et al. (2009).

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Will the restoration of canopy-forming brown algae keep up the pace with climate changes?

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Anthropogenic impacts and rapid environmental changes are causing unpredictable shifts in Mediterranean canopy-forming brown algae, leading to their regression or loss. Thus, there is an urgent need to set standards and guidelines to optimize the restoration, in order to grant the persistence of marine forests in the future. Outplanting represents an innovative technique for restoring Fucales, although its implementation consists of a set of delicate steps (Falace et al. 2018; De La Fuente et al. 2019), which need to be tailored to the specific requirements of the target species.

Here, we report the results gathered both in the Ligurian Sea and the Gulf of Trieste during the first two years of the EU project ROCPopLife. The two sites are subject to different regimes of human impact (being the Ligurian site in a good environmental status, while the Adriatic site in an urbanized context), but both experienced abrupt and extreme climate events.

In the Ligurian Sea, in July 2018 clay tiles with *Cystoseira amentacea var. stricta* Montagne germlings (~3 mm) were screwed to the rocky shore. After 2 months in the field, over the 40% of the tiles were covered with juveniles of ca. 8 mm. However, 6 months after the tile deployment, an unprecedented storm surge severely affected the restoration performance, leading to the loss of the 80% of the tiles. The remaining tiles hosted 3 to 6 cm-long juveniles, which reached 6-9 cm in length after 1 year.

In the Gulf of Trieste, a heatwave of 2.65 °C in February 2019 caused a premature reproductive event in *Treptacantha barbata* (Stackhouse) S. Orellana & M. Sansón, so that the species was found fertile almost 3 months earlier than usual (Bevilacqua et al. 2019). This anomalous event was exploited for carrying out a restoration event, and the same procedure was carried out in May. However, the number of released zygotes was 10 times lower and the germling length after 4 weeks of culture was ¼ with respect to the previous anomalous event. Once in the field, half of the tiles from the February event were protected against herbivores; starting from the fourth week, length was significantly lower in unprotected tiles, and, after 3 months, the 36% of the unprotected tiles resulted empty because of grazing effects.

Since both predictable site-specific stressors and anomalous climatic events can severely affect the restoration actions, the challenge is now to optimize the restoration process (from the monitoring of the donor populations to that of outplants) in order to overcome the negative effects of unpredictable impacts.

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Microcalcareous seaweeds a sentinel of trophic changes and CO_2 trapping in transitional water systems

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One of the main effects of eutrophication processes in transitional water systems is the abnormal development of nuisance macroalgae (Morand, Briand 1996). During the fast growth period they trap significant loads of carbon, nitrogen and phosphorus that return in the environment when the biomass exceeds collapse. In eutrophic environments, production and decomposition processes alternate quickly every year with more or less intense phenomena, rapidly changing most of the parameters of the water column and surface sediments and affecting benthic communities and macrophyte assemblages. The most obvious consequence is the disappearance of aquatic angiosperms (seagrasses and aquatic plants) and many sensitive macroalgae which are replaced by tionitrophilic taxa or, in the worst conditions, by phytoplankton and cyanobacteria (Sfriso et al. 2007; Munari, Mistri 2012). Among the sensitive macroalgae some microscopic epiphytic species, a few microns thick and 50-200 µm in width, belonging to the genera *Hydrolithon*, *Pneophyllum* and *Melobesia* are strongly affected by these environmental changes. The frequent pH fluctuations recorded in the water column prevent the presence and growth of these taxa. Their disappearance or appearance are the fastest response to the deterioration or improvement of the environment. These microscopic macroalgae, which grow as epiphytes on leaves of the aquatic angiosperms and thalli of larger algae, respond faster than seagrasses and aquatic plants to environment changes appearing or disappearing within a few months or in a few days during hypo-anoxic conditions which lower the pH below the average values that occur in environments of good-high ecological quality. They are present in all the transitional environments of the Mediterranean Sea, resulting in an excellent bioindicator of trophic changes (Sfriso et al. 2009). Contrary to aquatic plants that once disappeared take several years to recolonize the environment, because of the seed size of some millimeters, these macroalgae produce gametes (2-6 µm) or spores (20-60 µm) easily removable by tides and able to quickly recolonize the environment. Therefore, the search for their presence/absence in the environment is a fast and cheap tool to monitor the trophic status of transitional areas, especially the environment's tendency with months or years in advance.

Here the results obtained by analysing 11 macrophyte variables (total macroalgal taxa, macroalgal biomass, per cent macroalgal cover, number of sensitive macroalgae, number of small calcareous macroalgae, per cent cover of *C. nodosa*, *Z. marina*, *Z. noltei*, *R. cirrhosa*, per cent cover of aquatic angiosperms and the Macrophyte Quality Index) and 10 environmental parameters: (dissolved oxygen (%DO), transparency, salinity, pH, ammonium, nitrite, nitrate, dissolved inorganic nitrogen (DIN), reactive phosphorus (RP), Chlorophyll-*a*) in 257 sites of the main Italian TWS (Venice: 550 km^2, Grado and Marano: 158 km^2, Valli di Comacchio: 110 km^2, Po delta: 178 km^2, Pialassa della Baiona: 11km^2, Lesina: 51 km^2 and Orbetello: 27 km^2, with a water surface accounting for ca. 78% of the total one) are presented. pH was the most correlated parameter to the presence of these species whose number decreased as its values lowered. At pH values <7.93 no microcalcareous taxa were detected, whereas the highest number of taxa was recorded at pH values >8.35.

Therefore, microcalcareous macroalgae are an excellent sentinel of pH changes. This parameter in TWS is strongly affected by the trophic status showing the lower values in dystrophic areas where environmental conditions are bad-poor. As consequence the absence of microcalcareous taxa is also the best sentinel to distinguish degraded environments from those of good-high ecological status.

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Preliminary study for the mass cultivation of *Porphyra dioica* J. Brodie et L.M. Irvine

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The use of macroalgae for human nutrition is constantly increasing, especially in Europe (FAO 2018). The causes are numerous and could be related essentially to the high nutritional values of algae. Among these, the species of the genera *Porphyra* and *Pyropia*, known commercially as "nori" (Japan) or "laver" (Northern Europe), have the highest commercial value ($ 523 per wet metric tonnes, Fao 2018). Since the interest in nori has grown considerably in the western world, the need to identify a highly productive species among European ones, which is able to support an industrial cultivation, has attracted the attention of researcher on *Porphyra dioica*, present in all of Northern Europe with a lower distribution limit at low latitudes and considered a species suitable for aquaculture (Pereira et al. 2004). This species, like the others of the same genus, is characterized by a life cycle in which laminar gametophytes alternate with microscopic filamentous sporophytes (*conchocelis* phase) (Brodie, Irvine 1997). The aim of the present research was to study the influence of different environmental parameters on thalli growth to improve the knowledge on mass cultivation methods for *P. dioica* in Europe. This work is part of the project "Evaluación de recursos ficológicos de interés industrial en costas gallegas", which aims to improve large-scale production of algae of industrial interest. For this study thalli of *P. dioica* were collected along the north-western coasts of Galicia, Spain. The blades were transferred to the laboratory at a low temperature, cleaned from epiphytes and cultured at 14°C. The main target was to test the effects on biomass yield of photoperiod (12L: 12D, 16L: 8D and 8L: 16D) and irradiance (250 µE m^{-2} s^{-1}, 140 µE m^{-2} s^{-1} and 70 µE m^{-2} s^{-1}), and to test the "free-living" growth conditions on the *conchocelis* phase, traditionally cultivated on a calcareous substrate. To support the obtained data, the health status of the gametophytes was evaluated by the Fv/Fm index (variable fluorescence/maximum fluorescence of chlorophyll). Among tested culture conditions, the highest growth of thalli was obtained at the photoperiod 16:8 and the light intensity of 140 µE m^{-2} s^{-1}. The further increase of light intensity (250 µE m^{-2} s^{-1}) caused a reduction in the growth due to a high light stress, as shown by the Fv/Fm index. The cultivation of the *conchocelis* phase in free-living conditions gave excellent results.

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Marine phytoplankton database of Latium coastal waters (Middle Tyrrhenian Sea – Italy): checklist and Self Organizing Maps analyses of data collected from 2002 to 2017

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First information on phytoplankton assemblages from Latium coast date back to the 1980s and referred to limited areas such as that facing Latina (Massera-Bottazzi et al. 1980) or the Tiber river mouth area (Puddu et al. 1983). Later studies mainly focused on dominant microphytoplankton taxa (Congestri et al. 2006) and harmful algae (Congestri et al. 2001), with particular attention to the toxin producing species (Congestri et al. 2004, Bianco et al. 2006, Congestri et al. 2006, Congestri et al. 2008). The Regional Agency for Environmental Protection of Latium - ARPA Lazio carried out several monitoring programs of marine coastal waters, comprising the assessment of microphytoplankton, as required by the national regulation in the framework of European Conventions or Directives. These included: i) the Monitoring Program for Protection of the Costal Marine Environment (L. 979/1982) according to the Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean, conducted from June 2002 to January 2007 and from August 2008 to July 2009; ii) the monitoring activities (D.Lgs. 152/06) required by the Water Framework Directive 2000/60/EC that started in 2009 and is still ongoing; iii) the monitoring program (D.Lgs. 190/10) prescribed by the Marine Strategy Directive 2008/56/EC that started in 2015 and is still ongoing. Finally, surveillance activities (D.Lgs. 116/08), were conducted in compliance with the Directive 2006/7/EC concerning the management of bathing water quality with a special focus on the harmful algal blooms. Characterization of microphytoplankton in terms of taxonomic composition was performed with light and electron microscopy and relative abundances were assessed according to the Utermöhl method. This work aimed to the harmonization of data collected over time during multiple monitoring activities by providing a revised, extended and updated list of the phytoplankton *taxa* recorded in about 1200 samples from marine coastal waters of Latium Region thus implementing a validated local phytoplankton database. In order to characterize the microphytoplankton assemblages we performed statistical analysis based on the unsupervised Artificial Neural Networks (ANNs), in particular the Self Organizing Maps (SOMs), this was chosen for the intrinsic heterogeneity of the dataset itself. SOMs analyses allowed to group the samples according to the relative phytoplankton composition and abundance data. Thus, a preliminary assessment of the "physiognomy" of phytoplankton along the Lazio coasts was provided. We expanded the floristic list through the observations of net samples as well as samples collected in the case of occasional water discoloration, thus providing a checklist consisting of 293 taxa at least at genus level, including 35 harmful species, that will be a reference for current and future monitoring programs.

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Occurrences of phytoplankton bloom along coastal marine areas of Apulia Region (Italy)

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Algal blooms have been documented along the west and east coasts of Puglia Region. A review of bloom occurrences in coastal marine areas of Apulia Region (Italy) from 2013 to 2019 points out a total of 80 cases have been reported. The cases have been described after reports received by citizens or by competent authorities, or during planned sampling in the framework of monitoring programs required by European Directives (WFD 2000/60/CE and MSFD 2008/56/CE). Independently of major coast length, the reports of algal blooms indicate their predominance along the Southern Adriatic/Otranto canal coasts of Apulia especially the northern part and in the ports. Majority of the blooms reported along the Southern Adriatic/Otranto canal of Apulia are caused by Diatoms and small phytoflagellates, whereas diatom and Dinoflagellates blooms prevail along the Ionian coast. There have been 23 causative species responsible for blooms, of which *Leptocylindrus minimus*, *Skeletonema marinoi*, *Fibrocapsa japonica*, *Pseudo-nitzschia delicatissima* and *Noctiluca scintillans* are the most common. In the last two year also the species *Margalefidinium polykrikoides* has determined recurrent blooms, especially along the Ionian coasts of Puglia region. Most of the blooms occurred during spring or summer period. In coastal water, this can be due to increase of input of nutrients coming from sediment or from diffuse or point sources from terrestrial area that provides a competitive edge for blooming of phytoplankton species.

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Phytoflagellates blooms below the Antarctic land fast ice: Changes in functioning of a coastal Antarctic ecosystem?

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In late spring 2016 water samples, below annual fast ice from 0-100 m, were collected in a coastal area in Terra Nova Bay (Ross Sea) from December to January (2015-2016). We investigated the temporal distribution of temperature, salinity and phytoplankton composition. A massive bloom dominated by flagellates, < 15 µm of length, were observed in the phytoplankton community below the land fast ice of the Ross Sea. This group was numerically similar and/or dominant compared to diatoms during the studied period, representing from 40% to 91% of the total phytoplankton community. Flagellates blooms showed a high temporal variability in species composition: i) the Bolidophyceae *Pentalamina corona*, known for the Antarctic pelagic environments, was found for the first time under the ice with unusually high concentrations; ii) the chrysophyte *Ochromonas* spp., generally found in Antarctic lakes, was observed for the first time in the sea; and iii) the haptophyte *Phaeocystis antarctica*, was reported for the first time in the under ice system in high concentrations. To date the driving factors of this anomalous event are unknown, but the presence of a typical fresh waters species can be only assign as a signal of an environmental change related to a strong input of continental waters. In sigh of this, the dominance of small flagellates during late spring and early summer could have important implications on trophic relationships in Antarctic waters since can directly affect grazing and nursery of zooplankton species.

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Arctic phytoplankton diversity and distribution from the TARA Oceans Polar Circle campaign

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The interdisciplinary TARA Oceans Polar Circle Expedition (May-October 2013) aimed at studying the planktonic ecosystem, from viruses to zooplankton, based on samples collected at 20 stations across the Arctic Ocean. Within this project, we studied phytoplankton diversity and distribution in samples collected by Niskin bottles (total phytoplankton) and plankton nets (20-180 µm size-fractions). Total phytoplankton abundance and the relative abundance (RA) of microphytoplankton species were estimated using light and electron microscopy.
A total of 199 taxa were identified: 55 Dinophyta, 119 Bacillariophyta, 7 Prymnesiophyta (of which 4 coccolithophores) and 18 taxa belonging to other groups. Phytoplankton abundance and species composition greatly varied among sites and even among stations from the same areas. Diatoms were dominant in the northern Baffin Bay, in the Chukchi Sea and at one station of the Kara Sea, which showed the highest phytoplankton density (1.83×10^6 cell·L^{-1}) due to a bloom of *Chaetoceros* spp.. Species belonging to the genus *Chaetoceros* were the most abundant diatoms also in the northern Baffin Bay and in the Chukchi Sea. Pennate diatoms, including *Fragilariopsis* spp. and the colonial species *Fossula arctica*, were only abundant at one station of the Kara Sea. Prymnesiophytes, most probably non-colonial stages of *Phaeocystis pouchetii*, showed a peak (> 90 %) in the Norwegian Sea, whereas other undetermined small flagellates were dominant at several sites. Dinoflagellates were never abundant across the area investigated, whereas coccolithophores were only recorded at stations influenced by Atlantic waters. Phytoplankton communities grouped based on their geographic position and temperature. Typical Arctic species (as *Chaetoceros gelidus*) were recorded over the whole study area, while strict sea-ice species (e.g., *Fossula arctica*) were only found in in the Kara and Laptev seas, characterised by ice or melted ice. Conversely, cosmopolitan (e.g., *Chaetoceros diadema*) and boreal-tropical (e.g., *Oxytoxum scolopax*) species were mainly recorded in areas influenced by Atlantic and Pacific waters. These results are in line with previous studies on Arctic phytoplankton and concur to provide a sound baseline to detect possible trends in the planktonic system as a consequence of climate change.

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Distribution and trends of harmful algal blooms (HABs) in the Mediterranean Sea

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The Mediterranean Sea (MS) is characterized by oligotrophic offshore waters and coastal waters ranging from almost pristine to heavily impacted. Main resources are tourism and fisheries, whereas aquaculture is more developed in the western MS and continental Greek coasts. Based on a literature search, we have gathered two databases, one on the distribution of toxic microalgal species and the other on the impacts of harmful events (either toxic or linked to discolorations and mucilages). These databases represent a contribution to Global HAB Status Report, an assessment of HABs around the world (Hallegraeff et al. 2017, Zingone et al. 2017), and will be available within the Ocean Biogeographic Information System (OBIS) of the IOC IOC-UNESCO's International Oceanographic Data and Information (IODE) system (http://obis.org).
Information on MS HABs dates back to the beginning of the 1800, with reports of 'mare sporco' (mucilages) in the Adriatic Sea. Since then, mucilage events have been reported at several sites, at times concerning the benthic environment. Discolorations (e.g., *Chattonella subsalsa, Prorocentrum cordatum, Noctiluca scintillans* and *Alexandrium minutum*) have been recorded since the midst of the last century, often occurring in restricted areas or harbors (e.g., *A. taylorii*). Mucilages and discolorations are a major issue in touristic areas in summer. Although more than 70 potentially toxic species are detected in the MS, toxicity is less frequently reported and is mainly related to the dinoflagellates *Dinophysis* (e.g., *D. sacculus* and *D. fortii*) and *Alexandrium* (*A. minutum* and *A. pacificum*), with an important impact on aquaculture. *Pseudo-nitzschia* blooms are widespread, but domoic acid in shellfish rarely exceeds regulatory levels. In the SW Spanish coasts, closures in shellfish growing areas are also related to blooms of *Gymnodinium catenatum* and *Pseudo-nitzschia australis*. Fish kills are probably less sporadic than reported, representing a problem along the southern MS coasts and in the Ebro river delta. In the easternmost MS, blooms mainly affect the Golden Horn Estuary in the Sea of Marmara, sometimes associated to discolorations. More recent and recorded at several northern MS sites are the blooms of the benthic dinoflagellates *Ostreopsis*, a species also found along the southern MS coasts. Several new records of *Gambierdiscus* and *Fukuyoa* in the western and eastern MS raise concerns about the possible risk of ciguatera, a syndrome so far mainly reported for subtropical areas. A recent entry is the dinoflagellate *Vulcanodinium rugosum,* which is responsible for the accumulation of pinnatoxins in shellfish in different lagoons of the French MS. New entries are also *Azadinium* species. Besides the obvious increase of *Ostreopsis* impacts over the first decade of 2000, a higher number of harmful species and some increasing trends are noticed, in some cases related to increased monitoring activities. Yet no trends for toxic events have been detected in some well monitored areas, such as the French coasts, while some decreasing trends are also reported, such as *Alexandrium minutum* disappearing from its type locality, the Harbour of Alexandria. Overall, the main risk deriving from HABs appears to be related to their impacts on tourism, which represents a large part of the marine environment economy in the Mediterranean Sea.

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Distribution and potential toxicity of benthic harmful dinoflagellates in waters of Florida Bay and the Florida Keys

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Ciguatera Fish Poisoning is one of the most relevant seafood-borne illnesses worldwide caused by the ingestion of fish species claimed to bioaccumulate lipophilic ciguatoxins in the food web (Friedman *et al*., 2017). Ciguatoxins, and other related biotoxins such as maitotoxins, are produced by benthic dinoflagellates of the genera *Gambierdiscus* and *Fukuyoa*, generally recorded in association with *Prorocentrum*, *Ostreopsis*, *Coolia* and *Amphidinium* species (Berdalet, Tester 2018). Indeed, despite the number of studies on benthic dinoflagellate assemblages in tropical and subtropical areas, attention is often given almost exclusively, on species belonging to the genera *Gambierdiscus*/*Fukuyoa* and *Ostreopsis*, which are usually studied individually, while the whole benthic dinoflagellate community has been rarely investigated (e.g. Berdalet, Tester 2018).
In this study, the distribution and abundance of benthic dinoflagellates was explored in northern and eastern Florida Bay and along the bay and ocean sides of the Florida Keys. Florida Bay is negatively impacted on increasing eutrophication and changes in land use and water management practices (Briceño, Boyer 2010). In contrast to the number of studies conducted in Florida Bay with respect to the nutrient regime and planktonic microalgae, toxic benthic dinoflagellates have been comparatively understudied.
Sampling occurred in April and in October 2014, i.e. at the end of the dry and wet seasons, respectively. Samples of macrophytes were collected in 20 stations distributed in 4 distinct regions of Florida Bay and treated for counting and identification. Isolates were brought into culture and their toxicity was tested with oyster larvae bioassays.
Seven genera were detected, including *Prorocentrum*, *Coolia*, *Ostreopsis*, *Amphidinium*, *Gambierdiscus*, *Fukuyoa* (all included potentially toxic species) and *Sinophysis*. Mean abundances of the benthic dinoflagellate taxa estimated over the sampling period were not statistically different between the seasons (except for *Sinophysis*).
Moreover, this study documented the first record of *Coolia santacroce* in the Florida Keys.
Prorocentrum species represented the most abundant taxa (maximum abundances 10^4 cells g^{-1} fw), representing 37% of the entire dinoflagellate community, mainly represented by *P. rhathymum*, followed by *P. lima*, P. *hoffmannianum* and *P.* cf. *emarginatum*. *Coolia*, *Ostreopsis* and *Amphidinium* represented 21, 20 and 16% of the BHABs, respectively. On the contrary, *Gambierdiscus* were sporadically recorded representing only 5% of the entire dinoflagellate community. Results showed that certain benthic dinoflagellates were able to detach more easily than others from the benthic substrate and hence occupy the planktonic zone, i.e. *P. rhathymum*, *Gambierdiscus*., *P. hoffmannianum*, *P. lima* and *Coolia* (from the most detachable to the less one). On the contrary, *Ostreopsis* cells have a predominantly benthic behavior, but they can be transferred in water column only under certain environmental conditions. The distribution of these benthic dinoflagellates increased with water temperature and nutrient (especially phosphate) availability.
The toxicity tests revealed clear toxic effects for *Gambierdiscus caribaeus*, whose abundance exceeded 1000 cells g^{-1} fw at some sites, posing a risk of ciguatera fish poisoning in this area.

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First occurrence of *Margalefidinium* cf. *polykrikoides* blooms in Ionian Sea, Italy

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Harmful algal blooms (HABs) are natural phenomena, however some microalgal blooms can cause harm to humans and other organisms. These HABs have direct impacts on human health and negative influences on human wellbeing, mainly through their consequences to coastal ecosystem services (fisheries, tourism and recreation). These events can be favoured by anthropogenic pressures and eutrophication in coastal areas (Sarkar 2018). Global warming and associated changes in the oceans could affect HAB occurrences and toxicity as well, although forecasting the possible trends is still speculative and requires intensive multidisciplinary research (Wells et al. 2015). Dynamics of HABs vary from one site to another, depending on the hydrographic and ecological conditions but also the complexity of the algal life cycle, which is composed of discrete life stages whose morphology, ecological niche, function, and lifespan vary (Glibert et al. 2018). *Margalefidinium polykrikoides* (Margalef) F. Gómez, Richlen & D.M. Anderson 2017 is a cosmopolitan dinoflagellate notorious for causing fish-killing HABs. The red tide forming dinoflagellate genus *Margalefidinium* appears to be expanding globally, as well as blooming and/or causing more economic losses within its previously reported geographic distribution (Kudela, Gobler 2012). *Margalefidinium* has an adaptive capability conducive to rapid colonization of newly opened ecological niches, which may partially explain the apparent global expansion of its geographic range and bloom frequency. This species has a wide distribution in the Asian and European waters, it is responsible for high mortalities of wild and farmed fish and causes large economic losses in the Pacific, Atlantic, and Indian oceans (Matsuoka et al. 2008). In the Mediterranean Sea, the Italian coast is affected since the late 1990s and the Black Sea since 2001 (Zingone et al. 2006). In this work, we described an exceptional yellow-brownish discoloration of the waters in the shallow Porto Cesareo Bay (Mediterranean, Ionian Sea, Italy) in the July - August 2018 caused by a bloom of *M*. cf. *polykrikoides*. Cell densities reached 9.1 x 10^6 cells L^{-1} during the initial outbreak. A second bloom was observed about three weeks later reaching 6.7 x 10^5 cells L^{-1}. The assessment of the anthropogenic pressures and expected impacts on the marine-coastal waters was carried out. The study of live specimens showed great variation in cell size and shape, different cyst morphotypes were also found in the water samples and in the sediment. For the first time, we followed the life cycle of *M.* cf. *polykrikoides* in natural samples. The high densities of this species did not lead to fish die-offs in the bay, however it caused consequences on ecosystem and in turn, on tourism. The need for improving risk assessment to manage and prevent the occurrence of harmful blooms is globally enhancing. This is particularly true for dominant HAB-causing species after their persisting with low abundance in the phytoplankton assemblages but are worldwide considered as expanding their distribution, as the case of *Margalefidinium polykrikoides*.

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High Throughput Sequencing reveals marked seasonality for dinoflagellates and a distinctive winter assemblage at LTER-MC (Gulf of Naples, Mediterranean Sea)

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Dinoflagellates are important components of marine planktonic food webs acting as primary producers, consumers, symbionts or parasites. Environmental metabarcoding assessments have demonstrated that dinoflagellates often dominate marine planktonic communities both in terms of abundance and diversity. However, little information is available on dinoflagellate species dynamics and their occurrence in time. In this study, we investigated the temporal variation of the dinoflagellate community at the Long Term Ecological Research station LTER-MC in the Gulf of Naples (Mediterranean Sea) using the V4 barcode region of the 18S rRNA gene on 48 dates, sampled between 2011 and 2013. Dinoflagellates represented 32.7% of reads and 5.7% of ribotypes of total protists. A total of 96 different genera were retrieved throughout the samples, including 85 species that have never been recorded in the Gulf of Naples and 26 potentially toxic species. The new records are mainly represented by parasites (i.e. *Amoebophrya ceratii*, *Blastodinium* sp., *Chytriodinium* sp.), symbionts (*Gymnoxanthella radiolariae*, *Pelagodinium* sp.) and small-sized species (e.g. *Azadinium obesum*, *A. spinosum*, *Biecheleria brevisulcata*, *B. tirezensis*, *Heterocapsa pygmaea*). Overall, naked dinoflagellates, especially belonging to the genus *Gyrodinium* (42.51%) and to the Gymnodiniales *sensu stricto* (20.25%), dominated the dataset in terms of numbers of reads. Multivariate analyses revealed three principal seasonal clusters associated to winter, spring-summer and late summer-autumn conditions. LEfSe (LDA Effect Size) analyses identified a series of taxa that characterized the three seasonal clusters: 30 taxa for winter, 18 for spring-summer and 19 for late-autumn. Extended Local Similarity Analysis (eLSA) performed on a reduced dataset (547 ribotypes representing 74.5% of the reads with a similarity to a reference sequence higher than 97%) displayed a single connected network structure. Within the network, three statistically supported modules were detected based on the topology. These modules matched with winter, spring-summer and late summer-autumn communities highlighting the existence of well-structured seasonal assemblages. Heterotrophic dinoflagellates, including parasites, dominated the winter module, while chloroplastidic dinoflagellates were more abundant in spring-summer. Our results indicate robust communities succeeding over the seasonal cycle and highlight the existence of a distinct and specialised winter assemblage, which contrasts the general view that dinoflagellates are typically associated to summer conditions.

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Microalgae: a sustainable way for cattle wastewater recycling

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The MICROBE project combines engineering and scientific expertise to propose a circular and integrated process that uses zootechnical wastewater for the cultivation of microalgae. This represents a biorefinery approach applying microalgae to treat cattle wastewater with the double benefit to reduce the high nutrient loads in wastes and to produce microalgal biomass to ultimately use as a source of bioenergy. Microalgae cultivation for biofuel production represents a sustainable approach that overcomes the major obstacles associated with high volumetric productivity, use of edible feedstock and arable land coupled to the bioremediation of wastewaters and CO_2 mitigation (Gismondi et al. 2016; Abomohra, Elshobary 2019; Rugnini et al. 2019).
The first step of this project was to collect, analyse and characterise the zootechnical wastewater from an organic cattle breeding company (Morani, Rome, Italy). Physical-chemical characteristics of wastewater were studied, both on raw materials and after sequential filtrations with sieves from 2.0 mm to 0.125 mm. Results showed high concentrations of nitrogen and phosphorus (600-800 mg L^{-1} and 100-200 mg L^{-1}, respectively) and the presence of different heavy metals (such as Al, Cu, Ni, As, Fe). The evaluation of the functional microcosm through *Next Generation Sequencing* (NGS) was also performed. Then, the ability to grow in the filtered wastewater of robust green microalgae strains of the species *Tetradesmus obliquus* and *Desmodesmus* sp., selected for their tolerance to variable environmental conditions, was tested. The optimisation of growth conditions was, at first, performed testing different dilutions of wastewater, from 100% to 10% using single species cultures. Then we further lowered the concentrations, using dilutions of 25, 12.5 and 5%, for both monoalgal and mixed cultures (microalgal consortia) as we observed that raw cattle wastewater as medium, without any pre-treatment, required higher dilution levels to enhance light penetration and reduce the turbidity. Growth evaluation was conducted through optical density measurements, chlorophyll *a* extraction and biomass production. Nitrogen and phosphorus analyses were performed to evaluate the removal ability of the selected microalgae in this nutrient-rich wastewater. The scale-up phase of the process will be also considered from laboratory scale to pre-industrial scale photobioreactors (50 L each), designed for this project.
Data demonstrated high variability both in terms of growth parameters and nutrient values, probably due to high concentration of organic matter, suspended solids (35.7 g L^{-1}), bacteria and toxic contaminants present in cattle wastewater. The microalgal consortia, *T. obliquus* and *Desmodesmus* sp., seemed to be less affected by growth condition at the dilution of 12.5%, with the ability to remove more than 70% of total nitrogen and 98% of total phosphorus. Harvested biomass at different dilutions was then employed for lipid extraction. Fatty acids methyl esters (FAMEs) suitable for biodiesel production were obtained by *in-situ* transesterification. So far, results showed that the higher lipid percentage (% dry weight of the biomass) was found for the species grown at wastewater percentage lower than 25%, with FAME profiles showing carbon chains ranging from C10 to C18.
The project MICROBE characterised by a multidisciplinary working team/group (biologists, microbiologists, chemists, IT and industrial engineers) requires knowledge and innovation in all the steps of the biomass production process (from wastewater collection to lipid extraction) with the final goal to develop a sustainable "zero-waste" model of wastewater recycling.

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Removal of total and extra-cellular cyanotoxins from *Microcystis aeruginosa* using chlorinated compounds

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Cyanobacterial massive proliferations known as "blooms", are a naturally occurring phenomenon in freshwater bodies, whose frequency has globally increased over the past decades, mostly due to eutrophication and climate changes. Several species are able to produce toxic compounds, named cyanotoxins, which result hazardous for aquatic organisms. Humans may be exposed to them as well, mainly through recreational activities or consumption of contaminated water, indeed cyanotoxins presence in water for drinking purposes is considered as a major public health issue. The most widespread cyanotoxins are microcystins (MCs), hepatotoxic molecules counting more than 80 congeners, including the most toxic known variant MC-LR. Due to its high toxicity, a guideline value of 1.0 μg L^{-1} for MC-LR in drinking water has been suggested by World Health Organization, and it has been also adopted in Europe (Directive 98/83/EC). The removal of toxic compounds in drinking water plants is usually operated through oxidation, especially with chlorinated compounds; however, few data are available concerning cyanotoxins removal efficiency and the effects on cyanobacterial cells using these oxidants.

The aim of this study was to evaluate, after 1 h treatment, the capability of sodium hypochlorite (NaClO) and chlorine dioxide (ClO_2) to remove MCs produced by the cyanobacterium *Microcystis aeruginosa*, one of the most common species in freshwater bodies. *M. aeruginosa* was maintained in batch cultures under laboratory conditions and tested for its toxicological profile via LC-MS/MS. To assess the oxidants' effect on cells viability, inhibition of the photosynthetic efficiency was measured with a PAM fluorimeter. The concentration at which the two oxidizing treatments induced a reduction of 50% of the photosynthetic efficiency (EC_{50}), with respect to the control, was calculated showing that NaClO can exert its effect at a lower concentration than ClO_2 (EC_{50}: NaClO = 0.26 mg L^{-1}; ClO_2 = 0.55 mg L^{-1}). Finally, both oxidants at a low (0.5 mg L^{-1}) and high (2.0 mg L^{-1}) concentration were used to evaluate total and extra-cellular cyanotoxins' removal in *M. aeruginosa* cultures. NaClO showed a significantly higher removal efficacy, reducing total and extracellular MCs at both concentrations with respect to ClO_2 (ANOVA, $p < 0.05$). On the contrary, ClO_2 at the lowest concentration didn't succeed in MCs removal and determined an increase of extracellular toxins fraction compared to the control (ANOVA $p < 0.05$). Only the highest concentration lead to a reduction of about 60% of total MCs, however the remaining toxins were released in the medium, suggesting that ClO_2 could promote the release of cyanotoxins from cells and increase water hazard, especially when raw un-filtered waters are treated. NaClO was also found more effective in the degradation of main MCs congeners, including the most toxic MC-LR.

Preliminary results of this study showed that NaClO is a strong oxidant that can be successfully applied at low doses for cyanotoxins removal in drinking water plants, while ClO_2 could promote cell lysis and toxins release into the water. These results suggest that oxidant choice is a crucial step for the optimization of drinking water treatments.

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Recovery of rare earths and metals from spent fluorescent lamps by *Galdieria sulphuraria* (Rhodophyta)

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The disposal and recovery of WEEE is a problem that governments are taking note of and affects the entire population as it presents critical issues from an economic, environmental and health point of view (European Commission 2014). The current technologies for the recovery of rare and precious metals from WEEE are of two basic types: a) pyrometallurgical treatment, which does not meet eco-sustainability criteria, due to the high energy consumption and emission issues; b) hydrometallurgical treatment, which although highly selective and with low energy costs, provides a recycle with a high degree of impurities and does not solve the critical issues with respect to eco-sustainability. In the last decade, recovery of metals using bioprocess technology has been one of the most promising technologies due to low management costs and low environmental impact. *Galdieria sulphuraria* is a polyextremophilic red alga able to proliferate in strongly acidic, naturally metal-rich environments which developed unique mechanisms of metal tolerance (Iovinella et al. 2018; Minoda et al. 2015). We assessed the ability of *G. sulphuraria* to remove rare metals (yttrium, europium and gadolinium) from spent fluorescent lamps (bioleaching) under different acidic solutions; pH in the experiments was a critical factor, with an optimum sorption at pH ranging from 4 to 6. The capacity of simultaneous removal of rare metals from a mixed solution (cerium, europium, yttrium and terbium) was also investigated; *G. sulphuraria* was able to bioaccumulate all tested metals, with an uptake increasing as pH increased, and ranging from 30 to 56%.

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The results of the ARPA monitoring on the qualitative and quantitative consistency of marine phytoplankton

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In Italy, the Regional Agencies for Environmental Protection (ARPA) play a leading (sometimes exclusive) role in the institutional monitoring of surface waters, whether these are freshwater, transitional or marine ones, including those with a specific intended use pursuant to Legislative Decree n. 152/2006 and subsequent amendments and additions. These monitoring activities are also provided for by European Union Directives, including 2000/60/EC "Water Framework Directive", 2006/7/EC "Bathing Water Directive", and 2008/56/EC "Marine Strategy Directive", implemented by the Italian State by the respective Legislative Decrees n. 152/2006 as amended, n. 116/2008 as amended and n. 190/2010 as amended, for each of which monitoring activities are currently carried out by the ARPAs. As regards marine waters, in many of the aforementioned activities the phytoplankton monitoring is relevant, carried out by all the Environmental Agencies of the coastal Regions according to standard methods and protocols established at national level. Considering only the Apulia Region, ARPA currently monitors the phytoplankton communities in 42 stations of 39 marine-coastal water bodies according to Legislative Decree n. 152/2006 and in 22 stations up to 20 km from the coast according to Legislative Decree n. 190/2010, in addition to assessing the presence of the epibenthic microalga *Ostreopsis* cf. *ovata* in 20 sites corresponding to as many bathing waters according to Legislative Decree n. 190/2010 (Ungaro 2019). The frequency is different according to the type of monitoring/regulation, but it can be estimated that every year the ARPA Puglia laboratories carry out phytoplankton analysis on a total of about 800 samples; on a multi-year scale and with reference to the five-year period 2013-2017, only for the monitoring of the 39 regional marine-coastal water bodies (carried out every two months) the Apulian Agency analyzed about 1000 samples for the quali-quantitative determination of phytoplankton (Zingone et al. 2010). In the same period and for the same type of monitoring, more than 550 taxa were identified in the examined samples, over 400 of which at species level and about 150 at genus level; among the species some potentially harmful - as indicated in the IOC-UNESCO list (Moestrup et al. 2019) - have been found, which contributed about 10% to the total number of identified phytoplanktonic ones. More in detail, from the elaboration of data on a multi-year scale it appears that the two most representative groups of marine phytoplankton, namely diatoms and dinoflagellates, share much of the diversity of the Apulian seas, both with about 43% of the species on the total number of the examined samples; instead, in terms of abundance (cells/liter) the same two groups account for about 40% and 4% of the total, respectively. However, as foreseen and known in the literature, in the period considered the relative distribution and abundance of phytoplankton species was not always homogeneous in the investigated macro-area, showing variations on time and space scales. In conclusion, based on the above it is easy to understand how the huge amount of data on the qualitative and quantitative distribution of marine phytoplankton - information currently collected by the ARPAs in a standardized manner in all Italian coastal regions - can represent an invaluable asset for Italy and the scientific community, contributing to a more effective assessment of biodiversity and quality of the marine environment, always on condition that monitoring is kept active over time.

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First survey of epibenthic diatom communities on marine phanerogams and invertebrates from Omani coasts

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Complexity pervades biological systems at any scale: from microbes to higher organisms, from individuals to populations and from communities to ecosystems. Biodiversity and community structure are strongly influenced by the complexity of those relationships, in addition to the interplaying between multiple environmental conditions. On the other hand, complexity in microcommunities has not been solved yet. For instance, the role of substrates in shaping the diversity and structure of biofouling communities is virtually unexplored Benthic microalgae living in shallow coastal regions give a reliable contribution to the dynamics of the aquatic ecosystems, in terms of primary production, oxygenic activity, and trophic processes (Mac Intyre et al. 1996). Among benthic microalgae diatoms are reported to colonize sponges (Cerrano et al. 2004a, b), hydrozoans (Bavestrello et al. 2008; Romagnoli et al. 2006), bryozoans (Wuchter et al. 2003), crustaceans (Ikeda 1977), bivalves (Round 1981), and vertebrates (Round 1981; Round et al. 1990), with a high degree of specificity for some hosts. The species composition of epibenthic diatom communities seem to be influenced by the nature of substrate and by their biogeographic distribution. On the other hand, these diatoms communities appear to be composed by a limited number of genera, that can be considered fully adapted to the epibenthic lifestyle. Moreover, ecological studies on epibenthic diatom communities based on a rigorous taxonomic approach are very rare due to the small size of such organisms. Indeed, most of the species have average size less than 20 microns with taxonomic characters not resolvable in light microscopy, so the scanning electron microscopy (SEM) results the only possible approach for a correct quantitative analysis of such floristic communities. Our study, entirely based on scanning electron microscopy (SEM) investigation of diatom communities in undisturbed conditions gave us the first data on the hidden biodiversity of diatom assemblages associated to macrophytes and different invertebrates including sea urchins, gastropods, crustaceans and bivalves along Omani coasts in terms of functional classes and species composition. Preliminary results demostrated that all invertebrates and macroalgae collected along the Batinah coastal areas of Oman hosted benthic diatom communities which in both the case constituted the major element of their epibenthic microalgal flora. Significant differences in terms of cell density, specie composition and communities structure were observed between the diatom communities of invertebrates and macroalgae which appear quite similar within sampling locations analyzed . More in detail, cell densities of macroalgae diatom communities resulted more than one ten times higher than those of invertebrates with mean values approximately ranging 1000-4000 cell/mm^2 in the former versus 100 - 400 cell/mm^2 in the latter. Noteworthy is that the specie composition of macroalgae diatom communities was characterized by the complete dominance of adnate genus *Cocconeis* (represented by four species). Claim and gastropod diatom communities seem to be dominated still by the adnate genera *Amphora* and *Cocconeis* with a higher contribution of the former in claim and of the latter in gastropods respectively. Corals and sea urchins seem able to actively contrast diatom settlement by means of self-excreted mucilaginous matrices that trap the individual cells. Nevertheless, some genera of motile (*Mastogloia*, *Navicula*, *Diploneis Nitzschia* and *Pleurosigma*) and erect (*Achnanthes*, *Licmophora*) diatoms manage to colonize this complex and dynamic habitat.

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Preliminary data on the bioactivity of *Phaeodactylum tricornutum* Bohlin lipidic extract on Brown Adipocyte Tissue cells

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In the frame of my PhD research project, aimed to investigate the content and diversity of diatom bioactive molecules as candidates to be exploited in biotechnological applications, we focussed on lipidic extract potential against human obesity and related metabolic disorders.
Brown adipose tissue (BAT) is a mitochondria-rich tissue with high oxidative capacity and, when fully activated, synchronizes mainly glucose and fatty acid catabolism to finally dissipate the mitochondrial proton gradient through Thermogenin (UCP1). Recently, the inverse correlation between the presence of BAT and obesity and type 2 diabetes has been pointed out. BAT has gathered increasing attention as a therapeutic target for fighting human obesity and other metabolic disorders (Lettieri-Barbato 2019).
Diatoms can accumulate up to 25% of the dry biomass and are capable to produce large amounts of high quality PUFAs, as essential ω3 fatty acids (eicosapentaenoic, EPA, and docosahexaenoic, DHA, acids) with applications in dietetics, food supplements, therapeutics and for the treatment of chronic inflammation, atherosclerosis, hypertension and cancer.
In this work the model diatom *Phaeodactylum tricornutum* Bohlin was mass cultivated, batch culture, in indoor photobioreactors (30 L) at 25 °C, irradiance of 80 μmol photons m^{-2} s^{-1} and 12:12h L/D cycle. The biomass was harvested at the stationary phase (day 21) by settling and centrifuging, then freeze-dried and, subsequently, treated with a chloroform:methanol solution (2:1 v/v) following Bligh (1959) in order to extract the total lipids. Quali-quantitative characterization of extract fatty acids was performed by Gas Chromatography equipped with a Flame Ionization Detector (GC-FID).
Results showed that the total lipid content of *P. tricornutum* biomass, even in absence of stress conditions was high, 17.29% ± 1.50%. The fatty acid profile indicated that EPA (C20:5) was the most abundant fatty acid, 20.59%, followed by docosanoic (C22:0, 20%), cis-9- acid (C16:1, 17.27%), and hexadecanoic (16:0, 14.87%) acids.
Due to the role of PUFAs in human metabolism, counteracting insulin resistance, tissue inflammation and obesity, we assessed the metabolic effect of *P. tricornutum* lipidic extract on BAT cells, after 48h treatment, by means of Western Blot analysis. Data showed that the extract administration modulated the expression of key proteins involved in the thermogenesis pathway, such as the Adipose Triglyceride Lipase (ATGL) and Thermogenin (UCP1). Taken together these results prospect an anti-diabetic activity of BAT stimulated by *P. tricornutum* lipidic extract.

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Biodiversity of diatom community on corals and seagrasses from Saudi Arabian coasts of the Arabian Gulf: a taxonomical, ecological and environmental approach

L.Rabaoui, J.H. Moritz, M.A. Qurban, V. Perricone, V. Roviello, M. De Stefano

In equatorial and tropical marine waters, the rates of primary production of microalgal communities associated with seagrasses and seaweeds are comparable or sometimes higher to those of the phytoplankton component. These benthic microalgal communities are mainly represented by a limited number of diatom genera, belonging to different growth forms that can be considered fully adapted to the epibenthic lifestyle. In spite of the important ecological role played in the food chain, the biodiversity of diatom communities on seagrasses and seaweeds is still poorly studied and highly underestimated while that on corals has been virtually neglected as there are only few studies on this topic in the literature. Miller et al. (1977) reported tens of diatom genera associated with hard corals in Florida, besides those associated with the co-occurring seagrass *Thalassia testudinium*. More recently, pennate diatoms were reported colonizing corals of the genus *Porites spp*. on the Australian Great Barrier Reef (Diaz-Pulido, McCook 2002).

Our study, entirely based on scanning electron microscopy (SEM) investigation of diatom communities in undisturbed conditions, present the first data of biodiversity of diatoms associated to phanerogams and hard corals along the Saudi Arabian coasts of the Arabian Gulf. Sampling stations were selected on the basis of the co-presence of the seagrass (*Halodula uninervis*, *Halophila ovalis*, *Halophila stipulacea*) and coral species (*Acropora* sp., *Porites* sp. and *Platygyra* sp.) selected as optimal substrates for benthic diatom communities. Preliminary results of SEM floristic and quantitative analysis confirm the presence of benthic diatom communities in both, seagrasses and coral species collected. Taxonomical analysis allow us to identify 38 diatom species in seagrasses and approximately 30 species in corals. Although the composition in diatom species does not vary significantly between phanerogams and corals within each sampling location, this is not true for their total cell abundances (cell/mm2), which vary greatly between the former and the latter respectively. Indeed, the total abundance values of diatom communities on seagrasses ranged from a minimum of 1618 to a maximum of 11863 cells/mm^2 while those found on corals do not exceed hundreds of cells/mm^2 thus resulting in 10 to 100 times lower. *Halodula uninervis* was generally the most epiphytized phanerogam species.

More than 70% of diatom species appear to be present both on seagrasses and corals in sampling locations where the two substrates coexist. However of the approximately 38 species constituting the seagrasses diatom community, only those belonging to the genus *Cocconeis* (mainly *Cocconeis neothumensis var. marina, C. scutellum var. posidoniae, C. scutellum var. scutellum, C. stauroneiformis*) strongly dominate the communities in terms of abundance. *Cocconeis* dominance was indeed always manifested in the diatom communities of all *Halophila* species analyzed in each sampling sites reaching maximum values of over 10,000 cells/mm2, approximately one hundred times higher than those of other genera not exceeding all together 250-300 cell/mm2. Seagrasses leaves shown at low magnification in Scanning Electron microscopy appear fully covered by a uniform monolayer of *Cocconeis* cells generally dominates by the species *C. neothumensis* var. marina characterized by cells ranging between 10 and 40 μ.

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Morphological and molecular characterization of *Pseudo-nitzschia* spp. in the NW Adriatic Sea

S. Giulietti, S. Accoroni, T. Romagnoli, S. Bacchiocchi, C. Totti

Pseudo-nitzschia species are common representatives of the northern Adriatic diatom communities and occur throughout the year often causing intense blooms (abundances up to 10^6 cells/l). Of the 52 known species (Guiry, Guiry 2019), 26 are recognized to produce Domoic Acid (DA) involved in Amnesic Shellfish Poisoning (Lundholm 2019). The existence of cryptic and/or pseudocryptic species (e.g. *P. delicatissima* complex and *P. pseudodelicatissima* complex) makes identification at the species level problematic (Lundholm et al. 2003, 2006). Indeed, the frustule ultrastructure analysis by Transmission Electron Microscope (TEM) is fundamental but it is not always resolutive (Kim et al. 2015). The aim of this study is to characterize *Pseudo-nitzschia* species, during 18 months of sampling, in the NW Adriatic Sea. Sampling was carried out with monthly frequency in a LTER (Long Term Ecological Research) site, i.e. the coastal station of the Senigallia-Susak transect (SG01). Niskin samples were analyzed under inverted microscope for the abundance estimation. Net samples were collected at the surface to set up cultures for molecular, ultrastructural (TEM) and toxin analyses. One hundred and twenty-three strains were set up throughout the sampling period. The analysis of abundances revealed that species of both *P. delicatissima* and *P. pseudodelicatissima* complexes co-occurred almost the entire investigation period with the highest abundances in May ($2.8*10^6$ cells l^{-1}) and June ($1.9*10^6$ cells l^{-1}), respectively. Among the *P. seriata* group, *P. pungens* and *P. fraudulenta* have been recorded in winter ($6*10^3$ and $1.4*10^4$ cells l^{-1}, respectively). The allochthonous species *P. multistriata* has been found only in winter and autumn ($6*10^3$ cells l^{-1}). The molecular (LSU marker) and electron microscopy (TEM) analyses confirmed the presence of *P. pungens*, *P. fraudulenta*, *P. delicatissima*, *P. calliantha* and *P. mannii*. To date, no toxins were detected in 24 tested strains.

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The response of *Fucus virsoides* (Fucales, Ochrophyta) to a glyphosate-based herbicide exposure: ecophysiology and metabolomics approach

A. Falace, S. Kaleb, S. Felline, L. Del Coco, S. Fraschetti, F.P. Fanizzi

Glyphosate-based herbicides (GBHs) are recognized as sources of water pollution and several studies have investigated their effects on aquatic organisms. Yet, only few investigations have been performed on marine macroalgae (Falace et al. 2018; Felline et al. 2019).
We carried out different mesocosms experiments to test the responses of *Fucus virsoides* exposed to Roundup® solutions, the most common commercial formulation of GBHs. We firstly tested the effect of a continuous exposure (6 days) and the potential of recovery after a short exposure (24 h), combining GBH with and without nutrient enrichment, simulating a runoff event. Then we studied both the metabolomics responses and the effect on primary production of *F. virsoides* fronds exposed to different concentration of Roundup®.
Our results show deleterious effects of GBH on *F. virsoides*, independently from the duration of exposure and the presence of nutrients. Furthermore, alteration in the metabolomic profiles of exposed thalli compared to controls were detected, with the decrease in the aromatic amino acids (phenylalanine and tyrosine), an increase in shikimate content
Our results suggest that marine primary producers could be largely affected by the agricultural land use, this asking for further studies addressing the ecosystem-level effects of glyphosate-based herbicides in coastal waters.

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Natural recolonization of *Posidonia oceanica* ten years after the laying of a submarine gas pipeline

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Marine areas are facing significant increasing threats, which include biological, physical, chemical pollutions and habitat destruction, all together causing loss of biodiversity. In order to mitigate seagrass loss and renovate ecosystem functions, different restoration tools have been elaborated (Cunha et al. 2012). Here we report on the spontaneous *Posidonia oceanica* (L) Delile recovery in front of the harbor of Ischia (Punta San Pietro, Gulf of Naples, Italy) after its partial destruction to set up a submarine gas pipeline between the island and the land (Torregaveta, Monte di Procida, Italy)(Cotugno et al. in press). The trench, 300m long and dredged between 7.5 and 5.5m depth, was filled with rubbles after the deployment of the pipeline. The continuous flux of ramets from the adjacent stands favored a natural recolonization. Ten years later (2009-2019), the spontaneous *Posidonia* recovery was mapped, combining the use of underwater photogrammetry technique and GIS (Abadie et al. 2018), and the structure of the associated community was analyzed. Preliminary results are here referred to the deepest portion of the trench located at 7.8m depth. On an area of 334 m^2, a total of 121 new *Posidonia* patches, corresponding to 12% of coverage, was detected. Higher values of shoot density and richer vagile community (i.e. nr of species, abundance and diversity) were found in comparison with historically established stands with the same geographical exposition around the island of Ischia (Gambi et al. 1992; Buia et al. 2000; Vasapollo 2009; Garrard 2013). Data seems to indicate that a higher habitat complexity as well as a patch effect respect to continuous stands may favor a relatively rich vagile fauna. It is worth to note that spontaneous recruitments of detached plants from nearby meadows were successful on rubbles only and not on sandy areas, providing evidence of the importance of the substrate type (Di Carlo et al. 2006), mainly at shallow depths, where environmental conditions (i.e. water movement and sedimentation rate) may be not appropriate for the plant anchoring and establishment.

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Seaweeds: the sticky fly paper for microplastics in transitional water systems

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The topic of microplastic contamination in the environment has increasingly raised the level of attention of many researchers about the quantities and effects that these contaminants could produce in the environment. A lot of information was collected on water, soil, sediments and organisms around the world, highlighting the ubiquity of these particles but few data are provided on macroalgae, one the main transitional water system (TWS)producers. To fill this gap an investigation was carried out quantifying microplastic contamination on the seaweeds in 9 sites scattered in the Venice Lagoon. The three dominant macroalgal species of each site were collected and processed in duplicate. For each sample, 5 g of fresh seaweed were shaken for 30 min in 100mL of 50mM Na_2EDTA, NaCl 30‰ to extract the particles glued to the seaweed surface. The extract was treated overnight with NaOH 1% at 40°C to remove interfering compounds and filtered on GF-F fiberglass filters (0.7µm). The filters were stained by Nile red (Maes et al. 2017) and particles were counted with a stereomicroscope. Concurrently, 1mL of the algal extract was retained for the esopolysaccharide (EPS) determination, by ethanol 75% precipitation and phenol-sulphuric acid analysis (Dubois et al. 2002). The analyzed seaweeds were: *Agardhiella subulata, Chaetomorpha linum, Cystoseira barbata, Codium fragile* subsp. *fragile, Gracilaria gracilis, Gracilariopsis longissima, Laurencia obtusa, Sargassum muticum* and *Ulva rigida*. The microplastic contamination on the seaweed surface on average was 9.7 items g fw^{-1} with peaks up to 58 items g fw^{-1} in front of the Lido of Venice. The average coefficient of variation (CV) for different species within the same station (CV: 0.70) was lower than the average CV for the individual species in different stations (CV: 0.79). This means that the average microplastic content of the seaweeds was more likely bound to the station of origin than the species from which was extracted. Moreover a linear correlation (r^2=0.52) was found between the EPS superficial content in 13 macroalgal samples and the microplastics adherent to the same samples. A linear increase of microplastics in the seaweeds with an EPS content higher than 0.7 mg g fw^{-1} was recorded. Esopolysaccharides act as a glue that traps the microplastics flowing and settling in the water column making the seaweeds a “sticky fly paper” for microplastics. However also the EPS clearly displayed a station specific pattern more than a species specific pattern. Macroalgae during their life cycle integrate the contamination from microplastics in the water column and could be used as a good indicator of the overall contamination present in the water column. Moreover, macroalgae undergoing the action of grazers, transfer the adherent particles to the trophic chain favouring the transfer of microplastics from the water column to marine organisms.

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Bioactive compounds from *Ulva australis* Areschoug collected in the North Adriatic Sea

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Ulva australis Areschoug is a green seaweed belonging to the family Ulvaceae (Chlorophyta) widely distributed along the coasts of the Yellow Sea and the Bo Sea of China, and recently recorded in the North Adriatic Sea and in the Venice Lagoon (Italy). This species, previously known as *Ulva pertusa* Kjellman, is often misidentified as *Ulva rigida*, due to its cryptic morphology. *U. australis* tolerates a wide range of salinity, temperature and water quality, and grows rapidly in nutrient rich habitats. These characteristics have rendered it the classification of invasive species. *U. australis* is habitually used in Chinese traditional medicine for the treatment of hyperlipidemia, sunstroke, and urinary diseases.

To increase knowledge on the possible biotechnological uses of *U. australis*, this work explored this species as a potential source of bioactive products with multiple applications. For this purpose, *U. australis* was collected in the Chioggia inlet area (Lagoon of Venice, Italy) and evaluated for its fatty acids methyl esters profile (FAME), its chemical profile in terms of total contents of phenolics (TPC), flavonoids (TFC) condensed tannins (CTC) and carbohydrates (TCC). Extracts were further evaluated for *in vitro* antioxidant activity and for inhibition of enzymes related to neurological disorders (acetylcholinesterase: AChE, butyrylcholinesterase: BuChE), skin hyperpigmentation (tyrosinase), obesity (lipase), diabetes (α-glucosidase and α-amylase) and skin ageing (elastase).

Harvesting this seaweed for commercial purposes could be an efficient cost-effective alternative to control or at least mitigate the effects of its invasive presence. In this perspective, the concept of "eradication by utilization" could be reasonably practised in the North Adriatic Sea and in the Venice Lagoon for the management of *U. australis*.

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Killing them gently; control of phototrophic biofilms growing on stone monuments using plant products

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Although lithic archaeological sites and confined environments are relatively harsh environments for microorganisms to inhabit, they are still subjected to biodeterioration by microorganisms able to attack rocks and give rise to a microbial succession (cyanobacteria, bacteria, microalgae, mosses and fungi). These organisms can survive and even grow on minimal amounts of light, water and nutrients, mainly supplied by the substrate and unwitting visitors. Both structural and aesthetic damage to these valuable surfaces occur as a result of physical and chemical processes governed by the biofilm, which result in discoloration and material impairment. Standard physical and chemical treatments to remove biofilms can themselves cause surface damage as well as being health hazard. Moreover, the employment of chemical biocides seems to be selective for resistant microorganisms. Therefore, more sustainable and safer practices are being sought.

Rome, 64 AD, Emperor Nero ordered the reconstruction of his residence, with the Domus Aurea forming part of the project to transform Rome into a new city. The main part of the building was located on the Palatine and Oppian Hills and was famous for its sumptuous decoration in which gold and precious stone coverings were added to the stuccos, paintings and coloured marbles, while mosaics, normally restricted to floors were also placed in some of the vaulted ceilings. Nero's successors were embarrassed of the Domus' opulence and so stripped it of its marble, its jewels and its ivory within a decade. Within 40 years, the Domus Aurea was completely obliterated and filled in with soil. Surprisingly, this in-filling ensured the survival of the wall paintings by protecting them from damp.

After 20 years of excavation and restoration it was opened to the public in 1999, only to close six years later due to detachments and security problems. The doors opened again to the tourist in February of 2007 with restricted visitation. However, due to artificial lighting and high humidity levels many surfaces have been heavily infested by both heterotrophic and phototrophic biofilms. So now, among the main restoration efforts, the disinfection of biodeteriogens growing on the walls and ceilings has become fundamental. Data on the biocidal efficacy of some plant-based products is showing great promise, so it was decided to carry out tests on biofilms sampled at the Domus Aurea using the essential oil from *Lavandula angustifolia,* alcohol extracts from *Glycyrrhizza glabra* leaves (Trifolio-M GmbH) and *Capsicum* sp, singularly or mixed. Biofilms samples were collected in 'room 93' from an undecorated wall and were then homogenised and inoculated in agarized BG11 growth medium. Observations showed that the cyanobacterium *Scytonema julianum* was the dominant species, and has often been described from other hypogean environments, such as Roman Catacombs, and known to deteriorate substrate integrity by dissolution of minerals from the substrate and the precipitation of calcium carbonate on its sheaths. Identification of bacteria by r-DNA16S sequencing revealed the presence of Proteobacteria (6 spp.), Actinobacteria (2 spp.) and Bacteroidetes (1 sp.). Three fungal strains were also isolated and are to be identified. The biofilms were treated twice with the extracts on day 1 and 5, and the photosynthetic response of the biofilm was followed for five days with a mini-PAM portable fluorometer. Photosynthesis is highly susceptible to this kind of treatment, so measurements of rates were used as a proxy for cell health. Changes in photosynthetic activity of the samples treated with the extracts were compared to control biofilms receiving no treatment. Results showed that the essential oil of *L. angustifolia* and *G. glabra* leaves extract at 30% had the highest photosynthesis inhibition potential, followed by *G. glabra* extract 10%. *Capsicum* extract was the least efficient. These first results are encouraging enough to continue beyond the laboratory trials in the hope to realise an eco-friendly, non-toxic and sustainable strategy for the conservation of lithic cultural heritage.

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Molecular mechanisms regulating sexual reproduction and mating type determination in the diatom *Pseudo-nitzschia multistriata*

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Pseudo-nitzschia is an important genus of marine diatoms responsible for blooms in coastal and oceanic waters. We selected *Pseudo-nitzschia multistriata* as a model system to explore the process of sexual reproduction, which in diatoms is linked to the formation of large-sized cells within the cell size reduction/restitution cycle. *P. multistriata* is a heterothallic species in which sex is induced when cells of opposite mating type get in contact. We sequenced the *P. multistriata* genome (Basu et al. 2017), defined its meiotic toolkit (Patil et al. 2015) and elucidated the gene expression changes occurring in the opposite mating types during the early stages of sexual reproduction (Basu et al. 2017). To further refine our knowledge of this process, we are continuing our investigations of the gene expression changes occurring at additional stages, when gametes and F1 cells are present. Moreover, we are focusing on a set of genes that are preferentially expressed in one or the other of the two mating types. These mating type-related genes, identified using a transcriptomic-based approach, play a role in defining the mating type and in governing cell behaviour during sexual reproduction. Importantly, we identified structural differences in the genomic region for one of these genes, and demonstrated that it acts as the sex determinant in *P. multistriata* (Russo et al. 2018).

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Chromium tolerance in *Scenedesmus acutus* M. mediated by sulfur uptake

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Sulfur (S) is essential in the synthesis of important defence compounds such as the small cysteine-rich peptides, phytochelatins, metallotioneins and reduced glutathione involved in both metal chelation and in scavenging potential oxidative stress as a result of ROS production induced by Chromium (Cr) exposure (Volland et al. 2012). Hence sulfur availability can lead to an increased capacity to cope with biotic and abiotic stresses, a phenomenon known as SIR/SED (Sulfur Induced Resistance or Sulfur Enhanced Defence). Moreover, the tolerance to Cr seems strictly connected to sulfur metabolism (Pereira et al. 2008). Freshwater green algae have different sulfate (SO_4^{2-}) transporters located on the cellular membranes and various oxide anions (e.g. chromate, selenate) can directly compete with SO_4^{2-} at the site of transport. Once SO_4^{2-} is transported into a photosynthetic eukaryotic cell, it must be routed into the plastids for reductive assimilation, through an ABC-type chloroplast envelope-localized holocomplex composed by four proteins: SulP1 SulP2, Sbp and Sabc.

In this work we identified two genes coding for H^+/SO_4^{2-} co-transporters in two strains of freshwater algae *Scenedesmus acutus* (F. J. F. Meyen) with different Cr (VI) sensitivity. The two genes were named *SaSULTR1* and *SaSULTR2*. Albeit very similar, the aminoacidic sequences shows some differences in the pocket and in the regulative STAS domain in the protein 3' end. Moreover, we have completely identified *SaSulP1* and partially *SaSulP2* genes, coding for the envelope-targeted transmembrane proteins forming the channel of the chloroplast ABC transporter, which in their turn are bound to a sulfate-binding protein (Sbp) and to an ATP-binding protein (Sabc) on the cytosolic and the stromal side respectively (Takahashi et al. 2012).

In order to define the role of these transporters in Cr detoxification, we analysed their expression level by RT-qPCR, in both strains of *S. acutus* (wild type, wt vs chromium-tolerant, Cr-t). Previous data showed that S starvation induced in both *S. acutus* strains, a transient increase of Cr(VI) tolerance associated to an increased capacity of sulfur uptake and assimilation leading to an increase in cysteine synthesis during the recovery in standard medium (Gorbi et al. 2007). The analysis was thus performed on algae pre-cultured for 3 days in standard (+S) or S deprived medium (-S) and subsequently transferred into standard medium or in standard medium supplemented with 1and 2 mg Cr(VI)/l, supplied as potassium dichromate ($K_2Cr_2O_7$).

In +S condition a different regulation of the plasma membrane transporters was observed in the two strains. In fact, in the wt *SaSULTR2* expression was significantly higher than in the Cr-t strain and *SaSULTR1* was expressed only after Cr treatments. In Cr-t strain instead, both *SaSULTR1* and *SaSULTR2* were expressed in control condition, and increased their transcription in presence of 2 mg Cr(VI)/l. As far as the chloroplast transporter, while *SaSulP2* was quite stable in both strains, *SaSulP1* was negatively affected by chromium in the wt and induced by the metal in the Cr-t. Upon S starvation, a strong increase was observed in the transcripts of all the considered genes in both strains, but while in the wt their levels rapidly decreased after nutrient resupply, in the Cr-t strain *SaSULTR1* transcription remained elevated as well as that of *SaSulP1* even in the presence of 2 mg Cr(VI)/l.

The differential expression between wt and Cr-t strain of all the investigated genes, in response to different cultural conditions, seems directly linked to the enhanced sulfate uptake/assimilation pathway shown by Cr-t strain and putatively involved in its Cr(VI) tolerance. The up-regulation of *SULTRs*, following S starvation, may have two direct consequences which can strongly enhance Cr-tolerance: (i) decrease in Cr uptake due to the induction of high affinity sulfate transporters; (ii) a greater sulfate uptake and sulfur availability inside the cells for the synthesis of sulfur-containing molecules with the consequent increased capacity to cope with intracellular chromium, either through chelation and compartmentalization or through an enhanced antioxidant response.

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