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Environmental Stresses on Coastal Marine Algae

**1D.1**

**GROWTH AND PHOTOSYNTHETIC RESPONSES OF PICOCHLORUM OKLAHOMENSIS TO PHOSPHORUS AND SALINITY STRESSES**

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Photosynthetic organisms rarely experience optimal growth conditions in their natural habitat, and at any given time, two or more physical and chemical variables are likely to be suboptimal. The extreme environment of the Great Salt Plains (GSP) results in low algal biomass, such that natural selection is likely driven by survival of multiple abiotic stresses rather than rapid growth and biotic interactions. The objective of this study was to determine the effect of combined salinity stress and phosphate limitation on growth and photosynthesis in the green alga *Picoclorum oklahomensis* isolated from the GSP habitat. Algal cells were grown in batch cultures under phosphate sufficiency or phosphate limitation at salinities of 10, 50 and 100 ppt. in artificial seawater (AS 100) medium. Cells were physiologically characterized by initial growth rates, cell yields, photosynthetic light-response curves (oxygen evolution), pigment composition, and the chlorophyll fluorescence parameters F\(_{v}/F_{m}\), \(\Phi_{PSII}\), qP, and NPQ. Low phosphorus exhibit smaller inhibition by 100 ppt salinity relative to 10 ppt. Thus, high salinity appears to be reduced under low nutrient conditions. P resulted in qualitative differences in physiological response. The overall P stress response indicates reversible photoprotection. The interaction between salinity and phosphorus is with a general stress response that concurrently protects against several abiotic stress factors.

**1D.2**

**COUPLING BETWEEN THE CELL CYCLE AND THE VERTICAL MIGRATION IN INTERTIDAL MUDDY MICROPHYTOMATOS**

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Coastal areas are characterised by a high biological productivity which is mainly supported by estuarine intertidal mudflats. The high productivity of intertidal mudflats is due to their intensive microbial activity generated by autotrophic microalgae (MPB, microphytobenthos) and heterotrophic prokaryotes. The dynamics and extent of MPB primary productivity is very much based on a unique feature in the plant kingdom: movement. At our latitudes and in muddy sediment, MPB is quasi exclusively constituted of motile (‘epipelic’) diatom species which can endorse vertical migration through the sediment as a function of the tidal cycle and of the light climate. During diurnal emersion MPB can form a dense photosynthetic biofilm at the surface of the sediment. At high tide, MPB migrates down the sediment and stays in complete darkness until the next diurnal emersion occurs. It is believed that this migration rhythm divides the MPB production between two clear periods: photosynthesis during emersion and division during immersion. Although largely accepted, this hypothesis has never been deeply investigated. This is the purpose of this work. We harvested migratory MPB from fresh muddy sediment (from the
coastal area of La Rochelle) for which we measured the cell cycle phases thanks to flow cytometry. By using chemically synchronised monospecific cultures of common epipelagic diatoms of different size, ‘small’ (Navicula phyllepta), ‘medium’ (Cylindrotheca closterium) and ‘large’ (Pleurosigma sp.), we were able to distinguish the different phases of the cell cycle of each size group. Furthermore, by applying an inhibitor treatment together with different light regimes, we were able to differentiate the effect of migration and/or of the light climate on the cell cycle of MPB. Results are emphasised to highlight the importance of the environmentally driven migration rhythm of MPB on the production of coastal intertidal mudflats.

1D.3
EFFECT OF TURBULENCE REGIME IN THE PERSISTENCE OF DIATOM DERIVED POLYUNSATURATED ALDEHYDES IN SEAWATER

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During the last decades there has been a growing interest regarding the study of the ecological function of polyunsaturated aldehydes (PUAs) released by diatoms in oceanic waters. These metabolites have been proposed as a chemical defence for diatoms and an important role in the community succession of diatom blooms has been postulated. Toxicity tests of different diatom derived aldehydes at different concentrations over other non producing diatoms and other phytoplankters have been performed by different researchers. Experimental cultures used for toxicity tests are usually gently agitated by aeration, magnetic agitation or both, neglecting the volatile nature of the PUAs. We used 2E-4E-decadienal as a model PUA. As other polyunsaturated aldehydes Decadienal shows an absorbance maximum at 274 nm and a specific molar absorption coefficient of 3.1 104 L/cm mol in methanol solution. We used this property to follow the time evolution of two initial concentrations of 2E-4E-Decadienal (15 and 7 µM) in experimental cultures prepared in natural filtered and sterilized seawater and maintained at different turbulence regimes: Control (without any agitation), Areation (8.2 and 0.8 ml s⁻¹) and agitation (magnetic stirrer at 60 and 100 r.p.m). Decadienal dissappeared from the cultures exponentially, with an average rate of 0.0027 ± 0.0005 h⁻¹ at control conditions. This rate was not significantly different from that obtained at both agitation regimes (0.0028 ± 0.0005 h⁻¹). However, decadienal disappeared at a rate of 0.172 ± 0.021 h⁻¹ and 0.212 ± 0.075 h⁻¹ when cultures were aerated at 0.8 and 8.2 ml s⁻¹ respectively. This implies a time of persistence of decadienal less than 10 h for an initial concentration of 7 µM, a time much lower than the 48–72 h generally used for toxicity test on microalgal growth rates. Experimental and ecological implications of the observed rates are discussed.

1D.4
SPATIAL DISTRIBUTION OF POLYUNSATURATED ALDEHYDES IN THE GULF OF CÁDIZ (SW IBERIAN PENINSULA)

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Diatoms represent one of the most important primary producers in the ocean and constitute a major component of the coastal phytoplankton. These eukaryotic algae are able to produce and release an array of biologically-active metabolites such as the oxipilins. Among the oxipilins, the polyunsaturated aldehydes (PUAs) constitute a relevant group of volatile compounds, highly reactive. These metabolites have been proposed as a chemical defence for diatoms and recently are being reported teratogenic effects on different species groups. To date, however, most research has been developed in experimental cultures, and there is scarce information from field studies. Further analyses of the occurrence and environmental control of these compounds in nature become necessary to understand the ecological significance of these chemicals and its environmental regulation. In this work, comparative analysis of the potential spatial distribution of PUAs has been performed through three transects sited in the Gulf of Cádiz (SW Iberian Peninsula). Moreover we
The Water Framework Directive suggests the use of abundance and species composition of macrophytes to assess the ecological status class of coastal waters. In the case of the Mediterranean Sea, two ecological quality indices based in seaweed communities have been proposed: the EEI (Ecological Evaluation Index), and the CARLIT (cartography of littoral and upper-sublittoral rocky-shore communities). In this study, both indices have been applied and compared in the Mediterranean rocky shores of Andalusia (Southern Spain). For this purpose, 20 sites were sampled along Mediterranean coast from Tarifa (Province of Cádiz) to Despeñaperros (Province of Almería) (approx. 750 kilometres length). Algae in three replicates of 15 x 15 cm quadrats were collected. In addition, littoral communities were identified in the nearest coast. The correlation of values between EEI and CARLIT indices was significant (p-value > 0.05). However, EEI overestimated the ecological status class in some polluted places, where the effects of the local industry in the seaweed community were perceptible. This overestimation was related to the classification of the pollution resistant species Corallina spp., as sensitive species (ESG I). Furthermore, CARLIT values showed a clear trend increasing from the West to the East. In Andalusian coast there have been observed marked differences between reference sites attributed to the transition from the Atlantic Ocean to the Mediterranean Sea. For this reason, different references sites were used to calculate ecological quality ratios for CARLIT. Preliminary results suggested the division of Andalusian coast in three regions: 1) from Gibraltar Strait to Calaburras Cape; 2) from Calaburras Cape to Guardias Viejas; 3) from Guardias Viejas to the limit of Andalusia with the province of Murcia. This may have significant biogeographycal consequences, at least for the classification of water masses in the southern Spain Mediterranean coast.

**1D.5**

ASSESSMENT OF ECOLOGICAL STATUS IN SOUTHERN MEDITERRANEAN SPAIN USING MACROALGAE ASSEMBLAGES

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The present study is being developed in the framework of a research project dealing with exotic seaweed Caulerpa racemosa on the coast of the Croatian Adriatic Sea. Preliminary analysis focus on diatom assemblages existing in surface layer of sediments and from stones sampled on the coast of the Adriatic Sea affected by Caulerpa racemosa. Studied samples were taken monthly during summer season 2009 in areas of Caulerpa racemosa near Dubrovnik (Southern Adriatic, location Orsula, 42°37'49.45"N; 18°8'3.81"E) at the depth from 10 m to 14 m. Processed material (hydrogen peroxide treated) was studied by means of light (LM) and scanning electron microscopy (SEM). Identified assemblages of diatoms in sediment and on rocks were not as taxonomically rich and diverse as epiphytic diatoms on Caulerpa racemosa. The biraphid genera (Amphora and Diploneis) were represented by a high number of species, among others: Amphora acutiuscula Kützing, Amphora bigibba var. interrupta Grunow, Amphora coffeaeformis (C.A. Agardh) Kützing var. coffeaeformis, Diploneis smithii (Brébisson) Cleve, Diploneis weissflogii (A. Schmidt) Cleve. Monoraphid genus Cocconeis is known to be benthic with the raphe valves firmly attached to rocks, plants, animals or any other substrates. Cocconeis representatives constitute communities with high specimen abundance and species richness in the selected samples. Several forms of unclear taxonomical affinity were identified assemblages of diatoms in sediment and on rocks.
also observed. The group consists of taxa relatively scarcely identified in the world or absent in the literature. The results of detailed taxonomical studies of the collected material could be of great interest to diatom research.

1D.7
NITRATE AFFECTS PHOTOACCLIMATION AND OXIDATIVE STRESS IN CYSTOSEIRA TAMARISCIFOLIA IN SOUTHERN SPAIN

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Cystoseira species in the Mediterranean Sea are used as biological indicators of the high ecological status of coastal waters. Photosynthetic activity as in vivo Chl a fluorescence i.e. maximal quantum yield (Fv/Fm) and electron transport rate (ETR) as estimator of production was evaluated in Cystoseira tamariscifolia collected on rocky shores and in tidal pools of the eulitoral coast of Málaga (Southern Spain). After sampling, macroalgae were grown for 7 d out-door in 1.5 l UV transparent vessels under two levels of nitrate: 50 µM (high) and 5 µM (low). In addition, photosynthetic pigments, antioxidant activity (DPPH method), photoprotective compounds (phenols) and C: N ratio were measured after short-term (3 days) and medium-term stress (7 days). Both Fv/Fm and ETR at the beginning of the experiment were higher in algae collected from the rocky shores (high sun exposure) than that in algae growing in tidal pools (low sun exposure due to the immersion). Algae growing at high nitrate availability presented higher photosynthetic production during daily cycles than that under low nitrate level. After 7 d, photoinhibition at noon-time was produced but again it was higher in low nitrate compared to high nitrate grown algae. Algae incubated to increased irradiance in the short term (2 h) presented rapid decrease of photosynthetic yield and after transferring to shade condition, the recovery was also very fast. High dynamic photoinhibition, high non-photochemical quenching and high antioxidant capacity related to the accumulation of phenolic compounds explain the higher capacity of acclimation to high irradiances in Cystoseira tamariscifolia. Additionally, high nitrogen supply favoured the photoprotection related to the high photosynthetic production and high accumulation of phenolic compounds.

1D.8
APPLICABILITY OF ABC CURVES USING DIATOM ASSEMBLAGES ALONG ENVIRONMENTAL GRADIENTS

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Abundance/Biomass Comparison or ABC curves were initially introduced by Warwick (1986) for marine macrobenthic communities (polychaetes), and have recently received considerable attention for their ability to detect disturbed environmental conditions. The approach is based on the observation that undisturbed communities are characterized by large, slowly growing species with a long life span, thus when plotting k-dominance curves for both abundance and biomass the biomass curve is expected to lie above the abundance curve. As disturbance increases so does the percentage of smaller, opportunistic species, thus the two curves become similar, possibly overlapping each other more than once. Under intense disturbance a few small bodied species with short lives dominate numerically and their biomass curve lies below that of the abundance. Although Warwick & Clarke (1994) advise caution on the use of this approach on communities other than polychaetes, some attempts have been performed for fish, bird, and plankton assemblages with promising results. The present application of ABC curves on periphytic diatom assemblages along environmental gradients in a Mediterranean lagoon (Messolongi) aims to investigate the applicability of this approach to algal assemblages known for their sensitivity as environmental indicators. The results of the present study indicate that this method is efficient in detecting environmental stress due to nutrient inputs and salinity changes using diatom assemblages. Under conditions of strong variability in the lagoon environmental conditions, the source of disturbance could not always be inferred with safety. However, useful insights can be obtained when using this approach in conjunction with other community analysis methods such as multi dimensional scaling ordination. The response of coastal periphytic diatom
assemblages to disturbance, in terms of abundance and biomass, is discussed.

1D.9

MIGRATION VS PHOTOACCLIMATION IN MICROPHYTOBENTHOS

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In tidal estuaries microphytobenthic communities alone, are responsible for more than fifty percent of the carbon fixation in this environments, this high primary production is achieved despite living in an extreme and ever changing environment (the intertidal mudflats). The existence of high efficient photoprotective mechanisms, both physiological and behavioural, help these organisms in tackling environmental stress.

In this work we tested the hypothesis that the migratory behaviour be conditioned by long term photoacclimation in cultures of microphytobenthos (Navicula sp.). To this intent we built a custom migratory chamber where diatoms could move according a light gradient produced by a linear filter and a light source. The results show a photoaccumulation pattern evidencing a clear avoidance of extreme low or high light levels, and with maximum photoaccumulation values being found around ~50-70 µmol m⁻² s⁻¹ for the low light photoacclimated cells and ~100-150 µmol m⁻² s⁻¹ for the high light photoacclimated cells. These values are very close to the values of Ek from the light curves, which indicate that migration helps algae choose the optimal light levels, according to their photophysiology.

1D.10

EFFECT OF TSUNAMI AND COASTAL UPLIFT OF FEBRUARY 2010 ON CULTIVATED AND NATURAL POPULATIONS OF GRACILARIA CHILENsis

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The earthquake of February 2010 in Chile has had a huge impact on benthic intertidal populations of the region of Concepcion, including algae. Two different types of populations of Gracilaria chilensis coexist in Chile, natural populations attached to rocky substratum and populations associated to soft bottoms that generally correspond to farms. Two previous samplings from 2004 and 2009, from the impacted region of Concepcion and the non-impacted region of Puerto Montt, were completed by a sampling in 2010 three months after the event (at least two floating and two natural populations before and after the quake in each region). The frequency of haploid versus diploid individuals was checked in each population and in addition five microsatellite markers were analyzed. In Mars 2010, two natural populations from Concepcion had disappeared and only a few individuals from the estuary of Tubul, the largest cultivated population in Chile, had survived and had been already used to create new vegetatively propagated stands. Our results first show that both phases are always present in natural populations but not in farms. While the Puerto Montt farms show a consistent tendency to be dominated by diploid individuals (almost 100%), in Concepcion we observed farms dominated by diploids or by haploids. Possibly because very few time have passed since the probable bottleneck occurs, no changes in the genetic diversity of the surviving populations from Concepcion where detected.

1D.11

TWENTY YEARS COMPARATIVE STUDY OF THE MARINE MACROALGAL IN THE LITTORAL OF TIMANFAYA NATIONAL PARK

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Coastal regions are currently subject to environmental pressures of natural and anthropogenic origins. These stresses, especially those from human sources, generate changes in the structure and composition of the marine benthic assemblages, producing even losses in the biodiversity. In this sense, the coast of Timanfaya National Park,
Lanzarote (Canary Islands) constitutes a suitable place for the conservation of benthic communities, as well as to develop researches on macroalgal assemblages because human perturbations not only are restricted by legal framework but also by natural geologic and oceanographic constrains. We have studied the marine macroalgal assemblages along the intertidal zone of the National Park during the spring of 2010 reproducing the sampling protocol done by a similar study done in the area twenty years ago. A total of 102 taxa of macroalgae were identified in the 2010 survey, which represent approximately a 15 % of the marine flora of the Canary Islands, distributed as follows: 53 % red algae, 27.5 % brown algae, 15.5 % green algae and 4 % blue-green algae. Although the number of species recorded is widely similar to the previous study, the floristic composition varied moderately between this study and the previous one. Variation was notable mainly within the red algae (similarity of only 43 %) whereas it was lower for the blue-green algae (75 % of similarity), brown algae (57 %) and green algae (62 %). To interpret these floristic changes it is necessary to consider diverse environmental factors in the selected area such as the role of temperature increase during this time period. Although the coast of the Timanfaya National Park seems to retain its marine macroalgal communities in good condition, a long-term monitoring programme is necessary to determine the effects of global change in its marine ecosystem.

1D.12
BIOMECHANICAL PROPERTIES OF MACROALGAE IN THE VICINITY OF A PROPOSED HARBOUR: IMPLICATIONS FOR COASTAL MANAGEMENT

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Biomechanics is the discipline that studies the mechanical properties of living tissues. It is considered a useful tool in Marine Ecology, as mechanical properties appears to be important traits in the occurrence and the distribution of benthic organisms. Biomechanical properties of macroalgae are dynamic and plastic within a species and subjected to changes in environmental conditions, especially the hydrodynamic forces impinging the shore. An expansion of the Tarifa harbour is projected in the Gibraltar Strait (Southern Spain), which may affect the hydrodynamical conditions in the proximity of the project. The surrounding area is protected under the figure of Natural Park of Gibraltar Strait. That includes extensive rocky platforms (intertidal and subtidal) as well as subtidal communities of Laminariales and Tilopteridales. Thus, pre-operational values of biomechanical properties of macroalgae have been measured as reference values for the future project management. Sixteen macroalgae species (both intertidal and subtidal) were selected and subjected to two different tests (bending and tearing) in a universal testing machine. The following mechanical properties were estimated: maximum force before breaking, breaking stress, breaking strain, work of fracture, and modulus of elasticity (in bending and tension). Overall, subtidal species showed elastic, tough and stretchy thalli, whereas intertidal ones evidenced different strategies to deal with forces associated to waves. These tests will enable to relate the hydrodynamic environmental conditions to the species-specific mechanical traits. These results will led to the inclusion of specific mitigation measures in the project and to examine further changes in biomechanical properties of macroalgae in the monitoring programme if some environmental impacts of the project under operation must be addressed.

1D.13
TEMPERATURE AS A REGULATION FACTOR OF EXTERNAL CARBONIC ANHYDRASE IN INTERTIDAL MACROALGAE

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Most macroalgal species present adaptative mechanisms that let them live in limited conditions of CO₂ named Carbon Concentrating Mechanisms (CCM). One of its principal components is the external carbonic anhydrase (eCA), a metaloenzyme that catalyze the interconversion between CO₂ and HCO₃⁻ in the periplasmic space and whose accumulation is induced at low CO₂ concentrations. In the present study we propose the
existence of a possible regulation of eCA by temperature. As temperature decreases CO₂ concentration in seawater becomes higher due to an increase in solubility, thus it would be expected to find lower activity of eCA; however, comparing different studies of eCA activity in Mediterranean, Atlantic and polar macroalgae we observed an average increase of enzymatic activity with a decrease in seawater temperature. Therefore eCA would participate in the process of adaptation to low temperatures. We estimated activity and quantity of the enzyme eCA in seven species of Mediterranean Atlantic intertidal macroalgae (two Rhodophyta, three Chlorophyta and two Heterokontophyta) at three different temperatures (25°C, 15°C, 9°C). Growth rate and optimal quantum yield (F₀/Fₘ) were measured as control variables of cellular viability in these conditions. eCA activity was significantly higher at 9°C than at 25°C in four of the seven species analyzed, while immunodetections showed an increase in protein accumulation at 9°C in four out of five species where signal was detected. Growth rate was positive in all cases and F₀/Fₘ were not affected by temperature in three of these species, while the rest were slightly affected at 9°C. These results suggest that, at least in some macroalgae, eCA would be involved in the process of acclimation to low temperatures, increasing CO₂ fixation and hence decreasing accumulation of electrons in the photosynthetic electron transport chain.

1D.14
VARIABILITY OF CYMODOCEA NODOSA AND ZOSTERA NOLTII: MORPHOLOGICAL, STRUCTURAL AND DYNAMIC PARAMETERS

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The species Cymodocea nodosa and Zostera noltii often coexist in the N. Aegean, Greece, coastal waters forming extensive meadows of high productivity. The morphological (leaf length and width), structural (shoot density, leaf and rhizome-root biomass) and dynamic (leaf area index, % of broken-grazed leaves, leaf length frequency) parameters were studied in a shallow (2.5–3.5 m) meadow of Epanomi, outer Thermaikos Gulf. The aim was (1) to establish a basic line knowledge for the spatial growth pattern of the species, (2) to estimate the meadow degradation due to industrial, agricultural and urban sewages using CymoSkew index. Twenty quadrates (25 x 25 cm) were destructively sampled in total by scuba diving means in summer 2010. All data have been analyzed using non-parametric and parametric nested ANOVA in two spatial scales (100’s of meters-site, 10’s of meters-area). Both species of the meadow showed statistically significant differences (P < 0.05) or not in their morphological, structural and dynamic features at different spatial scales indicating that their growth pattern is patchy. CymoSkew index (2.96), a quantitative expression of Cymodocea nodosa leaf length asymmetry, has indicated the meadow as moderately degraded. This result is in accordance with the perturbation pattern of the outer part of Thermaikos Gulf, one of the most polluted Greek coasts.

1D.15
COPING WITH UV STRESS: THE ROLE OF CELL DEATH PROTEASES IN THE MICROALGAE DUNALIELLA TERTIOLECTA

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The effect of different UV stress treatments on the accumulation and activity of proteins traditionally regarded as “cell death proteases” such as caspase-like proteins (CLs) and metacaspases (MCs), as well as photosynthetic activity and number of dead cells, were evaluated. For this purpose, Dunaliella tertiolecta cultures were grown at 120 μmol m⁻² s⁻¹ in continuous PAR until they reached mid log phase. They were transferred to four different irradiance treatments: PAR (P), PAR + UVA (PA), PAR + UVA + UVB (PAB) and UVA + UVB (AB), where P = 120 μmol photons m⁻² s⁻¹, UVA = 17 W m⁻² and UVB = 1 W m⁻² (unweighted). We observed a decrease in the optimal quantum yield (F₀/Fₘ) under UV, followed by a recovery after 48 h in PAB and AB treatments, but not in PA. The highest values for yield recovery where obtained without PAR.
However, evidence for cell death occurring in the cultures was not found as indicated by SYTOX-green stain. The accumulation pattern for CLs 1, 3, 8 and 9, suggest that these proteases are present in the cells in a constitutive manner and UV did not have any effect in their accumulation. CL enzymatic activities were measured by using specific fluorescent substrates and the irreversible inhibitor Boc-D-AMC. The increase in CL-9 activities at 144 h was detected (except in PAB), but UV did not cause any increase in CL-8, suggesting the activation of the proteolytic cascade due to nutrient limitation. We also analysed type II MCs accumulation by western blots. PAB treatments demonstrated a correlation between UV stress and accumulation of these proteins. The maximal accumulation took place at 24 h in all cases, corresponding to the maximal effect of UV in Fv/Fm. Our results suggest the involvement of MCs in UV induced stress, acting as stress proteins, and related to cellular survival rather than to cell death.

1D.16

MPA MANAGEMENT IN THE AZORES: INTEGRATING BIOLOGICAL, DEPTH AND GEOLOGICAL DATA

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In the Atlantic the use of macroalgae for the assessment of ecological quality of coastal waters has focused on multimetric approaches based on littoral seaweed community features, namely the species richness (FSL/RSL) tool developed in the British Isles and the CFR tool in north Spain. However, intertidal rocky shore seaweed communities in the Macaronesian archipelagos are dominated by turfs and lack most of the large and abundant fucoids that are common in more northern countries. Therefore, neither of the two methods applies perfectly to Macaronesian shores. In an attempt to intercalibrate the results obtained by implementing the FSL/RSL and the CFR tools with Azorean seaweed communities, an alternative model is proposed building on their common features and adapting them to this region’s specificities. The proposed adaptations envisage the need to implement such tools across Macaronesia and compare to other North Atlantic shores as set out by EU guidelines (Water Framework Directive and Marine Strategy Framework Directive). However, the calibration of this method is quite difficult in the Azores where pollution is almost absent and its possible impacts diluted by the effect of strong wave action. Such a scenario forces one to work only with the top end of the quality scale, without management plans. The present work presents results on the use biotope methodology for the characterization of islets inside MPAs and the importance of this tool in ecosystem based management.

1D.17

AN INDEX BASED ON SEAWEED COMMUNITIES TO MONITOR COASTAL ECOLOGICAL QUALITY IN MACARONESIA

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the bottom end to calibrate its boundaries and validate its applicability, and it therefore needs to be tested in other geographical areas and under several pollution conditions.

1D.18

REGULATION OF D1 ACCUMULATION AND PSBA EXPRESSION IN DUNALIELLA TERTIOLECTA UNDER UV EXPOSURE

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The effect of different UV treatments on D1 protein accumulation and its gene (PsbA) expression, concentration of photosynthetic pigments (including xanthophylls cycle) and photosynthetic activity (estimated as changes in the optimal quantum yield of fluorescence (Fv/Fm) and electron transport rate (ETR)), were studied under chronic UV exposure during 7 days. Cells were exposed to four UV different treatments when cultures reached mid log phase: PAR (P), PAR + UVA (PA), PAR + UVA + UVB (PAB) and UVA + UVB (AB), P = 120 μmol photons m⁻² s⁻¹, UVA = 17 W m⁻² and UVB = 1 W m⁻² (unweighted). A general decrease in Fv/Fm was observed under UV but after 48 h small recovery was observed in PAB, but not in PA. However, the highest values for Fv/Fm recovery were obtained without PAR. Similar results were obtained with ETR and data paralleled the progressive decrease in O₂ production due to photoinhibition. In addition, ROS production was enhanced by UVB, specially at days 1 and 3, showing a negative correlation with PsbA gene expression and D1 accumulation (P < 0.05). D1 initially decreased during the first day but after 3 days in PAB, cultures recovered and reached initial values. Regarding pigmentary behaviour, chlorophyll a and b were only different under AB, presenting a pattern typically found in dark adaptation, and the content of neoxanthin and lutein followed the same trend as chlorophylls. The effect of UV on the xanthophylls cycle was also studied, remarking that the de-epoxidation state dropped down with maximal differences after 48 h in PAB, and 24 h in PA and AB. Our results demonstrate that D1 repair is enhanced by UVB in combination with PAR, and that such D1 induced-turnover is regulated by the rate of ROS accumulation and the efficiency of xanthophylls cycle, since ROS inhibit PsbA mRNA translation in this.

1D.19

INFLUENCE OF TEMPERATURE ON ALLELOPATHIC INTERACTION BETWEEN OSTREOPSIS CF. OVATA AND COOLIA MONOTIS

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Proliferation of the benthic dinoflagellates, Ostreopsis spp. are reported in many tropical and temperate regions worldwide. In the last decade, blooms of O. cf. ovata have become increasingly frequent in the Mediterranean Sea and resulted in relevant benthic biocenosis sufferings and human health problems. Besides putative palytoxin, new compounds, namely ovatoxins, have been recently isolated from different strains of O. cf. ovata through an in-depth high resolution (HR) LC-MS investigation. Along the Mediterranean coasts, Ostreopsis spp. are generally present in association with other benthic species, namely Coolia monotis and Prorocentrum spp. C. monotis is a species quite common in temperate and tropical waters, where it has been frequently detected at low concentrations during warmer periods. It is reported to produce a toxin, named cooliatoxin that is believed to be structurally related to yessotoxin based either upon the molecular weight and symptoms induced in mice; however, its structure is still uncharacterized. Moreover, the Mediterranean strains have never reported toxic effect. It is therefore interesting to investigate the interaction between the two species at different environmental conditions, especially temperature. Adriatic strains of the two
C. raciborskii are a freshwater cyanobacterium that produces a potent toxin (cyln-
dospermopsin, CYN) and is responsible for the
formation of severe blooms in many tropical and
subtropical Australian water reservoirs. The prolif-
eration of C. raciborskii in these environments and
the related water toxicity has been observed to be
strongly influenced by physical, chemical and bio-
logical parameters. Nevertheless, how these para-
eters affect the growth of C. raciborskii and
regulate CYN production and ecological selection
of strains with different toxic characteristics has
been scarcely studied in the laboratory. In an
attempt to fill this gap in our knowledge, I studied
the effects of irradiance on five Queensland
strains of O. cf. ovata cells grown in presence or absence of C. monitis were detected; the hypothesis of an
involvement of these compounds in competition
mechanisms is discussed.

1D.20
EFFECTS OF PHOTON FLUX ON
THE TOXIC CYANOBACTERIUM
CYLINDROSPERMOPSIS RACIBORSKII

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Cylindrospermopsis raciborskii is a freshwater cyanobacterium that produces a potent toxin (cyl-
dospermopsin, CYN) and is responsible for the
formation of severe blooms in many tropical and
subtropical Australian water reservoirs. The prolif-
eration of C. raciborskii in these environments and
the related water toxicity has been observed to be
strongly influenced by physical, chemical and bio-
logical parameters. Nevertheless, how these para-
eters affect the growth of C. raciborskii and
regulate CYN production and ecological selection
of strains with different toxic characteristics has
been scarcely studied in the laboratory. In an
attempt to fill this gap in our knowledge, I studied
the effects of irradiance on five Queensland
strains of C. raciborskii (AWT 205, NPD, CS
506, CS 509 and CS 510) and characterised by
different toxicities. Initial experiments on cells
exposed at 10, 40 and 90 μmol photons m\(^{-2}\)s\(^{-1}\)
showed that while all the strains can tolerate light
as low as 10 μmol photons m\(^{-2}\)s\(^{-1}\), only the toxic
strain AWT 205 can grow at the higher light
regimes where it exhibits enhanced growth.
Since CYN production is strongly related to cell
growth, light levels may alter the toxicity of
AWT 205. The faster growing cells also showed a
reduction of the overall cellular pigment content as
might be expected by growth at high photon flux.
This plasticity of the light harvesting apparatus in
strain AWT 205 suggests that a change of the
photosynthetic apparatus performance is
occuring under different photon flux. These
results suggest that strain AWT 205 better
manages alterations of light availability compared
with the other strains, making it a better
competitor for light. Therefore, we can speculate
that the photon flux characterizing a natural
ecosystem could play a key role in the regulation
of the ecological selection of different C. raciborskii
strains.

1D.21
INTERTIDAL MACROALGAE
ASSEMBLAGES IN RELATION TO
ENVIRONMENTAL FACTORS

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Effects of water motion, light, turbidity, salinity
and solids suspension on the composition of
rocky intertidal communities of the Abra de Bilbao
and adjacent coast (N Spain) were surveyed
five times over the period 2000–2008 in nine loca-
tions. Species cover was visually estimated on five
replicated quadrats at three different tidal heights
(0.5, 1 and 3 m above the lowest astronomical tide).
Abiotic measures were also recorded at each loca-
tion. The three assemblages identified on the basis of
the PCO ordination analysis were discriminated
according to the SIMPER routine by the following
species: (i) Bachelotia antillarum, Ulva prolifera and
Fucus vesiculosus in very sheltered sites with high
surface turbidity levels; (ii) Caulacanthus ustulatus,
Gelidium pusillum and Ralfsia verrucosa in shel-
tered and exposed sites with low surface turbidity
levels; (iii) Corallina elongata, Codium decorticatum
and Caulacanthus ustulatus in semi-exposed to very
exposed sites with moderate to low surface turbid-
ity. Distance-based linear models (DISTLM rou-
tine) were used for examine the relationships
between biological and environmental variables in
order to build a parsimonious model. Water
motion, turbidity, light and salinity showed signif-
icant relationships with species-derived multivari-
ate data cloud (based on Bray-Curtis), explaining
the first variable individually 43.7% (P < 0.001) of
the variation in community structure. The first two
variables together explained 48.8% (P < 0.001)
and subsequently variables add very little to this
and were not statistically significant in the condi-
tional test (P > 0.09 in all cases).
1D.22

EFFECT OF LIGHT ON VERTICAL MIGRATION AND PRODUCTIVITY OF COLD-WATER SUBLITTORAL MICROPHYTOBENTHOS

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Microphytobenthos (MPB) are principal primary producers in shallow marine habitats, and their activity significantly affects exchange of solutes between sediment and water column. While MPB have been studied extensively in tropical and temperate regions and in intertidal zones, studies on sublittoral MPB from cold waters, especially from high latitude regions like the Arctic, are very scarce. We conducted simultaneous measurements of back-reflectance and oxygen to monitor vertical migration and oxygen microenvironments in MPB from sublittoral sediments in Helgoland (North Sea, Germany) and Kongsfjorden (Svalbard, Norway). The measurements were done during variable illumination that mimicked diurnal light variations in situ. Generally, MPB from both sites migrated up during illumination and down during the dark. The migration upwards during increasing illumination was faster than migration downwards during decreasing illumination, and the start of migration was delayed relative to the onset of changes in illumination. These patterns were represented as a “migration loop” when plotting the measure of vertical MPB distribution against light intensity. The hysteresis of this loop depended on the rate of change in illumination as well as on the relative durations of the light and dark periods, and reflected the link between migration velocity and light stimulus. Qualitatively similar loops were also observed when oxygen inventory, calculated by depth-integrating the measured oxygen profile, was plotted as a function of illumination. The present study contains observations on three brown algae in nature from Turkey: Myrionema-like microthalli of Cladosiphon, Microspongium gelatinosum-like microthalli of Scytosiphon lomentaria and Hecatonema terminale-like microthalli of Punctaria. The taxa were collected from Ayvalik (Aegean Sea) and Dardanelles (Sea of Marmara).

1D.23

OBSERVATIONS ON THE SOME MARINE BROWN ALGAE IN NATURE FROM TURKEY

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The present study contains observations on three brown algae in nature from Turkey: Myrionema-like microthalli of Cladosiphon, Microspongium gelatinosum-like microthalli of Scytosiphon lomentaria and Hecatonema terminale-like microthalli of Punctaria. The taxa were collected from Ayvalik (Aegean Sea) and Dardanelles (Sea of Marmara).

1D.24

EFFECTS OF SIMULATED ENHANCED OR REDUCED MIXING ON PHYTOPLANKTON FROM THE NORTHEAST ATLANTIC

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In order to manipulate the oceanic phytoplankton growing conditions by simulating enhanced or reduced mixing in the surface layers, so-called translocation experiments were performed during the Stratiphyt cruise (July–August 2009), from Las Palmas (Spain) to Reykjavik (Iceland). In total 5 experiments were performed, during which nutri-
ent-rich water from 500 m depth and nutrient-poor water from 5 m below the Deep Chlorophyll Maximum were mixed. Each bottle also received unfiltered surface water as the inoculum of the natural phytoplankton community. The incubation bottles were placed on deck stabilized at surface water temperatures, half of them in high light (15% of surface PAR), and half of them in low light (8% of surface PAR). Phytoplankton responses were followed by flow cytometry (cell numbers, growth) and chlorophyll fluorescence (F₀, biomass), whereas photopigment and DNA samples were collected to assess changes in species composition. A clear distinction was seen.

1D.25

EFFECTS OF CADMIUM STRESS ON FRUSTULE DEFORMATION AND DNA DAMAGE IN NITZSCHIA PALEA (KÜTZING) W. SMITH 1856

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Nitzschia palea (Kützing) W. Smith 1856 is a unicellular diatom which has a wide distribution all over the world, especially in fresh waters and soil. In our study freshwater Nitzschia palea community isolated from soil and growth in Diatom Medium (DM) in laboratory conditions with 24 hours illumination. Cells treated with cadmium acetate which is a highly toxic element and generally carried by industrial wastes to freshwater environment. Cells exposed to 3 concentrations of cadmium acetate (0.1 mM, 1 mM, 5 mM and a control group) for 7, 24 and 48 hours. After every treatment cells were prepared for scanning electron microscopy (SEM) and RAPD-PCR analysis (Random Amplified Polymorphic DNA-Polymerase Chain Reaction). The effect of cadmium on frustule deformation was determined by SEM photography and the genetic deformation by RAPD-PCR. Dose dependent frustule deformation was observed especially with 1 mM and 5 mM concentrations. Duration also effected the cell wall deformation. The RAPD technique was used to detect DNA damage. The main changes observed in the RAPD profiles resulted in both appearance and disappearance of different bands and variation of their intensity. In our opinion, cadmium affected the cell wall structure and it may be lead inhibition of silica biosynthesis on genetic levels.
2D.1
GUIDELINES FOR RISK MANAGEMENT OF CYANOBACTERIA IN ITALIAN WATERS DESTINED TO HUMAN CONSUMPTION

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There is growing evidence that the spatial and temporal incidence of harmful algal blooms is increasing in freshwater basins, posing potential risks to human health, ecosystem sustainability and water supply. The management of cyanobacteria and cyanotoxins in source and by treatment water is one of the priority issues in the research activity of many countries. In the framework of a national program supported by the Italian Ministry of Health, a research group has been constituted to develop safety plans and alert levels framework for the control of drinking water in Italy. Consistently with WHO strategies and based on existing knowledge on toxic cyanobacteria in our country, the Italian guidelines were developed to provide updated information on monitoring methodologies, early warning and risk management of cyanotoxins in water for human consumption. This also included sampling requirements, recommended actions in response to threshold breaches and treatment options to provide a graduated response to the onset and progress of a cyanobacterial bloom in source water. Particular attention was paid to data on the spreading of *Planktothrix rubescens* in 36 Italian lakes and reservoirs, which winter bloom values in some cases exceeded $50 \times 10^9$ cell L$^{-1}$ with an associated microcystin-LR (MC-LR) equivalent concentration higher than $34 \times 10^3$ µg L$^{-1}$. The alert levels were defined by the value of a parameter based on the cell number of the blooming cyanobacterial species and the concentration of its toxin in Italian basins. Each value represents a level of risk to drinking water and results in an associated level of response from increased monitoring to notification to health authorities. In case of microcystin contamination, restriction in the use of drinking water starts when concentration exceeds 1.0 µg L$^{-1}$ of MC-LR equivalents at the consumer tap and it is associated with emergency plans run by local authorities.

2D.2
THE TOXIGENIC PLANKTONIC GONYAULACALEANS IN GREEK COASTAL WATERS: DIVERSITY AND DISTRIBUTION

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In the framework of the Greek HAB monitoring programme, more than 3,500 integrated water samples were examined under UV epifluorescence microscopy, after staining with fluorescent brightener, for the detection of potentially toxic thecate planktonic dinoflagellates. The samples were collected from sites located at the major bivalve molluscs’ production and/or harvesting areas along Greek coasts (Alexandroupoli & Kavala coasts, Thermaikos, Maliakos, Amvrakikos, Evoiko and Saronikos gulf) during 2009 and 2010. At least twelve *Alexandrium* species were identified based on key morphological characters, such as the plates 1’, 6”, sp, sa and Po. The
vegetative cells of no less than five Alexandrium species as well as Proctoceratium reticulatum, Lingulodinium polyedrum and Gonyaulax spinifera are reported for the first time in Greek waters. The highest diversity of the genus Alexandrium was recorded in Maliakos gulf (11 taxa); two of these Alexandrium taxa were found exclusively in this area. Saronikos and Thermaikos gulfs followed with 9 and 8 Alexandrium species, respectively. The most common taxa were A. andersoni in Amvrakikos gulf (15,000 cells L

orea) and A. minutum in Alexandroupoli coasts (40,000 cells L

orea). A. ostenfeldii/peruvianum species, only one taxon, forming chains, was detected only in this locality. The most abundant taxa were A. taylori in Alexandroupoli coasts (40,000 cells L

orea) and A. andersoni in Amvrakikos gulf (15,000 cells L

orea). Although Gonyaulaccean populations rarely displayed elevated abundance levels, distinct distribution patterns were recognized. The majority of Alexandrium species was recorded the period from late winter to late spring. Nevertheless, A. ostenfeldii/peruvianum occurred mainly during winter, while A. pseudogoniaulax, A. tamarense and A. minutum displayed maximum abundances during summer and autumn. Among the identified Alexandrium species, only A. ostenfeldii/peruvianum and A. leei were associated with the detection of toxicity in bivalve molluscs (spirolides and/or PSP toxins).

2D.3
EVALUATION OF THE ANTIPROTOZOAL ACTIVITY OF ASPARAGOPSIS EXTRACTS AGAINST LEISHMANIA INFANTUM

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Several species of marine macroalgae produce bioactive compounds. Among these, halogenated molecules produced by some red algae are largely known for their antibacterial, antiviral, anti-inflammatory and cytotoxic activities. Therefore, applications of macroalgal products are more and more frequent both in human and veterinary medicine. Particularly halogenated compounds from Asparagopsis taxiformis (Bonemaisonales, Rhodophyta) are active against several pathogens, such as Leishmania spp. (Kinetoplastida, Euglenozoa). Leishmaniasis is a disease with a worldwide distribution affecting both humans and animals. There is increasing awareness that drug treatment can be complicated by variation in the sensitivity of Leishmania species to drugs, variation in pharmacokinetics, and variation in drug-host immune response interaction. Crude extracts of A. taxiformis from the Strait of Messina were tested against L. infantum (MHOM/IT/80/IPT1), the agent of Leishmaniasis in Europe. Preliminary results showed that extracts had remarkable antiprotrozoal activity, revealing such algae as a great source of natural antiprotrozoal products.

2D.4
PARTIAL PURIFICATION AND CHARACTERIZATION OF A CALCIUM-DEPENDENT ALKALINE PHOSPHATASE FROM THE CYANOBACTERIA ARTHROSPIRA PLATENSIS (SPIRULINA)

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In the present work, Triton X-114 (TX-114) is used to extract and partially purify alkaline phosphatase (ALP) from a membranous fraction of Arthrospira platensis containing cell wall, plasma membrane, thylakoids and sheath. TX-114 has a double effect: solubilizing cell components to liberate the enzyme and, after phase partitioning, removing chlorophylls and other pigments present in the crude extract. The recovery of the enzyme in the aqueous phase suggests the overall hydrophilic character of these enzymes. ALP was kinetically characterized at pH 11.0 using p-nitrophenyl phosphate as substrate, giving a Km value of 1.7 mM. Orthovanadate was seen to be a competitive inhibitor of ALP, with a Ki of 0.8 mM. Moreover, in the presence of 70 μM EDTA, while the addition of Ca

++ reverted this inactivation; these results indicate that ALP from A. platensis is a calcium-dependent metalloenzyme. When the effect of Ca

++ was investigated in detail, a value of 0.067 μM−1 for the
affinity constant was obtained. The enzyme was histochemically localized in the cytoplasm, cell wall and sheath using the ELF method. It is assumed that the same enzyme is either soluble in the cytoplasm or in some way “trapped” in the cell wall or in the sheath. ALP localization within the sheath and the subsequent release of P may benefit the neighbouring cells surrounding this layer.

2D.5

BIOENERGETIC STRATEGY FOR THE BIODEGRADATION OF P-CRESOL BY THE GREEN ALGA SCENEDESMUS OBLIQUUS

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Phenols are considered environmental pollutants due to their potential toxicity. p-Cresol is widely used in disinfectants and fumigants, in the manufacture of synthetic resins, in photographic developers, and in explosives. It is highly toxic, corrosive, causes nervous system depression, and is a naturally occurring metabolic product that is formed from tyrosine by bacteria under anaerobic conditions. Different concentrations (0, 0.15, 0.3, 0.5, 0.75, 1, 1.5, 2 and 2.5 mM) were tested for their biodegradability by the microalga S. obliquus in closed systems of 100 mL total volume (50:50 culture:air), under continuous light (50-60 μmol m⁻² s⁻¹), without any exogenous carbon supply (only 0.034% CO₂ in the 50 mL of the available air). p-Cresol seemed to function as an alternative carbon source, since the biodegradation took place (0.15 mM was completely biodegraded within 5 days), by splitting the methyl group, converting it to methanol and in turn CO₂. Increasing p-cresol concentration resulted in an increase in biodegradation rate in the first day, while the total biodegradation percentage decreased. P-Cresol concentrations >1.5 mM decrease the photosynthetic reaction centre density, increase the functional antenna size and that leads to the reduced photosynthetic efficiency. Concentrations <1.5 mM functioned beneficially leading to a decrement of antenna size, an increment of reaction centre density and higher net photosynthesis. p-Cresol as alternative carbon source led to 20 times better growth in 0.3 and 0.5 mM compared to control, while in concentrations >0.75 mM there is no difference compared to control. In concentrations >0.75 mM the microalga offered more energy in biodegradation and less in growth, while in concentrations <0.75 mM growth and biodegradation could both be accomplished with the available energy.

2D.6

PROGRAM OF SELECTION OF NEW SPECIES AND STRAIN OF MICROALGAE WITH HIGH POTENTIAL FOR LIPID PRODUCTION

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Currently, more than 10% of the 30.000 known species of microalgae have not been yet physiologically and biochemically characterized and no more than 10 species are presently cultured at large scale. This means that microalgae are a group of organisms still to explore that every time they are having more importance in pharmaceutical industry, agroindustrial develops, and energy. To investigate the real potential of microalgae for lipid production, screening techniques must allow the analysis of a great number of samples in a short period of time. In this sense flow cytometry is a technique that gives information in a matter of minutes of a great amount of data at cellular level (size, complexity, viability, composition, etc), making possible the effective establishment of cellular indicators to developed a program of “massive and automated screening”. On the other hand, microcultures reproduce significantly (P=0.99) of approach the growth in the large scale cultures, without the limitations derived from handling great volumes. Another important parameter like the extractability of fats, allow the evaluation of more suitable strains for industrial lipid production. Our goal was selecting strains potential for biodiesel production. We developed a system based on microcultures to optimize time and resources. Using this approach a total of 33 strains of microalgae have been studied of which 4 of them were selected as high potential strains for the production of biodiesel.
**2D.7**

**CLADOPHORA RUPESTRIS (CLADOPHORALES, CHLOROPHYTA): A POTENTIAL SOURCE OF ANTIBACTERIAL COMPOUNDS**

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Recent studies have shown that seaweeds are a good source of structurally novel secondary metabolites with antibacterial, antialgal, antifungal and antitumoral properties. Therefore, natural products from marine algae appear as a promising alternative valuable source of new compounds for drug development. It is also noteworthy that seaweeds often produce salt-stable antimicrobial compounds useful in the control of either fish or shellfish pathogens in aquaculture where the high-salt conditions might reduce antibiotic efficacy. In this investigation the antimicrobial activity of Cladophora rupestris (Linnaeus) Kützing lipidic extract was assayed and its chemical characterization was carried out by gas chromatography and multinuclear and multidimensional NMR spectroscopy. The seaweed was collected in the Mar Piccolo of Taranto (northern Ionian Sea, Italy), washed, dried and powdered. The powdered material was subjected to Soxhlet extraction using chloroform/methanol (2:1, 55–60°C, 24 h). Five milligrams of extract were dissolved in ethanol and assayed for antimicrobial activity using the Kirby Bauer method (1966). Fatty acid composition was determined according to Budge and Parrish (2003). Analysis of fatty acid methyl esters was performed by gas-liquid chromatography. Cladophora rupestris lipidic extract showed a bacteriostatic activity against the two human pathogens Enterococcus sp. and Streptococcus agalactiae as well as all the tested Vibrio species (Vibrio ordali, Vibrio fluvialis, Vibrio salmonicida, Vibrio vulnificus, Vibrio cholerae non O-1, Vibrio metchnikovii). The 1H NMR spectrum in CDCl3 of algal lipid fraction showed the characteristic signals of saturated (SAFAs) and unsaturated fatty acids (UFAs) as well as other metabolites. The fatty acids profile of C. rupestris showed that palmitic acid methyl ester (16:0) was the predominant saturated fatty acids suggesting that it could be responsible of the observed antibacterial activity. These results are interesting considering the resistance against common antibiotics developed by bacteria and the need to control fish and shellfish diseases due to vibriosis.

**2D.8**

**LARGE SCALE CULTIVATION OF THE ALGA TRACHYDISCUS MINUTUS – A SOURCE OF HIGH AMOUNT OF EPA**

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The variation of fatty acid content and composition of the eustigmatophycean microalga Trachydiscus minutus in a large-scale thin-layer photobioreactor was analysed (12 m², 170 L). Two cultivation runs were accomplished two times during both spring and summer 2010 and each cultivation took approx. 20 days. After concentration of final biomass (dry weight 10.95 g l⁻¹) with food-grade natural polyelectrolyte flocculant, we gained the algal slurry of the concentration of 40.5 g l⁻¹ of dry weight. Total lipid content of the strain was 20.5 % (on dry weight basis). EPA was the major fatty acid and its concentration was 44.4 % of total fatty acids. Arachidonic acids, linoleic acid and palmitoleic acid accounted for over 22 % of total fatty acids. The proportion of all unsaturated fatty acids was almost 70%. These results show that T. minutus is nearly a top producer of EPA.

**2D.9**

**CLONING OF KENNEDY PATHWAY GENES FOR ENHANCED STORAGE LIPID PRODUCTION IN TRANSGENIC CHLORELLA**

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Posters
Expecting with the fact that the global petroleum reserves in the known oil field reservoir is dying out in next 50–60 years. Attention on searching for an alternative fuel is now an urgent matter to encounter. Biodiesel is deemed as one of the solutions to solve the problem which is capable making from a variety of feedstocks (i.e., vegetable oil and animal fat), however, the limited supply of these feedstocks impede the further expansion of biodiesel production. Microalgae have been recognized as potential good source for biodiesel production by either of their high oil content or the rapid biomass production, but not the both. Therefore, to assure the biodiesel production platform can be established base on the microalgae approach, the unicellular eukaryotic green algae *Chlorella*, known for its high growth rate but moderate lipid content, is chosen as the model to proof the concept that the lipid content can be boost up to an acceptable level while still maintaining its high growth rate can be achieved. To do this, we have developed a system for the expression of recombinant proteins in *Chlorella* for high lipid production, in turns to enhance the productivity. In this presentation, we will show the modulation of several enzymes, including glycerol-3-phosphate acyltransferase (GPAT), lysophosphatidic acid acyltransferase (LPAA), phosphatidic acid phosphatase (PAP) and diacylglycerol acyltransferase (DGAT), in Kennedy pathway can effectively increase the storage lipid (triacylglycerols; TAG) content up to two folds than that of the wild type in 7 days cultivation. In addition, the individual and the interactive effects of sodium acetate and sodium oleate on the lipid production of the new recombinant strain will also be discussed.

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**2D.10**

**INFLUENCE OF *ANABAENA* SP. PCC7120 ON THE GROWTH AND DEVELOPMENT OF ENERGETIC PLANTS**

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Cyanobacteria are one of the oldest and most abundant organisms on our planet. They have evolved sophisticated biosynthetic pathways to produce a rich and wide spectrum of bioactive natural products. Another important property of Cyanobacteria is their nitrogen fixation ability which can be used to enrich the soil in this significant compound. Application of cyanobacterial cells suspensions as a biofertilizer might increase the yield of economically important plants and decreases or eliminate the usage of fertilizers. The objective of this work was to study the influence of *Anabaena* sp. PCC7120 cells suspension on seeds germination and seedlings growth and development of three plant species used in renewable energetics: *Panicum virgatum*, *Sida hermaphrodita*, *Zea mays*. *Anabaena* sp. PCC7120 was cultivated in 250 ml Erlenmeyer’s flasks with BG11 medium in vegetation room at 27°C, with constant lighting. Cultures used in this experiment were in a log growth phase and had a known number of cells. For experimental purposes BG11 substrate was replaced with distilled water. In order to test germination ability and dynamics, 50 seeds were placed on each Petri dish (six repetitions) on two layers of tissue paper moistened with 2.7 ml of cells suspension. Germination took place in the dark. Cotyledons and radicals growth dynamics were tested in Phytotoxkit on substratum moistened with 10 ml of cells suspension. Distilled water was used for the control experiment. Chlorophyll content and phosphatases activity were measured. We observed that the treatments influenced the percentage of germinated seeds, growth and development of seedlings. Research where sponsored by Ministry of Science and Higher Education in Poland, Grant No. N N304 102940. This publication was prepared under the project “Bioenergy for the Region – Integrated programme for Ph.D. students development”, co-financed by the European Union under the European Social Fund.

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**2D.11**

**INVESTIGATION OF CYANOBACTERIA MOLECULAR IDENTIFIED AND TOXIN ISOLATED FORM IZMIR THERMALS**

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Toxic cyanobacteria (blue-green algae) are well reported in rivers, lakes and marine environments, but the toxin production of cyanobacteria in hot springs is largely unexplored. Therefore, the present study investigated the presence of toxic cyanobacteria and cyanotoxins in 5 different hot springs (Balcova, Zeytindali, Karakoc, Sifne and Gulbahce) in Izmir. 16S rDNA sequences from 10 filamentous cyanobacteria were obtained and phylogenetic tree from these sequences and sequences available in GenBank was constructed. Ten cyanobacterial cultures isolated from thermal places in Izmir, *Spirulina subalsa*, *Geitlerinema*, *Lyngbya*, *Oscillatoria*, *Pseudoscillatoria*, *Phormidium* sp. had toxic activity confirmed using microcystin enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) with specific primers targeted to the condensation domain of mcyA gene. The results of an ELISA revealed that cyanobacteria contained microcystin at detection limits of 0.16 ppb. The gene *mcyA* from the microcystin synthesis complex was detected in five samples. This is the first report of the presence of microcystin thermophilic cyanobacteria in Izmir.

**2D.12**

**BIOCONVERSION OF PROGESTERONE BY THE MICROALGA MICROCHAETE TENERA**

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Microorganisms including various species of microalgae are able to bioconvert chemical substance into useful metabolites. The first application of cyanobacteria in biotransformation of steroids goes back to 1986 when Abul-Hajj and Qian studied the ability to convert steroids using different algal strains. Since then, many studies on this feature of microalgae have been performed and a variety of products have been obtained. Currently, steroids and their derivatives have found extensive applications, such as anabolic, hormone replacement, progestational, antitumor agents, and oral contraceptives as well as sedatives. Research in the field of biotransformation is being continued for development of newer and more useful steroid analogues that are synthesized with great difficulty through chemical reactions. In the present study, a local isolated strain of *Microchaete tenera* Thuret ex. Bornet, belonging to the family of Microchaetaceae, was applied to convert progesterone to some steroid derivatives. The bioproducts were isolated, purified and then identified as 21α-hydroxyprgn-4-en-3-one, 21β-hydroxyprgn-4-en-3-one, 6α-hydroxyprgn-4-en-3,20-dione and 6β-hydroxyprgn-4-en-3,20-dione on the bases of spectral analyses. The study also showed that the best concentration of starting material, temperature, photoregime and the influence of CO2 partial pressure on the production of bioconverted metabolites were 0.25 g l−1, 25°C, continuous light and 2.0 ± 0.1 % v/v, respectively. Time course study of the biotransformation exhibited the production of all metabolites at highest concentrations in the 5th day.

**2D.13**

**EFFECT OF LIGHT AND NUTRIENT FLOW ON POLYSACCHARIDE AND LIPID CONTENT OF CULTURED HYDROPUNTIA AND ULVA**

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*Hydropuntia cornea* and *Ulva rigida* were intensively cultured in 90 L tanks under different light conditions: (1) outdoor under direct sunlight and (2) outdoor inside a greenhouse (80% PAR + 10% UVA + 0% UVB), at the CBM-ULPGC culture facilities (Gran Canaria, Canary Islands). Nutrients, mainly inorganic N-ammonium and P-phosphate, were provided by fish (*Sparus aurata*) wastewaters. Nutrients flow dynamic was established as follows: 4 weeks under continuous nutrients flow and then 2 weeks with running seawater. In a second experimental period, nutrients dynamic was inverted: 2 weeks with running seawater and then 4 weeks under continuous nutrients flow. Under these combined effects of light and nutrients flow dynamic, algal yields and growth rates, ammonium and phosphate biofiltration capacities, pigments content and maximal quantum yields (Fv/Fm) of chlorophyll fluorescence of PSII, as stress indicator, were determined. Biomass obtained from the different treatments was mainly evaluated as source of acid
polysaccharides and lipids, particularly triglycerides. Results revealed how combined effects of light qualities and nutrients flow dynamic have a significant effect on the growth characteristics (mostly affected by light) and biomass quality (mainly affected by nutrients dynamic) obtained from seaweed grown in fish biofiltration units.

2D.14

OPTIMIZATION OF HYDRODYNAMIC AND LIGHT CONDITIONS FOR CHLORELLA SP. IN A THIN LAYER PHOTOBioreACTOR

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Biomass yield expressed as gDW m⁻² day⁻¹ and photosynthetic activity as in vivo chlorophyll a fluorescence of photosystem II i.e. maximal quantum yield (Fv/Fm) and electron transport rate (ETR) as estimator of production were determined in Chlorella sp. growing outdoor in a thin layer photobioreactor (4 m²). Different residence times changing the algal culture fluxes and/or the height of the culture layer were applied in order to maximize the photosynthetic production and biomass yield. The photobioreactor is divided in four different compartments: (A) basin to homogenize the culture before circulating through (B) the planar exposure area, (C) culture tank and (D) dark area consisting of tubes and pump connecting C and A compartments. Relation between photosynthesis and biomass yield was analyzed under different light exposures taking into account the light attenuation coefficient (kₐ), incident irradiance and in vivo chlorophyll a specific absorption coefficient by phytoplankton (aphₗ). The combination of light attenuation by the culture (shading effect) with different residence times resulted in a maximization of algal production. This was due to the decrease of photo-inhibition during high irradiance periods. These results are discussed regarding their potential use for algal biomass production aiming at biodiesel or biogas production.

2D.15

ALGOBANK-CAEN, A FIFTY YEAR-OLD MICROALGAL CULTURE COLLECTION

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Algbank-Caen is the microalgal culture collection of the University of Caen Basse-Normandie (France). The collection consists of strains of marine, brackish water and freshwater microalgae that can be provided for research, education and industry. Algbank-Caen is a public non-profit culture collection dedicated to ensuring the long-term conservation, the quality and the distribution of these biological resources and to making available a maximum amount of information relating to the identity and origin of each strain. Initiated in early 1960s with a small number of local strains, the collection contained strains mainly from the English Channel and later from the Mediterranean, but strains from diverse locations around the globe have since been included (Norway, Tahiti, Morocco...). Through sampling missions, collaborations and taxonomic research, the collection has gathered a large number of strains, covering a large diversity (132 genera and 157 species), essentially Haptophyta (43.4%) but also Chlorophyta (14%), Bacillariophyta (11.9%) or Rhodophyta (9.4%). A first strain list was published over 25 years ago (Billard 1987). These last years, a set of new strains including notably several Botryococcus and Porphyridium strains has been added to the catalogue. The collection now also contains most of the strains routinely used in aquaculture (Isochrysis galbana, Nannochloropsis spp., Phaeodactylum tricornutum...) and a number of potentially toxic strains from diverse algal groups (Gambierdiscus toxicus, Alexandrium minutum...). Recently, the collection has been moved to a new laboratory devoted to Algbank-Caen and a new website (www.unicaen.fr/algobank) is now available for ordering (online catalogue and cart) and strains taxonomical details (online iconographic database).

2D.16

TOXICITY AND BIOTRANSFORMATION OF CHLOROPHENOLS BY THE MARINE MICROALGA TETRASELMIS MARINA: A QSAR STUDY

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The pollution of coastal sea waters with chlorinated phenols is of environmental concern because these compounds are toxic to organisms of different trophic levels in the food chain, are recalcitrant to treatment and may bioaccumulate in aquatic organisms. Chlorophenols exert a toxic effect on microorganisms by disrupting energy transduction, either by uncoupling oxidative and photosynthetic phosphorylation or by inhibiting electron transport. It has been previously shown that the marine microalga *Tetraselmis marina* is able to metabolize 4-chlorophenol and 2,4-dichlorophenol. In the present study the acute toxicity (expressed as effective concentration EC\textsubscript{50}) of the different mono and di-chlorophenols to *T. marina* was obtained experimentally, on a maximum biomass production basis. The results were subsequently analyzed using a quantitative structure-activity relationship (QSAR) method that was based on physicochemical properties of chlorophenols. The molecular descriptors employed in the analyses were: n-octanol/water partition coefficient (log P), molecular connectivity index (\(1\times\nu\)), molecular refractive index (Rm), steric parameter (\(\Sigma D\)), angular parameter (A), melting point (F) and electronic parameters of Hammet (\(\sigma_1, \sigma_2\)). The obtained EC\textsubscript{50} values ranged from 21 to 750 \(\mu\)M. The toxicity of chlorophenols increased with the number of substituted chlorine atoms, except for the case of double substitution on ortho-positions (2,6-dichlorophenol). QSARs based on the angular parameter (A) combined with any of the other descriptors were established with \(R^2\) ranged from 0.85 to 0.94. The angular parameter (A), which expresses the hindrance in the neighborhood of phenolic hydroxyl on positions 2 and 6, was found to provide satisfactory descriptions for the toxicity. Diversification of chlorophenols toxicity was controlled mainly by electronic or steric factors. The microalga ability to detoxify chlorophenol isomers was also investigated. The position of the chloro-substituent in the aromatic ring has an important effect on the microalga ability to conjugate glucose to chlorophenols. The microalga detoxified all mono- and di-chlorophenols with the exception of 2,6-dichlorophenol.

2D.17

**DISTRIBUTION AND POTENTIAL PRODUCERS OF CYLINDROSPERMOPSIS IN WESTERN POLAND**

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Cylindrospermopsis (CYN) is a known from severe toxicity that may affect the liver, kidneys, spleen, heart, thymus, or intestine of humans and animals, suspected also to be carcinogenic. In Poland for the first time this toxin was reported three years ago from two shallow hypereutrophic lakes, however the source of this toxin was not fully determined. We have studied distribution of CYN in 36 lakes situated in three western district of Poland. Integrated water samples for chemical and phytoplankton analysis were collected from surface layer of polimictic and epilimnion of stratified lakes at the beginning and the end of summer. Genetic analysis of the presence of *cyrJ* gene (578 bp) was performed to establish the occurrence of cylindrospermopsin-producing cyanobacteria. CYN concentration was detected using screening ELISA test followed by HPLC-DAD and LC-MS methods. Molecular study revealed the presence of *cyrJ* gene in DNA samples isolated from 18 lakes. ELISA tests revealed presence of CYN in 15 lakes. CYN concentrations were in the range of 0.12 – 3.36 \(\mu\)g L\(^{-1}\). The ELISA results were corroborated by LC-MS. In several samples the presence of CYN could be further verified by HPLC-DAD and LC-MS/MS spectra. Among potential cyanobacteria producers in these lakes *Cylindrospermopsis raciborskii* and *Aphanizomenon gracile* were the most common; however the share in phytoplankton biomass of the later one was much higher. Preliminary studies on isolated from these lakes cyanobacteria cultures showed presence of *cyrJ* gene in *A. gracile* culture indicating this cyanobacterium as a probably one of the major producers of CYN in Polish lakes.
The financial support for this study was provided by Polish Ministry of Science and Higher Education through research grant No. N N 304 020 437 and Tekes—the Finnish Funding Agency for Technology and Innovation grant No. 40193/09.

2D.18

TOXIN PRODUCTION BY PSEUDO-NITZSCHIA SPECIES ISOLATED FROM GREEK COASTAL WATERS

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A total of 82 Pseudo-nitzschia cultured strains, isolated during the last three years from six major shellfish production areas along Greek coasts, were tested for toxin (domoic acid-DA) production. These strains were assigned to ten Pseudo-nitzschia species and two unidentified morphotypes (a P. pseudodelicatissima-like and a P. delicatissima-like). The cultures were sampled once, during late-exponential to stationary phase, and analysed by HPLC coupled to an ultraviolet photodiode array detector (UV-DAD). ‘Whole-culture’ DA was detected in six P. pseudodelicatissima (out of the seven tested), in one P. pungens var. pungens (out of the nine examined) and in one P. galaxiae (out of the twelve analysed) cultures. The measured concentrations displayed considerable variation, ranging from detectable but unquantifiable amounts to 3.18 ng ml⁻¹. This study constitutes the first record of DA production by the nominal variety of P. pungens and the second by the species P. pseudodelicatissima and P. galaxiae.

2D.19

STUDY OF TWO UNCLASSIFIED PSEUDO-NITZSCHIA MORPHOTYPES FROM THERMAIKOS GULF, GREECE

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Two Pseudo-nitzschia delicatissima-like and two P. pseudodelicatissima-like strains were isolated from Thermaikos gulf in October 2007 and September 2008, respectively. Their morphological (assessed with transmission electron microscopy-TEM) and genetic (sequences of the internal transcribed spacer 2 region of rDNA-ITS2) variation were evaluated. ITS2 secondary structure comparisons were also conducted. The P. delicatissima-like strains [named (07)10A2 and (07)10A3] presented subtle morphological discrepancies from P. delicatissima sensu stricto and P. arenensis (poroids and girdle bands structure) while ITS2 secondary structure analysis (presence of a compensatory base change-CBC) supported this distinction. This morphotype most probably infers to cryptic (or most likely pseudo-cryptic) speciation within P. delicatissima species complex. The P. pseudodelicatissima-like strains [(08)9B2 and (08)9A7] were morphologically and genetically differentiated by other similar species (P. pseudodelicatissima, P. cuspidata, P. caciantha, P. calliantha and P. mannii). However, ITS2 secondary structure comparison with P. calliantha and P. mannii (the genetically closest to them species) did not sustain this distinction (absence of a CBC). The P. pseudodelicatissima-like morphotype along with the P. calliantha and P. mannii species probably form a complex of species that constitutes a case of potential incipient speciation.

2D.20

PHOTOSYNTHETIC AND RESPIRATORY RESPONSES OF GRACILARIA PARVISPORA FROM THE GULF OF CALIFORNIA

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Photosynthetic and respiratory responses (P-E curves) of *Gracilaria parvispora* (Ohmi) Papenfuss from the southeast Gulf of California were studied under various irradiance conditions at four temperature (20, 25, 30, 35°C) and four salinity (25, 30, 35, 40 psu) combinations. The alga showed acclimation in its photosynthetic and respiratory responses to tropical temperature as well as to oceanic salinity. A significant positive effect of temperature on light-saturated photosynthetic rate (P<sub>max</sub>) was observed for all salinities tested. Photosynthetic rates for treatments at 20 and 25°C were lower (≤9.2 mg O<sub>2</sub> g<sup>DW</sup> h<sup>-1</sup>) than for treatments at 30 and 35°C (>12 mg O<sub>2</sub> g<sup>DW</sup> h<sup>-1</sup>). *G. parvispora* from the Gulf of California showed limited tolerance to low salinities (25 psu) and low temperatures (20°C), and behaved rather as a tropical than a warm-temperate species. The interaction between temperature and salinity was found to be significant (ANOVA, *P* < 0.05). Responses to salinity indicated adaptation to oceanic salinity. Photosynthetic responses were lower at 25 psu than at higher salinities. The lowest P<sub>max</sub> values (6.2 to 8.2 mg O<sub>2</sub> g<sup>DW</sup> h<sup>-1</sup>) were observed at the lowest salinity (25 psu) regardless of temperature. Compensation and saturation irradiances (26-170 and 57-149 μmol photon m<sup>-2</sup> s<sup>-1</sup>, respectively) indicate adaptation to lower irradiances in shallow (1-2 m depth) habitats, where turbidity can be high, and the capacity of shade adaptation has been developed. Results suggest that mariculture efforts of *G. parvispora* would be limited by low temperatures in the Gulf of California from December to March, and indicate that this species will probably not be able to spread further in the Gulf of California due to low temperatures (<15°C) in the upper part of the Gulf in winter.

### 2D.21

**ENVIRONMENTAL FACTORS AND PHYCOCYANIN ASSOCIATED WITH MICROCYSTIN VARIATION IN LAKE PAMVOTIS**

*(GREECE)*

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Microcystins are the most commonly detected cyanotoxins in most freshwater systems and are produced by several genera of cyanobacteria, including *Microcystis, Anabaena, Nostoc, Planktothrix, Oscillatoria*, and *Nodularia*. Cyanobacterial mass accumulations attract the attention of water authorities and utilities, environmental and health agencies, and water-user groups, since they present water-treatment, supply, conservation, and health problems. In recent years, field surveys on microcystins have been extensively carried out in different parts of the world, while few studies addressed the significance of local environmental conditions on microcystin dynamics. Those environmental factors associated with microcystin variation could be an essential step to predict toxic events and protect public health. Also, recent studies suggest that measurement of phycocyanin concentration could be a valuable monitoring tool for analyzing the cyanobacterial concentration in freshwaters. The purpose of this paper is to describe seasonal variations of intra- and extracellular microcystins in the Mediterranean shallow Lake Pamvotis and the effects of environmental factors on microcystin dynamics. Lake monitoring was taken place during the year 2008, concerning total microcystin concentration, phycocyanin concentration, along with environmental parameters. *Microcystis* was the dominant cyanobacterial genus during the monitoring period, with the exception of June and July, when the dominant cyanobacterial genus was *Anabaena*. Total microcystins (intra- and extracellular) measured with ELISA, ranged from 0.98 μg/L (in winter) to 15.42 μg/L (in autumn). Intracellular microcystins composed 72.9% of the total microcystins. The total microcystin concentration was positively correlated with temperature, suggesting that elevated temperature favors higher microcystin concentrations. Total microcystin concentration was also positively correlated with Soluble Reactive Phosphorus, while a stronger correlation was revealed with phycocyanin, than with chl-a. Linear regression between microcystin concentrations and phycocyanin concentrations, suggests that evaluation of phycocyanin content would serve as a specific early warning signal for microcystin occurrence and also as an effective monitoring tool in Lake Pamvotis.
2D.22
FIRST FINDING OF A SPIRULINA SP. STRAIN (ETS-11) FROM THE EUGANEAN THERMAL DISTRICT

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A cyanobacterium strain, recognized at the light microscope as a Spirulina sp. was isolated from a population of cyanobacteria which colonized the muds of hydrothermal tanks of the Euganean District (Padova, Italy), at a temperature of 55°C. This cyanobacterium, cultured on BG11 medium, at 30°C under a light intensity of 10 μmol m⁻² s⁻¹ with 12 h photoperiod, has been called ETS-11 (where ETS is the acronym for Euganean Thermal Springs). The morphological and ultrastructural analyses evidenced that the cyanobacterium trichomes were blue-green, 0.7-0.8 μm wide, regularly spirally coiled with left-handed rotation. The coil thickness was 1.7 μm and the distance between coils was 250 nm. Trichomes produced expanded extracellular mucilaginous masses. The cells exhibited thylakoids peripherally arranged and parallel to the cell wall. The photosynthetic pigments analysis showed high level of phycocyanin (over 80% of total phycobiliproteins). Initial molecular analyses, based on the 16S rDNA gene, showed a 97% identity of our strain ETS-11 with Spirulina sp. CCC Snake P. Y-85 isolated from Yellowstone National Park (USA) hot springs. Moreover, preliminary analyses suggested that Spirulina sp. ETS-11 might have a diazotrophic activity.

2D.23
EVALUATION OF THREE ALGAE AS FEED ADDITIVES IN JUVENILE HYBRID TILAPIA (OREOCHROMIS SP.)

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The aim of this study was to evaluate the effect of replacement of fish meal with three algae (Gracilaria cornea, Ulva rigida and Scenedesmus almeriensis) meal on the growth and non-specific immune response in juvenile hybrid tilapia (Oreochromis sp. rocky mountain white). Chemical characterization of the algal biomass (crude protein, and lipid contents, fatty acid profile, carbohydrates, C:N ratio, ash, moisture) was carried out. The three experimental diets were formulated with this algal biomass in order to substitute 25% of fish meal (seaweed weight of total feed weight). A control diet was used, without inclusion of any seaweed. The standard length and wet body weight of the total population were recorded following four weeks after feeding and four parameters of the innate immune humoral response were evaluated: respiratory burst activity of macrophages, plasma lysozyme, anti-trypsin activity and alternative pathway of serum complement activity (ACH50). Growth of tilapia fed on control or diet supplemented with the three algae was similar. Anti-trypsin activity was similar in fishes feeding on control diet and algal supplemented diets. However, a significant increase in ACH50 was observed in fish fed on the diet supplemented with G. cornea and S. almeriensis. On the other hand, feeding tilapia with the three algae enhanced respiratory burst activity of phagocytic cells and plasma lysozyme. These results show i) that the three algae can be used to replace fish meal in diets of juvenile hybrid tilapia, and ii) that S. almeriensis derivatives can be used as an immunostimulant.

2D.24
ALGAL BIOMASS PRODUCTIVITY AND NUTRIENT REMOVAL EFFICIENCY OF INDOOR CULTURES IN WASTEWATER

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Domestic wastewaters are potential resources for production of microalgae and low-cost biofuels as by-products. This study investigated algal biomass productivity and nutrient removal by green algae
grown during treatment of municipal wastewater supplemented with CO₂. Preliminary studies have been conducted on the freshwater chlorophyte, Scenedesmus sp. in batch cultures. The aim of the research was evaluate municipal wastewaters as growth medium for algae cultivation and test the feasibility of an algal nutrient removal system. Laboratory experiments compared the algal biomass production in the primary and in the secondary wastewater effluent. The primary wastewater effluent reported a good growth yield of 2.1 g L⁻¹/C₀, in terms of Volatile Suspended Solids (VSS); nutrient removals was 77% for ammonium and 100% for phosphorus, in 3 days, and 99% for ammonium, in 7 days. Algal biomass has been characterized in terms of protein, total polysaccharides, total fatty acid content and elementary C:N ratio to evaluate which kind of biofuel is more convenient on an energy balance basis. Further studies on the marine eustigmatophyte Nannochloropsis gaditana in batch cultures are in progress; in addition the primary wastewater effluent will be used for semicontinuous indoor cultures with different hydraulic residence times (HRTs) to obtain the maximum biomass productivity and nutrient removal rate.

2D.25
OPTIMIZATION OF LIPID PRODUCTION IN MARINE MICROALGA NANNOCHELOROPSIS SP. (EUSTIGMATOPHYCEAE)
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Lipids are a broad group of naturally occurring molecules which includes fats, waxes, sterols, fat-soluble vitamins, monoglycerides, diglycerides, phospholipids, and others. Microalgae contain lipids and fatty acids as membrane components, storage products, metabolites, and sources of energy. Given high productivity of microalgae, justified by the shorter generation time and higher oil content as compared to crops (up to 80% on dry weight), the potential contribution that microalgae can give on a large scale fuel oil production is clear. In this study, we compared the cultivation of two different Nannochloropsis strains, N. oceanica CS-246 and N. sp.2 (local isolate), for maximal lipid productivity. From growth curves of strains, growth rates (μ = 0.57 and 0.48 day⁻¹) and doubling times (29.18 and 34.65 hours) were calculated for N. sp.2 and N. oceanica CS-246, respectively. For the induction of fatty acid accumulation, we exposed exponentially growing cultures to nitrogen starvation under high light intensity (350 μmol photons m⁻² s⁻¹). The highest average dry weight (DW) productivity (after 3 days) was 0.74 and 0.63 g/L/day for N. sp.2 and N. oceanica CS-246, respectively. The highest biomass total fatty acids (TFA) content was 48.55 and 47.72 % DW with the highest average productivity of 0.45 and 0.39 g TFA/L/day for N. sp.2 and N. oceanica CS-246, respectively. Preliminary research done in our lab demonstrated that N. sp.2 was relatively more stable than N. oceanica CS-246 when cultured outdoors. Thus, N. sp.2 is a promising candidate to be used for outdoor applications for modelling an efficient mode of biomass and lipid production.

2D.26
INHIBITORY EXTRACTS OF SOME BROWN ALGAE FROM TURKEY
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In this study methanolic extracts of some brown algae (Hincksia mitchelliae, Liebmannia leveillei, Scytosiphon lomentaria, Dictyota dichotoma, Cystoseira barbata) that were collected from Urla, Dardanelles and Iskendrun Bay of Turkey have been studied for their inhibitory activities against pathogenic microbes (Aeromonas hydrophila, Bacillus subtilis, Staphylococcus epidermidis, Enterobacter aerogenes, Escherichia coli and Enterococcus faecalis), in vitro.

1D.27
GROWTH OF GREEN ALGA DESMODESMUS ON INDUSTRIAL WASTE CONTAINING SEVERAL RARE EARTH ELEMENTS
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Rare earth elements (REEs) are an important component of industrial products namely electronics. As the export of REEs from primary producer is recently minimized, the requirement of recycling of REEs from industrial waste is in a center of interest of researchers and developers all around the world. One possibility of recycling is using microorganisms. We tested the green alga *Desmodesmus quadricauda* for a growth on the composition acquired from industrial waste. The composition containing ytterbium and europium was added to the mineral liquid medium as a powder in concentrations 0–1%. The composition containing lanthanum was first extracted with 80% nitric acid and then added to liquid medium in concentration 0.1–0.3%. The pH was adjusted to 7.0 by NaOH. The accumulation of REEs in algal cells was analyzed using ICP-MS. Cultures of *Desmodesmus quadricauda* were able to grow on composition containing ytterbium and europium up to concentration 1%. The growth of algae was even stimulated. The toxic threshold concentration for lanthanum nitrate was 0.2%. To conclude, the green algae can grow in presence of REEs from industrial waste. They could be the promising way for REEs recycling. Project was supported by the Grant Agency of the Czech Republic (grant no. 525/09/0102) and by Institutional Research Concept no. AV0Z50200510.
DNA Taxonomy: Bar Coding and Species Delineation

3D.1
MOLECULAR AND MICROSCOPIC EVIDENCE PLACE MICROALGAL SPECIES OF CHARACIOPSIS INTO THE EUSTIGMATOPHYCEAE

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Characiopsis Borzi is a large genus traditionally included in the Xanthophyceae, but two of its species have been previously reassigned to the class Eustigmatophyceae and placed into a new genus Pseudocharaciopsis typified by the species Pseudocharaciopsis texensis, later synonymized with Pseudocharaciopsis minuta. To clarify the taxonomic position of other species, a large sample of microalgae collected from Portuguese waters, isolated into culture and maintained at the ACOI culture collection as Characiopsis spp., was further studied by light and electron microscopy and by sequencing the 18S rRNA gene. For most cultures the ultrastructure of cells and zoospore flagellar apparatus showed typical eustigmatophycean features. Molecular analysis of the 18S rRNA gene of 28 ACOI strains confirmed the hypothesis of them being eustigmatophytes and raised further questions. At least two Characiopsis-like lineages became evident. One lineage comprising a majority of the Characiopsis strains studied is related to the previously characterized P. minuta UTEX 2113 (the authentic strain of P. texensis), whereas the other consists of the remaining Characiopsis strains found to be related with Pseudocharaciopsis ovalis and Pseudellipsoidion edaphicum in a separate group at a distant position within the eustigmatophyte phylogeny. Both morphological characteristics of the cells and molecular data support the existence of several species within the Pseudocharaciopsis minuta-related lineage. One of these species has been identified as Characiopsis borziana, the type species of the genus Characiopsis. Our results therefore indicate that the genus Characiopsis should be formally transferred to the Eustigmatophyceae. Furthermore, the genus Pseudocharaciopsis should probably be considered a junior synonym of Characiopsis and a new generic assignment is needed for P. ovalis and perhaps some other Characiopsis species.

3D.2
PHYLOGENETIC ANALYSIS BASED ON ITS SEQUENCES SUGGESTS RE-STRUCTURING OF D. VIRIDIS (CHLOROPHYTECEAE) TAXON

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Dunaliella viridis (Chlorophyceae, Dunaliellales) belongs to the Section Viridis of the subgenus Dunaliella. This halophilic species is able to tolerate hypersaline environments such as saltworks and it is widely distributed throughout the world. It has been stated that its taxonomic position needs to be investigated since several varieties and forms based largely on morphologic characteristics (cell size) have been described. In order to try to highlight the taxonomic status of this taxon we analyzed the Internal Transcribed Spacers (ITS) sequences (including the ITS2 secondary structure analysis) of sixteen D. viridis strains. Both the primary sequence analysis (ITS1 + ITS2) and the ITS2 secondary structure analysis positioned the majority of D. viridis strains in two different clades, revealing great heterogeneity and paraphyly. Unexpectedly, D. viridis var. pamelloides (CCAP11/34) and Dunaliella viridis var. euchlora (CCAP19/21) only shared a similarity of
54.4%, suggesting that these strains can not belong to the same species. *Dunaliella viridis* var. *euchlora* (CCAP19/21) was positioned in a different lineage of the other *D. viridis* specimens, but was positioned within the subgenus *Dunaliella*. On the contrary, *D. viridis* var. *palmelloides* (CCAP11/34) was positioned outside the subgenera *Dunaliella*. These results indicate that this strain is phylogenetically different from *D. viridis* and from the other *Dunaliella* species analyzed. Furthermore, the detection of at least three compensatory base changes (CBC) between *D. viridis* var. *palmelloides* (CCAP11/34) and the other *Dunaliella* strains suggest that this strain is a new species. In this sense we purpose the re-name of this strain as *D. palmelloides*. In conclusion, the primary sequence (ITS1 + ITS2) and the ITS2 secondary structure results suggests the re-structure of the of *D. viridis* taxon.

**3D.3**

**DETECTION OF ALIEN SPECIES IN TWO ITALIAN COASTAL LAGOONS USING THE DNA BARCODING TOOL**

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The introduction of non-indigenous species across the major European seas is a dynamic non-stop process. A wide range of vectors such as aquaculture, shipping (fouling, ballast water), lessepsian migration, aquarium trade and fishing nets are currently introducing a plethora of alien marine species into indigenous assemblages. In the Mediterranean Sea, coastal lagoons, such as Thau Lagoon in France, Venice Lagoon and Cape Peloro lagoon in Italy, are well-known points of introduction for marine macrophytes. In shallow lagoons and estuaries, eutrophication processes facilitate the development of macroagal biomass. During a census of macroagal diversity into Venice and Cape Peloro lagoons, the DNA barcode revealed cryptic diversity within the genera *Ulva* (Chlorophyta), *Hypnea* and the *Polysiphonia* complex (Rhodophyta). Introduction of alien species by the vector of oyster shells was highlighted by characterizing epizoic juvenile of algae. DNA barcoding demonstrated as a quick and efficient tool for the accurate identification of alien species and for the evaluation of their vectors.

**3D.4**

**MARINE BENTHIC DIATOM FLORA FROM KUMEJIMA ISLAND, THE RYUKYU ISLANDS, JAPAN**

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Kumejima Island (26°20’27”N 126°48’18”E) is a small island among the Ryukyu Islands in Japan. Due to Kuroshio, the strong warm current, flowing over the island, yearly average temperature of surface water is 22.8. It is the subtropical zone, with coral reefs that are generally known to being rich in biodiversity. Present study focuses on diatom flora of Kumejima Island, giving the better insight in the coral reef ecosystem. Samples of benthic diatoms were collected from various substrates, *Zostera japonica* (Magnoliophyta), *Boodlea coacta* (Chlorophyta), *Plocamium telfairiae* (Rhodophyta) and coral sands, in several places of Kumejima Island. All of the samples were analyzed with the use of light and scanning electron microscopies. Total number of 91 taxa in 34 genera were recorded from the 4 samples. *Amphora longa*, *Cocconeis* spp. and *Halamphora* sp. are dominant in some of the places. Morphologic and taxonomic comments are given for some of species. Additionally new species of *Hyalosira* was discovered, but we need further studying.

**3D.5**

**A STEP FORWARD TO DEFINE THE BANGIA COMPLEX IN EUROPE: INSIGHTS FROM DNA BARCODING**

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The Bangia complex has an intricate taxonomic and nomenclature history and includes species notoriously difficult to distinguish on morphological ground. In 1973 Geesink stated that the freshwater species *Bangia atropurpurea* (Roth) C. Agardh was conspecific with the marine species *Bangia fuscopurpurea* (Dillwyn) Lyngbye. However, subsequent karyological and DNA sequence data proved that both North American and European freshwater populations are relatively homogeneous among them but distinct from marine isolates. In 2007 a monotypic genus was described, *Bangiadulcis* W.A. Nelson, to include the freshwater species, but this new name is a superfluous synonym of *Bangia Lyngbye 1819*, being both based on the same generitype species *B. atropurpurea*. At present, *Bangia* is the accepted name of the freshwater genus and further studies are needed to determine the name and circumscription of the genus that includes *B. fuscopurpurea*. Furthermore, recent studies have shown that the last entity includes two cryptic species in north Atlantic. DNA barcoding data obtained from Mediterranean populations in the present study suggest the presence of another cryptic species within the Trebouxiophyceae. Based on its 18S rRNA gene sequence, this isolate is distantly related to *Nannochloris eucaryotum UTEX 2502, Chlorella minutissima C-1.1.9 and C. minutissima SAG 1.80 (≤97.6% 18S rRNA gene pairwise similarities). As these strains formed a problematic sister group within the Trebouxiophyceae (at this time neither can be definitively assigned to any existing genus) the taxonomic revision of this group was proposed based on the morphological and molecular characteristics of the isolates.

### 3D.6

**CHLOROPARVA PANNONICA GEN. ET SP. NOV. (TREBOUXIOPHYCEAE, CHLOROPHYTA) AND PROBLEMS SURROUNDING ITS CLOSEST RELATIVES**

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We describe *Chloroparva pannonica* Somogyi, Felföldi & Vörös gen. et sp. nov., a new trebouxiphycean picoplanktonic alga isolated from a turbid, shallow soda pan in Hungary. The cells are spherical to oval, less than 2 μm in diameter, with simple ultrastructure typical to small green algae. More detailed morphological studies were carried out by transmission electron microscopy. The phylogenetic position of the new chlorophyte confirms the proposal of a new genus within the Trebouxiophyceae. Based on its 18S rRNA gene sequence, this isolate is distantly related to *Nannochloris eucaryotum UTEX 2502, Chlorella minutissima C-1.1.9 and C. minutissima SAG 1.80 (≤97.6% 18S rRNA gene pairwise similarities). As these strains formed a problematic sister group within the Trebouxiophyceae (at this time neither can be definitively assigned to any existing genus) the taxonomic revision of this group was proposed based on the morphological and molecular characteristics of the isolates.

### 3D.7

**UNEXPECTED VARIABILITY WITHIN THE XANTHIDIUM ANTILOPAEUM (DESMIDIALES) SPECIES COMPLEX**

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*Xanthidium antilopaeum* is taxonomically one of the most problematic desmids. Over 70 infraspecific taxa of this species have been described since the 19th century, many of them having problematic taxonomical value. In the present study, we evaluated the phylogenetic position of 29 strains of *Xanthidium antilopaeum* and morphologically similar taxa (*X. cristatum, X. fasciculatum, X. aculeatum*) on the basis of molecular data to reveal the phylogenetic structure of the complex. The majority of strains used was isolated from a variety of European locations, several strains were obtained from culture collections. Apart from the molecular analyses, the morphology of the strains was analyzed using geometric morphometric methods and scanning electron microscopy of cells. Molecular
phylogenetic analyses based on \textit{trnG} intron, \textit{coxIII} gene and ITS rDNA sequences revealed thirteen well established clades generally well corresponding to the morphological data. Several infraspecific taxa (\textit{X. antilopaeum} var. \textit{basiorum}, \textit{X. antilopaeum} var. \textit{incrassatum}, \textit{X. antilopaeum} var. \textit{laeve}, \textit{X. antilopaeum} var. \textit{planum}, \textit{X. antilopaeum} var. \textit{polymatum}, \textit{X. cristatum} var. \textit{uncinatum}) should be better kept as separate species, on the other hand, some subspecific taxa, like e.g. \textit{X. antilopaeum} var. \textit{depauperatum} or \textit{X. antilopaeum} var. \textit{hebidarum}, probably do not have any taxonomical value.

3D.8
TAXONOMIC REEXAMINATION OF CHAROPHYTES (SECT. HARTMANIA)
BASED ON MORPHOLOGICAL AND MOLECULAR DATA
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The morphological and genetic variation in four species of section \textit{Hartmania} R.D. Wood from Poland was analyzed. The plants were initially classified using morphological features into four species: \textit{C. baltica}, \textit{C. hispida}, \textit{C. polyacantha} and \textit{C. rudis}. Fourteen quantitative characters were used in a principal component analysis to determine groupings among the species and discriminant analysis to determine the morphological features that best separated the groups. In addition, AFLP analysis was used to compare genetic similarity among the species. \textit{C. hispida} and \textit{C. rudis} comprise one molecular group whereas \textit{C. polyacantha} and \textit{C. baltica} differed considerably. In the discriminant analysis, the results show that \textit{C. hispida} and \textit{C. rudis} (on morphological criteria) are only partially distinguishable.
4D.1

POLYPHASIC CHARACTERIZATION OF A COCCOID DIAZOTROPHIC CYANOBACTERIUM FROM EUGANEAN THERMAL SPRINGS

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The muds of the hydrothermal tanks of the Euganean District (Padova, Italy) are colonized by populations of cyanobacteria, both coccoid and filamentous, which can adapt themselves to drastic and sudden changes of the water temperature. This research deals with ETS-10, a cyanobacterium isolated from a thermal mud and maintained in BG11 medium at 30°C, 12 h photoperiod with a photon flux rate of 10 μmol m⁻² s⁻¹. The cells of this organism, often in 2-4 celled groups, were spherical or hemispherical (10–15 μm in diameter) and surrounded by a colourless mucilaginous envelope. The thylakoids were numerous, slightly fasciculate, regularly distributed over the whole cell volume. All these morphological and ultrastructural features were similar to those described for strains belonging to the genus *Chroococcus*. The finding of *nif*H and *nif*D genes in its genome and the ability of growing in a medium without organic nitrogen proved that ETS-10 was able to fix nitrogen. The analyses performed on cultures grown at different temperatures showed that the thermo tolerance of ETS-10 was quite limited, being the organism able to live at 45–47°C only for short time. The phylogenetic analyses, based on the 16S rDNA and *rbcL* genes, showed that *Chroococcus* genus, as so defined, was polyphyletic. In particular, the obtained topologies were congruent and indicated the inclusion of ETS-10 in a clade with most of the *Chroococcus* species. The comparison of ETS-10 gene sequences with those available in GenBank and obtained in our laboratory from other *Chroococcus* species of international culture collection, suggested that ETS-10 could be considered a new species of the genus *Chroococcus*. Further analyses, using other marker genes, will be performed to better define if ETS-10 is actually a new species belonging to this genus, for which we would suggest the name *Chroococcus aponinus*.

4D.2

CHARACTERIZATION OF DSCBR GENE IN THE HALOPHILE MICROALGA, *DUNALIELLA* SP.

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CBR (carotenoid biosynthesis related protein) was initially isolated from the halophile microalga, *Dunaliella salina*, which had high homology with early-light-induced protein (elip) of plant. The Dscbr gene was isolated from the *Dunaliella sp*. and Dscbr gene has high homology with cbr of *D. bardawil*. Dscbr was named after. Gene coding for Dscbr is 976bp long, and composed of 190 amino acids. The expected molecular weight of Dscbr was 19.9 kDa. When the expression profiling of Dscbr protein under various salt concentration and different light condition were performed by western analysis, Dscbr from *Dunaliella* sp., *Dunaliella bardawil*, and *Dunaliella salina* CCAP 19/18, were only expressed in the cells cultured at the concentration of 4.5 M salt, but not in high light stress condition. Dscbr gene protein seems to be considered as salt stress protein; however, the exact fuction of this protein was not knwon. We, therefore, transformed Dscbr into non-halophile alga, *Chlamydomonas reinhardittii* to investigate the fuction of this gene and the results will be dicussed.
4D.3 EFFECTS OF LIGHT SPECTRUM ON PIGMENT CONTENT AND PHOTOSYNTHESIS ACTIVITY IN SYNECHOCOCCUS STRAINS
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Picoplanktonic organisms show a lot of adaptations which enable them to spread in aquatic environment and to dominate and occupy the niches inaccessible for other photoautotrophs. The experiments were conducted on three different picocyanobacterial strains from the genus *Synechococcus*: BA-120, BA-132 and BA-124. The strains were isolated from the coastal part of the Gulf of Gdańsk (The Southern Baltic) and are maintained as unialgal cultures in Culture Collection of Baltic Algae at the Institute of Oceanography in Gdynia (http://ocean.ug.edu.pl/~ccba/). *Synechococcus* strains were exposed at 10 μmol photons m⁻² s⁻¹ of different light spectrum: red, green, blue and white. To determine the capacity of investigated strains for chromatic adaptation the concentration of photosynthetic pigments and photosynthetic activity were analyzed. Chlorophyll a, carotenoids and phycobilins concentration was determined spectrophotometrically and the composition of carotenoid pigments was analyzed by HPLC. The measurements of photosynthesis rate were carried out using Clark oxygen electrode, and chlorophyll fluorescence was measured using Pulse Amplitude Modulation (PAM) by Hansatech FMS 1 fluorimeter. Photosynthetic activity was characterized by the course of photosynthetic light response curves (P-E), which illustrate the compensation (Pₐ) and saturation irradiances (Eₛ), the initial slope of photosynthetic curves (α), maximum rate of photosynthesis (Pₘₐₓ) and dark respiration (R). Moreover, the values of chosen fluorescence parameters were analyzed. Fv/Fm (maximum PSII quantum efficiency) and ΔF/ΦPSII (effective PSII quantum efficiency) can provide additional information concerning the photosynthetic apparatus. Each of the strains indicated the best growth and the best photosynthetic activity at full spectrum of light (white). However, comparing different range of light spectrum, BA-120 and BA-132 (both phycoerythrin-rich) strains showed favorable growth at green light, while BA-124 (phycocyanin-rich) showed that red light was better for its growth.

4D.4 VARIATIONS OF BIOMASS AND ASTAXANTHIN OF MARINE CHLORELLA SPP. CULTURED UNDER THE DIFFERENT COMPOSITIONS OF RED AND BLUE WAVELENGTH BY LIGHT EMITTING DIODE
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The main issues of microalgal resources are focused on how to enhance efficiently the biomass and functional biochemical substances (anti–oxidant) for algal industrialization such as fish feeding, human food, supplemental nutrients and pharmaceutical products, as well as environmental applications such as bioremediation, biofuel and reduction of greenhouse gas (CO₂) emissions. *Chlorella saccharophilia*, *C. salina*, *C. stigmaphora* and *C. vulgaris* were cultured by the different compositions of red:blue (R₂:B₁; R₃:B₁; R₄:B₁), just red or blue wavelengths, respectively. The cells of *C. vulgaris* grew very well just at the beginning culture for 5 days without any LED compositions, while the ones of *C. stigmaphora* grew better after culture of 5 days than *C. vulgaris*. The photosynthetic efficiencies measured by PAM fluorometry was the highest in *C. salina* (about 0.7) cultured at R₂:B₁, the ones was the lowest in *C. stigmaphora* at R:B (3:1). The cell density of *C. salina* was the highest under the 1000 us of LED pulse in 4R:1B (7 × 10⁶ cells/ml). The amount of astaxanthin in *C. salina* was higher (66 mg/dry wt. g) under the switching from R₂:B₁ to R₃:B₁ after culture of 5 days than that under the all red light (27 mg/dry wt. g) and the all blue light (36 mg/dry wt. g). To industrialize *Chlorella* spp., the switching composition of LED wavelength could be efficient to enhance the biomass and these kinds of functional substances such as astaxanthin as anti-oxidant.

4D.5 ENZYMATIC CHARACTERIZATION AND DISTRIBUTION OF δ-CA: A POTENTIAL INDICATOR OF PAST pCO₂
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Photosynthesising life in the ocean constitutes approximately 50% of the global carbon fixation, thus making marine algae an important sink for CO₂. However, the accumulation of inorganic carbon by eukaryotic marine phytoplankton is limited by the diffusion of CO₂ aqueous in water and the dehydration kinetics of bicarbonate to CO₂. These unfavourable conditions and the low biochemical affinity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) for CO₂ have driven marine phytoplankton to develop a panoply of carbon concentrating mechanisms (CCMs).

Several isoforms of CAs are known to exist in an integral role in CCMs of marine phytoplankton. However, the restriction of δ- and ζ-CA to marine algae is particularly interesting in terms of how these organisms evolve to circumvent carbon limitation in the ocean. To date, efforts to fully characterize the enzymatic properties of δ-CA have been unsuccessful. The lack of kinetic data has cast a reasonable amount of uncertainty on the role of this protein as a functional CA. In this study, we demonstrate unequivocally that TWCA1 from *Thalassiosira weissflogii* is a bona fide CA and is catalytically functional in the hydration of CO₂ and hydrolysis of p-nitrophenyl acetate (esterase activity). We also explore the evolution of δ-CA via its distribution and diversity in a variety of marine phytoplankton species (diatoms, coccolithophores and dinoflagellates) using degenerate primers designed based on published sequences. Results show a unique relationship between the adaptation of RuBisCO (using Phylogenetic Analysis of Maximum Likelihood, PAML) and the emergence of δ-CA.

**4D.6**

**STUDY OF SUPEROXIDE DISMUTASE AND CATALASE ENZYMATIC ACTIVITIES IN PHYTOPLANKTON UNDER UV EXPOSURE**

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*Dunaliella tertiolecta* (Chlorophyta) and *Gymnodinium sp.* (Dinophyceae) cells were grown at 90 μmol photons m⁻² s⁻¹ PAR (12 h light: 12 h dark photoperiod) until they reached mid log phase. At this point, four replicate cultures were transferred to continuous PAR + UVA + UVB (PAB) (PAR: 90 μmol photons m⁻² s⁻¹ UV treatments: 8.5 Wm⁻² UVA and 1.5 Wm⁻² UVB, unweighted) while four other control cultures remained with the same photoperiod during 7 d for *D. tertiolecta* and 2 d for Gymnodinium. Under less than 12 h of continuous UV, *Gymnodinium* cell abundance decreased while the number of dead cells, revealed by SYTOX-green stain increased. After 24 h, 100 % of the cells were dead and thymine dimmers were formed in the DNA. Reactive oxygen species (ROS) accumulation sharply increased during the first 24 h, whilst *Fv/Fm* dropped in both control and treatment cultures. SOD activity did not change with time and although CAT activity augmented in both treatments, cells still suffered from the UV stress, and ROS accumulation paralleled the increase in caspase-like enzymatic activities. *D. tertiolecta* behaviour was opposed to the dinoflagellate. Cell numbers increased regardless the UV treatment, no dead cells were observed and ROS accumulation was very low. SOD and CAT activities generally remained slightly higher under continuous UV and were more efficient at detoxifying the cells than *Gymnodinium*. *Fv/Fm* showed a decrease within time but then remained constant. Surprisingly, thymine dimmers were not formed. Caspase-like activities did not change significantly and once more their constitutive role in the cell as housekeeping proteins is demonstrated. Results suggest that this species has a great capacity to scavenge and deal with ROS, skipping over cell death. On the other hand, the dinoflagellate is a much more sensitive species to UV, not able to detoxify ROS efficiently, hence committed to cell death.

**4D.7**

**THE MOLECULAR BASIS OF INORGANIC CARBON UPTAKE MECHANISMS IN THE COCCOLITHOPHORE *EMILIANIA HUXLEYI***

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The mechanism of dissolved inorganic carbon (DIC) acquisition in coccolithophores is relatively unknown. By culturing *Emiliania huxleyi* at constant CO$_2$ and constant pH, we can start to disentangle the individual components of the carbonate system (CO$_2$, HCO$_3$/CO$_3^{2-}$, and pH) and understand their importance in coccolithophore biology. This study probes the gene expression of putatively key inorganic carbon and proton transport genes of cells cultured at constant CO$_2$ varying DIC/pH and constant pH varying CO$_2$/DIC. The gene expression profiles of target genes including several carbonic anhydrases, HCO$_3$/CO$_3^{2-}$ transporters, proton pumps and proton driven exchangers were investigated using quantitative reverse transcription PCR. Results show that a number of genes including a putative bicarbonate transporter, two carbonic anhydrases and several proton transporters are up-regulated under low DIC conditions. The expression of genes related to carbon transport is independent of CO$_2$/pH concentration but strongly correlates to CO$_2$ and HCO$_3$/DIC concentration. Changes in pH appear to have a limited effect on expression of most of the investigated genes. The resulting data provides a key insight into the inorganic carbon uptake mechanism of *E. huxleyi* and its regulation.

**4D.8**

THREE-DIMENSIONAL ANALYSIS OF TRANSIENT MEMBRANE COMPARTMENT DURING CYTOKINESIS IN BROWN ALGA, *SILVETIA BABINGTONII* (FUCALES, PHAEOPHYCEAE) BY ELECTRON TOMOGRAPHY

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New cell partition membrane was built by fusion of Golgi-derived vesicles (GVs) and flat cisternae (FCs) in brown algae. Transient membrane configuration on cytokinesis was studied in zygotes of *Silvetia babingtonii* by dual-axis electron tomography. After mitosis, a long and narrow membrane structure with a diameter of 20 nm was found along endoplasmic reticulum (ER) near cytokinetic plane. The structure will be an intermediate FC, since a part of it became broad like a FC. GVs fused to the edges of FCs arranged parallel to the cytokinetic plane, and then a complicated architecture was produced. Fringes, which were shaped by fusions of GVs from a central FC, extended the neighbouring to connect, thus giving membranous network (MN). In some places along the cytokinetic plane, conversion of the MNs to broad membranous sacs (MSs) was occurred. The MSs united each other to form a new cell partition membrane. A joint between the MSs swelled and possessed many invaginations of membrane. Coated pits were frequently observed at that stage. Complete new cell partition membrane had no membrane penetration and swelling regions. By analysing of changes in volume and surface area of membranous structure from MSs to a complete cell partition, it is considered that excess membrane material must be recycled by coated pits.

**4D.9**

THE EFFECT OF BREFELDIN A ON THE SYSTEM OF CONTRACTILE VACUOLES IN *MESOSTIGMA VIRIDE* LAUTERBORN (STREPTOPHYTA)

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The contractile vacuole (CV) is an osmoregulatory organelle which is found in many freshwater protists. The cycle of a CV is divided into two phases: the diastole (enlargement of the CV until a sphere is formed) and the systole (expelling of the fluid from the cell). *Mesostigma viride* is the only known streptophyte flagellate. Based on LM, cells possess an average of 8 CVs. During the CV cycle they fuse with each other and sometimes tubular connections between different vacuoles are
temporarily formed. Close contact zones between the CV and flagellar groove membrane were detected by TEM. These contact zones are probably the sites of water expulsion. In addition, a large number of clathrin coated vesicles (CCV) and pits are associated with the CVs. The number of CCVs was relatively higher on large than on small vacuoles, indicating that the vesicles might play a role in the regulation of water uptake and termination of the diastole (Buchmann and Becker 2009). For a more detailed investigation of the function of these vesicles we use Brefeldin A (BFA). BFA is a well-known inhibitor for a GTP-exchange factor (GEF) required for the formation of clathrin- and COPI-coated vesicles in the secretory pathway. Upon BFA treatment the CVs of Mesostigma swell up and form one or several large vacuoles. Finally the cells burst. We expect that CCVs in the system of CVs will be missing upon BFA treatment. To address this question we currently perform a TEM analysis and a 3D-reconstruction of BFA treated cells.

4D.10
NEW CELL-WALL POLYSACCHARIDES IN CHAROPHYTIC ALGAE
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It is ~460 million years since plants first colonised the land and it is generally accepted that the charophytes represent the closest living relatives of the land plants. The polysaccharides of angiosperms, pteridophytes and bryophytes have been well documented (Popper and Fry, 2003; 2004). However, more recently, the surprising presence of the cell-wall enzyme activities XET and MXE in charophytic algae (Fry et al., 2008) but lack of biochemical evidence for their substrate xyloglucan has revealed gaps in our knowledge of the composition and nature of the wall polysaccharides in charophytes. Few charophytic polysaccharides have been described to date (Sarkar et al., 2009). Charophytes possess cellulose and homogalacturonan (Proseus et al., 2008) but their hemicelluloses have not yet been characterised in detail. Charophytic walls contain certain unusual monosaccharide residues not routinely found in land-plants, e.g. 3-O-methylrhamnose and 3-O- methylgalactose (Popper and Fry, 2003). Previous work with enzymic digestion failed to detect isoprimeverose residues (characteristic of xyloglucan) in Coleochaete, Klebsormidium and Chara (Popper and Fry, 2003). Although immuno-techniques revealed xyloglucan-like polysaccharides in Chara (Domozych et al., 2009) and Spirogyra (Ikegaya et al., 2008), the specificity of the antibodies used is not sufficiently well characterised as to distinguish true xyloglucan from possible unknown related structures. Much remains to be discovered about charophyte cell walls: information that would promote our understanding and appreciation of the ‘primordial’ land-plant cell wall. We present here the non-cellulosic monosaccharides released by acid hydrolysis of the walls of representatives of all five orders of charophytes: Coleochaetales, Charales, Klebsormidiales, Zygnematales and Chlorokybales. Furthermore, the informative enzyme ‘Driselase’ was used to test for the hemicellulose xyloglucan. Walls from all five charophytic orders were fractionated into pectins, hemicelluloses and cellulose, and the composition of each fraction as revealed by acid and enzymic hydrolysis is here presented. Acknowledgement: Supported by the Leverhulme Foundation.

4D.11
EFFECT OF SALINITY, LIGHT INTENSITY AND NITROGEN AVAILABILITY ON LIPID PRODUCTION BY NANNOCHLOROPSIS SP.
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The eustigmatophyte microalgae of the Nannochloropsis genus are widely used in aquaculture as live food primarily due to the beneficial LC-PUFA composition featuring high content of the ω3 eicosapentaenoic acid (EPA). Under certain stressful conditions, these microalgae accumulate high content of storage lipids triacylglycerols (TAG), rendering them also as a promising organism for biodiesel production. We examined the responses of batch cultures of Nannochloropsis sp. to combined alterations in salinity (13, 27 and 40 g/l NaCl) and light intensity (170 and 700 μmol photons/m2/s) on nitrogen-replete and nitrogen-depleted media. On the nitrogen-replete medium, increases in light intensity and salinity enhanced the biomass and lipid contents of the culture, mainly due to augmented formation of TAG. Even, the percentage of EPA in total fatty acids increased with salinity. On the nitrogen-depleted medium, increases in light intensity and salinity enhanced the biomass and lipid contents of the culture, mainly due to augmented formation of TAG. Even, the percentage of EPA in total fatty acids increased with salinity.
acids (TFA) was reduced, and TAG reached 25% of biomass, yet, the EPA biomass and culture contents were retained relatively high. We show that choice of cultivation regimes may render Nannochloropsis sp. to produce simultaneously two major valuable components, EPA and TAG, while sustaining relatively high biomass growth rates. Under nitrogen starvation, the highest TFA content of biomass and average lipid productivity were achieved at 700 μmol photons/m² s and 13 g/l NaCl. Combination of high salinity, light intensity and nitrogen depletion was detrimental to TAG accumulation, and resulted drastic decrease in TFA content and average lipid productivity. We suggest that decreased TAG biosynthesis is associated with deviation of photosynthetic carbon allocation toward production of organic osmolytes or other storage components to cope with the increased salinity. It is also possible that increased intracellular sodium ion concentration and ROS production might have negative impact on the activity of fatty acid and TAG biosynthesis enzymes.

4D.12
DIFFERENTIAL REGULATION OF HEAT SHOCK PROTEINS AND CYTOCHROME P450 GENES IN SYMBIOTIC DINOFLAGELLATES UNDER THERMAL STRESS

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With current trends in global climate change the survival of coral reefs is dependent on their ability to adapt to heat stress conditions. When exposed to elevated temperatures cellular changes occur within the tissues of reef-building corals and their microalgal endosymbionts affecting the functionality of the coral-algal symbiosis. Unicellular photosynthetic dinoflagellates of the genus Symbiodinium are the most common endosymbionts of reef-building scleractinian corals, living in a symbiotic partnership known to be highly susceptible to environmental changes. Here, we identified members of two major Heat Shock Protein (HSP) families, Hsp70 and Hsp90, in Symbiodinium sp. (clade C) and the evolutionary relationship with other known HSPs from Phylum Dinoflagellata. Regulation of HSPs gene expression was analysed in samples of the scleractinian coral Acropora millepora subjected to elevated temperatures progressively over 18 hours (rapid thermal stress) and 120 hours (gradual thermal stress). Differential gene expression profiles observed for Hsp70 and Hsp90 suggests diverse role of these molecular chaperones during heat stress response. Heat stress resulted in a comparable expression pattern of Symbiodinium Hsp70 and Hsp90 genes from culture and in hospite, indicating their independent regulation from the host. In addition, we describe the presence of three new cytochrome P450 (CYP) genes from the reef-building coral endosymbiont Symbiodinium (type C3) and changes in their expression during exposure to severe and moderate heat stress conditions. The initial up-regulation of expression of CYP genes at moderately elevated temperatures (26°C and 29°C) was followed by a decrease in expression under the greater thermal stress conditions at 32°C. Both rapid heat stress and gradual heat stress at 32°C resulted in 50% to 90% decreases in CYP gene transcript abundance. Cytochrome P450 monoxygenases are involved in the first line of an organism’s chemical defense. Our results suggest that within the coral-alga symbiosis under heat stress conditions there is production of chemical stressors and/or transcriptional factors that regulate the expression of CYP genes. The findings of this study will provide better insight into changes occurring in coral endosymbionts under heat stress that will underpin our knowledge about stress responses in coral dinoflagellates and their ability to tolerate global changes occurring in the environment.

4D.13
PHYTOCHELATIN SYNTHESIS IN THE PHANEROGAM CYMODOCEA NODOSA

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Thioles are sulfuric analogous of alcohols and include phytochelatins (PT) with cystein and glutathione (GSH) being the most common peptides
in plants. In the presence of metals (Hg, Pb, Cu) thioles form chelating complexes. Phytochelatins are polypeptides different from metallothioneins (MT) of mammals and their presence is linked to the presence of metals. Photo-autotrophs are the main entrance for heavy metals in the trophic chain with humans and animals as the final receivers. Reduced glutathione, GSH (γ-glu-cys-gly) exists interchangeably with the oxidised form, GSSG. In plants, its physiological importance is expressed as sulphur metabolism and defence. It is the precursor of the phytochelatins, which are essential in sequestering heavy metals. Heavy metal toxicity poses major environmental and health problem. Cadmium is a non-essential heavy metal, toxic to cells at very low concentrations. Cadmium ions displace Ca\(^{++}\) or Zn\(^{++}\) in proteins and may cause oxidative stress. Furthermore the concentration of essential, but at high concentrations toxic, metals such as Cu\(^{++}\), Zn\(^{++}\), Fe\(^{++}\) is strongly controlled. We present data on phytochelatin variation in a confined meadow of *Cymodocea nodosa* in the Thessaloniki Bay that exhibits an increasing pollution level.
Molecular and Cellular Responses in Algae Induced by Changes in the Environment

5D.1

GROWTH DYNAMIC OF OSTREOPSIS OVATA BLOOMS IN LIGURIAN GULF (WEST MEDITERRANEAN)

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In this last decade Ostreopsis ovata always appears along the coast of the Ligurian Gulf (West Mediterranean sea). After a harmful algal bloom appeared in the Gulf of La Spezia during 2006, an investigation was carried out to evidence the critical factors that could favour O. ovata growth in order to prevent its development. We have weekly monitored both the density of the cells of O. ovata and the abiotic parameters: from spring to autumn the data were collected continuously in water and air. In our results the increased values of light and temperature are determinant in the phase of major proliferation of O. ovata. Contemporary, the toxic effect of O. ovata was observed on the growth of Dinophilus gyrociliatus by ecotoxic tests. The preliminary results suggest that O. ovata toxicity would depend both by microalgae density and its growth phase, and that it could be harmful for the polychaetes living in temperate coastal hard bottoms. Moreover, the presence of some cyanobacteria species could be implicated in the toxicity of the microalgae blooms and therefore, their determination could be useful to have a clearer picture of the O. ovata bloom dynamic in the Mediterranean Sea.

5D.2

METABOLIC PROFILING OF THE CHEMOSPHERE OF THE MARINE GREEN MACROALGAE ULVA AND ITS ASSOCIATED BACTERIA

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We aim to determine the waterborne metabolites released by the green macroalgae Ulva mutabilis and its associated bacteria under standardized conditions in order to understand their mutualistic interactions mediated by infochemicals. For the detection of exo-metabolites extraction of large volume of water is required in order to determine short term shifts and the plasticity of the chemical sphere. Using solid phase microextraction metabolites of Ulva culture medium can be easily extracted and used for metabolic profiling by mass spectrometry. Samples from cartridge enrichments were directly analyzed by liquid chromatography (UPLC) or gas chromatography (after derivatisation) coupled with a time-of-flight mass spectrometer (TOF-MS). In laboratory experiments we inoculated the defined seawater medium with axenic gametes of U. mutabilis. It is known that Ulva is not able to grow without certain bacteria. However, the morphogenesis of U. mutabilis can be induced upon addition of specific bacteria. Here we show that the exo-metabolic fingerprint depends on the bacterial community associated with U. mutabilis. This explorative approach, when combined with chemometric analysis, enabled us to distinguish between various bacterial
associations with *U. mutabilis*. We suggest that this compound release resembles the multiple interactions between *Ulva* and its associated bacteria. The exo-metabolome studies will hence help to decipher the implications of release patterns of potential infochemicals.

5D.3

**PHOTOSYNTHESIS OF CYSTOSEIRA SPP. AND CLIMATE CHANGE: ACCLIMATION AND VULNERABILITY TO STRESS FACTORS**

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*Cystoseira* spp. (Phaeophyta) are important elements of the marine vegetation in the Canary Islands, playing both a structural and a functional role in its coastal habitats. *Cystoseira* forests present high biodiversity, the complexity of associated trophic chain levels and elevated primary production. Recent field data confirms the reduction of their distribution, suggesting a shift in the physiological status and biomass production of these marine forests. In order to analyze the vulnerability of these key communities to climate change scenarios, photosynthetic responses were measured in several *Cystoseira* species (*C. humilis, C. tamariscifolia, C. compressa* and *C. abies-marina*) along a depth gradient by using in vivo chlorophyll fluorescence (i.e., maximal quantum yield (Fv/Fm)) as estimator of physiological status and photoinhibition, and electron transport rate (ETR) as estimator of photosynthetic capacity. The effect of increasing solar radiation was studied in situ by two different experimental approaches: A) along diurnal cycles and B) after the transplantation to high solar irradiance exposure followed by incubation under shade conditions (recovery phase). The photoprotection and photodamage outputs were related to algal zonation; intertidal *Cystoseira* species presented very active mechanisms of dynamic photoinhibition and the accumulation of phenolic compounds were also faster compared to species growing in the subtidal zone. The decrease of photosynthetic yield after short high irradiance exposure was lower and the recovery was higher in intertidal species such as *C. tamariscifolia* and *C. humilis*. *Cystoseira* species in the intertidal zone presented high resistance to high solar radiation, by the accumulation of phenolic compounds and high antioxidant activity (DPPH), compared with subtidal species such as *C. compressa* and *C. abies-marina*, which showed a slower acclimation response.

5D.4

**ELEVATED TEMPERATURE PARTIALLY COUNTERACTS UV PHOToinHIBITION IN THALASSIOSSIRA WEISSFLOGII**

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Outdoor experiments were carried out in Patagonia (Argentina) to assess the interactive effects of ultraviolet radiation (UVR) and temperature on three cellular targets: carbon assimilation, Chl *a* fluorescence of PS II, and Rubisco activity/gene expression, using the cosmopolitan diatom *Thalassiosira weissflogii* as a model organism. Exponentially growing cultures were exposed to solar radiation with or without UV-A (320–400 nm) and UV-B (280–320 nm) radiation at two temperatures – 20 and 25°C. Experiments lasted for whole daily cycles or for 1 hour centered around local noon. Samples incubated at 25°C exhibited significantly less UVR-induced inhibition of carbon fixation and photochemical quantum yield as compared to those incubated at 20°C. In addition, at 25°C Rubisco activity and gene expression were significantly higher than at 20°C. The higher Rubisco activity and gene expression were correlated with decreased dissipation of excess energy (evaluated via non-photochemical quenching, NPQ, and the de-epoxidation (DEPS) state). Our data indicate that an increase in temperature, would partially counteract the negative impact of UVR by increasing the response of metabolic pathways, such as those involved in Rubisco.
5D.5

EFFECTS OF ELEVATED CO₂ LEVELS ON GROWTH AND BIOCHEMICAL COMPOSITION OF TWO SEAWEED SPECIES

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Seaweeds cultivated in land-based tank systems in high densities often suffer carbon limitation, which reduces growth rates and productivity. In integrated multi-trophic aquaculture systems however, fish respiration leads to increased dissolved inorganic carbon levels in the water. Studies on this subject have shown contrasting results on the effects elevated inorganic carbon levels might have on seaweed growth and biochemical composition. In this study, the chlorophyte Ulva rotundata and the rhodophyte Gracilaria longissima were cultivated at high nutrient levels in medium aerated with two different CO₂ levels: normal (ambient, 0.035–0.040 %) and enriched (0.24–0.26 %). Photosynthetic efficiency and growth rates of the algae were monitored during 2–3 weeks and samples were taken of the water and analysed for dissolved inorganic and organic carbon. At the end of the experiment, algae were harvested and analysed for C and N, carbohydrate, lipid, ash and protein content. No difference in growth rates was found between normal and enriched treatments for G. longissima, whereas U. rotundata increased growth rates by 10–15 % in the enriched treatment. Total C content showed no differences between treatments for G. longissima and only a slight increase in the enriched treatment for U. rotundata. However, a significant increase in carbohydrate content was observed for both species. The results are discussed with respect to the carbon ecophysiology of the species and the potential of using them in integrated multi-trophic aquaculture systems.

5D.6

PHYSIOLOGICAL ADAPTATIONS OF MACROPHYTES KEY SPECIES TO COASTAL LAGOONS IN A CLIMATE CHANGE SCENARIO

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Benthic macrophytes have been proposed as one of the key biological elements to determine the ecological quality status in coastal waters as they could reveal the long-term effects of natural and anthropogenic environmental changes on marine benthic organisms. A total of 4 key species were selected from representative communities in the Mar Menor coastal lagoon and Mediterranean coastal waters, including Heterokontophyta (Cystoseira compressa (Esper) Gerloff & Nizamuddin and Padina pavonica (Linnaeus) Thivy), Rhodophyta (Chondrophyccus tennerrimus (Cremades) G. Furnari, Boisset, Cormaci et Serio) and the seagrass Cymodocea nodosa (Ucria) Asch. The main objective was to study the physiological response and the acclimation capacity of these species comparing the behaviour of lagoon benthic macrophytes versus those from coastal waters of Mediterranean. The physiological status of the key species was assessed by using in vivo chlorophyll fluorescence (maximal quantum yield [Fv/Fm], electron transport rate [ETR]), photoprotective compounds (xanthophylls cycle pigments, phenolic compounds, mycosporine-like amino acids), oxidative stress indicators (lipoperoxides and DPPH radical oxidation) and stoichiometry (C: N). Exposure-recovery experiments were conducted in order to determine the acclimation capacity of these species to higher UV irradiances. In general, our results showed higher photosynthetic capacities (ETRmax) in species grown in Mediterranean coastal waters or stations more influenced by Mediterranean waters than in the lagoon of Mar Menor. However, these species showed lower acclimation capacity as the exposure to increased irradiances during several hours provoked higher decreases in the Fv/Fm.
(photoinhibition). The photoprotective strategies varied among species. Then, the individual and interactive effects of environmental variables as well as the competition for light and nutrients between species are important processes that determine the structure of these benthic communities and allow predicting the capacity of adaptation of these species to Climate Change.

5D.7
EXTRINSIC FACTORS INFLUENCING PHLOROTANNIN LEVELS – EXPERIMENTAL OR OBSERVATIONAL DATA?
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Phlorotannins – polyphenolic compounds, found only in brown algae (Phaeophyceae), are believed to play defensive role against herbivores and in absorbing ultraviolet radiation. Their popularity among the scientists from various fields is currently on the increase due to their promising potential use in therapeutics, medicine and antifouling applications. Phlorotannin concentrations vary among and within the species, depending also on the age and size of a plant. Additionally, various extrinsic factors, such as grazing activity of herbivores, nutrient availability and exposure to atmospheric conditions are responsible for short time changes in the phlorotannin content. Most of studies on phlorotannin variations have been focusing mainly on experimental data. While factual dependencies in the natural environment exist between those secondary metabolites and physicochemical conditions, they have been discussed by only a few scientists. In the current research we try to face that problem and compare the data recorded in the field with phlorotannins in the plants from the same area. Samples of Sargassum thunbergii and Sargassum fusiformis (Fucales) were collected monthly throughout the year at the eastern coast of Boso Peninsula, Japan. Total phlorotannin contents were compared with the data of various extrinsic factors such as: water and air temperature, nutrient levels and UV radiation recorded at the sampling time. Our results show that previous purely experimental research on phlorotannin variations in controlled conditions might be misleading with regards to natural environment.

5D.8
CLIMATE CHANGE IMPLICATIONS FOR THE NUTRITIONAL QUALITY OF ALGAE

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Algae are important primary biomass producers at the base of the aquatic food chain worldwide and their lipid and fatty acid profiles determine the nutritional quality of algae for organisms at higher trophic levels. In this study the effect of increased temperature (as a result of climate change) on the lipid and fatty acid composition of Scenedesmus obliquus was examined with a special emphasis on the nutritional quality of this alga. The fatty acid patterns show a profile which is characteristic for chlorophytes with the domination of palmitate (16:0), oleate (18:1n-9) and hexadecatetraenoate (16:4n-3) as well as two nutritionally essential fatty acids, 18:2n-6 (linoleic) and 18:3n-3 (linolenic) acids. The distribution of fatty acids among individual lipid classes was typical of that found for C16:3-types of photosynthetic organisms. The major chloroplast glycolipids, MGDG and DGDG as well as the chloroplast phospholipid, PG, were enriched with linolenic acid. The high levels of the essential fatty acids were also found in two non-chloroplast phospholipids, PC and PE. A temperature rise from 20°C to 28°C resulted in an overall decrease in the level of fatty acid unsaturation. The level of linolenic acid together with two other n-3 fatty acids, 16:4 and 18:4, decreased significantly, whereas the levels of linoleic and palmitic acids increased as a result of the temperature shift. S. obliquus accumulated appreciable amounts of TAG as storage lipid and its content increased by 40% when the growth temperature rose. Our data clearly indicated that a rise in global environmental temperature will change the nutritional quality of algae. As a consequence of such alteration, the transfer of fatty acids from algae to organisms at higher trophic levels may also be affected by elevated temperatures with an overall influence on the nutritional and energy status of whole freshwater food-web chain.
PHOTOPROTECTION MECHANISMS AGAINST UV RADIATION IN HETEROCAPSA SP. ARE INFLUENCED BY NITROGEN SUPPLY

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The photoprotective and acclimation capacity against ultraviolet radiation (UVR) was assessed for the dinoflagellate *Heterocapsa* sp. The effects of UVR and N availability on photosynthetic activity and on the accumulation of photoprotective substances as mycosporine-like amino acids (MAAs) and xanthophyll cycle pigments were analyzed. Cells were cultivated under two different light treatments, photosynthetically active radiation (PAR) and PAR + UVR, and at two NaNO₃ concentrations, mid nitrogen (MN, 0.1 mmol L⁻¹) and high nitrogen (HN, 1 mmol L⁻¹) for 6 d. MAA and photosynthetic pigment contents as well as maximum quantum yield of fluorescence (Fᵥ/Fₘ) and electron transport rate (ETR) were analyzed at the initial time and after 3 and 6 d of experimentation. Fᵥ/Fₘ decreased due to UVR and N limitation. N enrichment reduced the deleterious effect of UVR on photosynthesis. The content of photosynthetic pigments and MAAs were higher at HN than at MN supply and a positive effect of UVR on MAA and pigment accumulation was observed, suggesting that under HN conditions the deleterious UVR effect is counteracted by MAAs. Under N limitation thermal energy dissipation takes place by the xanthophyll cycle, i.e., an increase of the de-epoxidation degree was observed under these culture conditions. However, UVR seems to favor diadinoxanthin accumulation, thus no photoprotection through xanthophyll cycle seems available in cells exposed to PAR + UVR and MN. We conclude that not only MAA accumulation but also N availability are very important to determine the photoprotective capacity against UVR of *Heterocapsa* sp.

CONSEQUENCES OF PHOSPHORUS LIMITATION ON MARINE PHYTOPLANKTON COMMUNITY ASSEMBLAGES AND ACTIVITY DURING SPRING TIME

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Understanding how phytoplankton dynamics in marine systems are linked to nutrient cycles has always been a challenge for aquatic ecologists. For the western part of the Dutch Wadden Sea, the actual studied area, long term time series show an increase in soluble reactive phosphorus (SRP), available form of P often in the form of phosphate. In this paper we try to settle the argument whether the western Wadden Sea experiences P-limitation during spring bloom and its influences on the phytoplankton community. The experimental design chosen is a short term nutrient enrichment experiment (called also bioassay) using confined natural phytoplankton communities) with 3 possible nutrient limitations: +NP; +NSi; +PSi; +NPSi, and a control without any addition of nutrient. In order to investigate changes in phytoplankton community, several physiological parameters are measured: maximum quantum efficiency of PSII (Fᵥ/Fₘ), alkaline phosphatase in order to detect a possible P stress in single cells (APA with molecular probe ELF-97), flow cytometer (FCM) pigments compositions and phytoplankton activity with a specific groups primary producers with phospholipids fatty acids (PLFA) labeled with carbon stables isotopes. From the beginning of the bioassays experiments, low concentrations of P are found. The phytoplankton biomass results showed an increase in the bioassays involving addition of P. In addition, the Fᵥ/Fₘ values increased in the treatments with P and decreased APA. The PLFA patterns revealed different succession of phytoplankton community in the different bioassays but all seemed to be P limited.

THE EFFECT OF LIGHT AND NUTRIENT ON GROWTH IN CULTURES OF TRIPALMA LAEVIS (PARMALES, HETEROKONTA)

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A small siliceous species of marine phytoplankton order Parmales (Heterokonta, 1) are abundant in sub-artic and Antarctic seas. Parmales alga (identified as *Tripalma laevis* from the morphology) isolated for the first time by Ichinomiya et al. (2) from Oyashio Current in the western North Pacific has been enabling us to study the physiological properties of this algal group using a laboratory culture system. We have established the stable culture in an artificial sea water medium (Aquil, 3) after slight modifications (4), and the effects of light intensities and nutrient concentrations on the growth were studied using batch cultures. Cultures were maintained under fluorescent lamp light with light/dark (12 h/12 h) cycles at 5°C, and the growth rate (m day⁻¹) was estimated from the increasing in cell numbers during the exponential growth phase. The highest growth (0.45 day⁻¹) was obtained under the light intensity at around 100 μmole m⁻² sec⁻¹, but a significant growth (0.23 day⁻¹) was observed as low as 5 μmole m⁻² sec⁻¹. The nutrient concentrations that provided the maximum growth rate were about 10⁻⁴ M, 10⁻⁵ M and 10⁻⁶ 10⁻⁷ M for nitrogen (NaNO₃), phosphate (K₂HPO₄) and iron (Fe-EDTA), respectively. The growth rate was almost the same at the concentrations of silicon (Na₂SiO₃·9H₂O) between 10⁻⁴ to 10⁻⁶ M, but it was slightly reduced at 10⁻⁷ M. The environmental factors that affect the abundance of Parmales algae in the natural habitat will be discussed.

5D.12

THE EFFECT OF DESICCATION STRESS ON THE FILAMENTOUS GREEN ALGA *ZYGNEMA*

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We investigated desiccation tolerance of the filamentous green alga *Zygneuma* (Zygmenatophyceae, Streptophyta). *Zygneuma* typically occurs in shallow freshwater pools and on the wet soil where it often forms massive mats. As these habitats can easily dry out, *Zygneuma* is supposed to possess adaptations that enable it to survive without water supply for longer periods of time. We tested six different strains of *Zygneuma*, including strains from the Arctic and Antarctic where drought is important environmental stress. Fresh (i.e. growth phase) and old (i.e. starved) cultures were desiccated under three different treatments: (1) 9 days of desiccation under fluctuating (12-h period) relative air humidity (RH) of 96–99%, (2) 9 days of quick desiccation at RH = 40 % and (3) combination of both treatments (1) and (2).

There was a distinct difference in survival between fresh and old cultures. The fresh cultures did not survive desiccation in any of the treatments; however, at least a small proportion of cells from old, starved cultures were able to survive in all treatments. The old cells also differ from the fresh ones in their morphology – they possess a lot of storage material and thicker cell walls. Whether and how these last-mentioned properties affect the cell desiccation tolerance in *Zygneuma* remains a question.

5D.13

IS GAMETOGENESIS AND ZOOSPOROGENESIS IN MICROALGAE REGULATED BY PHYTOCHROME?

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The reproduction patterns in some microalgal species of Chlorophyceae (*Botryosphaerella sudetica*, *Neochloris vigensis*, Bracteacoccus minor) and Eustigmatophyceae (*Trachydiscus minutus*) were investigated. Under external conditions allowing maximal growth these microalgae reproduce asexually producing autosporas or aplanospores. However, appropriate manipulation with external conditions led to the turnover of the reproduction pattern – production of zoospores or gametes. Light was the crucial factor in the reproduction in all tested species. Production of zoospores and gametes was inhibited by light whereas nitrogen had no effect on reproduction. Motile cells emerged when algae were cultivated in darkness; time of dark treatment necessary for zoosporogenesis/gametogenesis differed substantially among tested species. I studied the morphology of motile cells, the mode of sexual reproduction, and the efficiency of both motile stages production and mating events. In order to gain detailed insight into reproduction patterns I have selected *Botryosphaerella sudetica* for investigations under different treatments. Non-actinic red light (RL) applied in the early phase of darkness (2–4 hours) suppressed both zoosporogenesis and gametogenesis, however long-time cultivation under RL had
the same effect as darkness. Dynamics of zooids production was rather fluctuating. The effect of RL and darkness indicates an involvement of the phytochrome in reproductive regulation. We may hypothesize that the active form of phytochrome (Pₜ) blocks both gametogenesis and zoosporogenesis and that Pᵣ reverses to the inactive form (Pᵣ) either under red light treatment or spontaneously in darkness. To consider hypothetical informatory role of light in zoosporogenesis/gametogenesis appropriately, further investigation is needed.

5D.14

TOXIC EFFECTS OF CADMIUM ON SELECTED METABOLIC PROCESSES IN ANABAENA SP. PCC 7120 DURING NITROGEN STARVATION

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In filamentous cyanobacteria like Anabaena sp. PCC7120 metabolic processes of oxygen photosynthesis and nitrogen fixation are mutually exclusive and spatially separated into two distinct cell types but occur simultaneously during aerobic cell growth. N₂ fixation reaction takes place in specialized cells – heterocysts. They differentiate from vegetative cells in response to the deprivation of combined nitrogen. Heterocysts lose activity of PSII and of photosynthetic CO₂ fixation, acquire supplemental envelope layers and undergo other morphological and metabolic modifications to create anaerobic conditions for functioning of oxygen-labile nitrogenase complex. Heterocysts also lose activity of PSII and of photosynthetic CO₂ fixation, acquire supplemental envelope layers and undergo other morphological and metabolic modifications to create anaerobic conditions for functioning of oxygen-labile nitrogenase complex. Heterocysts also have specific hydrogenases and oxidases that contribute to the generation of ATP for the demanding diazotrophic metabolism. Cadmium is toxic at very low concentrations and it is not physiologically essential for most living organisms. Cadmium is considered an extremely significant pollutant due to its high toxicity and great solubility in water. It is commonly found in contaminated ecosystems. In metalloenzymes it can substitute for other metal ions like zinc(II), calcium(II) and copper(II). Cadmium(II) is a powerful enzyme inhibitor and shows a very strong affinity to structures containing sulfhydryl groups. The objective of this work was to study the effect of cadmium on the Anabaena sp. PCC7120 growth on medium without combined nitrogen. Anabaena sp. PCC7120 was cultivated in 250 ml Erlenmeyer’s flasks on modified BG11 medium without nitrogen. Cultures were incubated at 27°C for 28 days. Five different concentrations of cadmium(II) were applied in this experiment (0.5; 1; 2; 5; 10 mg l⁻¹). Cell growth was determined by measuring optical density (OD) at 680 nm and 720 nm. Morphology of cells was inspected under the light microscope. Chlorophyll fluorescence, nitrogenase activity, phosphatases (pH 7,5 and pH = 6,0) activity, cell count and dry mass were measured. Cadmium influenced the growth of Anabaena PCC7120. Exposure to contaminant also affected the activity of phosphatases and nitorgenase.

5D.15

ASSESSMENT OF HEAVY METALS (CD, CO, CR, CU, MN, NI, PB AND ZN) IN WATER, SEDIMENT AND THALLI OF FRESHWATER ULVA IN POLAND

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Studies of the Ulva taxa describe these green macroalgae as good indices of increased concentration of chlorides, nitrogen and heavy metals in seawaters. The aims of this research were to investigate the ability of the freshwater Ulva to the biomonitoring and the use as an bioindicator of heavy metals contamination in lakes and rivers. Three fractions of samples: water, sediment and Ulva thalli were collected from Nielsba River and Malta Lake in the summer season of 2010. Additionally, a chemical composition of waters in which Ulva thalli appeared was measured. In our study ICP-MS method was used. The mean metal concentrations in the freshwater Ulva from lake were decreased in the following order: Mn > Cr > Ni > Zn > Cu > Pb > Co > Cd and in the sediments: Mn > Cu > Pb > Zn > Cr > Ni > Co > Cd, whereas in water the ratio was found to be Ni > Cr > Mn > Zn > Cu > Co, Cd, Pb⁵⁺. The relative abundance of these heavy metals in the algae from river decreased in
the following order: Mn > Zn > Cr > Ni > Cu > Pb > Co > Cd, in the sediment: Mn > Zn > Cu > Pb > Cr > Ni > Co > Cd and in the water: Ni > Cr > Mn > Zn > Pb = Cu > Co, Cd > Cu > Co, Cd BDL. Only Mn concentration in the algae from river showed a significant positive correlation with concentration in the water. There were both positive and negative correlations among some metals in Ulva, water and sediments. Our results are confirming the high ability to the bioaccumulation of heavy metals, especially Mn by freshwater Ulva. The study was supported by the Polish Ministry of Education Science grant No. N N304 0134 37. Co-author - Andrzej Rybak was also supported by Operational Program Human Capital (OP HC-8.2.2) co-financed by the European Social Fund of the EU.

5D.16 LIGHT AND TIME DEPENDENT EXPRESSION OF CALVIN CYCLE ENZYMES IN THE DIATOM PHAEODACTYLM TRICORNUTUM

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Diatoms have experienced a fascinating complex evolution, merging characteristics of red algae and heterotrophic eukaryotic cells via secondary endocytobiosis. They are ecologically very important algae, being responsible for about 20% of total global primary production. Elucidating the mechanism of their highly efficient CO₂ fixation is a challenging task and the insights gained may have a high potential for practical use in genetic engineering, biotechnology and industry. Interestingly the Calvin Cycle of diatoms reveals striking differences in terms of regulation compared to the respective metabolic pathway in green algae and land plant plastids. The capacity for light-induced redox-regulation by thioredoxins is notably limited in diatoms - so far, redox-regulation was only shown for a single enzyme of the Calvin Cycle in diatoms. To investigate how the Calvin Cycle in diatoms may be regulated, we determined the diel expression transcript levels of some Calvin Cycle enzymes in the diatom Phaeodactylum tricornutum at different light/dark conditions via quantitative Real Time PCR, allowing us to characterise the changes in regard to light dependency and circadian control. We identified the plastidic glyceraldehyde-3-phosphate dehydrogenase (GAP C1) and the phosphoribulokinase (PRK) to be strongly regulated in P. tricornutum at the expression level. Interestingly in higher plants and green algae these two enzymes are typically co-regulated by forming a redox-sensitive complex with a protein called CP12, which apparently is missing in most diatoms.

5D.17 METABOLITE PROFILING INDICATES INTRASPECIFIC VARIABILITY IN THE ACCUMULATION OF COMPATIBLE SOLUTES IN THE HAPTOPHYTE ALGA EMILIANIA HUXLEYI

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The marine Haptophyte alga Emiliania huxleyi plays an important role in global biogeochemical cycles through its production of calcium carbonate coccoliths, although the biological role of calcification remains unclear. Using a combination of nuclear magnetic resonance and liquid-chromatography mass-spectrometry techniques, we have characterised the major metabolites of several calcifying and non-calcifying E. huxleyi strains to examine metabolic links to calcification. We examined both haploid and diploid cells throughout their growth cycle and have attempted to identify the major compatible solutes. In accordance with previous research, our findings indicate that both dimethylsulphoniopropionate (DMSP) and cyclohexanetetrol (CHT) are major osmolytes in E. huxleyi. However, the presence and abundance of these two compounds is highly dependent on strain and life cycle phase. A clear distinction is observed in stationary phase cultures, with most diploid strains accumulating DMSP, whereas the majority of haploid strains accumulate CHT and cyclohexanepentol (CHP). We have also identified other compatible solutes, including sugar alcohols, cyclitols and glycine betaine which show significant variation between strains. We conclude that the composition of compatible solutes in E. huxleyi is highly variable. Each strain accumulates at least four or five primary compatible solutes and their abundance varies significantly with culture age, which suggests that environmental factors such as...
pH, light and nutrient availability may influence the accumulation of different compatible solutes. As each of these compatible solutes has different costs and benefits to the organism and different impacts on the cell’s environment, unravelling the complexities of this intraspecific metabolic variability will provide important information for our wider understanding of phytoplankton physiology.

5D.18
SENSING ENVIRONMENTAL STRESS CONDITIONS AND REGULATION OF CELL FATE VIA REDOX SIGNALING IN DIATOMS
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Diatoms form an important group of algae in the oceans, being responsible for about 20% of global primary production. Nevertheless, the molecular basis for their ecological success in oceans is still unresolved. Accumulation of Reactive Oxygen Species (ROS) has been implicated in various responses to changes in environmental stresses in animals, plants and marine phytoplankton. While high level of ROS results in induction of hallmarks of Programmed Cell Death (PCD), moderate levels of ROS act as an acclimation signal to induce mechanisms of resilience to environmental stress. Here we report the use of the redox-sensitive Green Florescence Protein-roGFP as a novel sensitive reporter for oxidative stress and its role in inducing acclimation versus PCD in responses to a wide range of stresses and chemical cues in marine diatoms. We currently investigate how spatial and temporal distribution of ROS production on the subcellular level may be regulated under diverse abiotic and biotic stress conditions and in response to chemical signaling. Diatom transformants exhibited a wide dynamic range allowing high sensitivity to small redox changes in response to various stimuli. Furthermore, roGFP enables accurate intracellular monitoring of ROS and targeting to specific organelles, thus overcoming the pitfalls of the commonly used redox-sensitive fluorescent dyes. We expose transformants of the genetic model diatom *Phaeodactylum tricornutum* to different environmental stress and measure the degree of oxidation of roGFP in various cellular organelles and at the population level response. This was coupled with monitoring cellular markers for PCD, in order to gain important insights into the involvement of ROS in PCD versus acclimation mechanisms in diatoms. We propose the potential use of roGFP expressing diatoms as “environmental redox sensors” and aim to gain important insights of the acclimation of phytoplankton to the ever-changing marine environment.

5D.19
THE IMPACT OF COUMAPHOS ON THE ANTIOXIDANT METABOLISM IN *ULVA* SP.
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Coumaphos is one of several organophosphates used in the process of wool scouring. Effluents from this process reach coastal waters without any treatment, carrying organic substances with half-lives of up to 5 years in soils and sediments, and several months in water. Consultation with a Māori elder from Bluff identified an obvious loss of coastal life, from shellfish to seaweeds, along several parts of the coast of the South Island of New Zealand. These observations were made over the past 40 years, along a coastal area fed by the Oreti River catchment. A number of industries such as three wool scourers, two meat and freezing works, saw milling and intensive sheep and dairy farming are or were situated within that catchment. Water eco-toxicity tests are commonly conducted on invertebrates and fish, but not on plants and algae, neglecting the less obvious impacts toxin contamination can have on primary producers. For the people of Ngāi Tahu the seaweeds *Durvillaea antarctica* (Rimurapa), *Porphyra* sp. (Ngāi Tahu Claims Settlement Act) and *Ulva* sp. are of traditional importance, so we are investigating the ability of these seaweeds to cope with organic pollutants. This is the first report of the impact of Coumaphos, on *Ulva* sp. Enzymatic and non-enzymatic antioxidant levels were measured along a time course experiment over a period of seven days. For the assessment of free radical development and oxidative damage hydrogen peroxide and lipid hydroperoxide levels were measured.
5D.20

ALLELOPATHY IN *SYNECHOCOCUS* SP.: EFFECT ON ALGAL AND CYANOBACTERIAL MONOCULTURES

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Allelopathic interactions are occurring in all aquatic habitats and among all groups of aquatic primary producers. Allelopathy may also play an important role in interspecific competition and contribute to cyanobacterial bloom maintenance (Suikkanen, 2008). In this study, the influence of allelochemicals on the growth, chlorophyll fluorescence and photosynthesis irradiance curves (P-E) of three different phytoplankton species: green alga *Chlorella vulgaris*, cyanobacterium *Nodularia spumigena* and diatom *Skeletonema marinoi* was investigated by addition of cell-free filtrate of *Synechococcus* sp. cultures grown under different light conditions (10, 100 and 190 \(\text{mol photons m}^{-2}\text{s}^{-1}\)). The examined strains were isolated from the Baltic Sea and are maintained as unialgal cultures in Culture Collection of Baltic Algae (http://ocean.ug.edu.pl/c24ccba/).

To determine the effect of the investigated factor and their interaction on allelopathy activity of examined strains, one-way analysis of variance ANOVA (Tukey’s HSD) were carried out. The culture density was estimated microscopically and by measurement of optical density at 750 nm. The measurements of photosynthesis rate were carried out using Clark oxygen electrode and chlorophyll fluorescence were measured using Pulse Amplitude Modulation fluorometry. Addition of cell-free filtrate from *Synechococcus* sp. cultures grown under varied irradiance inhibited *C. vulgaris*, *N. spumigena* and *S. marinoi*. The highest drop of growth, chlorophyll fluorescence and P-E curves of analyzed species were observed after the addition of cell-free filtrate obtained from *Synechococcus* sp. cultures grown under varied irradiance inhibited *C. vulgaris*, *N. spumigena* and *S. marinoi*. The highest drop of growth, chlorophyll fluorescence and P-E curves of analyzed species were observed after the addition of cell-free filtrate obtained from *Synechococcus* sp. cultures grown at 190 \(\mu\text{mol photons m}^{-2}\text{s}^{-1}\). The diatom *S. marinoi* was the most sensitive to the influence of cyanobacterial allelochemicals. At the seventh day of experiment the number of cells in *S. marinoi*, *N. spumigena* and *C. vulgaris* cultures constituted respectively 40%, 70% and 80% of their control. This work suggests that picocyanobacteria *Synechococcus* sp. can inhibited the growth, chlorophyll fluorescence and P-E curves of all tested strains: *C. vulgaris*, *N. spumigena* and *S. marinoi* and the production of allelopathic substances is dependent on light intensity.

5D.21

IDENTIFICATION OF PROTEINS INVOLVED IN OSMOREGULATION IN *CHLAMYDOMONAS REINHARDTII* (CHLOROPHYTA) USING AN INSERTIONAL MUTAGENESIS APPROACH

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The CV is an osmoregulatory organelle that expels water out of the cell. Little is known about the proteins involved in this osmoregulatory action. Therefore, an insertional mutagenesis using a hygromycin resistant gene (pHyg3, Berthold et al., 2002) and *Chlamydomonas reinhardtii* CC3395 was performed. About 1000 mutants were screened for defects in osmoregulation. Mutants will be analyzed by RESDA-PCR (Restriction Enzyme Site-Directed Amplification PCR), to determine which gene is affected and causes the defect in osmoregulation. In addition, osmoregulatory mutants will be characterized regarding growth in media of different osmotic strength and CV activity using video microscopy.

5D.22

PHOTOSYNTHESIS AND CALCIFICATION IN CORALLINE ALGAE (RHODOPHYTA: CORALLINALES)

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Coralline algae (Corallinales) are important primary producers in coral reefs. They are also important sources of inorganic carbon and carbonate sand regardless of their morphology or life style (e. g. attached, free-living, articulated, crustose). Crustose forms have a relevant role as they can act as reef cementers and builders. Their high Mg-calcite (more soluble form) content of their cell walls makes them highly vulnerable to ocean acidification. Combined effect of elevated sea temperature and acidification (global threats) may produce a strong impact on this community.
On the other hand, local threats such as nutrient enrichment (e.g. phosphorus) can inhibit calcification. An evaluation of the magnitude of each threat on the coralline community is needed, especially, for the development of prediction models. Three different coralline morphs have been selected in a Mexican coral reef lagoon (crustose, articulated and rhodoliths) to perform a photo-physiological description prior to experimentally analyze the effect of temperature, pH and phosphorus availability in mesocosms. In this contribution we show preliminary results describing differences among the three morphotypes in light absorption properties (capacity and efficiency), and in photosynthesis and calcification rates. Differences in light absorption explain niche partitioning in the reef environment among the three morphs. Simultaneous determinations of photosynthesis and calcification and the use of inhibitors allow understanding differences and similitudes among morphs in their association and their mechanistic interdependence.

5D.23
PHOTOPERIODIC REGULATION OF RECEPTACLE FORMATION IN SARGASSUM HORNERI USING CLONAL CULTURED THALLI
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We developed a clonal culture for Sargassum horneri (Turner) C. Agard. Regenerated vegetative thalli were obtained using lateral branches excised from a thallus grown from a single embryo under short-day (10 h light and 14 h dark cycles, SD) conditions. The lateral branches excised from a SD-regenerated thallus became vegetative thalli again as far as they were cultured under SD. The regenerated thalli were able to enter the reproductive phase within a short time (ca. 42 days) at a small size (main branch length < 50 mm) when they were cultured under long day (14 h light and 10 h dark cycles, LD) conditions. Suppression of blade formation at the apices of the branches was a distinctive morphological change at the beginning of reproductive phase; elongation of the branches without blades was followed by differentiation of receptacles bearing conceptacles on their surface. Release of motile sperm from mature conceptacles was observed. The apex of the receptacle had an ability to interconvert between the reproductive and vegetative phase because the leaves sprouted again when transferred from LD to SD. We examined the effects of day-length and performed night-break (NB) experiments using different light quality (red-, blue- and green-light) to determine light effects on receptacle formation in S. horneri. The presence of critical night length (between 10 and 11 h dark) and induction of receptacle differentiation by SD with NB strongly suggest reproductive regulation of S. horneri is a photoperiodic long-day response. An NB with blue- and green-light was effective for reproductive induction, but not red-light. This suggests blue- and green-light photoreceptors, but not phytochrome, are involved in the photoperiodic reproductive response of S. horneri.
6D.1

THE ENIGMATIC GENETICAL AND GEOGRAPHICAL DIVERSITY WITHIN GENUS *MICROCOLEUS* (CYANOBACTERIA)

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The genus *Microcoleus* Desmazières ex Gomont occurs frequently in aerophytic biotopes all around the world. In this study, we investigated two species: *M. vaginatus* and *M. steenstrupii*. Studied strains were isolated from different habitats in the Czech Republic – puddles, mineral spring and wet soil. The 16S rRNA gene, 16S-23S ITS sequence data and the morphological characterization were obtained. The phylogenetical analysis of 16S rRNA gene confirmed polyphyletic origin of the genus *Microcoleus* and revealed a significant similarity of our isolates to those deposited in the GenBank, although these sequences originated from soil crusts in the United States. An analysis of 16S-23S ITS helices uncovered a broad diversity within *M. vaginatus* and *M. steenstrupii* cluster. Helices of the Czech studied strains were compared with published data and it revealed following assumptions. Czech *M. steenstrupii* strains showed a significant difference to the American one. In contrast, Czech *M. vaginatus* was not clearly distinguished from the American. Moreover, Czech strains of *M. vaginatus* were found highly variable. Finally, an ancestral geographical distribution of *M. vaginatus* was reconstructed using S-DIVA software (Statistical Dispersal-Vicariance Analysis) based on 16S rRNA gene. This reconstruction demonstrated that *M. vaginatus* ancestors originated more likely from the Europe than from the United States.

6D.2

GREEN, COCCOID PICOPLANKTON FROM MESOTROPHIC OCEANIC AREAS: A NEW CLADE OF GREEN ALGAE.

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The phylogeny, morphology and ultrastructure of a new clade of green algae is presented. The group, here labeled ‘clade VIIA’ to distinguish it from the six previously known clades of ‘prasinophytes’, comprises a number of cultures from the Center for Culture of Marine Phytoplankton (CCMP) and Roscoff Culture Collection (RCC) obtained from the Mediterranean Sea, Pacific Ocean, Indian Ocean and Atlantic Ocean as well as environmental sequences obtained mostly from the Pacific Ocean, but also from other regions such as the Mediterranean Sea and even temperate coastal areas. Clade VIIA is sister to *Picocystis salinarum* that was described from a hypersaline habitat. We present phylogenies based on SSU rDNA sequences from a number of cultures and environmental sequences, and morphology and ultrastructure of the culture RCC 287. The cells of cultured strains are spherical, measuring 2–4 µm, and contain one cup-shaped chloroplast. The cells are usually solitary, but they may aggregate to form colonies. Chloroplast thylakoids may
occur singly or be arranged into stacks. There is no pyrenoid, but one or more large starch grains may be present in the chloroplast. Cells have a cell wall consisting of several layers. The composition of the cell wall is unknown. Strain RCC 287 possesses only carotenoids that are typical of the Chlorophyceae. This contrasts with *Picoeystis*, which contains alloxanthin (typical of cryptomonads), diatoxanthin (typical of heterokonts), and monadoxanthin (typical of euglenoids) in addition to the pigments commonly found in green algae. The clade VIIA species appear to be typical of warm mesotrophic oceanic areas.

6D.3
DIFFERENT TELOMERIC MOTIFS IN CHLAMYDOMONADALES ALGAE

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The ends of linear eukaryotic chromosomes are capped by nucleoprotein structures called telomeres. They are composed of repetitive telomeric sequences and telomere-specific proteins. Telomeres are conserved structures and telomeric sequence motifs could be useful as additional criteria for classification. On the other hand, the widespread distribution of algae in different branches of tree of life offers unique chance to study telomere evolution. The telomeric repeats are typically synthesized by telomerase, a special reverse transcriptase, which use its own RNA subunit as a template determining directly the sequence of telomeric DNA. Using telomere-repeat-amplification-protocol (TRAP), we have detected telomerase activity in different Chlamydomonadales species. To identify telomeric motifs in tested algae, the products of TRAP assays were cloned and sequenced. On the basis of their telomeric motifs, the Chlamydomonadales could be divided into the major groups possessing either TTTTAGGG telomeric repeats previously found in *Chlamydomonas reinhardii* or the *Arabidopsis*-type telomeric sequences (TTTAGGG), the predominant telomeric motif in green algae. In the particular Chlamydomonadales species, their phylogenetic positions based on the rDNA sequences were compared with the occurrence of TTTTAGGG and TTTAGGG telomeric motifs in these algae. Acknowledgements: This work has been supported by the Grant Agency of the Czech Republic (521/09/1912).

6D.4
DIVERSITY AND EVOLUTION OF TELOMERIC SEQUENCES IN GREEN ALGAE

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Telomeres are an essential part of eukaryotic chromosomes and despite the highly conserved mechanism of telomere synthesis, the actual nucleotide sequence of telomeric repeats exhibit a remarkable variation among different eukaryotic lineages. Our aim was to investigate the diversity and evolution of telomeric sequences in green algae. By exploiting public and our own culture collections, we have selected a set of green algal strains from several major groups (Chlorophyceae, Trebouxiophyceae, Ulvophyceae, Klebsormidiophyceae, Zygnemophyceae) that satisfied the criteria of axenicity and good growth in laboratory conditions. Telomeric sequence data were obtained by sequencing products obtained by the Telomerase Repeat Amplification Protocol (TRAP) or by Southern hybridization of genomic DNA with telomeric probes. We further sequenced the 18S rRNA gene and/or the ITS region for most strains to verify their presupposed taxonomic identity and to enable defining their phylogenetic position among green algae. Our preliminary results suggest that representatives of the class Trebouxiophyceae and a subset of strains affiliate
to the class Chlorophyceae exhibit the same telo-
meric sequence as a majority of land plants,
which can thus be considered as a probable plesio-
morphic state for the whole group of
Chloroplastida (Viridiplantae). However, we
found that several representatives of the order
Volvocales within the Chlorophyceae possess a
novel telomeric sequence different from that gen-
eral for the green kingdom. Phylogenetic analyses
aimed at pinpointing the evolutionary switch to the
new telomeric sequence within the Volvocales will
be presented. Furthermore, our results also help to
clarify the identity of some strains. For example,
the strains SAG 1.72 and SAG 34.72 are both cur-
rently maintained under the species name
Chloromonas actinochloris in the SAG collection,
but they differ in morphological characteristics,
in the sequences of the ITS region and in the telo-
meric sequences, suggesting that they represent two
separate species. Acknowledgements: This work
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7D.1
GENOME EXPRESSION MODIFIED BY HERBIVORY AND RELATED ELICITORS IN ROCKWEED, FUCUS VESICULOSUS

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The brown alga *Fucus vesiculosus* is capable of inducible chemical defence. It produces metabolites that act against herbivores “on demand”, i.e. while it is being grazed on. The nature of these metabolites is largely unknown but two triggers of defence response have been proposed: oligo-alginate and methyl-jasmonate. To explore the genetic basis of inducible anti-herbivore defence in this ecologically important brown alga, we developed and applied a microarray. For design of the arrays, we employed 454 sequencing from pooled RNA from grazed and un-grazed *F. vesiculosus*. This resulted in 23099 gene models, which were spotted on the array. For tests on the arrays, RNA from three fully replicated defence induction experiments was harvested at different points in time. In one experiment, isopods grazed on the algae directly, the other two employed oligo-alginate and methyl-jasmonate. Feeding assays proved that all treatments in all three experiments induced a significantly reduced palatability of *F. vesiculosus* to isopods. The two chemical inductions modified genome expression substantially. For the oligo-alginate induction, this confirms that *F. vesiculosus* recognizes cell wall matrix oligo-saccharides, which also originate during grazing. Direct grazing by isopods evoked relatively little change in gene expression, although we may have missed the onset of the transcriptomic response. Overall, we demonstrate for the first time that the ecofunctional genomics approach allows to gain new insights into the underlying processes of defense in rockweeds.

7D.2
ALIGNMENT AND PHYLOGENETIC ANALYSES OF THE COMPLETE nrRNA OPERON USING THE SUESSIALES AS A TEST CASE

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Phylogenetic analyses in the Dinophyceae using nuclear-encoded rDNA have mostly employed either the SSU rRNA gene or the 5’-terminus of the LSU rDNA. Very few studies were based on both rRNA genes or even on the complete rRNA operon. We used the dinoflagellate order Suessiales as a test case to compare resolution and topologies of phylogenetic trees inferred from complete and partial rRNA operons. The experimental setup consisted of 20 taxa of the Suessiales and a dataset with approximately 5000 aligned characters. This alignment was split, and nine different partitions were analyzed. Each partition contained different genes or gene fragments. By comparison of the tree topologies as well as the bootstraps and posterior probabilities we found some interesting differences. The trees show topology differences for the SSU and for the shortest LSU rDNA partition, and the resolution was improved with increasing alignment length. The results suggest that the complete rRNA operon or at least a combination of SSU and partial LSU rDNA enhance resolution in molecular phylogenetic analyses of the Suessiales.
7D.3
CHARACTERISING SEX-RELATED PROTEINS FROM THE BROWN ALGA SCYTOSIPHON LOMENTARIA USING PROTEOMICS
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Many species within the Brown Algae (Phaeophyceae) have a complex life cycle including sexual reproduction. Before mating of gametes might occur, possible mating partners need to find each other. In addition they have to recognize each other as mating partners. In order to elucidate the mating processes of Brown Algae on the molecular level we characterized the proteome of gametes of Scytosiphon lomentaria. The life cycle of this species comprises an alternation of generations with isogametes. In addition S. lomentaria is closely related to Ectocarpus siliculosus, whose genome was published recently. Mating compatible male and female gametes of S. lomentaria were collected from the field site of Charatsunai, Muroran, Hokkaido, Japan. Protein purification was carried out and proteins were subjected to 2D-IEF/SDS-PAGE for separation. Then protein spots were excised and analyzed by mass spectrometry (nanoUPLC-ESI-Q-TOF). These data were compared to the annotated E. siliculosus genome data. New bioinformatic tools were developed for the identification of proteins and for the comparison of proteins deriving from male and female gametes. More than 400 protein spots could be detected and sequenced subsequently. Most of these proteins (>80%) could be identified using the Ectocarpus genome. Some of the non-identified proteins are species-specific for S. lomentaria. For them only de novo-sequences could be obtained so far. Furthermore the results indicate only low variability in the protein patterns of male and female gametes. But some proteins could be detected in one sex only.

7D.4
REDIRECTING CARBON FLUX TO TRIACYLGLYCEROLS PRODUCTION IN SYNECHOCYSTIS SP. PCC 6803
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Algae-based biodiesel is regarded as biodiesel of next generation due to non-food resource and high per-area productivity. Photosynthetic microorganisms that are capable of accumulating lipid as energy storage material are good candidates for biodiesel production, such as algae. Unlike algae, wild cyanobacterium has no ability to form triacylglycerol (TAG) because of lack of key enzyme for synthesizing triacylglycerols. However, this deficient can be improved by genetic engineering. In our study, diacylglycerol acyltransferase (DGAT) gene from Acinetobacter baylii ADP1 was introduced into Synechocystis sp. PCC 6803 to construct TAG synthesis pathway. The positive results of DGAT gene integration into genome and protein expression were verified via colony PCR and western blotting assay. On the other hand, the knockout mutant to block polyhydroxybutyrate (PHB) synthesis pathway was carried out for repartition of carbon flux in cyanobacterium. The mutant containing transformed DGAT gene and phaC knockout revealed oil droplet formation within cell by observing yellow-gold fluorescence after Nile Red staining. Lipid content and TAG production assay in wild type, DGAT expression mutant, phaC knockout mutant and combined one are still on-going.
8D.1

DIVERSITY AND BIOGEOGRAPHIC DISTRIBUTION OF CENTRIC DIATOMS IN EASTERN PART OF HUNGARY

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Centric diatoms are particularly characteristic of phytoplankton of lakes in spring but they are often abundant and sometimes dominant from early spring to fall in large slow rivers of Hungary. They are important for primary production and estimation of water quality. Centric diatoms of the River Danube have been regularly studied by light and electron microscopy, but there are currently only a few sporadic studies for lakes and other rivers. Centric diatoms of 176 different Hungarian waters situated on east side of River Danube were examined. Among them the largest was River Danube where more than 100 samples were analysed by SEM. The smallest ones are different creeks and ponds where only one sample was analysed. The distribution maps of species were created by ESRI ArcInfo 9.3 software. 49 centric taxa were found during this study from 11 genera. The average taxon number was 6 in a sample (7 in lotic, 5 in lentic habitats), the maximum was 40 (40 in lotic, 14 in lentic habitats) and the minimum was 1. _Stephanodiscus minutulus_ was the most frequent species (riches 57 % of all sites). 9 taxa were found more than 20% of sites, 18 taxa between 5–20% and 6 taxa only in one site. Some centrics were found only in lentic habitats (_Chaetoceros mülleri, Cyclotella comensis, C. gamma_) while some ones only in lotic habitats (_Stephanocostis chanticus, Thalassiosira incerta_) but most of them can be found in both habitats. Several lentic species (like _Cyclotella distinguenda, C. ocellata_) were also present in the phytoplankton of rivers and several lotic species (_Skeletonema potamos_) were also present in the phytoplankton of the lakes when they are in connection with each others. The diversity was highest in the large rivers. This study was supported by the National Science Foundation of Hungary (OTKA 68327).

8D.2

NITROGEN FORMS CONCENTRATIONS AS MAIN FACTOR DETERMINING COMPOSITION OF PHYTOPLANKTON AND BACTERIA ASSEMBLAGES IN THE EUTROPHIC LAKE

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Our studies were concerned on defining interrelations between phytoplankton, bacteria communities and environmental factors. We conducted our research in Góreckie Lake (eutrophic lake in NW Poland). Samples (in the number of 83) were collected in every month during whole year in vertical profile (0–15 m). We have analyzed biomass and frequency of phytoplankton (cyanobacteria and eukaryotic algae) and species structure of aerobic, facultative anaerobic and aerotolerant bacteria. We have also analyzed water chemistry of every sample (temperature, conductivity, pH, oxygen, chlorophyll a, phosphate and nitrogen concentrations). To determine environmental gradients for both microorganisms groups we have used multidimensional analyses (DCA, RDA). Most relevant factors determining development of phytoplankton were: water temperature,
conductivity, all forms of nitrogen and phosphates. While for bacteria oxidated nitrogen forms ($NO_3^-$, $NO_2^-$) and conductivity were most important environmental factors. Therefore, there is clear relation between cyanobacteria (*Planktothrix agardhii*, *Aphanizomenon flos-aquae*, *Limonothrix redekei*, *Pseudanabaena limnetica*) and bacteria groups (*Bacillus cereus*, *B. licheniformis*, *B. megaterium*, *B. pneumoniae*, *B. thuringiensis*). We also identified a second group of bacteria connected with high concentrations of chlorophyll *a* (mainly members of genus *Enterobacter*). Moreover, we found virus infecting several *Bacillus* species which can interfere in nitrogen cycle in the system. Our research helps to understand better the influence of water chemistry on planktonic water organisms, especially on the functioning of the microbiological loop in a eutrophic lake. Study was supported by Grant number NN303391136.

8D.3

**PHYTOPLANKTON DEVELOPMENT IN A HIGHLY EUTROPHIC LAKE FROM PAMPA’S PLAIN: A FUNCTIONAL APPROACH**

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During its annual development, phytoplankton communities undergo changes in both their quantity and specific composition. The most frequent species of a specific environment share common suitable attributes and they constitute assemblages that reflect autecological aspects of preference and tolerance. The present study aims to further knowledge of phytoplankton development in an hyper-eutrophic and polymeric reservoir from the Pampa’s plain (Paso de las Piedras Reservoir, Argentina) and in particular (1) to validate the Reynolds’ classification scheme and (2) to analyze the phytoplankton structure and dynamics in terms of functional groups in relation to abiotic variables. We carried out weekly or bi-weekly monitoring from January 2004 to June 2005 at two sampling stations (S1 and S3). Acribing the descriptive species to the associations outlined by Reynolds we found 15 of the proposed functional groups, these groups together accounted for more than 80% of the total biovolume during all the study period. The majority of the associations were typical of eutrophic-hypertrophic systems. Functional groups D, P, H1, F, J and C were the most important. With the exception of H1, all the dominant groups were characteristic of mixed environments. Sampling station S3 showed higher values of biovolume, there were also differences in the relative contribution of some groups to the total biovolume, since in S1 there was a major contribution of J group whereas in S3 there was a major contribution of K and H1 groups. In the same way, during July and August the major contribution to the biovolume in S1 was accounted for group C whereas at S3 was accounted for D. The CVA indicated that the functional groups were significantly more probable than random groups in both stations. The multivariate RDA showed that water temperature, reactive soluble phosphorus concentration and N:P ratio explained a significant proportion of the functional group variation.

8D.4

**THE AEROPHYTIC ALGAE FROM CAVES IN OJCÓW NATIONAL PARK (SOUTHERN POLAND)**

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The studies were conducted in the spring and summer 2008–2009 on the occurrence, distribution and endangerments of the aerophytic algae developing in the six caves (*Lokietka*, *Ciemna*, *Zbożecka*, *Sąpsowska*, *Krakowska* and *Biała*) of the Ojców National Park. In total 65 of algae taxa belonging to three taxonomic classes (that is *Bacillariophyceae*, *Chlorophyceae* and *Cyanophyceae*) were found. However ecological studies on cave algae are specific and rare, although caves represent an almost ideal natural laboratory for algological studies with practically constant ecological parameters. This undoubted diversity of caves habitats characteristics and values cause that they are exposed to numerous dangers (such as: water and air pollution, change of microclimate and destruction of algae and cyanobacteria growing on the caves walls). In the caves single mosses, liverworts, pteridophytes, vascular plants and mainly the aerophytic algae are most frequently occurring. These organisms often play a key role in the food webs and in the colonisation processes of rocky habitats causing the colourful effects on the caves walls. These processes are favoured by a usually stable environmental conditions prevailing in the caves. All these factors
cause that the caves often attract the cosmopolitan species, in consequence of which the native components are gradually eliminated.

8D.5
PATTERNS IN TAXONOMIC COMPOSITION OF EPIPHYTIC DIATOM ASSEMBLAGES FROM TERRA NOVA BAY (ANTARCTICA)

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Epiphytic diatoms are one of the key biotic elements of the Antarctic marine ecosystem. Their unusually high metabolic flexibility gives them a crucial role in the Antarctic trophic chain. Despite being fundamental to the ecosystem functioning, diatom communities associated with marine macroalgae are frequently ignored in the floristic studies of the area. Few attempts to investigate substrate preferences have been reported. The present study provides the first examination and description of the epiphytic diatom flora from Terra Nova Bay (Ross Sea, Antarctica). Material was collected during Italian Antarctic campaigns in the years 1989–2005. Samples of thalli of Iridaea cordata, Phyllophora Antarctica, and Plocamium cartilagineum were taken from different depths at five sampling sites localized along the shore. All observations have been carried out under the scanning electron microscope. The genus Cocconeis was the most common. Two identified, previously described and well known from the Antarctic waters species – Cocconeis antiqua and C. fasciolata, as well as small naviculoid species (most likely Navicula perminuta), appeared in abundance. The Cocconeis component of the assemblage was estimated to approach $10^6$ cells per cm$^2$ on foliose hosts such as Iridaea. Melosira adelie was the only non-pennate diatom to achieve any degree of dominance, although Paralia sol and Trigonium arcticum were common on some of the filamentous parts of host macroalgae. Our results indicate a strong relation between the diatom assemblages and the sampling site. There is no clear evidence of diatoms host specificity. However, the filamentous forms as well as topographic anomalies (e.g. colonies of hydroids or bryozoans) can provide a point of attachment for a wider range of taxa.

8D.6
ENVIRONMENTAL DRIVERS OF PHYTOPLANKTON IN A TURBID ESTUARY: NUTRIENT VS. LIGHT LIMITATION

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Nutrients and light are typically considered the most important drivers of phytoplankton growth in estuaries. Given that phytoplankton plays a critical role in estuarine ecosystems, a comprehensive understanding of how phytoplankton is regulated is needed to properly assess the impacts of eutrophication and other natural or human-induced perturbations. Our main goal was to evaluate the effects of nutrient and light availability on phytoplankton composition and growth in the Guadiana estuary, using microcosm experiments with natural phytoplankton communities collected in the freshwater zone of the Guadiana estuary, a sensitive and pristine estuary, where anthropogenic pressures have been increasing in the last years. Phytoplankton growth seemed to be nitrogen-limited throughout the productive period. During summer, cyanobacteria and the harmful dinoflagellate, Kryptoperidinium foliaceum, responded to N enrichment in the absence of Si. Indeed, the presence of K. foliaceum was observed for the first time in the freshwater tidal reaches of the Guadiana estuary. The significant increase on dinoflagellates and cyanobacteria growth in response to N enrichment in the absence of Si is alarming, because anthropogenic nutrient enrichments usually increase N and P, but not Si. Furthermore, relatively high N concentrations, up to 22 µM, were limiting to phytoplankton growth. These results should therefore be used as a management tool when establishing nutrient criteria and nutrient loading budgets to estuarine waters. Light limitation of phytoplankton growth occurred throughout the year and no photoinhibition was observed at least up to 615 µmol photons m$^{-2}$ s$^{-1}$. In the summer, co-limitation by nutrients prevented a positive response of phytoplankton to light enrichment. Diatoms were the most light-limited group, whilst cyanobacteria were the only group acclimated to low light conditions. High saturating irradiances, high light-saturated rates of primary production and low photosynthetic
efficiencies suggest that phytoplankton was not acclimated to low light conditions that prevail in the estuary.

**8D.7**

**COMPARISON OF THE DIATOM BIOTA AND THE WHOLE BENTHIC COMMUNITIES OF LAKE BALATON**

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Recent studies on benthic diatoms of Lake Balaton are sporadic and we poorly know their role in the whole community. Therefore a microscopic investigation was conducted on samples taken in three basins of the lake from four different substrata in June and August 2008 and then this was supplemented with the examination of bacterial and eukaryotic SSU rRNA genes by denaturant gradient gel-electrophoresis (DGGE) and sequence analysis. Cluster analysis and diversity profiles were used to characterize the community structure data gained by the results of the two assays. Diatom communities showed the separation of the samples from northern and southern shore, and northern ones had higher diversity values. These were also observed in the case of 16S rDNA patterns. This concordance may be due to the detection of plastid DNA of eukaryotic algae. However, diversity profiles of 16S rDNA data showed a tendency of decreasing diversity values with higher trophity levels that wasn’t characteristic to the diatoms. The 18S rDNA patterns showed grouping according to substrata. Based on these data epiphyton samples were grouped together with those of epilithon while in the case of diatoms they made a common cluster with samples of artificial substratum. The most abundant diatom species were *Achnanthidium minutissimum, Amphora pediculus, Cymbella exigua, Encyonopsis minuta, Staurosira grigorszkyi, Navicula cryptotenella* and *Nitzschia dissipata*. Cymbella exigua was found to be characteristic species of reed of Lake Balaton this is in concordance with the results of sequence analysis.

**8D.8**

**DIVERSITY OF TERRESTRIAL MICROALGAE (INCLUDING LICHEN PHOTOBIONTS) ALONG AN ALTITUDINAL GRADIENT IN A TROPICAL MOUNTAIN FOREST (ECUADOR)**

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Terrestrial habitats like epiphytic crusts and soils in tropical rainforests are among the least known habitats for microalgae world-wide. Recent studies of terrestrial green algae in tropical rainforest have almost exclusively concentrated on members of the Trentepohliales (Ulvothecaceae) and molecular methods have not yet been used to microalgae from these habitats. Therefore, recently we began a study the genetic diversity of tropical terrestrial green algae using mainly ITS-2 rDNA as DNA barcodes. Using PCR primers preferring green algal rDNA, an about 300 bp short fragment of the 3’-end of 18S rDNA is simultaneously amplified together with the complete ITS1, 5.8S and ITS2 rDNA regions. The short 18S rDNA fragment allows it to anchor the highly variable ITS2 sequences within a phylogeny, i.e. to allow identification roughly at the genus level. For the ITS2 conserved secondary structure models will be developed and used to search for compensatory base changes (CBCs) that have been found indicative to delineate biological species. With this approach we address the questions whether the same species as in temperate regions are dwelling in the tropics and whether tropical forest sites comprise higher species diversity than temperate sites. An extensive number of defined research plots within the Podocarpus National Park (Ecuador)
is investigated along an altitudinal gradient from 1000 – 3000 m above sea level. The molecular studies will also be complemented by cultures to assess the algal diversity as complete as possible. As a first result various species of the unicellular green algal genera *Heveochlorella*, *Coccomyxa* and *Chloroidium* were frequently found in epiphytic crusts.

**8D.9**

**CHAROPHYTE AND PHYTOPLANKTON COMMUNITIES IN CHARA-DOMINATED LAKES: ENVIRONMENT RELATIONSHIPS AND RESPONSE TO DEPTH OF WATER, LIGHT CLIMATE AND TROPHY**

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We investigated the importance of depth of water, light climate and water chemistry for charophyte and phytoplankton species in two depth mesotrophic lakes: Budzynskie and Wilczynskie. We recorded 11 species of charophytes and 87 taxa of phytoplankton. Both depth of water, depth of light penetration and water chemistry were found to be important factor for charophytes and phytoplankton species. Distribution of charophytes was connected with one clear gradient defined by light climate (underwater irradiance of PAR 400–700 nm) and depth of water. Three different environmental gradients determine occurrence of phytoplankton species: base-richness (Mg, Ca, Na, K, SO₄, and conductivity), trophy of water (N-total, NO₃, PO₄) and optical features of the water (DOM, CDOC, DOC). The average of phytoplankton taxa richness was higher in shallow sites with more species of *Chara* genus, then in monospecific stand of *Nitellopsis obtusa* or *Nitella opaca* in depth water. Our research helps to understand better interrelations between phytoplankton and charophytes communities and environmental factors in depth lakes.

**8D.10**

**SILICA-SCALED CHRYSOPHYTES FROM VIETNAM**

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Silica-scaled chrysophytes from tropical lakes, reservoirs and swamps located in Vietnam have been studied from January 2008 to June 2009. The use of electron microscopy technique allowed for the discovery of 26 species in eight reservoirs and in mangrove wetland. In the class Chrysophyceae, two species belonging to two genera (*Chrysosphaerella*, *Paraphysomonas*) were found. In the class Synurophyceae, 17 species and three varieties of the genus *Mallomonas* have been recorded, three species belonging to the genus *Synura* and a single taxon from the genus *Chrysosidium* were found. Twenty five taxa are reported for the first time from Vietnam. Three *Mallomonas* taxa are new to the science. Generally, flora of silica-scaled chrysophytes in the studied sites comprises species which were found in South-East Asia and other tropical regions before. Only *Mallomonas guttata* Wujek var. *simplex* K.H. Nicholls was observed in the tropics for the first time. Most of the identified species have a wide or cosmopolitan distribution. Among them *Mallomonas guttata* var. *guttata*, *M. peronoides* (Harris) Momeu & Péterfi, *M. portaeferreae* Péterfi & Asmund, *M. splendid* (G.S. West) Playfair emend. Croome, Durrorschmidt & P.A. Tyler, *M. kalinae* Rezacova occurs chiefly in tropical waters. Only *Mallomonas morrisonensis* Croome & P.A. Tyler and *M. favosa* K.H. Nicholls f. *gemma* Durrorschmidt & Croome are restricted to subtropical and tropical regions. The work was supported by RFBR grant 11-04-00420-a.
TERRESTRIAL GREEN ALGAE UNDER DIFFERENT LAND USES AND MANagements: A CULTURE-INDEPENDENT APPROACH

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Green algal communities, present in large amounts in the top few centimeters of soil, may be highly influenced by environmental factors. Therefore, we attempt at exploring the genetic diversity of green algae in various types of soils under different land use and management intensities and to establish key factors that determine the algal community structures. Using green algae-preferring PCR primers, 18S rDNA clone libraries were established from jointly sampled soil cores of 57 defined research plots within the three so-called German Biodiversity Exploratories (www.biodiversity-exploratories.de), exemplary large scale research sites to study the roles of land use and management for biodiversity. To identify the recovered algal clones, their sequences were grouped into OTUs (Operational Taxonomic Units), next closest relatives determined by comparisons with already available sequences and phylogenetically analyzed.

Green algae were the most diverse group in soils of the Exploratories. The predominant group in forest soil was Trebouxiophyceae (37 OTUs) whereas in grassland soils Chlorophyceae (31 OTUs) was more dominant. One Exploratory appeared dissimilar to the two others with respect to their grassland soil communities. Both grassland and forest soils exhibited OTUs that were found exclusively there, with grassland soils being more species rich than forest soils which may be explained by differences in pH. In forests, soil of the unmanaged plots exhibited less OTUs than those under management. Although gradients in the algal communities were revealed using multivariate statistics, they could be explained by management intensity only for one Exploratory. For seven forest plots of one Exploratory also the corresponding green algae on tree barks were studied, but no members of Chlorophyceae were recovered. While all tree bark OTUs were also retrieved from soils (but in lower frequencies), their total number was reduced (13 OTUs) compared to the soil samples of the same plots.

CHANGES IN SOIL ALGAL COMMUNITIES WITH LAND USE AND MANAGEMENT INTENSITY: A CULTURAL APPROACH

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Algal communities present in large amounts in the top few centimeters of soil occupy a key functional role as primary producers and their structure may be highly influenced by environmental factors. Therefore, we attempt to unravel the composition of algal communities from soils under different land use and management intensity. A culture-based approach including microscopy and rDNA sequencing of isolates was performed on jointly sampled soil cores of 57 defined research plots within the so-called German Biodiversity Exploratories (www.biodiversity-exploratories.de), exemplary large scale research sites to study the impact of land use and management on biodiversity. A total of 56 genera of eukaryotic algae were identified with the majority of them from the Chlorophyta, i.e. Trebouxiophyceae and Chlorophyceae. Several of our green algal isolates were phylogenetically and morphologically close to genera recently described only from tropical regions. The diversity data were analyzed using multivariate statistics to test the influence of physico-chemical parameters and land use intensity on the composition of soil algal communities. Among all three Exploratories, there was a general pattern of the differences between forest and grassland algal communities. The algal communities in grassland soils exhibited larger species richness than those in forests combined with a more frequent occurrence of filamentous Xanthophyceae, pennate diatoms and nostocalean cyanobacteria. Algal communities of forest soils were characterized by a considerably higher abundance of chlamydomonads (Chlorophyceae) and genera also inhabiting tree-bark. The intensity of land use was not reflected by significant changes in algal communities because the soil algal diversity was almost the same at intensively managed and unmanaged plots. In contrast, changes in the soil algal communities were significantly correlated with...
physico-chemical parameters such as pH and the carbon:nitrogen ratio.

8D.13

PHYTOPLANKTON IN THE AREA OF THE NERETVA RIVER MOUTH, NE MEDITERRANEAN (2003–2005)

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Despite recent work on the phytoplankton ecology of eastern Adriatic coastal waters, there remain sites that have not yet been investigated. Among these is the area of the Neretva River mouth (Middle Adriatic). Phytoplankton structure and abundance were investigated at one station at monthly intervals from March 2003 to July 2005. There were two peaks of microphytoplankton (cells longer than 20 \( \mu \text{m} \)) abundance in each year, one in spring (March 2004 and April 2005), and the other in July 2004 and 2005. Diatoms dominated the microphytoplankton abundance throughout the year. A main feature of the assemblages was the dominance of more than one species. This likely is owed to the frequent and rapid environmental changes typical of this dynamic ecosystem.

8D.14

DIVERSITY AND BIOLOGICAL PECULIARITIES OF GONYOSTOMUM ALGAE IN LITHUANIA

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Gonyostomum algae were found in 36 Lithuanian lakes of more than hundred studied in 2008–2010. Species occurred mainly in small, closed, and surrounded by swampy banks lakes with pH values ranging from 4.28 to 7.81, conductivity – 13–242 \( \mu \text{s cm}^{-1} \). Two species were described based on morphological characteristics. Gonyostomum semen was the most spread (recorded in 32 lakes) and abundant, bloom-forming (\( > 10 \text{ mg L}^{-1} \)) species comprising over 50% of phytoplankton biomass in twelve lakes. Dependence of the tested microalgae to G. semen species was proved with 99% reliability in three lakes after comparison of amplified 18S rRNA gene with sequences deposited in GenBank also. Another species from this genus, Gonyostomum cf. intermedium showed low productivity (abundance up to 8.6 thous. cells L\(^{-1}\), biomass up to 0.15 mg L\(^{-1}\)) in four lakes. Diurnal vertical distribution of the migratory alga G. semen was studied in two stratified lakes. 70–75% of raphidophytes biomass was cumulated in the upper 0–4 m water layer. G. semen exhibited a upward migration towards the light early in the morning until midday and sink downward through the water column in the afternoon. Vertical, diurnal and seasonal G. semen cell size variations were evaluated. Low temperature and illumination, increased hydrogen sulfide in the anoxic hypolimnion layer could determine heterotrophic algae nutrition, simultaneously the smaller algal cells to compare with the ones combining autotrophic and heterotrophic nutrition in the upper layer. The diurnal algal size variation could be related to raphidophytes vegetative longitudinal and perpendicular division. Specific bacteria and several yeast species developed in the Gonyostomum lakes. Microorganisms were distinguished by their adhesive and biocidal features; therefore they were able to survive in the toxic features-possessing microalgae environment as well as could affect expansion and competition of Gonyostomum.

8D.15

FRESHWATER RED ALGAE IN LITHUANIA: SPECIES DIVERSITY AND ECOLOGY

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Taxonomy and ecology of the highly specialized and specific freshwater Rhodophyta group in Europe is much less studied than the other systematic groups of algae. Data about species diversity and the distribution of freshwater red algae in Lithuania are also scarce. Thirteen freshwater red algal species of 6 genera (Audouinella, Chroodactylon, Batrachospermum, Hildenbrandia, Lemanea, Thorea) have been described from Lithuanian streams and lakes. Species of section Batrachospermum: B. arcuatum Kylin, B. boryanum Sidorot, B. confusum f. anatinum (Sirodot) S.A. Stewart & M.L. Vis, and B. gelatinosum (L.) De Candolle mostly concentrated in 1st–4th order
clear water streams with current velocity 0.02–0.9 m s⁻¹, slightly acidic and alkaline water (pH 6.3–8.6), temperature being 1.0–23.0°C, and water specific conductance 229–780 μS cm⁻¹. B. borystenum and B. gelatinosum are common species in Lithuania. Species of section Turfosa: B. keratophyllum Bory and B. turfosum Bory de Saint-Vincent appears in brown water (pH 3.7–7.6) tolerates large range of conditions: water pH 7.4–8.8, temperature 0.75–22.0°C, relative high water specific conductance 5–30 μS cm⁻¹ and current velocity 0.5–1.3 m s⁻¹. Chroodactylon ornatum (C.A. Agardh) Basson is a rare species in Lithuania. Obtained in deep alkaline (pH 8.35–8.7) lakes with a relatively high water specific conductance (275–328 μS cm⁻¹). The species was found growing atypically – directly on wooden bridge constructions, forms single, clearly visible 2–5 mm tufts. In presentation will be discussed some morphometric features in which Lithuania populations different from other populations in the other regions.

8D.16
FIRST DATA ON A CULTURE COLLECTION OF FRESHWATER ALGAE FROM SARDINIA
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The purpose of this project is to realize for Sardinia identification, selection and in vitro cultivation of photosynthetic microorganisms from different habitats. The focus is to map the alga flora on a regional scale and identify habitats with elevated biodiversity and carry out an inventory of freshwater species at a regional level. The biodiversity of freshwater algae within a specific environment is normally considered in reference to the number of species present. Assessment of biodiversity in aquatic habitats is important for a number of reasons, including comparison of different natural habitats in relation to geographic, hydrological, and specific environmental parameters and to understand fundamental aspects of community structure dynamics. Species richness, genetic diversity and unmodified indigenous communities are all components of biodiversity. This collection was started in June 2010 at the Institute of Botany, Melkonian Laboratory, the University of Cologne, Germany, and later at the Botanical Department at the University of Cagliari. Today the collection comprises fifty non-axenic unialgal strains of prokaryotic and eukaryotic microalgae. A culture can be defined as an artificial environment in which algae grow. To obtain an unialgal culture, one species must be isolated from all the rest using the techniques for isolation and cultivation of microalgae followed Surek & Melkonian (2004). The collection is based on strains isolated from centre-east of Sardinia; currently the collection hosts species assigned to Cyanobacteria, Chlorophyta, Heterokontophyta, Streptophyta, Cryptophyta and Euglenophyta. Some strains of interest are currently being studied in further detail.

8D.17
DESMIDIOLOGICAL COMPARISON OF PEAT BOGS IN TWO MOUNTAIN REGIONS WITH DIFFERENT ENVIRONMENTAL BURDEN
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Two mountain regions of the Sudetes Mts (Czech Republic) have been investigated. Peat bogs of the Jizerské hory Mts represent ecosystems significantly damaged by human activities (especially in the second half of the 20th century). High level of air contamination led to acidification, deforestation and desiccation of the area. On the other hand, the Jeseníky Mts have been much less affected by these environmental changes. Thus, both regions seem to be suitable for comparative study of desmid communities which are well known as good indicators of habitat conditions and biological quality of wetlands. Within thirteen Sudetes peat bogs, 59 permanent plots comprising
Sphagnum lawns, bog pools and lakes have been repeatedly sampled. Desmid diversity, rare taxa occurrence, ecological preferences and indication of ecosystem stability have been studied and compared between the mentioned regions. The total of 93 taxa of desmids was found. Some of them are rare in the Czech Republic (Hyalotheca dissiliens var. iatraica Racib., Stauroastrum simionyi var. semicirculare Coesel, Staurodesmus spencerianus (Mask.) Teiling, Cosmarium furcatuspermum West & G. S. West, Cosmarium notabile var. transiens Insam & Krieger, Cosmarium subquadrans var. minor Symoens). Our results suggest that the recently recorded desmid flora reflects the gradual improvement of the peat bogs in the Jizerské hory Mts, which is not (yet) evident from the macroflora. In conclusion, the character and the value of both parts of Sudetes Mts are recently not significantly different.

8D.18
TRACKING GLOBAL CHANGES IN ANTARCTIC CYANOBACTERIA BIODIVERSITY
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With increasing concern regarding global climate change, evaluation of biodiversity (genetic, species, ecosystem), is necessary in order to track changes within the climate system: it affects interpretations of future global and especially polar climate change. In this context, Cyanobacteria diversity monitoring could be important as a possible biological indicator. Our hypotheses regard diversity and connectivity between diverse habitats across a range of geographic scales. Cyanobacteria have a cosmopolitan distribution in many extreme environments and they adapt to various stress environments, thus playing important roles in carbon and nitrogen cycles modifying morphology, metabolism and light-harvesting systems. By studying antarctic Cyanobacteria biodiversity, its adaptation and distribution at different spatial scales, we aimed at determining whether polar soil ecosystems could be more sensitive to anthropogenic climate change than temperate regions. Cyanobacteria were collected from several subregions of the Antarctica and genetically analyzed by 16S-ITS-23S and TrnL sequencing. Moreover, a data set of gene bank was included for phylogenetic analyses and different habitat was compared between them: Antarctic soil, Mediterranean Sea and lakes. By molecular markers Cyanobacteria species were identified and their phylogenetic analyses was related to their ecotypes characterization (different substrate and geographical coordinates). Results show their affinity of the aplotypes examined by Genbank sequence and their reciprocal relations in that region 16S (partial) and ITS (included trnIle and trnAla). Our results lead to support an independent phylogenetic dataset of ITS and TrnL producing concordant clusters.

8D.19
THE SPANISH BANK OF ALGAE: A CULTURE COLLECTION EXPLORING THE BIODIVERSITY OF MACARONESIAN MICROALGAE
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Macaronesia is worldwide considered a hotspot of biological diversity. Macaronesian microalgae, especially from non-marine habitats, however, have not been studied in any detail until now and therefore their biodiversity remains largely unknown. The Spanish Bank of Algae (Banco Español de Algas: BEA) is a culture collection of microalgae (including cyanobacteria) that has been established in 2003 and is registered with the World Federation of Culture Collections (WFCC) and the World Data Center for Microorganisms (WFCC-MIRCEN). In 2005 the World Intellectual Property Organization (WIPO) accredited BEA as an International Depositary Authority (IDA). The culture collection holds about 400 clonal strains of microalgae, mostly from Macaronesia and other subtropical regions. Currently, one third of the strains are axenic and efforts to cryopreserve strains and create a gDNA bank are underway. The collection covers a wide diversity of taxonomic groups and habitats, including
extreme habitats. Microalgae from all major groups are represented (Chlorophyta, Streptophyta, Dinophyta, Heterokontophyta, Euglenophyta, Cryptophyta, Cyanobacteria). The main objective of BEA is to collect, isolate, identify, and conserve the biodiversity of microalgae and cyanobacteria from Macaronesia and subtropical regions using a multidisciplinary approach. This includes the most up-to-date methods for isolation, culturing, preparation of axenic cultures, cryopreservation and identification, including fluorescence-activated cell sorting and DNA sequencing of nuclear and plastid barcoding regions. BEA also conducts training courses for students and academic and industrial professionals in modern techniques of algal sampling, isolation, purification, growth, conservation and applications.
ASSESSMENT OF THE CARLIT METHODOLOGY IN THE MEDITERRANEAN ROCKY WATER BODIES

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The Water Framework Directive (2000/60/EC) sets up a river basin and transitional coastal water masses management plan. Carlit method was devised by Ballesteros et al. (2007) to assess water bodies’ Ecological Quality Ratio (EQR). Key features of the method are rocky algal communities’ composition, geomorphological features and nature of the substrate. Over the last 4 years, our team assessed all the rocky water bodies of French Mediterranean shorelines, including Corsica within the Directive (over 4,500 kilometres of shore).

Abroad, we evaluated all 9 rocky water bodies of Malta and the entire coastline of Slovenia. In the latter, however, Carlit does not apply because of the tidal regime and the heavy coastal urbanization (95% of the 42-km coastline is artificial). In France, out of 37 rocky water bodies, 19 have a high ecological status, 9 are good, 7 are moderate and 2 are poor. In Malta, 7 have a high ecological status, 1 is good and 1 is moderate. In heavily modified and artificialised rocky water bodies, there may be a discrepancy between the relatively high EQR and the poor ecological status as assessed by experts. For example, in Monaco (FRDC10b), although 80% of the shoreline is artificial, the status as calculated through the EQR is good but the sole macroalgal communities would rank it as moderate. However, on a large time scale, an artificial substrate will tend to turn into a natural habitat suitable for algal colonisation. In order to obtain more coherent results in terms of ecological expertise, we propose to consider man-made structures natural. This would result in lower EQR for over-urbanized seashores. Further work is necessary in order to set up new reference zones better suited to regional features especially in North Western Mediterranean Sea.

DEVELOPING A GLOBAL SEAWEED NETWORK

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Seaweeds (red, brown and green algae) are important sources of food, industrial materials and, potentially, biofuels and biomedical compounds throughout the developed and developing worlds. There is an increasing demand from industry for more detailed taxonomic knowledge and reliable identification techniques of commercially important species, but we are still in the process of understanding their biodiversity and resolving their taxonomy. There is a great need to share algal knowledge and to link data for industrially-used species from around the world. Databases, notably AlgaeBase and EOL, provide a potential framework for this work, but a global effort to make information available and to develop molecular barcoding techniques for the identification of utilizable seaweeds is urgently needed. In January 2010, a workshop ‘Developing a Global Seaweed Strategy’ was held at the Natural History Museum, London. Delegates represented seaweed biodiversity and taxonomy, phylogenetics, the seaweed industry, economics, DNA barcoding, conservation and web-based information (AlgaeBase), with input from Africa (South Africa and Tanzania), USA, Canada, Brazil, SE Asia (Korea, Malaysia) and Northern Europe (France, Ireland, UK). The outcome of this meeting was a Global Seaweed Network: a global seaweed strategy, a document by the members of the Global Seaweed Strategy workshop which set out the project. The vision of the network is to develop a programme over the next 5–10 years that will
enhance and develop the global seaweed community into an internationally recognized and respected scientific body that can innovate, provide knowledge and tools for science, aquaculture, etc and enable economic progress and reach the wider public. In this talk, I will give an overview of this project on behalf of the steering group.

9D.3
VERTEBRATA LANOSA ON ASCOPHYLLUM NODOSUM: EFFECTS ON REPRODUCTIVE AND VEGETATIVE STRUCTURES

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The nature of the symbiosis between Ascophyllum nodosum and Vertebrata lanosa remains largely unresolved. To address this issue we conducted field observations on both vegetative and reproductive parameters of epiphyted and non-epiphyted fronds of A. nodosum. The main aim of this study was to evaluate the effects of the presence V. lanosa on vegetative and reproductive structures of A. nodosum. The samples were collected from Tor Bay Provincial Park, Guysborough County, Nova Scotia. Between April to July 2008, we haphazardly collected 80 non-epiphytized and 81 epiphytized A. nodosum fronds during low tides in the mid-intertidal zone where V. lanosa was common. A second sampling was made in late July 2011 collecting over 200 fronds of both A. nodosum’s populations. We detected significant differences in several analyzed variables: frond length (FL); dry mass (DM); number of primary branches (NPB); number of broken branches (NBB); length of segments added in 2007 and 2006 (LSA07)/ (LSA06); dry mass segmented formed in 2007, 2005 and 2004 (DMS07) (DMS05) (DMS04); number of receptacles added on segments formed in 2007 (NR07) and dry mass receptacles on segments of 2007 (DMR07). All of them were higher on epiphyted A. nodosum fronds. Also, we observed significant and positive correlations between number V. lanosa clumps and A. nodosum dry mass.

9D.4
BIOFILM CULTURES TO UNDERSTAND OSTREOPSIS BENTHIC HABS

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An incubator prototype specially designed for culturing aquatic phototrophic biofilms on substrata at controlled environmental conditions was used to perform semi-continuous flow microcosm experiments with microphytobenthos sampled during toxic Ostreopsis ovata outbreaks in summer along the middle Tyrhenian Sea coast. The system provided the possibility of simultaneously controlling environmental conditions and co-culturing biofilm organisms on a set of artificial substrata in a closed photobioreactor, providing a realistic assessment of community processes and maintaining biofilm integrity during sampling. The design allowed to study the effect of time, site, substratum of in situ growth and the role of key species in community development starting from natural inocula, cultured on polycarbonate slides, in a moving film of K/2 medium (50 L h−1 flow velocity) under controlled temperature (25°C) and light conditions (110 μmol photons m−2 s−1). Benthic dinoflagellates, diatoms and filamentous cyanobacteria constituted significant fractions, in taxon richness and biomass, of the natural biofilms formed on macroalgae and bryozoans used as inocula. Biofilm cells were all embedded in a common mucilage matrix (EPS, exopolymeric substances). Non-destructive, real time monitoring of biomass accumulation was obtained by recordings of transmittance values by light sensors positioned under the slides. Growth curves indicated that biofilms reached a mature stage in 40 to 50 days. Species accrual, growth and compositional shifts over time were monitored using a variety of microscopical technique, including confocal laser scanning microscopy. Initial adhesion and substratum colonisation appeared as stochastic processes, then community structure and physiognomy, especially of the photosynthetic fraction, visibly changed with time. Prevalence of cyanobacteria at late growth stages was detected in all cultures, which showed complex layering, inner voids and channels. HPLC on matrix sugars revealed variations in total amounts and
composition. No toxic compounds were detected in the final communities tested by LC-MS and MALDI-TOF MS techniques.

9D.5
DEVELOPING NANO-SCALE PHYSICAL MODELS TO IDENTIFY MECHANISMS OF PORE OCCLUSION FORMATION IN DIATOMS

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The species-specific biosilica cell walls of diatoms exhibit astounding diversity of morphologies, with structures that span scales from 5 nm to 0.5 mm. Under normal physiological conditions, the finest structures, pore occlusions, assemble and solidify into roughly deterministic, delicate patterns in a matter of minutes. These patterns are believed to be conserved within species and to be propagated faithfully between generations, but vary between species. However, there is evidence of variation in morphology and nano-scale structure with growth conditions. Nevertheless, very little is known about the biological and physicochemical mechanisms that direct nano-mesoscale diatom silica assembly. If the mechanisms are simple or synthetically imitable, or if they can be externally manipulated, then they are potentially of great interest to the nanotech industry. We are studying whether and how a variety of pore occlusion morphologies change in response to changes in growth conditions, including temperature, salinity, pH, and silica concentration. These experimental results will be interpreted in the context of a computational model which closely mimics the pore occlusion morphologies seen in a number of diatom species. Combining experimental and computational data, we aim to make testable predictions about the physicochemical mechanisms directing pore occlusion formation. This poster will outline our approach and present some preliminary results.

9D.6
MACROALGAE COMMUNITIES AS INDICATORS TO ASSESS WATER QUALITY IN THE STRAIT OF GIBRALTAR

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Macrocogalae constitute one of the biological quality elements to assess water quality, as required to implement the Water Framework Directive (WFD). Specifically, composition and abundance of aquatic flora is recommended as a key quality element to assess the ecological status of coastal waters in reference conditions. In the Strait of Gibraltar, very different conditions and pressures can be found ranging from bad to high Ecological Status Class (ESC). This zone is a transitional region between the Atlantic Ocean and the Mediterranean Sea. The sensitivity of one index proposed to the Atlantic region (Reduced Species List “RSL”) and two indices proposed for the Mediterranean region (CARtography LIToral “CARLIT”, and Ecological Evaluation Index “EEI”) were tested in six sites. Algal samples were collected in 15 × 15 cm quadrats (n = 3). In addition, cartography and a species list of the seaweed community was elaborated at each locality. All indices showed sensitivity to the gradient of anthropogenic pressures. However, the references used to apply the CARLIT index in the Mediterranean Sea were not accurate enough for this region. Therefore, based on data obtained in the El Estrecho National Park, new references set conditions were established. On the other hand, EEI showed the worst response because it overestimated the ESC in some polluted places. The most accurate indices to estimate the ESC in this region were RSL and CARLIT.

9D.7
DEVELOPMENT OF A NEW INDEX FOR THE QUALIFICATION OF MACROALGAL COMMUNITIES

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A protocol has been established in the framework of the Benthic Network of Brittany (REBENT) to evaluate the status of macroalgal communities on rocky shores. Two parameters are measured on the field at each bathymetric level corresponding to algal belts in sheltered sites: diversity of macrophytes and covering of each species. Raw data
are then analysed by pooling species in taxonomic, stratum (vertical layer) and structural (morpho-anatomical) groups. Based on these elements, a new index has been developed to estimate both the degree of organization of the canopy and the stability of seaweed communities. The status index of the canopy (IC) corresponds to the sum of three sub-indexes: IT which refers to the evolution degree of the canopy on a taxonomic basis, IS, which takes into account the repartition of algae into vertical layers (stratification), giving therefore an idea of the volumetric distribution of the seaweeds, and IO, which is related to the average level of functional organisation of the community. The index has been applied to both spatial and temporal variations in 14 sites of Brittany, showing its accuracy to point out both constitutive differences and induced evolutions within and between macroalgal communities.

9D.8
COLONIZATION OF MARINE PERIPHYTIC DIATOMS IN BOSNIA AND HERZEGOVINA, EASTERN ADRIATIC
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Bosnia and Herzegovina has a total sea surface area of 8 km² (http://www.mvteo.gov.ba) with a maximum depth of 27 m. The country has only 24.5 kilometers (15 mi) of coastline around the town of Neum which is the only coastal town in Bosnia and Herzegovina and an important tourism destination. Taxonomic and ecological studies of marine diatoms have been started, for the first time ever, in Bosnia and Herzegovina at the beginning of 2010. According to our preliminary data, 116 marine diatom taxa (both benthic and planktonic) within 51 genera have been found in the country. The aim of this study was to determine the colonization processes of diatoms on the surface of the glass substrate. For this purpose, samples were taken from the glass slides at 1 m depth during spring and summer 2011. Periphyton was sampled each two weeks during both seasons. Additionally, samples for physical-chemical parameters were taken to determine environment-diatom relationships.

9D.9
NEVER!! – A BLOOM OF TOXIC BLUE-GREEN ALGAE IN AN UPLAND LAKE IN SNOWDONIA, NORTH WALES, U.K
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The phytoplankton of Llyn Padarn has been studied over the past forty years. This lake is a typical glacially formed, elongate, piedmont lake. It is situated in the Llanberis pass, some 7 km from Snowdon, the highest mountain in England and Wales. This lake is c.3.2 km long with a maximum depth of c.27 m and average depth of 14.3 m and develops thermal stratification from late May-June until September-early October. Only in extremely severe winters (for North Wales e.g. 1986 and 2009) has the water surface frozen over. In the first year of study, 1970, the phytoplankton was dominated by the minute centric diatom Cyclotella glomerata but in subsequent years the dominant springtime diatom has been the common Asterionella formosa apart from in a couple of years when Asterionella has been subjected to severe Chytrid parasitism and been replaced by Tabellaria flocculosa, Fragilaria crotonensis or Synedra acus. After the spring diatom growth the lake then consistently passes through a clear water phase supporting nano-flagellates, most frequently Rhodomonas minuta. In summer, green algae have dominated the phytoplankton including Paulschultzia pseudovolvox and Ankistrodesmus falcatus with lower numbers of Eudorina elegans, Pandorina morum and Stauroastrum planktonicum. Tremendous blooms of S. planktonicum occurred in 1992, the lake appeared like bright green paint and this resulted in a High Court Injunction as the lake had become progressively more eutrophic with decreased water quality. Thus in 1995 phosphorus stripping (tertiary treatment) was introduced to the sewerage treatment plant at Llanberis. However, despite this, eutrophication became further enhanced in 2009 such that a huge bloom of the problematical toxic blue-green alga Anabaena flos-aquae developed forming a visible scum on the water surface from early June until October 2009. Aesthetically, the lake appeared hypertrophic and was toxic, thus all public access to the lake was precluded. This may well have contributed to a declining economic
situation in the Llanberis area. This is a well-known tourist region for mountaineering and climbing and the lake is used widely for leisure, fishing and water sports and is well renowned for its rare endemic fish, the char, a relic from the last ice age.

9D.10
REGRESSION OF CYMODOCEA NODOSA SEAGRASS BEDS AT GRAN CANARIA: AN HISTORICAL PERSPECTIVE

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Seagrass meadows have worldwide experienced a dramatic reduction in the last 30 years. In this study, we compared the shoot density, leaf length and biomass of the seagrass C. nodosa in 9 seagrass meadows at the oceanic island of Gran Canaria (Canary Islands, eastern Atlantic) between 1994–1995 and 2011. We show that the abundance of the seagrass C. nodosa has experienced a drastic decline that has been concurrently accompanied by an increase in the abundance of the green rhizophytic algae Caulerpa prolifera.

9D.11
REASSESSMENT OF THE GREEN ALGAL GENUS COELASTRELLA (SCENEDESMAECEAE, CHLOROPHYTA)

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The Scenedesmaceae form a prominent clade of autosporic solitary or coenobial coccoid green algae. Understanding the actual diversity and taxonomic identity of scenedesmacean species and genera have proven very difficult and molecular systematic analyses led to dramatic changes in the definition of scenedesmacean genera as well as the composition of the whole group as compared to traditional schemes based on morphology. One of the neglected lineages within the Scenedesmaceae is the genus Coelastrella, currently comprising eight species of mostly subaerial algae typically with meridional ribs on the cell surface. Possible existence of additional species is, however, indicated by GenBank 18S rDNA sequences from some strains currently placed into different genera (Scenedesmus, Asterarcys, Coelastrum). We have initiated a project on a systematic reassessment of the genus Coelastrella by studying an array of scenedesmacean strains obtained from culture collections and from our own isolation effort. We are employing morphological observations (light microscopy, SEM, TEM) and sequencing of the 18S rDNA and ITS2 regions to delineate genetically distinct groups and to evaluate stability and plasticity of their morphological characters. Our preliminary results indicate that the genus Coelastrella is far more diverse than previously thought and comprises a number species that need to be formally transferred from other genera as well as several species new to science.

9D.12
MORPHOLOGICAL COMPARISON OF TWO CENTRIC DIATOMS: STEPHANODISCUS TRIPORUS GENKAL ET KUZMIN AND S. VESTIBULIS HÅKANSSON, THERIOT ET STOERMER

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Stephanodiscus triporus Genkal et Kuzmin was described in 1978 based on a study of phytoplankton Volgograd Reservoir (Russia), using TEM. According to the description, this species has small-sized diameter 3.7–10.6 μm, 14-30 striae in 10 μm, 30-60 areolae in 10 μm. S. triporus differs from other species of the genus by the presence of three satellite pores around the valve face fultoportula. This planktonic species has been found in many reservoirs lakes, rivers of Russia and also in Morara River (Slovakia). Using SEM a few additional characteristics could be found: external opening of valve face fultoportula has a small elevation, external tube of rimoportula is longer and larger than spines, external openings of marginal
fultoportulae are surrounded by an arch ending at the edge of valve. Areolae in the valve centre are located randomly. The single row of areolae becomes double, triple or quadruple at the edge of valve face. There are marginal fultoportulae with three satellite pores below a spine on every second to fifth interstriae. The rimoportula is situated in the ring of marginal fultoportulae with well-developed labium oriented in different angle to interstria. *Stephanodiscus vestibulis* Håkansson, Theriot et Stoermer has been described in 1986 from an eutrophic freshwater canal (plankton, Lazy Lagoon, Iowa, USA), having marginal fultoportulae surrounding by an arch (porch-like structure or “vestibule”) and valve face central fultoportula with three satellite pores. *S. vestibulis* was later recorded in Michigan Lake (USA), Big, Manitoba and Ontario lakes (fossil, Canada), Brejo do Espinho Lagoon (fossil, Brazil), Sayama Pond (sediment core, Osaka, Japan), Natkong River (plankton, Korea), Greifen and Pfäffikon Lakes (Switzerland), Sèvre Nantaise River (epilithon, France), Voltoya River (epilithon, Spain), Balaton Lake and rivers Danube and Tisza (plankton, Hungary). *Stephanodiscus triporus* and *S. vestibulis* have several similarities in valve morphology; they are discussed in details on the poster.

9D.13

**ASSEMBLE – ASSOCIATION OF EUROPEAN MARINE BIOLOGICAL LABORATORIES**

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ASSEMBLE (www.assemblemarine.org) is an EU FP7 Access to Research Infrastructure Initiative comprising a network of marine research stations with sites across Europe as well as in Israel and Chile. The fundamental objectives if ASSEMBLE are to enhance transnational access to existing key infrastructures with respect to the provision of marine model organisms. Thus, this initiative comprises access, research and networking activities. ASSEMBLE infrastructures are of particular interest to the phycological community. The network includes 2 major algal culture collections (CCAP and RCC) whose resources can be accessed under this scheme (both on site and remotely). Also, most of the ASSEMBLE partner institutions provide access to coastal and oceanic algal habitats, with adequate lab facilities, sea-going equipment and scientific diving available to the user community.

On-site access can be applied for during 2 annual calls (submission deadlines usually February 15 and September 15).

9D.14

**ROLE OF THE CULTURE COLLECTION OF BALTIC ALGAE IN DISCOVERING UNIQUE PROPERTIES OF BALTIC MICROALGAE**

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The Baltic is a semi-enclosed sea and the world’s largest brackish water ecosystem. Its short period of existence, natural salinity gradient from marine to almost freshwater conditions and strong anthropogenic pressure, make it an exceptional environment for living organisms and very interesting field of studies on ecophysiological response to environmental stress. Microalgae play very important role as primary producers in all water ecosystems. Recently, their great potential for practical application in biomonitoring and biotechnology has also been acknowledged. However, this diverse group of organisms is still relatively poorly recognised, thus more research on the morphology, phylogenetics and ecophysiology is required, and culture collections of algae can provide valuable material for this purpose. The Culture Collection of Baltic Algae (CCBA) was established in 1980s and is located at the Institute of Oceanography in Gdynia. The main aim of the CCBA is to isolate, maintain and provide microalgal strains for use in research, education and biotechnology. The main emphasis is put on Baltic cyanobacteria, green algae and diatoms, however a number of strains were isolated from inland waters and obtained from other collections is also maintained in the CCBA. It includes some interesting taxa, e.g. strains which are typical for the region, harmful or potentially suitable for medical and industrial uses. The complete list of strains is available on the web page: http://ocean.ug.edu.pl/~ccba/. The research conducted at the Laboratory of Marine Plant Ecophysiology, Institute of Oceanography, is based on laboratory factorial experiments carried out on strains maintained at the CCBA. Among others, the effect of salinity, as one of the main factors having a great influence on the structure and functioning of the Baltic ecosystem, is studied in the context of morphological and
ecophysiological response of microalgae. Moreover, the autecological characteristics and allelopathic activity of picoplankton strains are investigated. Certain taxa are also applied in algal toxicity tests for determining toxic effects of xenobiotics like ionic liquids and nanosilver.

9D.15
GRATOL – ASSEMBLING THE GREEN ALGAL TREE OF LIFE.

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The GRAToL project will assemble a Tree of Life for the green algae, a diverse group of photosynthetic organisms that play important ecological roles in freshwater, marine and terrestrial habitats. A minimum estimate of diversity is just over 14,000 species, with members distributed on all continents and across virtually every habitat type on Earth. The GRAToL project includes over 25 participating scientists, collaborators, and trainees, who contribute taxonomic expertise on the different branches of the green algal tree of life, as well as extensive knowledge of data collection approaches, data analysis, and bioinformatics. We are using an approach that is a hybrid of PCR-based multigene sequencing, morphology and next-generation sequencing methods. The team will assemble a green algal tree of life by sequencing 10 genes for 425 taxa, sampling orthologs from the nucleus, mitochondria, and plastid genomes for phylogenetic analysis. A global analysis will be used to make a reclassification for all green algae. Next-generation sequencing is being for a subset of 16 taxa to study deep phylogenetic relationships in the green algae, identify new target genes for detailed analysis of particular groups, and examine organelar genome evolution in the green algae. The overall goal of this project is to prepare a new classification for all major branches of green algae, in addition to an analysis involving sister groups (red algae, embryophytes). This project will seek to integrate data with other AToL projects (red algae, heterokonts, liverworts and other embryophytes) to identify core genes for broad phylogenetic analyses. A public web site for GRAToL (www.gratol.org) is being developed that includes a library of images, resulting phylogenetic trees, and other resources. We will disseminate new phylogenetic analysis methods through free, open-source software. This research is funded by NSF GRAToL: Assembling the green algal tree of life (NSF 1036495 TO JLB).

9D.16
CONSERVATION OF SEAWEEDS SUSCEPTIBLE FOR PROTECTION IN GALICIA (NW SPAIN): A GENETIC APPROACH

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The flora of Galicia harbours a number of species of conservation concern because of their rareness and high ecological value. The red seaweeds Pseudopolyides furcellatus, a recently described autochthonous species, as well as Ahnfeltiopsis pusilla and Grateloupia lanceola, two species with populations isolated by more than 1000 km from their closest conspecifics, are example of this group that could also be susceptible of protection by the regional government. In this regard, while some information about the abundance and distribution of these macroalgae is available, the population structure and connectivity of regional demes remains unknown. This information could be very helpful to assess the conservation status of these species and to infer to what extent their regional populations are vulnerable to stochastic environmental and anthropogenic events. In order to fill this gap, we conducted several population studies using different neutral genetic markers (AFLPs and microsatellites). Our results confirm that AFLPs provide higher resolving power than microsatellites in species with low genetic diversity due to the larger number of markers analyzed. However, microsatellites are less sensitive to low quality of DNA, a relatively common problem with marine
macroalgae. Regardless of the marker, we found a number of similarities among the 3 species: low genetic diversity, significant spatial genetic differentiation at large scale (hundreds of km), and a clear isolation by distance pattern. However, the magnitude of the genetic differentiation among enclaves was species-specific, and advises against any quick extrapolation from one species to another even if they are relatively close taxa (Rhodophyta) with similar distribution ranges. Financial support was provided by Spain’s Secretaría de Estado de Investigación, Ministerio de Ciencia e Innovación (Project CTM2007-61011).

9D.17

STRUCTURAL RESPONSE OF MICROSCOPIC ALGAE TO ULVA MASS APPEARANCE IN INLAND WATERS

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In freshwater ecosystems, the Ulva mats development may influence the composition and quantitative structure of microscopic algae by changing habitat conditions through restraining accessibility of nutrients and light. This study describes the phytoseston assemblages of freshwater ecosystems, which grows at exposed habitats in high Ulva thalli occurrence. During summer seasons (2006–2010) material for phytoseston analysis has been sampled from different habitat types (lake, pond, river, streams) with Ulva thalli appearing on a large scale. Water samples were taken directly from under the Ulva patch as well as from the open water without macroalgae for comparison. Additional variables, such as salinity, pH, conductivity, nutrient and chlorophyll a concentrations were also determined. The particular sampling stations showed variations in the taxonomic structure of phytoseston assemblages connected with separate type of water bodies, the size of thalli patches of Ulva and the length of their appearance time in the summer. Ulva sites were characterized by distinct richer species diversity, total abundance and biomass when compared with the open water sites. It was observed that cryptophytes and some euglenoids dominated irrespective of the sampling station, while diatoms (mainly Cocconeis placentula) dominated in the Ulva sites. Green algae (e.g. Tetraëdron, Desmodesmus, Scenedesmus, Monoraphidium), Cryptomonas rostrata and Cyclotella radiosa revealed their dominant character within the zone of open water. Besides, our findings clearly show that Ulva thalli stimulated the rise in the share of epiphytic diatoms in the water and at the same time inhibited the green algae development in phytoseston communities (statistically significant correlations). The study was supported by the Polish Ministry of Education Science grant No. N N304 0134 37.

9D.18

MORPHOLOGY AND SYSTEMATICS OF A NEW SPECIES OF GRACILARIA (RHODOPHYTA) FROM THE MEDITERRANEAN SEA

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Gracilaria Greville (1830) is an important economic red seaweed genus being the major agarophyte resource in the world. It is characterized by a wide phenotypic variability and great species diversity. In many cases these features lead to the lack of clear morphological diagnostic criteria for species recognition making the systematic of this group very problematic. Progress in molecular techniques has led to solve many problems in species identification and to revise the systematics of the genus Gracilaria (Gurgel & Fredericq, 2004). Several markers of different DNA regions have been used to study phylogenetic relationship at the species level: the chloroplast encoded rbcL gene, the rbcL-rbcS intergenic spacer region, the mitochondrial cox1 gene, the cox2-cox3 spacer, and the nuclear internal transcribed spacer ITS. Here we report the characterization of a new species of this group identified through molecular analyses, using the rbcL gene and the rbcL-rbcS spacer, combined with morphological observations. This new entity was found on artificial rocky substrata of the low midlittoral and upper sublittoral zones of the Venice Lagoon during the period from March to July 2009–2010. Thalli exhibit green-yellowish pigmentation with pink shades and dense bushy and spiny branching in the distal portions. RbcL results indicate that our Venice specimen is conspecific
with another Gracilaria species (named Gracilaria-L102) reported from southern Sicily by Gargiulo et al. (2006) and not identifiable with any other species described until now for the Mediterranean Sea. The recent finding of this new species is probably not due to an extra-Mediterranean introduction, but is the consequence of its misidentification with other specimens whose morphologies are very similar: Gracilaria gracilis in the Venice Lagoon and Gracilaria conferta in Sicily.

9D.19

POPULATION STRUCTURE IN DESMIDS SPECIES MICRASTERIAS ROTATA AND M. THOMASIANA (ZYGNEMATOPHYCEAE)

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The existence of population structure in protists is a hotly debated topic. Protists often form huge populations, and because of their small body size, they are assumed to have high dispersal potential. These two factors led to the “everything is everywhere” hypothesis, which posits that protists have unlimited geographical ranges and therefore lack population structure (Finlay 2002). On the other hand, Weisse (2008) proposed that protists differentiation is based on the microhabitats. Most population studies have focused on heterotrophic protists, while studies on phototrophic protists are limited. These studies, which are based on molecular markers, generally show quite strong population differentiation linked by geographic distance. Desmids could comprise the protists group with differentiated populations as they embody the properties like sexual reproduction, diplontic life cycle and biogeographic pattern. The species Micrasterias rotata and M. thomassiana are well defined morphologically as well as by chloroplast intron sequences of RNA-Gly transfer gene (trnGucc). We have investigated 106 strains of M. rotata and 151 strains of M. thomassiana, obtained from SVCK culture collection and isolated from natural habitats across Europe, mainly from Czech Republic. We get cDNA of four nuclear low-copy markers and according to them we have designed specific primers for these genes. In the actin sequences we have amplified two exons and one intron regions. M. rotata strains were highly variable not only in intron sequences, but also in exon regions. The structure of M. rotata and M. thomassiana populations based on the low-copy nuclear markers (actin, gapC, gapA) will be presented. These preliminary results indicate that the unexpected variability among the populations of broadly distributed species M. rotata and M. thomassiana could be based on ecological parameters of the microhabitats rather than linked to the geographic distance.

9D.20

MACROALGAE AS BIOINDICATORS IN THE ADRIATIC SEA: THE APPLICATION OF CARLIT AND EEI METHODS

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A strong incentive to development of new bioindicator methods to assess the state of aquatic environment was declaration of EU Water Framework Directive. New methods that use marine macroalgae to measure the ecological status of benthic communities were published in the past decade, among them the Ecological Evaluation Index or EEI (Orfanidis et al., 2001) and CARLIT (CARTography of LITtoral, Ballesteros et al., 2007). CARLIT method quantifies communities with different sensitivity to disturbance on a macroscale by visual estimation of coverage on at least 50 meters of coastline. EEI quantifies total vertical coverage of species sorted into different functional groups on a microscale, from destructively collected samples (400 cm²). CARLIT method was developed and is currently in use in the Western, while EEI was developed and used in the Eastern Mediterranean Sea. The Adriatic Sea is a specific environment, with its eastern Croatian coastline complex and more than 6000 kilometers long. To find the most suitable method for bio-monitoring according to the WFD requirements, EEI and CARLIT indices were applied and tested from 2009 to 2011 on eight locations in the middle and southern Adriatic Sea. The results were evaluated separately for each method and compared to available pressure data. On the locations where both of the methods were applied, results are compared. The strengths and weaknesses of methods are outlined and problems in the application, as sea urchin grazing, are discussed.
Nongeniculate coralline algae are an outstanding group of marine benthic calcareous algae with a worldwide distribution from the poles to the tropics, and from the intertidal to 250 m. Key components of some marine communities, they display an ability to successfully colonize unsuitable habitats by building biogenic habitats (e.g. maerl beds). Their high plasticity leads to morphological convergences among phylogenetically distant taxa. As a result, the early 20th century experienced an inflation of described species, but later studies found that the taxonomic classification should be based on more accurate morpho-anatomical features. Lately, the advent of new molecular tools has revealed that the current taxonomic scheme is still unstable and problematic, and exposed the presence of cryptic species. In the particular case of maerl beds, two maerl-forming species are commonly cited for NE Atlantic: *Phymatolithon calcareum* and *Lithothamnion corallioides*. Recent studies have uncovered the occurrence of a new species (*Mesophyllum rhodoliformis*) in NW Iberian Peninsula (Galicia), suggesting that European maerl beds could be more biodiverse than previously thought. If their taxonomic status is still open to debate, the genetic diversity and structure of maerl beds is totally obscure. In this scenario, we recently started a research project aimed to investigate the population genetics of maerl beds in NE Atlantic with a focus on Galicia and French Brittany (two regions where maerl beds are particularly important habitats). As a first step, we have assessed the use of DNA barcodes to discriminate between the various species of maerl-forming algae. Additionally, we have investigated the feasibility of developing high-resolution molecular markers (microsatellites) using new, high-throughput sequencing approaches. Early results from these research efforts are presented here. Financial support: Spain’s Ministerio de Ciencia e Innovación (CTM2010-18787) and Xunta de Galicia (10MMA103003PR). VP acknowledges financial support from the British Phycological Society.
barcodes (COI-5P) as a benchmark for reliable species discrimination, we have determined the various species found across this range and assessed a number of morpho-anatomical characters as potential diagnostic features. Here, we present the results achieved with this work. CP acknowledges financial support by the grant Bolsas de investigación from Deputación da Coruña.

9D.23

EPiphytism in Dictyota dichotoma Population from Argentina

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Dictyota is one of the largest genera of the order Dictyotales (Phaeophyceae) that habitat template and tropical coasts. Dictyota dichotoma is characterized by parenchymatous and flabelled thalli, which grow from single apical cells transversally oriented. In Argentina as in other sites of the world, the epiphytic relationships on this host are scarce known. The main aim of this work is study the presence, abundance and seasonal variations of the epiphytic load on D. dichotoma. Samples were collected from subtidal zone by scuba driving during April 2008 to March 2009 in “Las Grutas” beach from Rio Negro Province, Argentina. Samples were fixed in formaldehyde 5% solution to posterior observation under OM. D. dichotoma thalli were divided in apical, medial and basal regions, where it was analyzed the epiphytism’s degree. Each of these sections was subdivided in other 5 consecutive subsections to determined possible epiphytic gradients of distribution.

D. dichotoma were colonized by 37 epiphytic organisms. The diatoms had higher representation in number of genera. The more abundant species were: Microchaete auriginea, Calothrix fusca, Grammatophora marina, Cocconeis sp., Stylonema alsidii, Audouinella secundata, Polysiphonia sp. and Acrochaete marchantiae. During January a higher amount of represented phylla was observed. During August the higher epiphytic abundance was registered, mainly represented by G. marina, M. auriginea and Cocconeis sp. During spring was observed the higher epiphytic load, accounted for M. auriginea. Medial host’s regions presented the higher number of represented phylla and epiphytic load in winter, mainly represented by G. marina, Cocconeis sp. and M. auriginea. In the other two regions, apical and basal, it was during spring. Moreover, it was observed a gradual increase of epiphytic load from subsections 1 to 5 in all three regions of host’s thalli.

9D.24

Crustose Epiflora of Subtidal Maërl Beds in the Continental South Portugal

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The Atlantic European maërl beds serve as refuges for a high number of taxa and stages of heteromorphic life histories that are represented under crustose forms. Some of them such as Cruoria cruoriaeformis and Halarachnion ligulatum stage (“Cruoria rosea”) are almost restricted to these subtidal habitats. In the recent years (2006–2009), it was carried out a research study about the distribution and associated flora of maërl beds in the Northwest of the Iberian Peninsula (Galicia) and South Portugal (Algarve). New records of crustose taxa were published from Galician beds. Here, we provide new data of this group of algae from the Algarve region (Armaçao de Pêra and Lagos-Portimão). The examined samples were collected by dredging and SCUBA diving at 13–23 m depth. We present new records for the Portuguese continental coast: Contarinia peyssonneliaeformis, Cruoria cruoriaeformis, H. ligulatum stage, Peyssonnelia armorica and Atractophora hypnoides stage (“Rhododiscus pulcherrimus”). The female reproductive structures of C. peyssonneliaeformis are also described. Other crustose species such as Peyssonnelia coriacea, P. dubyi and P. harveyana increase their distribution range currently reported on the Portuguese coasts. The diversity and composition of crustose flora obtained are also compared with those recorded in other Atlantic maërl beds from the Iberian Peninsula (Galícia) and neighboring regions. Financial support for this work was provided by Spain’s Ministerio de Educación y Ciencia and FEDER (CGL2006-03576/BOS).
9D.25

A REVISION OF *DASYA* C.AGARDH (DASYACEAE, RHODOPHYTA) IN THE WESTERN MEDITERRANEAN BASIN

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*Dasya* is a complex genus, with a great taxonomic and nomenclatural confusion that still remains unresolved. The revision of species growing in the west Mediterranean basin (from northwestern Italy and Algeria to the Iberian Peninsula) has evidenced the existence of eight taxa, one of which is described as new: *D. baillouviana*, *D. corymbifera*, *D. hutchinsiae*, *D. ocellata*, *D. patentissima* sp. nov. prov., *D. rigidula*, *D. rigescens* and *D. sessilis*. Some collections identified as *D. punicea* have also been demonstrated to belong to some of the above mentioned taxa, and therefore the presence of this species in this area needs to be confirmed. Synonymy and types are included for the accepted taxa, as well as a brief diagnosis that facilitates the identification. Furthermore, the taxonomic value of morphological characters traditionally used for taxonomy is discussed. Some of them are demonstrated to be not useful and are therefore discarded, whereas other new characters are shown to be of high diagnostic value. A preliminary phylogeny of the west Mediterranean taxa of *Dasya* is also reported, based on rbcL sequences (cpDNA). On the basis of the obtained trees, character state distribution patterns are evaluated, and conservative characters are identified. Two well-defined morphological and molecular groups are identified, the taxonomic significance of which is discussed.

9D.26

DEVELOPMENT OF A QRT-PCR ASSAY FOR MONITORING OSTREOPSIS CF. OVATA IN THE MEDITERRANEAN SEA

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*Ostreopsis* is a benthic/epiphytic dinoflagellate genus known to produce palytoxin–like compounds. In the last few years, serious cases of human intoxication associated with *Ostreopsis* spp. blooms have been recorded along the Mediterranean coastlines. Two species (*O. cf. ovata* and *O. cf. siamensis*) are being found with increasing frequency in the Mediterranean basin, but a correct identification of these species by microscopy is very difficult due to strong resemblance and the high variability of some morphometric features. For this purpose, we developed a quantitative real-time PCR assay for the identification and quantification of *O. cf. ovata* in field samples that were collected at the Conero Riviera, (northern Adriatic Sea) during 2009 year both at surface seawater and as epiphytic microflora on macrophytes. This method is based on the SYBR Green I real-time PCR technology and combines the use of a PLSUO standard curve (a plasmid containing the target LSU rDNA gene of *O. cf. ovata*) with a “gold standard” created with pooled crude extracts from environmental samples collected during the bloom to normalize the variability of LSU rDNA gene copy number per cells between analyzed samples. Based on their similar PCR efficiencies (95% and 98%, respectively), the exact rDNA copy number per cell was obtained in cultured and environmental samples. The sensitivity of the qrt-PCR assay was of cells g⁻¹ fw or 1⁻¹ in macrophyte and seawater samples, respectively, and the high reproducibility demonstrated the accuracy of the technical set-up in the complete ranges of quantification over time. The abundance of *Ostreopsis* was analyzed also by inverted microscope. Results were compared with qrt-PCR for validation method. This molecular approach may be considered alternative to traditional microscopy and applied for the monitoring of benthic toxic microalgal species in the marine ecosystems.

9D.27

PHENOLOGY OF THE ALIEN SEAWEED *GRATELOUPIA TURUTURU* POPULATION IN THE MAR PICCOLO OF TARANTO

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The Mar Piccolo in the Gulf of Taranto (northern Ionian Sea, southern Italy) was described as one of the most relevant transitional water system acting like a hot spot of alien species in the Mediterranean Sea. The basin was continuously monitored since 1986 in the framework of several research projects. As a result, the alien red macroalga Grateloupia turuturu Yamada (Rhodophyta, Halymeniales) was firstly observed in February 2007, probably introduced by the shellfish transfers occurring along the docks where the mollusc import-export firms are mostly placed. The observed population along the docks where the mollusc import-export firms are mostly placed. The observed population of G. turuturu showed a clear clumped spatial pattern in the Mar Piccolo with thalli settled both on natural (stones, mussel valves) and artificial (small ropes, plastic tubular nets for mussel culture, fishing nets) hard substrata. In particular, thalli were mainly observed in the eutrophic zone at the intertidal and upper sub-littoral level, down to 50 cm of depth. The vegetative and reproductive phenology, as well as the recruitment of the species in the basin, was analyzed by monthly field measurements carried out from October 2008 to October 2010 on thalli settled on natural hard substrata. The highest number of upright thalli and the longest blades were recorded from December to May. On the contrary, less numerous and shorter uprights (2 cm at most) were observed together with encrusting thalli from June to October. The longest blade (80 cm) was found in April 2009. Tetrasporophytes and gametophytes were found during February-March and March-June, respectively. During the summer-autumn and winter-spring periods, significant differences were observed in both mean number of thalli ($P < 0.01$) and mean lengths ($P < 0.05$) as well as in the blade length distributions ($P < 0.001$). The study is ongoing to verify if the species will become persistent.

**9D.28**

**NUMERICAL MODELLING OF ENVIRONMENTAL AND ANTHROPOGENIC EFFECTS ON THE DIATOM **Skeletonema marinoi** **

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The Northern Adriatic Sea is characterised by shallow waters and considerable fresh water input mainly coming from the Po River, representing a primary source of nutrients and pollutants. The ready availability of dissolved nutrients produces massive diatom blooms along the north-western Adriatic coast. This work, based on experimental and model data, investigates on the relationship between two selected environmental factors (temperature and light intensity) and the diatoms growth. To reproduce the exponential PAR decrease through the water column, under seasonal environmental conditions, batch cultures of the diatom *Skeletonema marinoi* were grown at four temperatures (10, 15, 20, 25°C) and three light intensities. 10 and 15°C were chosen as optimal diatom growth temperatures, while 20 and 25°C were selected to study the algal behaviour under extreme conditions as possible scenario expected under global warming. Finally, to test anthropogenic impact, the algal growth was investigated under increasing temperature conditions and in the presence of the herbicide Terbutylazine at 5 different concentrations (1, 5, 10, 20, 30 μg/L). This compound is frequently used in agriculture, especially in crops farming, and it is the most abundant herbicide found in Italian ground, river and marine coastal waters. The algal growth was monitored by measuring carbon, chlorophyll and polysaccharides cell content, nutrients consumption (nitrate and phosphate), photosynthetic activity, turbidity and cell abundance. Results show a strong dependence of growth rate on temperature and light intensity conditions. Under extreme temperature conditions a strong effect of Terbutylazine on algal growth is observed. Experimental data are compared with outputs of the zero-dimensional version of the Biogeochemical Fluxes Model (BFM). The BFM is a generic biomass based numerical model of the Carbon, Nitrogen Phosphorus and Silicon marine biogeochemical cycles. Model and observed data do not fit very well, indicating that future model simulations need to be improved.

**9D.29**

**PRELIMINARILY STUDIES OF INTER- & INTRASPECIFIC GENETIC POLYMORPHISM OF TWO THALASSIOIRALES SPECIES**

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The phylogeny of Thalassiosirales was recently reconstructed according to nuclear and chloroplast genes which markers also revealed the historical pattern of freshwater colonization, some genera however need additional revision. *Skeletonema potamos* is the sole member of *Skeletonema* genus which lives only in freshwater, it can be also found in rivers Danube and Tisza in Hungary, but the strait phylogenetic position of this species has been unclear. There are some questions about the taxonomy of *Cyclotella* genus as well, which is not a monophyletic group. *Cyclotella ocellata* is one of the characteristic diatom species in Hungarian freshwaters, but with its closest relative (*C. boda-nica*) it is well separated from all the other *Cyclotella* species into a distinct clade which includes all the species of *Stephanodiscus*, *Cyclostephanos* and *Discostella* genera. *C. ocellata* often shows high morphologic divergence within and among populations which makes more difficult to clear the inter- and intraspecific relationships and suggests the existence of cryptic species. To determine the accurate phylogenetic position of *S. potamos* useful tools are the nuclear genes of SSU and LSU rRNA and for higher resolution rbcL and psbC in chloroplast genome. In the case of *C. ocellata* we are testing amplified fragment polymorphism (AFLP) whether it is an appropriate method to reveal the assumed genetic polymorphism behind the different morphotypes.

**9D.30**

**FOTTEA – AN INTERNATIONAL PHYCOLOGICAL JOURNAL**

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**9D.31**

**SEXUAL REPRODUCTION AND HAPLOID PARTHENOGENESIS IN THE RAPHID DIATOM AMPHORA COMMUTATA**

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Sexual reproduction of the raphid diatom *Amphora commutata* was observed in the present study. We conducted crossing experiments with clones isolated from brackish water in the delta of the River Ebro, Spain, which showed that *A. commutata* is heterothallic. Successful pairs produced two auxospores, each of which formed an initial cell that subsequently underwent mitotic divisions. Sibling auxospores were separated and cultured from 10 parental pairs, i.e. 20 clones in total. Back crosses revealed that all the sibling pairs comprised opposite mating types. During the sexual reproduction of this species, gametes were released by each gametangium to fuse with those produced by the gametangium paired within it, belonging to the opposite mating type. We interrupted this process by isolating released gametes before they fused. Out of 25 gametes successfully isolated and transferred into culture wells, four became auxospores and gave rise to viable vegetative haploids, whose frustules were less heavily silicified than that of diploids. It was unclear from which parental clone the gametes were derived, because the parental pair was disrupted during gamete isolation. Ploidy was confirmed by a quantitative method using Feulgen staining. Then we crossed the haploid clones against the same parental diploids that had been crossed originally to induce gamete release. Each haploid clone could mate with only one of the parental diploids, most likely the one that did not give rise to the haploid’s founder gamete. In pairs of haploid
and diploid cells, each gametangium produced two gametes and of the four gametes thus formed, two fertilized each other to form viable F2 progeny, whereas the other two remained unfused or formed a zygote that aborted.

9D.32
THREE PENNATE DIATOMS FROM ISCHIA THERMAL DISTRICT (NAPLES, ITALY)
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Ischia thermal District (Naples, Italy) has been famous since Greek and Roman period for therapeutic purposes and to date the numerous establishments that compose it are attended by thousands of people. As the Euganean District (Padova, Italy), another Italian thermal environment, the surface of Ischia thermal muds is colonized by several organisms, among which cyanobacteria and diatoms. Several surveys have shown the important contribution of Euganean microorganisms to the anti-inflammatory and therapeutic properties of the mud (Tolomio et al. 1999; Lalli et al. 2004; Marcolongo et al. 2006) as well as characterized them (Ceschi-Berrini et al. 2004; Moro et al. 2007a; 2007b; Moro et al. 2010a, 2010b). On the contrary, Ischia thermal biodiversity has been less investigated, with only one study by Pitschmann dating back to 1969 and a recent survey by Sciuto et al. (2011). Here we report the characterization of three raphid pennate diatoms isolated by two tanks of Ischia thermal District, according to morphological (light microscopy and scanning electron microscopy) and phylogenetic analyses. The phylogenetic analyses have been carried out using the rbcL gene as a molecular marker. The three isolates represent different species from the pinnulariid, naviculoid, and nitzschiid groups. Their precise identification, according to both the morphological and molecular data, is discussed.

9D.33
SYSTEMATICS AND BIODIVERSITY: A JOURNAL DEVOTED TO WHOLE-ORGANISM BIOLOGY
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Systematics and Biodiversity is a quarterly, international, peer-reviewed life science journal published by Taylor & Francis for The Natural History Museum, London. The journal is devoted to whole-organism biology. The criterion for publication is scientific merit. Systematics and Biodiversity documents the diversity of organisms in all natural phyla, through taxonomic papers, while also addressing topical issues relating to biological collections, and the principles of systematics. It particularly emphasises the importance and multi-disciplinary significance of systematics, with contributions which address the implications of other fields of systematics, or which advance our understanding of other fields through taxonomic knowledge, especially in relation to nature, origins, and conservation of biodiversity, at all taxonomic levels. The Editor does not accept single species descriptions, monographs or serialised part-studies for publication. Taxonomic/systematic manuscripts must include modern methods such as cladistics, phylogenetic analysis, etc. Instructions for authors can be accessed at: http://www.tandf.co.uk/journals/TSAB. Manuscripts should be submitted to: http://mc.manuscriptcentral.com/tsab. The Editor is available to discuss ideas and/or the suitability of preliminary drafts of manuscripts.

9D.34
ECOLOGICAL DIFFERENTIATION OF CRYPTIC SPECIES WITHIN AN ASEXUAL PROTIST MORPHOSPECIES
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Taxa of microbial eukaryotes defined on morphological basis display a large degree of genetic diversity, implying the existence of numerous cryptic species. However, it has been postulated that genetic diversity merely mirrors accumulation of neutral mutations. We used a cosmopolitan, widely distributed morphospecies of asexual filamentous green algae - Klebsormidium flaccidum (Streptophyta) - as a case taxon to study the cryptic diversity in protists. The concatenated ITS rDNA + rbcL phylogeny of more than sixty European strains revealed the considerable genetic variability within the K. flaccidum morphotype. The results of inferred character evolution indicated the existence of strong phylogenetic pattern.
in at least two phenotypic characters. Moreover, the lineages exhibited strong ecological preferences to one of the three selected habitat types: natural subaerial substrata, artificial subaerial substrata and aquatic habitats. All above-mentioned evidences pointed to the existence of a high number of cryptic species within the single protist morphospecies. We suppose that the species turnover time and speciation rate in some lineages of small-sized protists could be comparable to that for macroorganisms, regardless of their huge population sizes. We consider that the permanent existence of genetically and ecologically well-defined cryptic species is enabled by the mechanism of periodic selection.

9D.35

POLYSIPHONIA IBERICA SP. NOV. (CERAMIALES, RHODOPHYTA) BASED ON MORPHOLOGY AND MOLECULAR EVIDENCE

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A new red algal species (Polysiphonia iberica) is described from the Atlantic Iberian Peninsula (Basque country, Galicia and South of Portugal), based on morphology and molecular evidence. It is confined to sciaphilic subtidal rocky bottoms and can be distinguished by its erect habit, 5–7 orders of dichotomous branching, axes 350–900 μm in diameter at the base, segments composed by 9–11 pericentral cells and 0.5–2 diametres long. Plants are bright red to brown-red in colour, 3–6 cm high, firm at the base but ultimate divisions are soft and flaccid. Cortication is only restricted to the base of large thalli. Plants are initially attached to substrate by a discoid holdfast with the tips of decumbent branches reattaching with secondary discoid holdfasts or rhizoids, which are cut off from pericentral cells. Lateral branches develop independently from trichoblasts or scar cells, which are absent. Tetrasporangia (25–40 μm in diameter) are placed in straight series of up to 15 segments. Sexual structures are unknown, even by culture in laboratory. The new species is assigned to Polysiphonia sensu lato based on these morphological features, but it shows intermediate characters between Neosiphonia and Polysiphonia sensu stricto. It resembles Neosiphonia in having holdfast discoid, erect indeterminate branches developed from the main axes and rhizoids cut off from pericentral cells, however P. iberica has lateral branch initials separated by naked internodal segments, trichoblasts absent and tetrasporangia in straight series, as it occurs in Polysiphonia sensu stricto. On the basis of the morphological features, it is assigned to Polysiphonia sensu lato, but it is not possible to include it in Neosiphonia neither Polysiphonia. Molecular data also indicate that P. iberica is closely related to the “multipericentral” group and/or “Neosiphonia” group rather than Polysiphonia group. Contribution to the Spanish projects CGL2006-03576/BOS (Ministerio de Educación y Ciencia, FEDER), CGL2009-09495/BOS (Ministerio de Ciencia e Innovación, FEDER) and KOPRI project PE11030 (Korea Polar Research Institute).

9D.36

ALIEN MARINE MACROPHYTES IN TURKEY

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A list of accepted alien marine macrophytes occurring on the coast of Turkey is presented. The list consist of 12 Rhodophyta (Red algae), 13 Phaeophyceae (Brown Algae), 6 Chlorophyta (Green algae) and 1 Magnoliophyta for a total of 32 taxa at specific and infraspecific level. Data concerning geographical and Mediterranean distributions and the possible vector of introduction of each species are also given.
ULTRASTRUCTURAL STUDY ON PLASMODESMATA OF BROWN ALGAE

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Multicellular organisms have intercellular connecting structures which play important roles in the cell-to-cell communication. Brown algae are known to have complex multicellularity and plasmodesmata (PD) as well as green plants. We conducted the ultrastructural study on PD of several brown algal species including Dictyota dichotoma, Laminaria japonica, Sytospthon lomentaria and Ectocarpus siliculosus. Brown algal PD were unbrunched simple microchannels with diameter of 10–30 nm. In contrast to PD of green plants, the desmotubule was not observed in PD of the samples analysed in this study. Brown algal cells have the thick cell walls. In Dictyotales, Fucales and Laminariales, dense PD gathered in the relatively thinner cell wall region to make the pit field. Especially in the Dityota and Laminaria, many membranous structures were closely associated with the pit field, which was thought to play some parts in the molecular traffic through the pit field. On the other hand, the pit field was not found in Ectocarpales, Desmarestiales and Sphacerariales, in which PD were dispersed in the cell wall. The pit field seemed to be developed in the species having multiseriate system while not in the uniseriate filamentous species. Therefore, it was assumed that occurrence of the pit field was related to the complexity of the body plan in brown algae. Additionally, the electron tomographic analysis of the cytokinesis of Dictyota revealed that the pit field was formed in the certain area of the cytokinetic diaphragm and the cell wall subsequently developed from the pit field. The determination of the position of PD in the cell wall would be important factor for the morphogenesis of the complex multicellularity in brown algae.

STRAINS OF THE GENUS TETRASELMIS FROM GREEK COASTAL LAGOONS OF THE IONIAN SEA

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Twelve strains of phytoplankton belonging to the genus Tetraselmis Stein (Prasinophyceae) were isolated from coastal lagoons of the Ionian Sea. Strains V 2-2 & V 2-3 were isolated from Vatatsa coastal lagoon part of the Kalamas River delta in the north-west coast of Greece. Strains R 1-1, R 1-2, R 1-3, R 9-2 & R 9-3 were isolated from the closed inner lagoon of Rodia part of the Louros River delta in the semi-closed embayment of Amvrakikos Gulf in the central-west coast of Greece. Strains T 3-1 & T 3-2 were isolated from the Tsoukalio coastal lagoon adjacent to the Rodia Lagoon communicating through a narrow and deep channel. Strains Mes 5, Mes 17 & Mes 20 were isolated from the Mesolongi salt pans of the wider coastal lagoon of the old Acheloos River delta in the opening of the Korinthian Gulf in the south-west coast of Greece. Following biochemical characterization (proximate analysis, fatty & amino acid profile) presented elsewhere, here we present results of photosynthetic pigment composition and morphometric characterization using Nomarski and transmission electron microscopy in an effort to identify the strains to the species level. According to results some strains were classified as members of Tetraselmis wettsteinii (Schiller) Throndsen, T. gracile (Kylin) Butcher. For the rest the pending molecular characterization based on certain RNA sequences (e.g. small ribosome subunit 18S) might aid in the clarification of their exact taxonomic position.

FRESHWATER DIATOMS OF ILE AMSTERDAM (TAAF, SOUTHERN INDIAN OCEAN)

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Ile Amsterdam (77°34’E, 37°47’S), located in the southern Indian Ocean close to the East Indian mid-oceanic ridge, is one of the most remote and isolated oceanic islands in the world. The island is entirely volcanic and constituted of a single cone culminating at 881 m of altitude. The isolated geographical position and special physical features have lead to the presence of a very typical diatom flora with 97 taxa belonging to 30 genera. Both cosmopolitan and endemic sub-Antarctic taxa were found. The most important taxa were *Frustulia lebouvieri* Van de Vijver & Gremmen, *Eunotia paludosa* Grunow var. *paludosa*, *Planothidium lanceolatum* (Bre´bisson) Lange-Bertalot, *Kobayasiella subantarctica* Van de Vijver & Vanhoutte and *Eunotia muscicola* Krasske var. *muscicola*. Both chemistry data and diatom distributions indicate that altitude, conductivity and pH are the major factors in grouping and separating the waterbodies on Ile Amsterdam. Based on the DCA and CCA, the freshwater bodies can be divided into 3 main groups: a first group of acid lakes and ponds occurring in the central part of the island, a second group of coastal streams and rivers with high salinity levels and finally, a third group containing the samples with the highest pH values and PO4 concentrations. The diatom flora reflects this division and seems to provide excellent response to the measured chemistry data. The results indicate that a specific diatom flora exists on the island and seems to be distinctly separated from the non-marine diatom flora in other parts of the sub-Antarctic Region. This survey was made possible with the financial support of the Institut Polair Français - Paul-Emile Victor (IPEV) in the frame of the Terrestrial Ecology program 136. Additional funding was provided by the Science Foundation, Flanders (project: G.0533.07 N) and by the Funding of Ministry of Education of Czech Republic; project No. MSM0021622416.

**9D.40**

**PORPHYRA SUBORBITICULATA (BANGIALES, RHODOPHYTA) IN NORTHERN SPAIN, AN ASIATIC SPECIES NEW TO EUROPEAN ATLANTIC**

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In recent years, the number of alien seaweeds reported from the Atlantic European region has increased, but it is predicted that use of molecular techniques will reveal the occurrence of more cryptic introductions. In the course of the taxonomic review of the genus *Porphyra* in the Iberian Peninsula (Spanish government project CGL2008-00932/BOS), we localized one population of *Porphyra suborbiculata* in Santander (Cantabria, Northern Spain). This species, originally described by Kjellman in 1897 from a specimen collected in Goto, Japan, is widely distributed in Asian waters (Japan, China, Korea, Vietnam and Philippines) and it has been reported as an introduction based on molecular evaluations to the shores of New Zealand, Australia, Pacific Mexico, Brazil and Atlantic USA. Iberian specimens were identified and confirmed as *P. suborbiculata* by comparing rbcL gene sequences with GenBank sequences of this species from New Zealand. Thalli collected from Northern Spain were small (up to 2 cm), shortly stipitate, pink to red in colour, monostromatic, and blades had dentate margins. It is a monoecious species, with male sori in small patches, and endosporangia with an irregular number of spores occurring near the blade margins. Reproductive blades were observed during all months it was present (January to June). It grows on rocks, barnacles (*Chthamalus* spp.) and limpets (*Patella* spp.) within the mid- to upper intertidal zones. This species is annual,
occurring from winter to spring. The population reached maximum densities in May (late spring), when the largest blades were observed, began to decline in June, and had completely disappeared by July.

9D.41
IDENTIFICATION OF THE MATING TYPE LOCUS IN PENNATE DIATOMS
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Diatoms have a unique diplontic life cycle characterized by gradual size reduction which ultimately leads to programmed cell death. The latter, which often leads to loss of strains and cultures, can only be avoided by sexual reproduction, which enables cell size restitution via a specialized type of zygote, called the auxospore. This essential link between cell size recovery and sexual reproduction is unique for diatoms. Until now however, not much is known about the molecular basis of sexual reproduction and sex determination in diatoms. Most sexual determination systems are genetically determined by a specific region, the so-called mating type (MT) locus. We are currently identifying the sex determination system of the pennate marine diatom *Seminavis robusta*. An F1 mapping population was made of 2 parental and 116 progeny cultures. AFLP analysis was performed on the mapping population using 54 different primer combinations resulting in 500 AFLP markers. High density linkage maps were constructed using these markers. This resulted in 28 linkage groups. The sex locus was integrated in the linkage maps by including the sex phenotype as a fictive marker. Illumina sequencing was used to identify sequence alterations between a bulk of the + mating type and a bulk of the - mating type strains of the F1 mapping population. The highest differences in allelic counts are currently being mapped on the *Seminavis* genome sequence which will allow identifying and characterizing the mating type locus. This will be followed by a functional characterization of the MT genes by RT-PCR and genetic transformation, and study of the evolution of the selected MT locus genes in related diatom species.

9D.42
SPECIES COMPOSITION, ZONATION AND BIOMASS OF SEAWEEDS IN KONGSFJORDEN, SVALBARD - A BASELINE STUDY
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Recent predictions indicate that the water temperatures in Fram Strait of the North Atlantic will increase until the end of this century by about 1.5°C in winter and 3.5°C in summer. As temperature is one of the most important factors controlling biogeographic distribution of seaweeds, distributional shifts are an inevitable effect of global warming. Here, we present data (from 1996) on species composition, zonation and biomass of seaweeds from Hansneset, Kongsfjorden on Spitsbergen, Svalbard (78°55’N, 11°56’E). The majority of species (71%) belonged to the Arctic-temperate distribution group, whereas 6% were endemic Arctic species and the remaining 23% were cosmopolitan species distributed from the tropics to the Arctic. The upper sublittoral (3–5 m depth) was characterised by the brown algae *Fucus distichus*, *Pylaiella littoralis*, *Chordaria flagelliformis*, *Saccorhiza dermatodea*, species of the genera *Acrosiphonia* and *Spongomorpha* (green algae) and *Devaleraea ramentacea* (red alga). The dominant species in the mid-sublittoral (5–15 m depth) were the brown algae *Alaria esculenta*, *Laminaria digitata* and *Saccharina latissima*. The red algae *Callophyllis cristata* and the brown algae *Desmarestia viridis*, *Sphacelaria plumosa* and *D. aculeata* occurred as undergrowth species. The lower sublittoral (15–30 m depth) was the brown alga *Alaria esculenta*, *Laminaria digitata* and *Saccharina latissima*. The red algae *Callophyllis cristata* and the brown algae *Desmarestia viridis*, *Sphacelaria plumosa* and *D. aculeata* occurred as undergrowth species. The lower sublittoral (15–30 m depth) was characterised by the red algae *Phycodrys rubens* and *Ptilota gunnari*. Both species also grew as undergrowth species in the mid-sublittoral. The endemic Arctic brown alga *Laminaria solidungula* was a rare species at our study site; it
is mainly found in the inner part of Kongsfjorden. The maximum wet biomass in the upper sublittoral was 4.5 kg m\(^{-2}\), in the mid-sublittoral 8.6 kg m\(^{-2}\) and in the lower sublittoral 0.9 kg m\(^{-2}\). In 2012, the study will be repeated at the same site in order to demonstrate possible changes in biodiversity, biomasses and zonation during a period of 16 years.

9D.43

PATTERN OF ALGAL DIVERSITY IN FISHPONDS CONTAMINATED WITH HEAVY METALS (UPPER SILESIA, POLAND)

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Pollution of freshwaters and sediments by potentially harmful metals is a serious problem in many regions of the world. The major sources of contamination are from mines. In Poland, many mines are located in Upper Silesia – southern part of Poland. This region is heavy impacted by anthropogenic activity. Algae can contribute to the movement of metals from the polluted water to the bottom sediments. Our studies were focused on the phytoplankton in five fish ponds, which are impoundments of the Matylda River. For more than 100 years, the Matylda River had been polluted by runoff containing zinc and lead ores from a mine in Chrzanów. Finally, the mine was closed in 1972. We found that sediments from four of these ponds (MM, MS, MD, MDo) are still highly contaminated with Cd, Zn and Pb. Their concentrations oscillate from several to a thousand times higher compared to the geochemical background. The sediments of the fifth pond (MK) have a lower level of contamination of heavy metals, because they were removed three years ago. We found that the phytoplankton composition was similar between the four ponds (MM, MS, MD, MDo) with contaminated sediments and diatoms, green algae, and occasionally cyanobacteria were dominant. This might be due to a higher tolerance to heavy metals by some of the species, especially diatoms and green algae. The phytoplankton composition of the pond (MK – sediments removed three years ago) was different, since chrysophytes were dominant. We did not find any significant differences in densities of algae between the fishponds. These results suggest that the removal of sediments in one of the ponds (MK) reduced the heavy metal loading, which resulted in a very different phytoplankton community structure but not their density. Thus, we conclude that heavy metals concentrations in sediments did not affect the growth of plankton communities, but probably modified their composition.

9D.44

PROBLEMS AND SOLUTIONS IN GRACILARIA SYSTEMATICS: AN EXAMPLE FROM G. BURSA-PASTORIS (GMELIN) SILVA

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With the spread of the DNA barcoding method (Hebert et al., 2003), more and more authors have shown how the use of molecular markers can shed light on the systematics of problematic seaweed taxa that cannot be solved with morphological characters alone. This is true also for members of the genus Gracilaria Greville (Saunders, 2009; Destombe et al., 2010). In fact, Gracilaria is a taxonomically challenging group because of structural simplicity, high morphological plasticity, and great species diversity. Classically, the identification of Gracilaria species has been based on gross morphological characters and incorrect applications have often led to misidentifications. Moreover, species delineation is problematic for the limitations of distinct morphological and reproductive characteristics (Pareek et al., 2010).

Here we focus on some Mediterranean entities, both preserved in herbarium collection of the University of Padova (Italy) and recently sampled, that have been attributed or are morphologically ascribable, respectively, to the species Gracilaria bursa-pastoris (Gmelin) Silva. Long since, it is known that G. bursa-pastoris, the current type species of the genus, is characterized by the presence of several morphotypes. This can lead to two possible errors: assignment of different morphotypes to distinct species or identification as G. bursa-pastoris of species actually different, thinking that they represent diverse morphotypes. The use of
rbcL-rbcS intergenic spacer as molecular marker has provided new insights on the different Mediterranean Gracilaria entities considered, revealing also the existence of a species, collected in 1869 and preserved in an historical herbarium, never sequenced before.

9D.45
ALGAE AND CARNIVOROUS PLANTS – RELATIONSHIPS?

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Charles Darwin was one of the first scientists to notice many living organisms in the bladders of Utricularia which were not killed by the plant. Twenty years later similar observations were made by Polish phycologists M. Raciborski who reported detailed information about the algae living inside and outside Utricularia purpurea traps. From that time, specially during last decade of 20th century, several authors had described algae associated with traps of various taxa of carnivorous plants. Now we know that the traps of carnivorous plants have a complex microbial food web with bacteria forming the main viable microbial biomass in their traps. The research problem generally goes into two directions: taxonomical and cytological studies of algae and physiological ones. The second ones give us the possibility of understanding interactions between a “plant-farmer” (carnivorous plant) and a “guest” (algae). Two independent opinions have been reported recently. One says that algae are digested in the carnivorous plant traps, especially in those which occur in oligotrophic waters and the other one says that algae settle inside the traps and develop either independently or as symbionts. The second opinion was checked by explorers from Austria, Czech Republic, Slovakia and by us. Our study is based on the material from the traps of various carnivorous plant genera; we found that, in this special environment, different taxa of algae are in good condition. The aim of this study was to investigate the biodiversity of algal flora occurring inside and associated with the traps of carnivorous plants. The work was carried out within the scientific project No. N 304 220135 supported by the Polish Ministry of Science and Higher Education.

9D.46
DIATOMS FROM SURUGA-BAY DEEP SEAWATER, JAPAN

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Deep seawater (DSW) is very well known of its stable law temperature, very low pollutants, but also to be rich in nutrients. Due to these features, enterprises and national fisheries scientific experiment stations all over Japan try to utilize it for various purposes. Macro algae and fish are cultivated in the several of these facilities with DSW running through the water tanks. While there has been number of studies focusing on morphology of diatoms growing in those tanks, no-one still has investigated the reason behind their abundance and origin. Current study focuses on this issue and tries to determine diatoms existence in the deep layer of the ocean. 250 mL, 500 mL, 1 L, 1.5 L of the sample of DSW collected at Suruga-Bay Deep Seawater Aquaculture Research Center of Shizuoka Prefectural Research Institute of Fishery, Japan (34°50’55”N, 138°19’24”E) that pumps the water from 397 m depth on December 21st, 2010 and March 4th, 2011 were filtered and observed with light and scanning electron microscopy. Additionally, 200 mL (five beakers of 40 mL each) was incubated for 14 days under 18°C with photoperiod of 14 L: 10D in order to see whether some species grow up. The full frustules were regarded as “survived in DSW”. Full frustules, dormant cells of Thalassiosira species and Rhaphoneis crinigera were observed. Additionally, the following taxa were observed in the incubated water: Chaetoceros (3 species), Ditylum (2), Nitzschia (4), Odontella (1), and Skeletonema (2). These results indicate that the observed species may survive in DSW under certain conditions.
A CYTOMORPHOLOGICAL AND MOLECULAR DESCRIPTION OF NEW MICROALGAL STRAINS FROM THE MALTESE ISLANDS

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The biodiversity of microalgae and cyanobacteria forming subaerial phototrophic biofilms in Maltese hypogea has been recently described. Amongst the strains isolated in culture, the non-heterocytous (Oscillatorialean) and heterocytous (Stigonematalean, Nostocalean) cyanobacterial strains were predominant. The predominant microalgae included chlorococcalean (Chlorellalean) and filamentous (Trentepohlialean) strains. These strains now form part of the Maltese Catacomb Culture Collection (MCCC) housed at the University of Malta. This collection is presently being enlarged to include cyanobacterial and microalgal strains from other environments such as caves and the biological soil crust (BSC). A combined approach was applied for an accurate assessment of taxonomy and phylogeny of strains in the culture collection. The ecology and cytomorphology of these microorganisms were described. Different gene fragments (16S and 18S rRNA) were then amplified and sequenced in order to describe these organisms at generic level. Since these DNA fragments are relatively conserved, the internal transcribed spacer (ITS) region was also considered to estimate relationships at the subgeneric level. A multigene approach was thus adopted in which sequences from different genes (including the 23S plastid and nuclear-encoded small subunit SSU rRNA genes) were taken into account for a number of strains. Phylogenetic trees were constructed using parsimony and Bayesian analyses. Some cyanobacterial and microalgal strains were found to cluster separately to the type species of the closest related taxa. Different phylogenetic trees were compared and these showed the significance of using the multigene approach in phylogenetic reconstruction. Moreover, the secondary structure of the SSU and ITS regions was elucidated and was found to be particular to some new strains. The consideration of different genes increased the robustness of the consensus phylogenetic tree. The combined use of DNA sequences provided a molecular basis for an accurate description of novel species.

REVIEW OF LAMINARIA RODRIGUEZII RECORDS IN THE ADRIATIC SEA

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Laminaria rodriguezii Bornet is an endemic deep water Mediterranean alga. In the Adriatic Sea it is observed only in Croatia. First detailed knowledge of the alga distribution was obtained in 1948–1949 during Hvar expedition. Using trawl net, the alga was collected on 15 transects in Jabuka Pit and Palagruža Island area where it was found isolated or together with Sargassum vulgare C. Ag., Halacrhcion spathulatum (J. Ag.) Kütz. f. luxurians Ercegović and Cystoseira discors C. Ag. ssp. latiramosa Ercegović. In expeditions between 1956 and 1961 the alga was collected using trawl nets on new locations near Jabuka Pit and most of the previous records were confirmed. During all these missions, algae were collected on transects which were between 120 and 260 meters deep. Since 1996 annual fishery expeditions using trawl nets have been established and cover also Jabuka Pit and Palagruža Island area. These expeditions recorded L. rodriguezii as a bycatch on just three transects near Palagruža Island. In 1998 a thallus was collected also near Palagruža Island by a grab on 118 m depth. With the aim to assess the status of L. rodriguezii, we explored ten transects in Jabuka Pit and Palagruža Island area using ROV in spring 2010 (Project “Brown algal biodiversity and ecology in the Eastern Mediterranean Sea” – TOTAL Foundation). Two thalli of L. rodriguezii were found only close to Island of Palagruža on 98 m depth in the area protected from trawling. Laminaria rodriguezii became exceptionally rare in the area of Palagruža Island or even completely disappeared in Jabuka Pit, most probably due to intensive trawling. Even if the fishing pressure in the area of Palagruža Island is smaller than in Jabuka Pit, the recent records of the alga were critically sporadic. A management plan should be developed to enable maintenance of L. rodriguezii in the Adriatic Sea.