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# 17<sup>th</sup> Scientific Conference of the Phycology Section [DBG]

**11.03.–14.03.2018**  
*Berchtesgaden, Germany*

## ORGANIZATION

A. Holzinger [University of Innsbruck]  
M. Schagerl [University of Vienna]



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S. 03-08

General  
introduction,  
information  
and important  
addresses

## COVER

Algal species number for Germany as a percentage information  
(Gutowski et al. 1998. *Trophiekartierung von aufwuchs-  
und makrophytendominierten Fließgewässern. Inf.ber. Bayer.  
Landesamt Wasserwirtschaft 4/98*)

- **Heterokontophyta** ~ 43%
- **Dinophyta** ~ 4%
- **Cyanobacteria** ~ 10%
- **Euglenophyta** ~ 9%
- **others** ~ 3%
- **green line** ~ 31%

## ORGANIZATION

A. Holzinger [University of Innsbruck]  
M. Schagerl [University of Vienna]

## LAYOUT

Abteilung eins [www.abteilungeins.com]

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01

# INTRODUCTION



## Dear Phycologists and Guests,

Welcome to the 17<sup>th</sup> Scientific Conference of the Phycology Section of the German Botanical Society in Berchtesgaden, Germany. More than 70 scientific contributions have been submitted with topics representing the whole breath of phycology. Talks will be given in seven specific sessions, posters will be displayed throughout the conference; additionally a poster session will be held on Monday afternoon. Young researchers are encouraged to present their Master theses and Doctoral thesis, which will then be evaluated for prizes. The prizes will be awarded by Regine Jahn (Berlin) during the conference dinner on Tuesday evening.

The municipality of Berchtesgaden is located in the south East of Upper Bavaria, and surrounded by beautiful mountain ridges. The first historical note dates back to around 1100 where the area was mentioned because of the rich salt deposits.

Much of the wealth derived from the salt mines, the first of which started to operate in 1517.

The salt is still mined and the brine transferred to Bad Reichhall for further processing. We were able to organize a visit to the salt mines on Tuesday afternoon, which will then be followed by the conference dinner in the “Maschinenhaus”, which formerly housed machines for the salt mining (details see below).

Together with other communities, Berchtesgaden belongs to the “Nationalpark Berchtesgaden”. In 1990, the UNESCO declared this Park to a Biosphere Reserve. The Nationalpark-Information Center “Haus der Berge” is offering several exhibitions, education programs and events. You will get free admission to the exhibition during your stay.

We hope that you will enjoy the Phycological Conference in Berchtesgaden. May this meeting bring new ideas, enthusiasm and friendships!

**Andreas Holzinger und Michael Schagerl**  
*organizers of this meeting*

### **Oral presentations**

Please make sure to upload your presentation files at the registration desk well in advance to your talk. All presentations will be uploaded onto computers provided by the in-house technicians; presentations will be deleted afterwards. Oral presentations are restricted to a time slot of max. 20 min (15 min talk, 5 min discussion). The schedule is tight and we ask you to prepare your talk accordingly. A laptop and a beamer will be provided, the system is running under the windows 7 platform. Please use powerpoint ppt/pptx-formatting or PDFs. If you implement video sequences, please carefully check that the file/video is working properly on the provided computers. The local technicians cannot guarantee for a smooth switch to Apple-systems, so we kindly ask for file conversion to the windows system.

### **Food**

During the conference, coffee, lunch and dinner/ice breaker will be provided from “Spiesberger’s Alpenküche” directly located at the conference venue “Haus der Berge”. The conference dinner (Tuesday evening) will be organized by “Gasthof Reichenbach”, Bergwerkstraße 81, Berchtesgaden. It is located in the “Maschinenhaus”, a former building of the salt mines with a very special ambience. Participants visiting the salt mines will be directed to the location, which is situated just nearby the salt mines entrance (others please organize the travel by your own).

### **Posters**

Formats up to A0 (841x1189 mm) are possible. Material for installing the posters will be provided. Please be available during the poster session on Monday afternoon, which is also a perfect opportunity to make new contacts and enjoy interesting conversations.

### **Awards**

Oral presentations of Diploma-, Master-, or Bachelor theses may join in the competition for the Algological Studies-sponsorship prize. For Doctoral theses, the E. G. Pringsheim-prize will be awarded. Also for Posters, there will be a recognition award provided.

Members of the jury in alphabetical order: Burkhard Becker (Cologne), Claudia Büchel (Frankfurt), Ilse Foissner (Salzburg), Martin Lohr (Mainz), Maria Mittag (Jena).

### **Visits of the Salt Mines**

A bus shuttle service is organized to the salt mines, departing on Tuesday, March 13<sup>th</sup>, at around 16:00 from the conference center to the salt mines located at Bergwerkstraße 83. As only a maximum number of 50 people is allowed to participate at one tour, the same bus will return again around 16:20 and take the second group to the salt mines. Both groups will be ready for the conference dinner in time. Please note: please organize the return to your accommodations individually (hiking, taxi,...). The visit to the salt mines is included in the conference fee.

## **Important addresses & phone numbers**

The conference will be held in the Nationalparkzentrum Haus der Berge

Hanielstraße 7  
83471 Berchtesgaden  
Telefon +49 86 52 / 9790 60-0  
hausderberge@npv-bgd.bayern.de  
www.haus-der-berge.bayern.de

*Free WLAN is available in the “Haus der Berge”  
the password is hdb83471*

**S. 09-20**

Timetable  
of the 17<sup>th</sup>  
Scientific  
Confer-  
ence of the  
Phycology  
Section

**02**

# ***PROGRAM***

**P** = E. G. Pringsheim  
**A** = Algological Studies-sponsorship  
**R** = poster recognition

## SUNDAY, 11.3.2018

### Arrival

13.00_	Relax
15.30	Registration
14.30_	Relax
15.30	Coffee "get together"
15.30_	Schagerl, M. & Holzinger, A.
15.45	Welcome and organization
15.45_	Brendel, U.
16.00	Information on "Nationalpark Berchtesgaden"

### Polar Algae (chair Karsten, U.)

16.00_	Karsten, U.
16.10	Introduction in the DFG priority Program "Antarctic Research"
16.10_	Bartsch, I. & Zacher, K.
16.30	Winter survival capacity of microscopic Polar kelp stages
16.30_	Monteiro, C.; et al.
<b>P</b> 16.50	Short-term transcriptomic responses to temperature and salinity stress in sporophytes of the kelp <i>Saccharina latissima</i>
16.50_	Li, H.; et al.
<b>P</b> 17.10	Physiological and transcriptomic responses of <i>Saccharina latissima</i> from the Arctic to temperature and salinity stress
17.10_	Lutz, S.; et al.
17.30	The diversity and functions of ice algal communities on the Greenland Ice Sheet
17.30_	Remias, D.; et al.
17.50	<i>Ochromonas</i> sp. DR75b ( <i>Chrysophyceae</i> ) isolated from alpine snow: physiological and biochemical characterization
17.50_	Schiller, J.; et al.
18.10	Heading northward to Scandinavia – <i>Undaria pinnatifida</i> in the northern Wadden Sea
18.30_	Relax
open end	Ice breaker and finger food followed by auction sale of phycological objects (auctinator Wilhelm, C.)

## MONDAY, 12.3.2018

### Physiology (chair Bilger, W.)

- P** 09.00 – Buck, J.; et al.  
09.20 – *The role of the four LhcX isoforms in photoprotection of the diatom Phaeodactylum tricornutum*
- P** 09.20 – Dautermann, O. & Lohr, M.  
09.40 – *Expansion of the Zeaxanthin Epoxidase-Family in Major Groups of Marine Algae*
- 09.40 – Diehl, N.; et al.  
10.00 – *Stress metabolite pattern in the eulittoral red alga Pyropia plicata (Bangiales) in New Zealand*
- P** 10.00 – Molchanova, M.; et al.  
10.20 – *Generation of storage polysaccharides in diatoms*
- 10.20 – Pescheck, F. & Bilger, W.  
10.40 – *Photoreactivation in green macroalgae*
- 10.40 – Relax  
11.00 – *Coffe break*
- P** 11.00 – Aigner, S.; et al.  
11.20 – *Ecophysiological characterization and abiotic stress tolerance of aquatic terrestrial and symbiotic Chlorella-(like) species*
- 11.20 – Procházková, L.; et al.  
11.40 – *Ecophysiology and morphology the of snow alga Chloromonas krienitzii (Chlorophyceae) from the High Tatra Mountains*
- P** 11.40 – Trumhová, K.; et al.  
12.00 – *Effect of frost on viability of conjugating green alga Zygnema (Zygnematophyceae)*
- P** 12.00 – Mundt, F.; et al.  
12.20 – *The dark part of life – Gene expression and relevant physiology under darkness in Cosmarium crenatum (Desmidiaceae Streptophyta)*
- 12.20 – Relax  
13.30 – *Lunch break*
- 13.30 –  
15.40 – *Poster session followed & coffee*

### Biodiversity & Phylogeny (chair Jahn, R.)

- P** 15.40 – Steinhagen, S.; et al.  
16.00 – *Conspicuity of the model organism Ulva mutabilis and the potential nuisance algae Ulva compressa (Ulvophyceae Chlorophyta)*
- 16.00 – Darienko, T.; et al.  
16.20 – *Molecular phylogeny and genetic variability among endosymbiotic green algae of various ciliates revealed by multiple gene analyses and AFLP technique*
- 16.20 – Mikhailyuk, T.; et al.  
16.40 – *New lineages of streptophyte algae (Streptophyta) from terrestrial habitats revealed by an integrative approach*
- 16.40 – Gottschling, M.; et al.  
17.00 – *The importance of the epitype concept for reliable species determination in protists such as dinophytes*
- P** 17.00 – Mora, D.; et al.  
17.20 – *Morphology and metabarcoding! A test with stream diatoms from Mexico highlights complementarity of identification methods*
- 17.20 – Skukan, R.; et al.  
17.40 – *DNA Barcoding for assessing distribution patterns in cryptic seaweed species: the “Codium” case study in the Bay of Biscay Northeast Atlantic Ocean*
- A** 17.40 – Van, A. T.; et al.  
18.00 – *Reconsidering the phylogenetic relationships of selected taxa within the Cymbellaceae (Bacillariophyta) with an integrative approach*
- 18.00 – Relax  
19.00 – *Dinner*
- 19.00 –  
open end – *General meeting of the members of the phycological society*

MONDAY

MONDAY



## TUESDAY, 13.3.2018

### Methods & Applied Phycology (chair Kroth, P.)

<b>P</b>	09.00 –	Dunker, S.; et al.
	09.20	<i>Image-based flow cytometry as a new valuable tool in phytoplankton research</i>
<b>P</b>	09.20 –	Madhuri, S.; et al.
	09.40	<i>A new strategy to complement AUREO 1a in TALEN knockout strains of Phaeodactylum tricornutum</i>
	09.40 –	Meichßner, R.; et al.
	10.00	<i>Aquaculture of Fucus-species in the Baltic Sea by means of vegetative reproduction</i>
	10.00 –	Sommer, V.; et al.
	10.20	<i>Restoration of potash spoil heaps by the establishment of biological soil crust algae</i>
	10.20 –	Jorde, F.; et al.
	10.40	<i>The Algae Crop Rotation principle as a potential basis for algae mass production</i>
	10.40 –	Relax
	11.00	<i>Coffe break</i>

### Algal Cell Biology (chair Lütz-Meindl, U.)

<b>A</b>	11.00 –	Niedermeier, M. & Lütz-Meindl, U.
	11.20	<i>Crystal formation in the alga Micrasterias in response to strontium and barium stress</i>
<b>A</b>	11.20 –	Steiner, P.; et al.
	11.40	<i>Stress induces mitochondrial fusion in the unicellular model alga Micrasterias</i>
<b>P</b>	11.40 –	Absolonova, M.; et al.
	12.00	<i>Local pH changes on the surface of Chara and salinity response</i>
	12.00 –	Hoepflinger, M. C.; et al.
	12.20	<i>Chloroplasts of characean internodal cells divide when immobilized at the cell periphery</i>
	12.20 –	Relax
	13.40	<i>Lunch break</i>

### Ecology & Global Change (chair Benning, L.)

<b>P</b>	13.40 –	Bozzato, D.; et al.
	14.00	<i>The Effect of Climate Change on the Carbon Balance in Microalgae</i>
<b>P</b>	14.00 –	Bernard, M.; et al.
	14.20	<i>Deciphering kelp-endophyte interactions</i>
	14.20 –	Graiff, A.; et al.
	14.40	<i>Model simulation of seasonal growth of Fucus vesiculosus in its benthic community under different global change scenarios</i>
<b>A</b>	14.40 –	Marquardt, A.; et al.
	15.00	<i>A first insight into the diversity of eukaryotic soil algae of alpine hummocky meadows</i>
	15.00 –	Sasso, S.; et al.
	15.20	<i>Chlamydomonas reinhardtii as a model system to study the interactions of microalgae with other microorganisms</i>
	15.20 –	Relax
	15.50	<i>Coffe break</i>
	15.50 –	Relax
	18.30	<i>Visit to the salt mines</i>
	18.30 –	Relax
	open end	<i>Conference dinner with award ceremonies of the Hans-Adolph von Stosch-Medal and student contributions</i>

WEDNESDAY, 14.3.2018

Physiology & Ressources (chair Hanelt, D.)

09.00 – 09.20	Mudimu, O.; et al. <i>Screening of microalgae and cyanobacteria strains for α-tocopherol content at different growth phases and the influence of nitrate reduction on α-tocopherol production</i>
09.20 – 09.40	Pierangelini, M.; et al. <i>Early-branching terrestrial streptophytes respond differently to light changes but are similar in temperature requirement</i>
09.40 – 10.00	Meier, L.; et al. <i>Genomic Insights into the Biosynthesis of Photoprotective Pigments in Porphyra: Carotenoids and Mycosporine-like Amino Acids</i>
10.00 – 10.20	Kurmayer, R.; et al. <i>Toward an understanding of secondary metabolic diversity among algal bloom-forming cyanobacteria</i>
10.20 – 10.40	Roach, T.; et al. <i>Managing light use efficiency in Chlamydomonas reinhardtii</i>
10.40 – 11.00	relax <i>coffe break</i>
11.00 – 11.20	Becker, B. & Wollenschläger, J. <i>Acetate Assimilation in Chlamydomonas reinhardtii</i>
11.20 – 11.40	Río Bártulos, C.; et al. <i>Genome editing in the diatom Phaeodactylum tricornutum methods and applications</i>
11.40 – 12.00	Lorenz, M. <i>The Nagoya-Protocol – do's and don'ts for phycologists</i>
12.00 – 12.10	Schagerl, M. & Holzinger, A. <i>Farewell</i>

MONDAY, 12.3.2018

Poster contributions in alphabetical order

Abarca, N.; et al. <i>Integrative taxonomy: Defining the core group of the genus Gomphonema with molecular and morphological methods</i>	Hartmann, A.; et al. <i>Marine Algae as sources for unique bioactive molecules with photo-protective potential and relevance as cosmeceuticals</i>
Bilous, O. P. & Ivanova, N. O. <i>Description of the current state of phytoplankton in the reservoir Sasyk (Ukraine)</i>	Heesch, S.; et al. <i>Towards a genetic map for the brown alga Saccorhiza polyschides</i>
Buchholz, C. M.; et al. <i>Detection of parameters influencing isotopic composition in kelps</i>	Hoef-Emden, K. <i>Chroomonas: To be or not to be</i>
Calvaruso, C. & Büchel, C. <i>Isolation of thylakoid subfractions containing PSII supercomplexes from Thalassiosira pseudonana</i>	Holzinger, A.; et al. <i>Metabolomic analysis of Zygnema sp. vegetative and pre-akinetete field samples from Svalbard</i>
Carrasco, D.; et al. <i>Establishing model systems for studying flagellate green microalgae in the marine environment</i>	Hotter, V.; et al. <i>The vegetation composition along a High Arctic moisture gradient with a special focus on biological soil crusts</i>
Glaser, K. <i>Taxon-Omics: Biological soil crusts as unique microecosystem represent a suitable model system to address taxonomy and cryptic diversity of microalgal key players</i>	Jacobs, M. J.; et al. <i>Experimental evolution in the sea ice diatom Melosira arctica monitored via chlorophyll florescence imaging</i>
	Jäger, S. & Büchel, C. <i>CD-spectra of isolated Thalassiosira pseudonana plastids and their reaction to different salt treatments</i>

R Klimešová, M.; et al. <i>What do we reveal by cloning of the mats of Trentepohlia (Ulvophyceae Chlorophyta)?</i>	Samolov, E.; et al. <i>Klebsormidium from biological soil crusts in Chile</i>
R Krämer, L. C. & Lohr, M. <i>Investigation of the early carotenoid biosynthetic pathway in algae</i>	Scheschonk, L.; et al. <i>High Arctic kelps maintain their photosynthetic functions throughout the polar night</i>
Kusber, W.-H. & Jahn, R. <i>Algal names and taxa – information needed vs. information provided</i>	Schubert, M.; et al. <i>Characterization of two CRY-DASH like proteins in Chlamydomonas reinhardtii</i>
Laeseke, P.; et al. <i>Temperature tolerance of the invasive red alga Capreolia implexa and migration potential along South American coasts under present day and future conditions</i>	Sommer, A.; et al. <i>Constitutive endocytosis in Chara internodal cells revealed by plasma membrane dyes and fluid phase markers</i>
Liesner, D.; et al. <i>Temperature effects on Laminaria digitata – Phenotypic plasticity and transgenerational effects in an isolated kelp population</i>	R Stephan, S.; et al. <i>Impacts of skyglow on phytoplankton species</i>
Pröschold, T. & Darienko, T. <i>Toward a monograph of non-marine Ulvophyceae using an integrative approach</i>	Zhou, H.; et al. <i>Towards establishment of a genetic transformation system in charophyte green algae – Zygenematophyceae</i>
Rybalka, N.; et al. <i>The Xanthophyceae – a well-studied algal group on the road to molecular oblivion?</i>	Zimmermann, J.; et al. <i>German Barcode of Life 2 (GBOL2) – eDNA metabarcoding of diatoms in the context of the EU Water Framework Directive (EU WFD)</i>

S. 21-94

Talks and  
posters in  
alphabetical  
order by  
first author

03

# ***ABSTRACTS***

**Abarca, N.**  
**Zimmermann, J.**  
**Mora, D.**  
**Skibbe, O.**  
**Jahn, R.**

Botanic Garden and Botanical Museum Berlin, Freie Universität Berlin,  
Königin-Luise-Str. 6–8, 14195 Berlin, Germany; n.abarca@bgbm.org

## Integrative taxonomy: Defining the core group of the genus *Gomphonema* with molecular and morphological methods

*Gomphonema acuminatum* s.l. – the type species of the genus *Gomphonema* – has long been recognized as a highly variable species-complex with broad ecological tolerances in which many species and varieties have been described. Taxonomic discrimination between taxa, however, is hard to unravel because of morphological similarities and high infra-specific variation. The current separation is mainly based on morphological characters of the valve, e.g., the pattern of the central area, density and branching of the striae, the form of the punctae, as well as the outline. In cases where morphological and ultrastructural characters are insufficient for species delimitation, molecular data can provide evidence on the significance of features which are important for integrative taxonomy.

In order to differentiate taxa and to assess potential cryptic species, infraspecific variation and biogeographical distribution patterns, uni-algal cultures identified as from the *Gomphonema acuminatum* complex were isolated from environmental samples from Germany, Spain, France, Faroe Islands, Korea, and Mexico. They were studied by light and electron microscopy and four molecular markers. The integrative taxonomic approach clarified which features – synapomorphies – belong to the core group and therefore which taxa do not belong to the core group.

<sup>1</sup>Department of Cell Biology and Physiology, University of Salzburg, Salzburg, Austria

<sup>2</sup>School of Physics,  
The University of New South Wales, Sydney, Australia

**Absolonova, M.<sup>1</sup>**  
**Beilby, M. J.<sup>2</sup>**  
**Sommer, A.<sup>1</sup>**  
**Hoepflinger, M. C.<sup>1</sup>**  
**Foissner, I.<sup>1</sup>**

## Local pH changes on the surface of *Chara* and salinity response

Internodal cells of salt sensitive *Chara australis* generate conspicuous patterns of acid and alkaline bands at their surface when exposed to light. It has been shown that this phenomenon is dependent on photosynthesis and it is assumed to result from inhomogeneous distribution/activation of proton pumps. Electrical measurements indicate that salinity inhibits the proton pump and opens putative H<sup>+</sup>/OH<sup>-</sup> channels over the cell surface. Based on these findings, we imaged the pH changes outside the cell wall using fluorescein isothiocyanate (FITC) coupled to dextran 70 in artificial fresh water and in

saline medium. In the early phase of saline exposure, we observed decline of the pH banding pattern and the transient appearance of bright alkaline spots. Some of the spots became fixed in space after longer exposure. The osmotic component of the saline stress did not affect banding or production of transient spots. ZnCl<sub>2</sub>, the main known blocker of animal H<sup>+</sup> channels, abolished both the spot formation in saline medium and the pH banding pattern in artificial fresh water.

Absolonova et al. Surface pH changes suggest a role for H<sup>+</sup>/OH<sup>-</sup> channels in salinity response of *Chara australis*. Protoplasma (in press)

<sup>1</sup>University of Innsbruck, Institute of Botany,  
Sternwartestr. 15, A-6020 Innsbruck, Austria

<sup>2</sup>University of Rostock, Institute of Biological Sciences,  
Albert-Einstein-Str. 3, D-18057, Rostock Germany

## Ecophysiological characterization and abiotic stress tolerance of aquatic, terrestrial and symbiotic *Chlorella*-(like) species

The colonization of terrestrial habitats was a pivotal event in earth history requiring numerous evolutionary innovations to facilitate survival in these new environments. Compared to their aquatic pendants, terrestrial algae are exposed to harsher and strongly fluctuating abiotic factors, including desiccation and intense irradiation. Green microalgae of the genus *Chlorella* colonize terrestrial habitats either free-living or as symbiotic photobionts in lichens. The aim of the study was to compare closely related *Chlorella*-like species isolated from different habitats like high-alpine (*C. vulgaris* ASIB BB67) and tundra (*C. mirabilis* SAG 38.88) soils as well as with species of symbiotic (*C. sphaerica* SAG 11.88) and aquatic (*C. vulgaris* SAG 211-11b) origin regarding their ability to withstand several abiotic stress factors, like temperature, desiccation and irradiation treatments including elevated UVAB/PAR-ratios. Phylogenetic

relationships were analysed and biochemical responses were elucidated by high pressure liquid chromatography to verify the presence of secondary compounds, supporting survival in their specific habitats. We found inter- and intraspecific differences regarding their ability to grow and produce oxygen under certain temperature regimes, whereas the high alpine isolate was most tolerant against higher temperatures in contrast to tundra and symbiotic *Chlorella*-like species showing stenotolerant ecology. The latter two species are also synthesising a mycosporine-like amino acid (Prasiolin) as UV-sunscreen, lacking in the high-alpine and freshwater *C. vulgaris* species. The presence of such specific secondary metabolites (chemosystematic markers), was used to confirm recent reassessments in phylogenetic taxonomy for this polyphyletic assemblage.

**Bartsch, I.; Zacher, K.**

Alfred-Wegener Institute for Polar and Marine  
Research, Am Handelshafen 12, 27570 Bremerhaven,  
Germany

## Winter survival capacity of microscopic Polar kelp stages

In autumn 2016 we initiated an overwintering experiment at Kongsfjorden/Spitzbergen (laboratory and in situ experiment) in order to investigate the capacity of kelp spores, gametophytes and microscopic sporophytes to survive the extended Polar winter darkness in combination with current and enhanced winter temperatures (0, 4 and 8°C) as well as the impact of different daylengths. Uni- and multicellular gametophytes of all 3 tested kelp species (*Saccharina latissima*, *S. nigripes*, *Laminaria digitata*) had a high ability to survive 6 month of darkness irrespective of temperature, but survival capacity in darkness of microscopic sporophytes was considerably reduced at 8 °C compared to 4 and 0°C. Post-cultivation (short daylength, low light) after the

winter treatment resulted in highest sporophyte recruitment from multicellular gametophytes. Cold-temperate to Arctic *S. latissima* recruited best at 8°C, but Arctic *S. nigripes* at 0°C. In situ sporophyte recruitment of all tested kelp species was extremely slow and macroscopically visible juvenile sporophytes became only apparent in August 2017 – 10 months after seeding. The results suggest a very good adaptation to the seasonal daylength regime and Polar darkness, but future elevated winter temperatures may change the recruitment potential of kelps.



**Becker, B.; Wollenschläger, J.**

University of Cologne, Cologne, Germany

## Acetate Assimilation in *Chlamydomonas reinhardtii*

*Chlamydomonas reinhardtii* (Chlorophyceae) is a well-studied model system in molecular and cellular biology. The genome is sequenced, lots of mutants are available and genes can be knocked out using various technologies. However, despite all this work on *Chlamydomonas*, we still do not know much about a peculiar trait of *Chlamydomonas reinhardtii*, only rarely present in green algae and embryophytes: Acetate assimilation. *Chlamydomonas* is able to grow heterotrophically using acetate as single carbon and energy source, as well as mixotrophically using acetate as single carbon source. A total of 7 potential acetate assimilation

enzymes, forming acetyl-coenzyme A from acetate and coenzyme A in a one or two step reaction, have been identified in the genome of *Chlamydomonas reinhardtii* CC503. The seven enzymes can be combined to 5 potential assimilation pathways (possibly localized in different subcellular compartments), suggesting that acetate assimilation plays an important role in natural life for *Chlamydomonas reinhardtii*. In this presentation we will present first results regarding the role of acetate assimilation during different growth conditions and during different phase of a *Chlamydomonas* cell culture.

**Bernard, M.<sup>1</sup>  
Peters, A. F.<sup>2</sup>  
Rousvoal, S.<sup>1</sup>  
Dartevelle, L.<sup>1</sup>  
Leblanc, C.<sup>1</sup>**

<sup>1</sup>Sorbonne Universités, UPMC Univ Paris 06, CNRS, UMR 8227, Integrative Biology of Marine Models, Station Biologique de Roscoff, Roscoff, France | <sup>2</sup>Bezhin Rosko, Santec, France

## Deciphering kelp-endophyte interactions

The marine brown macroalga *Saccharina latissima* is an important primary producer in temperate to cold northern hemisphere shores and an economically relevant seaweed with high industrial potential. Morphological changes - such as dark spots, twisted stipes and deformation of the blades - have been observed in wild populations and seaweed farms. The putative cause is the filamentous endophytic brown alga *Laminarionema elsbetiae*, which is highly prevalent in European *Saccharina* populations but has also been found occasionally in *Laminaria digitata*. *L. elsbetiae* is known to invade stipes and fronds of its hosts, however nothing is known about the molecular mechanisms of the interaction. To get further insight into the physiology of host-endophyte specific interactions, we set-up an experimental design to monitor the impact of the endophyte on growth of laboratory-grown kelp sporophytes.

First results revealed that co-cultivation of *L. elsbetiae* does not affect growth of its main host. On the contrary, growth of the occasional host *L. digitata* decreased significantly within less than a week when co-cultured with the endophyte. The detection of endophytic filaments in the kelp tissue by qPCR suggests that defence reactions against *L. elsbetiae* were triggered in *L. digitata*, but not in *S. latissima*. Transcriptomic analysis of endophyte-induced responses in both kelp species will help us to decipher the molecular bases of kelp-endophyte interactions.

## Description of the current state of Phytoplankton in the Reservoir Sasyk (Ukraine)

Nowadays, a proper using of water bodies is a very pressing problem that humanity faces. In this regard, the reservoir Sasyk, located on the north-west coast of the Black Sea near the delta of the Danube River in Ukraine is quite indicative. Sasyk is an anthropogenically altered water body with an area of 200–215 km<sup>2</sup> and a maximum depth of 3.2–3.3 m. Originally, Sasyk is a brackish water estuary (mineralization up to 16 ppt). After an unsuccessful desalination attempted in the 1980s, water salinity presently equals 0.3–2.7 ppt. Currently the reservoir is not suitable neither for water supply to the population nor for irrigation. The purpose of our study was to investigate the current state of the Sasyk reservoir by the taxonomic composition of the phytoplankton. Samples of plankton algae were taken at a depth of 0.5 m in

the summer period of 2013–2014. At present, we may conclude that 160 species of algae (163 intraspecies taxa) from 8 divisions, 13 classes, 27 orders, 44 families and 92 genera were identified during the studied period. It should be noted that a clear predominance of blue-green algae (*Aphanocapsa planctonica*, *Anagnostidinema amphibium*, *Merismopedia warmingiana*, *Merismopedia tenuissima* and *Microcystis wesenbergii*) as the dominant species complex that may indicate an unfavourable state of the reservoir.

## The Effect of Climate Change on the Carbon Balance in Microalgae

The Southern Ocean is an important sink for the atmospheric CO<sub>2</sub> due to the physical and the biological (photosynthetic) activity of phytoplankton. Some studies have investigated the photosynthetic activity of the benthic and pelagic producers, but there is only very scarce knowledge about the carbon losses due to respiration of phytoplankton. The reason for this lack of information is principally methodological limitations. In the light of expected changes of environmental conditions due to the climate change, the aim of the project is to investigate the range of variability of photosynthesis over respiration ratio (rP/R) in response to different growth conditions, namely: different temperatures, water salinity and in dependence on iron availability. In this respect, two key species of the Southern ocean are investigated: the diatom *Fragilariopsis cylindrus* and the prymnesiophyte *Phaeocystis antarctica*.

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## Detection of parameters influencing isotopic composition in kelps

Kelp derived detritus is a potential food item at the base level of marine food webs. A good knowledge of the factors that influence stable isotope composition in kelps is essential for reliable food web models: The variation of stable isotope composition was analysed in three kelp species growing in Kongsfjorden, Svalbard. The parameters considered were depth, age, and algal anatomy: Between 15 and 2.5 m depth, towards higher photosynthetic rate, *Alaria esculenta* was gradually enriched in  $\delta^{13}\text{C}$  while  $\delta^{15}\text{N}$  did not change. 283-year-old algae had significantly higher  $\delta^{15}\text{N}$  values in their blades than 586-year-old ones. A two factor analysis did not show any interactive effects between depth and age class. *A. esculenta* as well as *Saccharina latissima* and *Laminaria digitata* exhibited enrichment in heavy carbon isotopes in the blades

compared to stipes, while within each species  $\delta^{15}\text{N}$  values remained the same between blades and stipes. A more detailed analysis of kelp blades showed that independent of location in the fjord, year or season, and in all three species, young tissue was more enriched in  $^{13}\text{C}$  than meristem and that decaying apical tissue was depleted relative to young tissue. The degree of this  $^{13}\text{C}$  depletion varied with kelp species. Meristem tissue appeared most uniform compared to young and decaying blade tissues and its  $\delta^{13}\text{C}$  signature was characteristic for each species with no significant difference in  $\delta^{15}\text{N}$  values. Decaying tissue, which is closest to what becomes available to the food web as phytodetritus, showed no difference in  $\delta^{13}\text{C}$  between species.

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## The role of the four Lhcx isoforms in photoprotection of the diatom *Phaeodactylum tricornutum*

Diatoms possess a high capacity for qE (energy-dependent fluorescence quenching), the fast, proton-gradient and xanthophyll-cycle dependent part of non-photochemical quenching. This enables them to populate aquatic environments with fast changing light conditions, e.g. coastal or upwelling regions. Additionally, they can acclimate within hours to high light and low nutrient conditions by an enhancement of their NPQ capacity. Besides the amount of xanthophyll cycle pigments, Lhcx proteins define the qE capacity of diatoms and are expressed during the time of acclimatization. *Phaeodactylum tricornutum* owns four highly similar Lhcx isoforms, being specifically expressed under different stress conditions, of which we knocked out Lhcx1 and

Lhcx2. Interestingly, the Lhcx1 knockout strains are completely qE-deficient, indicating the pivotal role of Lhcx1 as a master-switch for qE, while Lhcx2 knockout lines demonstrate the importance of Lhcx2 for acclimatization to low iron conditions. By complementing both Lhcx1 and Lhcx2 KO strains with each of the four Lhcx proteins, respectively, we gain fundamental insights into the specific physiological properties in the qE process of each Lhcx protein.

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In contrast to higher plants, a diatom Photosystem II super-complex is still not characterized. The presence of a silica shell in diatoms cells poses the main obstacle to this study, since this makes the isolation of intact thylakoid membranes containing native state supercomplexes difficult. The silica shell is relatively stable and treatment with French press or sonication are required to release the organelles inside the cells. On the other hand, shearing forces can cause a complete disruption of the thylakoid membranes and the detachment of interaction partners of the PSII core. Nagao et al.<sup>[1]</sup> isolated for the first time oxygen-evolving PSII particles and they characterized most of the core and extrinsic proteins from marine algae *Chaetoceros gracilis*, but without detailed analysis of FCP interaction partners. The aim of this work comprises the analysis of PSII supercomplexes. Starting from isolated plastids from the centric diatom *Thalassio-*

## Isolation of thylakoid subfractions containing PSII supercom- plexes from *Thalassiosira pseudonana*

*sira pseudonana* according to Flori et al.<sup>[2]</sup>, a further sub-fraction of thylakoid membranes was performed. In order to identify FCPs (Fucoxanthin-Chlorophyll Proteins) that interact with PSII dimers, PSII supercomplexes should be isolated in a close to near-native state. BN PAGE and Western blot analysis were used to determine the native state and composition of protein complexes isolated from the thylakoid membrane subfractions.

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## Establishing model systems for studying flagellate green microalgae in the marine environment

The flagellated freshwater alga *Chlamydomonas reinhardtii* has been used for decades as a model organism to study different biological processes such as photosynthesis, behavioral responses along with flagellar function or light responses<sup>[1]</sup>. Very recently, it was also established as a model for biotic interactions with other microbes<sup>[2]</sup>. Here, we aim to establish molecular tools for two marine flagellate green microalgae to study their interactions with biotic and abiotic factors in the marine environment.

So far, we examined the properties of two isolates of *Chlamydomonas*, which were isolated from different marine environments. These are *C. euryale* from the yellow sea (Qingdao, China) and *Chlamydomonas* sp. from Nantu-

cket Sound (Massachusetts, USA). The growth conditions for both species have been characterized. Both strains grow in the same pH range of 5 to 9, but show different salinity preferences. Both strains have been recently sequenced and we have obtained the first genomic drafts. These data are now being used to develop gene cassettes along with resistance markers for transformation.

Moreover, we found that a secondary metabolite from a heterotrophic bacterium, orfamide A, which immobilizes *C. reinhardtii* by deflagellation also immobilizes *Chlamydomonas* sp.<sup>[2]</sup>. Thus, at least some of the biotic interactions of flagellate green microalgae may be similar in freshwater and marine habitats.

<sup>[1]</sup>Nagao, R. et al. Isolation and characterization of oxygen-evolving thylakoid membranes and Photosystem II particles from a marine diatom *Chaetoceros gracilis*. *Biochim. Biophys. Acts* (2007).

<sup>[2]</sup>Flori, S. et al. Plastid thylakoid architecture optimizes photosynthesis in diatoms. *Nature communication* (2017).

<sup>[1]</sup>Merchant, S. S. et al. The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. *Science* 318, 245–50 (2007).

<sup>[2]</sup>Aiyar, P. et al. Antagonistic bacteria disrupt calcium homeostasis and immobilize algal cells. *Nature Communications* 8, 1756 (2017).

## Molecular phylogeny and genetic variability among endosymbiotic green algae of various ciliates revealed by multiple gene analyses and AFLP technique

Endosymbiotic green algae are widely distributed in ciliates such as *Paramecium bursaria*, *Stentor polymorphus*, *Climacostomum virens*, *Coleps hirtus*, or *Euplotes daidaleos*, and have traditionally been identified as named or unnamed species of *Chlorella* or *Zoochlorella* or referred to as *Chlorella*-like algae or zoochlorellae. We studied several endosymbionts isolated from various hosts and geographical localities using an integrative approach (nuclear encoded small subunit and internal transcribed spacer regions of rRNA gene sequences including their secondary structures, morphology, physiology and virus sensitivity). Three *Chlorella*-like endosymbionts could be identified in various ciliates: *Chlorella variabilis*, *C.*

*vulgaris*, and *Micractinium conductrix*. The subdivision into species was highly supported by different phylogenetic methods and characteristic compensatory base changes in the secondary structures among the ITS-2 sequences. To detect the genetic variability below the species level, we analyzed the AFLP patterns (Amplified Fragment Length Polymorphism) using two pairs of restriction enzymes (EcoRI+MseI and EcoRI+PstI) and compared them with those of the free-living relatives. Strains with identical ITS sequences showed differences in AFLP pattern, which correlated with their geographical origin.

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The algae in the world's oceans are important both as primary producers sustaining the marine food web and as major contributors to global carbon fixation. While they share the core set of photosynthetic proteins with land plants, they differ notably in the carotenoids they use for photosynthetic light-harvesting and photoprotection. All these carotenoids have in common the presence of epoxy groups that are synthesized by enzymes termed zeaxanthin epoxidases (ZEP). In land plants and most green algae, ZEP catalyzes the epoxidation of zeaxanthin to violaxanthin and is encoded by a single gene. Conversely, many marine algae contain multiple copies of putative ZEP genes whose functions have not yet been studied in detail. We have developed a new tool for the functional characterization of algal ZEP genes by heterologous expression in a ZEP-deficient tobacco mutant. Here, we used this method for analysis

of ZEP candidates from several marine algae. HPLC analyses of pigment extracts from tobacco leaves expressing the different ZEP genes revealed that they all encode proteins with epoxidase activity, but that the enzymes differ markedly in their substrate preferences. The functional data were complemented by phylogenetic analyses of ZEP genes from all major algal lineages, giving insights into the evolution and functional differentiation of the ZEP family in marine algae.

## Expansion of the Zeaxanthin Epoxidase-Family in Major Groups of Marine Algae



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## Stress metabolite pattern in the eulittoral red alga *Pyropia plicata* (Bangiales) in New Zealand

Stress metabolite concentrations in response to ultraviolet radiation (UVR) and salinity changes were assayed in the red alga *Pyropia plicata* (Bangiales, Rhodophyta) over three seasons from April to November 2016 in the intertidal zone in Wellington, New Zealand. The mycosporine-like amino acids (MAA) shinorine and porphyra-334 were the quantitatively dominant UV-sunscreen compounds, and the total concentrations varied over the year between 5 and 14 mg g<sup>-1</sup> dry weight (DW), but neither UVR nor PAR had a significant impact on the MAA values. A UV-B stress experiment was conducted, but the MAA concentrations 6–8 mg g<sup>-1</sup> DW, did not change. This suggests, that *P. plicata* has sufficiently high UV-sunscreen concentrations and hence does not

respond to changes in UV-B radiation. *Pyropia plicata* contained three heterosides (floridoside, D- and L-isofloridoside), which are organic osmolytes. The seasonally total concentrations of these compounds varied between 203 and 1,226 mmol kg<sup>-1</sup> DW, with L-isofloridoside dominating all samples. A salt stress experiment showed an increase in the total heteroside concentrations in *P. plicata* with increasing salinities. However, floridoside was the most up-regulated heteroside under hypersaline conditions indicating its key role in osmotic acclimation. Our data indicate that *P. plicata* always contains various stress metabolites in consistently high concentrations which mitigate against environmental changes typical of the intertidal zone of New Zealand.

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## Image-based flow cytometry as a new valuable tool in phytoplankton research

The image-based flow cytometer ImageStream®X MK II was primarily developed for biomedical research. This new technique combines high-quality microscopic analysis with the high throughput methodology of flow cytometry. Based on their autofluorescence properties, phytoplankton organisms in the size range of 1–150 µm can be analyzed with the instrument. In comparison to existing flow cytometric methods, for each cell passing the laser, 12 images including brightfield, light scatter and fluorescence images are taken. Fluorescence images allow each species to be assigned to larger taxonomic groups and provide additional information about localization of fluorescence within cells. Brightfield images add multivariate morphological information, helping to identify cells on a species level. In combination with deep learning techniques, we aim to

automatize species identification and even try to determine the age of individuals. The measurements are done within minutes, this avoids artifact-inducing fixation procedures. Another valuable option provided is that the data matrix for each measurement can be archived and analysed at a later timepoint, increasing the efficiency of the process by separating sample measurement from data analysis. In general, the new technology adds powerful capabilities and features to the toolbox of phycological methods. We apply this image-based flow cytometry to 1) analyze phytoplankton diversity effects with experimental laboratory cultures, 2) monitor phytoplankton in the field and 3) establish automated high-throughput species identification tools.

## Taxon-Omics: Biological soil crusts as unique microecosystem represent a suitable model system to address taxonomy and cryptic diversity of mi- croalgal key players

On a global scale, biological soil crusts (BSC) are the most productive microbial biomass in arid and other extreme regions with various microalgal taxa as key components. BSCs are formed by different living organisms and their by-products, creating a microecosystem with microalgae as key players. The systematics of microalgae is currently changing from a morphological to a molecular phylogenetic species concept. Both systems disagree in taxonomical classification and species delimitation – a fundamental problem that needs to be addressed. The microalgal genera *Coccomyxa* and *Stichococcus* can be found in BSCs all over the world, indicating ecological key functions. However, their taxonomy is still not resolved, which hamper, for example, any biodiversity estimation.

A polyphasic approach based on molecular data analyzed by modern algorithms and supported

by morphological, ecophysiological and biochemical characteristics will result in a robust taxonomy as well as in the discovery and definition of new species. Therefore, existing strains from culture collections will be ordered and new strains of the respective genera will be isolated from various BSCs. Where missing, the highly variable ITS region and the chloroplast marker *rbcl* will be sequenced. Based on these data phylogenetic trees will be calculated and modern algorithms (like GYMC and PTP) for species delimitation will be applied. The results will be supported by chemotaxonomic markers (low molecular weight carbohydrates), habitat information, ecophysiological and morphological characteristics. This polyphasic approach will give strong evidence for a taxonomic revision of *Coccomyxa* and *Stichococcus*, thereby speeding up the naming process by defining species limits; new species will be described and, if necessary, morphological species merged.

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## The importance of the epitype concept for reliable species determination in pro- tists such as dinophytes

For unicellular organisms such as dinophytes, the biodiversity assessment started not until the late 18th century using light microscopy. Type material, particularly of older taxa, consists of specimens mounted permanently on glass or mica slides (a historically important example is the Ehrenberg Collection in Berlin) or of illustrations only. In many cases, type material is ambiguous and makes reliable species determination problematic because of various sources of error, including imperfect documentation of subcellular diagnostic traits or insufficient morphological differentiation within cryptic species complexes. For a correct application of such ambiguous scientific names, the Shenzhen Code (ICN) provides a tool for an epitype designation. In our ongoing research, we clarify the taxonomic identity of dinophyte species (such as *Durinskia oculata*, *Palatinus apiculatus* and *Scrippsiella acuminata*)

by collecting living material at type localities. After establishing living strains, species are DNA-bar-coded using rRNA sequences and investigated using modern light and scanning electron microscopy. Strains that are morphologically consistent with corresponding protologues are used for designation of interpretative epitypes in form of permanent slides for light microscopy. The significant difference from the historical types is that fully documented epitypes correspond to living material. Thus, epitypification is a key tool for a stable taxonomy and reliable species determination ensuring unambiguous link between a scientific species name, its protologue, morphology, ultrastructure, genetic characterisation and spatial distribution, all of which are of great importance especially for character-poor, unicellular organisms such as dinophytes.

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## Model simulation of seasonal growth of *Fucus vesiculosus* in its benthic community under different global change scenarios

Coastal marine ecosystems are under increasing threat from rapid environmental change. The single and combined impact of elevated seawater temperature and pCO<sub>2</sub> (1100 ppm) on the brown alga *Fucus vesiculosus* together with its associated community (i.e. epiphytes and mesograzers) was studied in mesocosm experiments between April 2013 and March 2014. Based on parameters and process rates measured in these experiments, a numerical boxmodel simulating the seasonal growth of *Fucus* in the Kiel Outdoor Benthocosms (KOBs) was developed. Nitrogen (N) and carbon (C) cycling in the KOBs were considered and relevant physiological and ecological processes were implemented, such as (1) Storage of C and N assimilates by *Fucus*, leading to a temporal decoupling of assimilation and growth. (2) Shading effects of epiphytes. (3) Grazing by different herbivores on both *Fucus* and epiphytes, but with species-specific rates and preferences. To run si-

mulations under present and global change scenarios (e.g. warming, ocean acidification) the model was forced with realistic atmospheric and hydrographic data of the Kiel Fjord. The model reproduces the magnitude and the seasonal growth cycle along with C and N content of *Fucus* in the KOB over one year under ambient conditions. Furthermore, the model simulates well the growth of *Fucus* in the mesocosms under different temperature and pCO<sub>2</sub> conditions. Overall, temperature effects are more pronounced than CO<sub>2</sub> effects. In the face of ongoing global change a synthesis of the existing knowledge on habitat-forming species and their sensitivities to changing environmental conditions is important. Thus, understanding of the ecological role of *Fucus* as primary producer, carbon sink, and ecosystem engineer in the coastal ecosystem of the Baltic Sea may allow for more reliable predictions into the future.

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## Marine Algae as sources for unique bioactive molecules with photo-protective potential and relevance as cosmeceuticals

Marine algae show an enormous biochemical diversity often including unique secondary metabolites with interesting bioactivities that could prevent or reduce adverse effects from ultraviolet radiation<sup>[1]</sup>. Those molecules are not only relevant for the organism itself, but could also be used for the treatment or the protection of human skin against harmful effects of UV radiation such as premature skin aging, inflammation, hyperpigmentation and skin cancer. During the past 5 years we were able to identify ecologically relevant novel natural products such as MAAs (Mycosporine-like Amino Acids) and Coumarins and could confirm that they have anti skin-aging properties. Through our investigations we developed new methodologies for the isolation, purification and structural elucidation of so far unexplored algal metabolites. In order to ensure their precise quantification in ex-

tracts, novel analytical approaches for the analysis of MAAs including an HPLC method using hydrophilic interaction liquid chromatography (HILIC) and a separation by capillary electrophoresis (CE) have been introduced<sup>[2]</sup>. Moreover, we were able to prove the anti-skin aging potential of MAAs which was measured in a previously validated collagenase assay indicating a dose dependent inhibition of the enzyme by all tested derivatives, with IC<sub>50</sub> values in the range of 19.40 µg/ml (*Asterina*-330) to 37.73 µg/ml (*Porphyra*-334)<sup>[3]</sup>, and even lower values for the Coumarins isolated from Dasycladales, Chlorophyta (16.98 µg/ml). In our ongoing project we will explore further uninvestigated species from diverse habitats in search for novel skin protective agents, which may have pharmaceutical or cosmetic relevance.

**Keywords:** Marine algae, MAAs, Coumarins, photo-protective effects, analytical approaches

<sup>[1]</sup>Thomas NV et al, *Mar Drugs* 2013;11: 146–164

<sup>[2]</sup>Hartmann A et al, *Mar Drugs* 2015;13: 6291–6305

<sup>[3]</sup>Hartmann A et al, *Planta Med.* 2015;81: 813–20

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## Towards a genetic map for the brown alga *Saccorhiza polyschides*

The brown algae are an ecologically and economically important class of organisms that developed multicellularity independently from plants, animals and fungi. The ERC SexSea project aims at sequencing the genomes of 20 brown algal species to allow genetic studies of their life history traits such as life cycles, sexual systems, and reproductive characters. One of the species of interest, *Saccorhiza polyschides* (Light-foot) Batters, a member of the order Tilopteridales, is one of the largest algae growing along warm-temperate European coasts. Kelp-like, its macroscopic sporophyte alternates in a diplohaplontic life cycle with microscopic dioicous gametophytes.

Genetic maps are important tools in molecular genetics, aiding, for example, the association of phenotypes with genes and thus the location of mutations, and providing the basis for positional cloning. To generate a genetic map for *S. polyschides*, a mapping population consisting of 147 gametophytes (73 females and 74 males) was raised in the laboratory from spores released from a single sporophyte collected on the northern coast of Brittany, France. Double digest Restriction Associated DNA sequencing (ddRADseq) was employed to genotype this population, and a de novo genetic map was constructed based on SNP markers. We present preliminary results, as well as discuss the merits and disadvantages of the method chosen.

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## *Chroomonas*: To be or not to be

Traditionally *Chroomonas* has been defined as a cryptophyte genus with phycocyanins PC 630 or 645, with a gullet as a cell invagination and with a periplast consisting of rectangular periplast plates. In molecular phylogenetic analyses *Chroomonas* proved to be paraphyletic. Sequenced DNA tags from the type material of the type species *Chroomonas nordstedtii* Hansgirg unequivocally identified subclade 1 as the real *Chroomonas* sensu Hansgirg.

Subclade 1, however, consists of species with ancestral types of periplast, i.e. with hexagonal periplast plates. The genus *Hemiselmis* separates subclade 1 from subclades 3 and 4. The latter subclades encompass lineages conforming to the traditional ultrastructural definition of the genus *Chroomonas*. If *Hemiselmis* is supposed to be maintained, all members of subclades 3 to 4 corresponding to this definition have to be renamed to establish a consistent systematics. DNA-containing specimens allow for an unequivocal identification, but may cause conflicts with accepted genus definitions.

## Chloroplasts of characean internodal cells divide when immobilized at the cell periphery

Unlike most higher plant cells, chloroplasts of characean internodal cells are immobilized in the cell cortex. During cell growth, chloroplasts divide perpendicular to the maximum cell wall strain and are therefore oriented as helical files along the plasma membrane. Actin filament bundles at the inner chloroplast surface generate endoplasmic rotational streaming via interaction with myosin-bound organelles. Upon mechanical disturbance, single chloroplasts or chloroplast files may detach from the cortex and are released into the streaming endoplasm. These endoplasmic chloroplasts are able to increase their surface and volume, but have never been observed to divide. Chloroplasts can also be removed from the cortex by local irradiation with intense UV

light. This treatment allows the formation of chloroplast-free “windows” that are repopulated within several weeks to months. This happens either via growth and division of chloroplasts, which starts preferentially from the downstream border of the window, or via reattachment of single endoplasmic chloroplasts. Within the window chloroplasts grow and divide parallel to the direction of endoplasmic flow. These findings indicate that chloroplasts of characean internodal cells are only able to divide when immobilized at the cell periphery. For further insights into the attachment as well as the division of chloroplasts we screened *Chara australis* cDNA libraries for proteins functionally involved in these mechanisms.

## Metabolomic analysis of *Zygnema* sp. vegetative and pre-akinetes field samples from Svalbard

*Zygnema* sp. is a genus of common streptophyte green algae that form conspicuous mats in the high Arctic. We collected field samples near Longyearbyen, Svalbard with a vegetative- (large vacuoles, bright green chloroplasts), and a pre-akinetes appearance (many storage compounds, thick cell walls) and screened for their metabolite composition by GC-MS. The samples were also characterized by light- and transmission electron microscopy.

We were able to detect 171 compounds out of which 116 metabolites were successfully identified. 83 compounds showed significant differences between the investigated groups. Most of the amino-acids detected like hydroxyproline, cysteine or glutamine were down-accumulated in the pre-akinetes samples. Among the detected free fatty acids, oleic acid (C18:1), linoleic acid (C18:2) and paullinic acid (C20:1)

were significantly up-accumulated in pre-akinetes. Most low-molecular-weight organic acids were down-accumulated in pre-akinetes. In contrast, sugars and sugar alcohols were found to be up-accumulated in pre-akinetes, particularly galactinol, gentiobiose, raffinose and trehalose. Monosaccharides like mannose and ribose were down-accumulated in pre-akinetes.

In summary, substantial differences in low-molecular-weight metabolites exist between vegetative cells and pre-akinetes. Whereas vegetative cells are metabolically more active, considering the abundance of amino acids and low-molecular-weight organic acids, pre-akinetes contain more osmotically active sugars and sugar alcohols, as well as storage compounds. This is helpful to surviving unfavorable environmental conditions like desiccation in summer and freezing in winter.



## The vegetation composition along a High Arctic moisture gradient with a special focus on biological soil crusts

Nowhere is the climate change impact more visible than in the Arctic. The Arctic winter temperature increase is two to three times of the planet's average – a phenomenon also known as Arctic amplification (Screen 2017). Especially the Arctic tundra is sensible to climate change, with complex microbial responses in the soil. However, Arctic soil and lake microbial communities have important ecological roles such as in the global carbon cycle. Therefore, a comprehensive understanding of the biodiversity and activity of soil and lake microbial communities is crucial to evaluate their role on ecosystem functions with a focus on the climate change impact. To study the diversity of microbes and plants we chose two study sites at the High Arctic island Svalbard (Knudsenheia and Ossian Sarsfjellet). In Summer 2017, we analyzed the vegetation co-

verage along an environmental gradient with increased water availability (dry, intermediate, wet) with a focus on biological soil crusts (BSCs) at both field sites. Furthermore, soil enzymatic activity measurements along the gradient were conducted. Regarding the observed vegetation coverage, both field sites were similar and showed a large proportion of BSCs and mosses. However, only cyanobacteria-dominated BSCs were found. Especially in Knudsenheia, lichens represented the dominant vegetation in the dry plots. The vegetation coverage and potential plant litter was further compared to the soil enzymatic activities along the moisture gradient.

## Experimental evolution in the sea ice diatom *Melosira arctica* monitored via chlorophyll fluorescence imaging

Ecologically interesting and relevant species do seldom have the beneficial ease of handling that is a feature of model organisms. Thus, it is not always possible to use standardized methods of monitoring growth and fitness during experiments. The sea ice diatom *Melosira arctica* is abundant under and within Arctic sea ice and does aggregate in situ as well as in culture, making it inaccessible to standard counting methods. This diatom is one of the major primary producers in the Arctic and we are interested whether it can adapt to climate change. We investigate its possible adaptation capacity to increased temperature and light, two factors we expect to increase in the Arctic when the ice and snow cover are reduced due to global warming. This experiment is set as a long-term experiment over a period of 12 months, to enable us to see not only changes in transcriptomics and short-term acclimation, but also in e.g. Methylation patterns of the DNA and long-term adaptation. To monitor *M. arctica* behaviour during the experiment, we used an imaging PAM (pulse amplitude modulation) which allows us to record a whole cul-

ture flask – a non-invasive approach that is needed mostly, because we cannot get representative and non-disturbed subsamples of our algae. We use the basic chlorophyll fluorescence during exponential growth for a relative growth curve and the chlorophyll fluorescence quantum yield after a saturation pulse (Fv/Fm) to estimate the fitness of our cultures.

The data that we could acquire with this method in the middle of our ongoing experiment already show that *Melosira arctica* is severely stressed by both, high light and high temperature with a stronger impact of high temperature than high light and a detrimental effect of a combination of both.

## CD-spectra of isolated *Thalassiosira pseudonana* plastids and their reaction to different salt treatments

Although the multi-protein-complexes taking part in photosynthesis are widely conserved and the basic mechanism is the same in organisms throughout different biological clades – i.e. light harvesting, transfer to photosystems, energy conservation – the way, the photosynthetically active membranes are organised is diverse. Cyanobacteria possess phycobilisomes and thus non-appressed thylakoids, higher plants show a lateral heterogeneity with membranes being separated into grana and stroma. In heterokonts thylakoids span the entire plastid in bands of three. However, it is still unclear, what the three-dimensional network exactly looks like and which forces are involved in maintaining it stable, but still dynamic. In this contribution, we show data of circular dichroism (CD) measurements on isolated diatom

plastids (isolation according to Flori et al., 2017). Szabo et al (2008) proposed that the main psi-type CD peak (polymer-or-salt-induced) can be correlated with the structural integrity (i.e. the multilamellar organisation) of the thylakoid membranes. Based on this suggestion, we show that the main peak's features are not only preserved during the isolation of the plastids, but also that they are sensitive to salt treatments. The data reveal two different effects of cations: First, ions stabilise the membrane arrangement against temperature-induced degradation and second, ions seem to affect vertical stacking of the membranes. Our experiments prove divalent cations to be more effective than monovalent ones. These findings imply a crucial role for cations in chloroplast architecture in diatoms. References: Szabó, M. et al, Photosynth Res 95 (2008) Flori, S. et al, Nat. Commun. 8 (2017)

## The Algae Crop Rotation principle as a potential basis for algae mass production

Microalgae producers often face the problem that outdoor photobioreactors become uneconomic for a specific period of the year, as growth rate and metabolite production of the algal strain used decrease when environmental parameters change to unfavourable conditions. Temperature and light are the most decisive factors in this regard. Within the FP7-EU-project PUFACChain numerous strains from the SAG and CC-Cryo algae collections were screened for best DHA and EPA producers. Two EPA producing strains, one being mesophilic, the other being cryophilic were compared with regard to their EPA productivities in relation to temperature and light. We could show that each strain has very different preferences in this regard. EPA yields in the mesophilic strain are best when grown at 20–30°C and at PAR photon flux densities 30–1,000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , whilst the cryophilic strain prefers temperatures between 4–20°C and 5–100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  respectively. It becomes obvious that at locations

in Middle or Northern Europe with strong differences in temperature and light between summer and winter, one of the strains finds its optimum conditions during the warmer, the other during the colder seasons. Alternating such two strains in the course of a year according to the prevailing environmental conditions, i.e. applying our proposed Algal Crop Rotation (ACR) principle, can result in an annual overall increase in productivity of such an algal mass production plant without applying any major external temperature or light regulation, and thus, without investing extra energy and costs. We modelled conditions at four European locations with strong differences in their climate from laboratory and pilot plant results and could show that the annual overall EPA-yield could be increased significantly when a cryophilic strain is used during the colder and darker seasons in Middle Europe (Hamburg) and in Northern Europe (Oslo, Trondheim).

## What do we reveal by cloning of the mats of *Trentepohlia* (Ulvophyceae, Chlorophyta)?

The genus *Trentepohlia* Martius 1817 is the best-known member of Trentepohliales, an order of subaerial green microalgae widespread in humid climates. Species of *Trentepohlia* form conspicuously coloured mats on natural and man-made surfaces. These mats attracted the attention of the early botanists who described the first species (Linnaeus 1753, Wiggers 1780). The taxonomy of *Trentepohlia* has been studied intensively since the 18<sup>th</sup> century. The species delimitation has been based mostly on morphological characters as observed in light microscopy: development of prostrate and erect axes, length and width of cells, shape of cells, and shape, size and arrangement of gametangia and zoosporangia (De Toni 1889, Hariot 1889, 1890, De Wildeman 1891, 1896, Printz 1920, 1939, Cribb 1970, Sarma 1986). Mats of *Trentepohlia* as observed in the field have always been perceived as populations consisting of one species, and as such their habit has played a major role in the establishment of several species (which were often

described by old botanists in terms of colour of the growth, smell, texture, thickness, specificity of habitat and type of substrate (Fischer, 1922; Printz, 1939). However, during our observations of field collections of *Trentepohlia*, we repeatedly observed the presence of two or more different morphotypes in the same mat. Furthermore, it is not uncommon to observe multiple morphologically dissimilar *Trentepohlia* thalli appearing in Petri dishes containing the sample of a piece of a single mat. These findings led us to re-consider the species homogeneity of trentepohlialean mats.

In this study we tested the possibility that mats of *Trentepohlia* in Europe are composed of more than one species using a combined approach based on morphological and molecular methods (sequences of the *rbcL* gene), and evaluated its importance for the species concept of this genus.

## Investigation of the early carotenoid biosynthetic pathway in algae

The early steps of carotenoid biosynthesis in photosynthetic eukaryotes encompass the reaction sequence from the colourless 15-cis-phytoene (the first carotenoid) to the red-coloured trans-lycopene (the last linear carotenoid) that is a central precursor of all major plant carotenoids. Land plants and cyanobacteria have been shown to need four different enzymes for this task, two desaturases and two isomerases. Phytoene desaturase (PDS) and zeta-carotene desaturase (ZDS) extend the conjugated double bond system in the molecule, while zeta-carotene isomerase (ZISO) and carotenoid isomerase (CRTISO) are needed for conversion of multiple cis-intermediates into their trans-isomers. Non-photosynthetic eubacteria, archaeobacteria and fungi that synthesize carotenoids, however, possess a phytoene desaturase catalysing the entire reaction sequence from phytoene to trans-lycopene.

By mining genomic and transcriptomic data, we found that algae in general use the same four enzymes as land plants for lycopene formation. We also found, however, that many algae in addition possess a protein with homology to the bacterial/fungal phytoene desaturase. Here, we have investigated the phylogeny, enzymatic activity and cellular localization of this protein from a red alga and a diatom.

## Toward an understanding of secondary metabolic diversity among algal bloom-forming cyanobacteria

The bioactive secondary metabolites produced by bloom-forming freshwater cyanobacteria can make freshwater hazardous. Individual genotypes contain an impressive metabolic diversity of toxic/bioactive peptides resulting in a variable spatial/temporal distribution of (non)toxic genotypes. Despite their importance to human wellbeing, such genotype diversity so far has not been mapped at scales relevant to nature. In this study, we genome-(re)sequenced ten strains of the genus *Planktothrix*, representing three distantly related phylogenetic lineages defined from a larger number of isolates (more than hundred strains), originating from shallow or deep lake habitats of temperate and tropical climatic zone from three continents (Europe, North America, and Africa). These high-quality draft genomes provide insights into location and physical proximity of individual peptide synthesis gene clusters located on the chromosome or on plasmids. All iso-

lates were analyzed for toxic/bioactive peptide synthesis gene clusters as well as the distribution and activity of transposable elements potentially influencing biosynthesis. In general toxic/bioactive peptide synthesis gene clusters are located on the chromosome, while plasmids carry biosynthesis genes in exceptional cases only. Vertical gene transfer is the overriding mechanism in biosynthesis gene acquisition or loss, while for the synthesis of some bioactive peptides the role of horizontal gene transfer either partial or in total between cyanobacterial genera could be shown. The high frequency of functional biosynthesis synthesis genes observed among bloom-forming *Planktothrix* species (*P. agardhii*/*P. rubescens*) but not among other morphologically similar *Planktothrix* species suggests a potential functional linkage between bioactive peptide production and the colonization potential and possible dominance in habitats.

## Algal names and taxa – information needed vs. information provided

The new International Code of Nomenclature for Algae, Fungi, and Plants (Shenzhen-Code) is covering all questions of naming algae in an appropriate way. It will be published in 2018 and will include some new provisions relevant to phycologists. One of them is the registration of scientific names and nomenclatural types which is being implemented as Phycobank ([www.phycobank.org](http://www.phycobank.org)) in Berlin (DFG JA 874/8-1). Furthermore, an overview will present state of the art of taxonomic databases that cover different fields of phycology related to fundamental and applied science, such as AlgaTerra

database (financed by BMBF), taxonomic checklists and Red Lists for algae in Germany (supported by BfN), the developments of the „Taxaliste der Gewässerorganismen Deutschlands“ (organized by UBA) and the „Harmonisierte Taxaliste für Phytoplankton, HTL“ for phytoplankton monitoring within the EU Water Framework Directive (financed by LAWA). A special focus will be on workflows mobilizing data, data exchange in data networks, and availability of information and workflows.

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## Temperature tolerance of the invasive red alga *Capreolia implexa* and migration potential along South American coasts under present day and future conditions

Species migrations under climate change are a global phenomenon and can pose threats to invaded ecosystems. *Capreolia implexa* (Rhodophyta) is a species native to South Australia and New Zealand and has recently been introduced to Chile. At the infested coastlines it has established in dense mats in the upper intertidal at several rocky shores some hundred kilometers apart. Due to its growth habit it is believed to be a strong competitor for space for other species. We conducted temperature tolerance (2–22°C) and freezing experiments (–20°C vs. 12°C, light vs. dark, submerged vs. exposed) to investigate physiological thresholds as a base for setting-up a distribution model to predict the potential spatial range for further migrations.

This model is accompanied by a Species Distribution Model based on available presence data to identify regions with conflicting predictions. We conclude that *C. implexa* has the potential to expand its distributional range much further along South-American coasts. Furthermore, climate change will have an impact on its realized and potential distributional range. Under the IPCC rcp85 scenario temperature conditions in the year 2100 will lead to a shift of the potential distributional range in South America as well as in the native range of *C. implexa* further southwards. This might also be connected to an overall loss of habitat in its native range. Under the rcp26 scenario conditions predicted changes in habitat suitability are much less pronounced.

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## Physiological and transcriptomic responses of *Saccharina latissima* from the Arctic to temperature and salinity stress

The Arctic region is currently facing substantial environmental changes. Melting of glaciers as a consequence of increasing temperature subsequently creates stressful environmental conditions, such as reduced salinity in coastal habitats of kelp beds. We investigated the physiological and transcriptomic performance of the sugar kelp *Saccharina latissima* from Kongsfjorden (Svalbard, Norway) over a 24-hour exposure at two salinities (20 and 30 psu) after a 7-day pre-acclimation at three temperatures (0, 8 and 15°C). The results demonstrate that the maximum quantum yield of PS II (Fv/Fm) at 15°C was significantly higher than at 0°C, but showed no difference at the two salinities. Pigment content exhibited similar response patterns. Salinity, however, affected gene ex-

pression much stronger than temperature. The highest number of differentially expressed genes (DEGs; DESeq2 with log2Ratio≥2), compared to the control at 8°C and normal salinity, was found in the specimens at 8°C and low salinity (1,374), followed by samples at 0°C and low salinity (1,193). The lowest number of DEGs appeared in the individuals at 0°C and normal salinity (274). Expression profile changes mainly focused on regulations of photosynthetic components and transport processes, as well as induction of ROS scavengers. On the physiological level, our findings indicate a high plastic performance of *S. latissima* at higher temperatures and lower salinities accompanied by significant gene expression modulation.



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## Temperature effects on *Laminaria digitata* – Phenotypic plasticity and transgenerational effects in an isolated kelp population

Transgenerational effects (parent environment effects on offspring traits) have recently gained attention as a means of fast response to changing environmental conditions under climate change. In temperate and polar rocky coastal ecosystems, kelps form the base of complexly structured and highly diverse species associations. This study investigates the potential for temperature-related within-generation and transgenerational plasticity in a geographically isolated population of the brown alga *Laminaria digitata* from the island of Helgoland (North Sea). We obtained separate strains (genetic lines) from fertile field material and assessed sporophyte growth and gametogenesis at 5 and 15°C. Rearing the resulting sporophytes in a full-factorial design at these two temperatures resulted in four temperature history treatments. A concluding experiment on growth,

photosynthetic characteristics (Fv/Fm, rapid P-I curves, pigments) and storage compounds of five parental strains should allow for the separation of phenotypic plasticity from genetic effects in the response to 5 and 15°C. Initial results indicate seasonality in the temperature response, with higher plasticity in material collected in spring than in summer. Gametogenesis was faster at 15 than at 5°C, but recruitment was higher at 5°C. A difference in morphology between sporophytes raised at 5 and 15°C indicates that the temperature during growth had a stronger impact on morphology than the temperature during gametogenesis. Here we will show preliminary results of whether performance is related to genetic background or phenotypic plasticity among strains.

## The Nagoya-Protocol – dos and don'ts for phycologists

Since the Nagoya Protocol (NP) on Access and Benefit Sharing (ABS) came into force in Oct 2014, greater attention on legal aspects of accession, exchange and use of genetic resources is required. ABS stands for the 'access to genetic resources and the fair and equitable sharing of benefits arising from their utilization'. Countries have sovereign rights over their genetic resources, including plants, animals and microorganisms, but also DNA. Utilization includes basic research such as sequencing or deposition of type material in public collections. Thus, ensuring legal clarity in how they can (or cannot) access and use genetic resources and resulting data has many implications on phycologists. Culture collections became main stakeholders to follow NP and national legislations and to raise awareness to users and depositors. Over 100 countries ratified the NP, but many did not implement ABS legislation yet. Primary information about global and national legislations is provided on the CBD website (Convention of Biological Diversity)

by the ABS Clearing House (<https://absch.cbd.int/>). The EU implemented NP legislation including a, register of collections'. Registered collections proved to meet specific criteria and their users demonstrate due diligence when sourcing material (e.g. cultures or DNA). They apply standardized procedures for accessing and exchanging genetic material and related information in line with CBD and NP. These include extensive documentation for incoming and outgoing material. Accurate data on origin and deposition needs to be stored. Multiple ABS documents from source and import countries, permits and material transfer agreements along the deposition line may accompany the material. SAG Culture Collection of Algae at Goettingen University currently expands its database and public catalogue to meet this demand and optimize service. On national level SAG participates in an expert panel defining criteria for capacity building and registration of collections.

# The diversity and functions of ice algal communities on the Greenland Ice Sheet

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The Greenland Ice Sheet (GrIS) is an important component of Earth's cryosphere. Due to the extensive area over which surface melting occurs, it can be regarded as the largest supraglacial ecosystem on Earth. Ice algae are the dominant primary producers on bare ice surfaces throughout the melt season. Ice algal pigments cause a darkening of the ice surface, which in turn decreases albedo and increases melt rates. Their role in changing melt rates has only recently been recognized, yet, we know little about their community compositions and functions. Here we present results of the microbial diversity in aerosol and surface samples collected on the GrIS during the 2016 and 2017 melt seasons. For the first time we performed high-throughput amplicon sequencing on ice algal communities along a 100 km transect across the "dark zone" of the GrIS. Our data reveal an extremely low algal

diversity, which was dominated by the ice algae *Ancylonema nordenskiöldii* and *Mesotaenium* sp. with minor contributions of Chlamydomonadaceae and Trebouxiaceae taxa. Oligotyping revealed hidden diversity that could not be detected by conventional clustering of OTUs. Oligotypes of the dominant taxa exhibit a site-specific distribution, which may be linked to differences in the extent of the melting. Furthermore, we present preliminary metabolomics data from varying environmental conditions (e.g., min. and max. light settings). These ,omics data were further cross-correlated with a range of physico-chemical boundary conditions (e.g., nutrients, carbon species, trace metals). Our results will help better understand distribution patterns and metabolic functions of ice algal communities that play a crucial role in the GrIS ecosystem.

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# A new strategy to complement AUREO 1a in TALEN knockout strains of *Phaeodactylum tricornutum*

The relation of mutations in genes and a certain phenotype can be verified by complementation tests. In genetically tractable diatoms like *Phaeodactylum tricornutum*, complementation of knocked out genes is difficult due to its diploid stage and the unavailability of sexual crosses in the lab. Therefore, we designed a new strategy to complement the AUREO 1a gene in TALEN-based (Transcription activator-like effector nuclease) bi-allelic knockouts of *P. tricornutum*. Aureochromes (AUREOs) are both blue light receptors as well as transcription factors, possessing both a LOV and a bZIP domain. Four orthologues of aureochromes have been identified in model diatom *P. tricornutum*, i.e. AUREO 1a, 1b, 1c and 2. Here we studied TALEN mediated AUREO 1a knockout mutants of *P. tricornutum* (Serif et. al 2017). A pPha-T1 vector with a new antibiotic cassette (different to that used for the knockout of AUREO 1a) was chosen to develop a complementation vector. In order

to avoid modification of the complementing gene via TALENs, the TALEN binding sites of this AUREO 1a gene were modified without changing the amino acid sequence using a codon usage table for codon optimization in *P. tricornutum*. The modified AUREO 1a gene along with its native promoter and terminator was transformed by particle gun bombardment in AUREO 1a TALEN knockout strains of *P. tricornutum*. We studied the genotypic and phenotypic characteristics of obtained putative complemented clones in comparison to wildtype. The expression of AUREO 1a in knock out strains was confirmed by Western Blotting showing successful complementation. We also found that AUREO 1a indeed acts as a repressor of high light acclimation as characterized by Schellenberger Costa B. et al in 2013.

## A first insight into the diversity of eukaryotic soil algae of alpine hummocky meadows

Hummocky meadows are a unique landscape form that mainly occurs in the Alps and are characterized by a wavy surface and a distinct soil composition. Their origin has been investigated for decades, but studies on their diversity have been rare. Biodiversity data about algae in the soils of the Hummocky meadows were absent so far. For a first insight into the diversity of eukaryotic soil algae we focused on the hummocky meadows near Mittenwald at the foothills of the Alps. From the soil of the pits DNA was extracted and a clone library approach targeting the ITS rDNA regions was used. In addition, the surface of a rock located inside the same hummocky meadows and covered with lichens was studied for comparison. Six primer combinations targeted to different groups of eukaryotic soil algae were used. A total of 261 clones were analysed and 131 algal ITS2 sequences were grouped into 41 OTU's (97% identity level). Comparisons with available reference sequences revealed

algal species already recorded from all around the world, but also numerous OTUs for which no highly similar reference sequences were available. Mostly members from Trebouxiophyceae (23 OTUs, e.g. *Diplopsphaera* spp., *Trebouxia* spp.) and Chlorophyceae (14 OTUs, e.g. *Chloromonas* spp., *Coelastrella* spp.) were recorded, but only three OTUs from Ulvophyceae and one from Klebsormidiophyceae (*Interfilum*). No overlap was found between the soil and rock samples indicating that there may be no exchange of algae between the two habitats. Three OTUs represented still unidentified chlorophytes previously recovered from alpine rocks. One ulvophycean OTU was highly similar (98%) to sequences from *Chaetrichon basiliensis* culture strains isolated from freshwater habitats, while the other two OTUs were only distantly related to known ulvophytes. The latter most probably represent new species.

## Aquaculture of *Fucus*-species in the Baltic Sea by means of vegetative reproduction

Brown algae of the genus *Fucus*, especially *Fucus vesiculosus*, are commonly used in cosmetics and food supplement products with an increasing demand in recent years. So far, the biomass needed for the fabrication of these products is supplied solely from harvesting of wild *Fucus*-stocks, e.g. in Norway, France and Ireland. In order to protect these wild stocks and establish a sustainable method for the generation of *Fucus*-biomass, we aim to develop an offshore aquaculture system for *Fucus*-species in the Baltic Sea. Thereby, we try to circumvent the very time-consuming and vulnerable step of sexual reproduction by the use of vegetative propagation, which is also exhibited by some wild *Fucus*-populations. In order to test this approach, cultivation experiments were performed in 2016 and 2017 on an experimental platform located on the Kiel fjord. A first experiment examined the ability of Baltic Sea *F. vesiculosus*, *F. serratus* and *F. evanescens* to grow vegeta-

tively without attachment to the natural substrate. Secondly, the potential for regrowth of cut thallus pieces of different sizes and parts of the thallus was tested. Both experiments successfully confirmed the feasibility of the approach by showing that Baltic Sea *Fucus*-species can be cultivated without substrate and have the ability to regrow from cut thallus pieces as long as these pieces contain a meristem. In a third experiment, the optimum density for cultivation of vegetative thalli was investigated and found to range between 1 and 2.5 kg/m<sup>2</sup>. These results are very promising for the further development of *Fucus*-aquaculture in the Baltic Sea.

## Genomic Insights into the Biosynthesis of Photoprotective Pigments in *Porphyra*: Carotenoids and Mycosporine-like Amino Acids

As intertidal species, *Porphyra* is frequently exposed to excessive photosynthetically active radiation (PAR) and potentially harmful UV-light. Carotenoids are important for protection of the photosynthetic apparatus under excessive PAR, while mycosporine-like amino acids (MAAs) protect against UV-related damage. Here, we have explored the genetic basis of the biosynthesis of carotenoids and MAAs in *Porphyra*. By mining genomic and transcriptomic data, we recovered the almost complete gene repertoire required for carotenoid formation in *Porphyra*. Consistent with the accumulation of lutein, *Porphyra* has two lycopene cyclases, one catalyzing the formation of beta-ionone rings whereas the other likely has the epsilon-cyclase activity needed for lutein synthesis. Our phylogenetic analyses indicate that epsilon-cyclases in Rhodophyta and Viridiplantae evolved independently by duplication

of their respective beta-cyclase genes and that the two lycopene cyclase genes in cryptophyte algae originate from their secondary plastids of red algal origin.

In cyanobacteria, the biosynthesis of MAAs involves the four genes MysA, MysB, MysC and MysD. *Porphyra* contains a gene encoding a MysA-MysB protein fusion also found in other intertidal red algae and in some chromalveolates, i.e., algae with secondary plastids of red algal origin. Although virtually absent from extant green algae, the fusion is also present in chlorarachniophytes and euglenophytes with secondary plastids of green algal origin. Moreover, *Porphyra* and related red algae contain a gene encoding a MysC-MysD fusion protein, and in *Porphyra* and *Chondrus* the MysA-MysB and MysC-MysD fusion genes are located next to each other on opposite DNA strands. Conservation of the MAA gene cluster and the gene fusions suggest that this arrangement provides a selective advantage and efficient MAA biosynthesis for red algae experiencing high UV irradiance.

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## New lineages of streptophyte algae (Streptophyta) from terrestrial habitats revealed by an integrative approach

Besides the Zygnematophytes, structurally simple members of the Streptophyta have been rather neglected. Therefore, our study investigated morphological, ultrastructural and molecular phylogeny of several uncommon strains of Streptophyta isolated from terrestrial habitats in America and Europe. Two new genetic lineages were found which possibly can be attributed to two separate genera. Combined 18S rRNA and rbcL phylogeny showed that one of these lineages nested inside Klebsormidiophyceae in sister position to *Hormidiella*, another separate lineage among known basal lineages of Streptophyta. The first new lineage is represented by algae with packet-like (sarcinoid) and filamentous branched thalli and a cell organization typical for Klebsormidi-

ophyceae. The second lineage is represented by an easily disintegrating filamentous alga which exhibits a cell coverage of unique structure: layers of submicroscopic scales of piliform shape covering the plasma membrane and exfoliate inside the mucilage envelope surrounding cells. All new taxa have general morphological similarity with known streptophytes like *Chlorokybus* and *Interfilum*, but differ in some essential morphological and ultrastructural features. Streptophyte algae are a group of great evolutionary interest as possible ancestors of higher plants. Therefore, discovery and further investigation of new taxa within this group will contribute to a deeper understanding of the evolution of basal Streptophyta lineages.



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## Generation of storage polysaccharides in diatoms

$\beta$ -glucans are common polysaccharides in algae, but also in plants, as structural components of cell walls, as signalling molecules, or as a storage compound for carbohydrates. Most Stramenopiles, including diatoms, synthesise soluble linear  $\beta$ -1,3-D-glucans with a  $\beta$ -1,6-D-branching, named chrysolaminarin, and store it in cytoplasmic vacuoles. The synthetic pathway of chrysolaminarin is not yet fully understood, and may differ considerably to starch production. A precursor for the chrysolaminarin is UDP-glucose, which is supplied by UDP-glucose pyrophosphorylase. A membrane-intrinsic glucan synthase transports UDP-glucose into the vacuole and

polymerizes it to a glucan backbone, while membrane-anchored transglycosylases provide side branches.

Glucan synthase is vacuolar membrane protein and its localisation has been demonstrated by GFP fusion protein expression (Huang et al., unpublished). The functional activity of the enzyme was studied by on posttranscriptional silencing lines. Reduction of the enzyme level leads to physiological changes, including lower chrysolaminarin deposition and higher lipid biosynthesis, depressed growth and raised photoprotection capacity.

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## Short-term transcriptomic responses to temperature and salinity stress in sporophytes of the kelp *Saccharina latissima*

Climate change is significantly impacting the structure and function of marine ecosystems worldwide with implications for species distribution ranges. In coastal systems, climate change may also alter other abiotic factors such as salinity, which may decrease due to increased glacial melting in the Arctic or precipitation in temperate regions. Despite the prime ecological importance of kelps (order Laminariales) which dominate rocky benthic ecosystems in temperate to polar regions, the acclimation mechanisms and transcriptomic responses remain understudied. Here, we investigate the physiological and transcriptomic responses in sporophytes of the sugar kelp, *Saccharina latissima* to salinity stress after acclimation to temperature and their interrelationships. Juvenile sporophytes of a strain from Roscoff, France were pre-cultivated at 8°C and 30 PSU for three months. After seven days of acclimation to 0°C and 15°C, sporophytes were exposed

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to a low salinity treatment (20 PSU) for 24 h. We established a reference transcriptome from all reads obtained through Illumina HiSeq. A total of 205 363 transcripts were assembled containing 135 959 “Trinity’s genes”. Gene expression is mostly driven by salinity stress than by temperature. The highest number of regulated genes, in comparison to the control, was found in response to the treatment 0°C low salinity (3003), followed by 8°C low salinity (1491) and 15°C low salinity (1158). Moreover, only few genes (168) were found to be differentially expressed in all low salinity treatments, showing that the response to low salinity is modulated by temperature. Growth, photosynthetic efficiency and pigment content were also impacted by stress.



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## Morphology and metabarcoding! A test with stream diatoms from Mexico highlights complementarity of identification methods

Diatoms are widely used as biological indicators, with several diatom-based indices developed to monitor environmental change. The conclusions that can be drawn from those indices rely on the taxonomical identity of the taxa found and their abundances, thus accurate species identification and abundance estimation are crucial in using diatoms as indicators. As a test case, the epilithic diatom communities in tropical streams from the Lerma-Chapala Basin, Central Mexico were evaluated by morphology and metabarcoding in order to compare how these two identification methods perform. In parallel, a taxonomic reference library was assembled based on clonal cultivation in order to increase the identification success of the metabarcoding method. The morphological analysis of environmental samples resulted in the identification of 205 infrageneric taxa in 43 genera, whereas the metabarcoding approach resulted in the

identification of 266 taxa in 35 genera. From the taxonomic assignments through metabarcoding, 23 were only possible due to the generation of our own regional taxonomic reference library, which consisted of 188 strains belonging to 70 species in 24 genera. The comparison of relative abundances of the morphology and metabarcoding methods showed big disparities between them, with the metabarcoding approach requiring improvements for a more accurate estimation of abundance. We conclude that the combination of morphology and metabarcoding methods increases the detection and identification of diatoms.

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## Screening of microalgae and cyanobacteria strains for $\alpha$ -tocopherol content at different growth phases and the influence of nitrate reduction on $\alpha$ -tocopherol production

Tocopherols (vitamin E) are only synthesized by photosynthetic organisms and have wide applications in cosmetics and as dietary supplements in human nutrition and aquaculture. Tocopherols from microalgae and cyanobacteria are rarely investigated, and little is known about their contents. Therefore, 130 strains of cultured microalgae and cyanobacteria have been analyzed for  $\alpha$ -tocopherol content under various culture conditions. The growth phase had a significant effect on content of  $\alpha$ -tocopherol. Maximal amounts were observed at the stationary growth phase. Reduction of

nitrate concentration in media caused an increased production of  $\alpha$ -tocopherol. The contents were significantly enhanced when the nitrate concentration was reduced to one fourth in culture media used. The content of  $\alpha$ -tocopherol was found to reflect phylogenetic relationships at the level of classes, with classes of Rhodophyta and Cyanobacteria accumulating the lowest contents. Within each class, contents varied widely at the species level emphasizing the importance of extensive screening procedures for the identification of strains with high  $\alpha$ -tocopherol contents.

## The dark part of life – Gene expression and relevant physiology under darkness in *Cosmarium crenatum* (Desmidiaceae, Streptophyta)

The Polar regions form unique habitats, characterized by extreme environmental gradients and conditions. Temperatures below the freezing point in combination with total darkness in winter and continuous light including relative high UV radiation during summer. The Desmidiaceae are distributed worldwide and form the largest taxonomic entity within the Zygnematophyceae. Many studies focused on photosynthetic performance, cell biology and growth patterns in this group. However, their molecular biology is still poorly understood.

A polar strain of *Cosmarium crenatum* was cultured under various abiotic conditions including darkness, high PAR, UVR and different temperatures. Photosynthetic activity was monitored by PAM fluorescence and growth was calculated using a coulter counter. RNA sampling for gene expression was performed initially, mid-term and

long-term (1 hour, 1 day and 1 week). A normalized reference transcriptome was established, consisting 28,992,600 reads. A total of 141,711 assembled transcripts were generated, containing 54,389 trinity genes. The transcriptome displays a high functional genomic coverage, including all major metabolic pathways i.e. carbohydrate, amino acid, lipid and nucleotide metabolism. To investigate gene expression underlying long-term darkness exposure, an Illumina HiSeq approach was applied. Under darkness, strong changes in gene expression were detected, 4,138 contigs declined and 3,767 induced. Strongest down-regulation was observed for genes encoding components of glycolysis and photosynthesis. Interestingly, the optimal quantum yield did not differ significantly between the darkness and control group after one week, where there were differences in growth patterns.

## Crystal formation in the alga *Micrasterias* in response to strontium and barium stress

The Earth alkali metals strontium and barium are present in low concentrations in soil and water. Because of their chemical similarity to Ca they compete with this ion for uptake in organisms which may lead to undifferentiated transport of these ions into cells. Desmids such as the unicellular model alga *Micrasterias denticulata* inhabit peat bogs and are closely related to higher plants. In the present study we investigate effects of SrCl<sub>2</sub> and BaCl<sub>2</sub> on cell growth, differentiation and ultrastructure of *Micrasterias* and we analyze distribution of these metals by means of TEM-coupled electron energy loss spectroscopy (EELS). Developing and non-growing cells of *Micrasterias* were exposed to artificially elevated SrCl<sub>2</sub> and BaCl<sub>2</sub> concentrations up to 21 d. Cells were high pressure frozen and freeze substituted for TEM and EELS analyses.

Concentrations between 250 µM and 2 mM SrCl<sub>2</sub> and between 250 µM

and 1 mM BaCl<sub>2</sub> led to physiological changes in *Micrasterias*, such as reduced vitality, decreased cell division- and growth-rates and altered photosynthetic- and respiratory activity corresponding to the applied metal. After 3 days exposure crystals in different size and shape appeared in surviving cells in dependence of the treatment. While addition of BaCl<sub>2</sub> led to small amorphous crystals, SrCl<sub>2</sub> induced formation of long spike-shaped crystals, which sometimes extended through the entire cell lumen. Different stages of crystal formation inside intracellular compartments were observed by TEM. Our EELS analyses provided evidence that the crystals contain inorganic BaSO<sub>4</sub> (barite) and Sr in an organic compound. These results were recently confirmed by Raman spectroscopy.

Our study indicates that *Micrasterias denticulata* belongs to the few organisms that are able to cope with Ba and Sr by forming differently shaped crystal. By removing the metals from the metabolism in this way, biomineralization serves as detoxification mechanism in this fresh water alga.

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## Photore- activation in green macroalgae

In contrast to higher plants and other macroalgae most green macroalgae do not employ UVB screening by absorbing pigments to protect themselves against UVB-induced cellular damages. However, this seems to be no disadvantage for them as representatives of non-UVB screening green algae, like *Ulva intestinalis*, can be found as major components of the eulittoral community populating highly irradiated habitats.

Interestingly, we found that in situ almost no UVB-induced DNA damage accumulates over a summer day or even a couple of sunny weeks in *U. intestinalis*. This strongly suggests that repair of UVB induced DNA dimers, especially by photoreactivation, should be of great efficiency in non UVB screening green macroalgae. To test this hypothesis, we compared the photoreactivation rate of *U. intestinalis* to that of *Cladophora* sp. which is a UVB screening green macroalga. Further, we started to investigate how mechanisms like acclimation and light dependency of repair activity control and modulate photoreactivation in green macroalgae.

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## Early-branching, terrestrial streptophytes respond differently to light changes but are similar in temperature requirement

Streptophyte green algae (SGA) originated ~700 million years ago and they are considered to be the ancestors of land plants. Several groups of SGA are currently known and distinguished in two evolutionary clades, the early- and late-branching streptophytes. Today, many members of SGA show adaptations to terrestrialization. We selected five uncommon early branching SGAs isolated from different geographical regions for ecophysiological investigations to elucidate their strategies for land colonialization. Particularly, we provide insights on photosynthesis regulation and acclimation under short- and long-term changes (1 week) of light conditions. Regulation of photosynthesis and respiration is

also studied in relation to temperature changes. Rapid light curves show different susceptibility of the photosynthetic apparatus to photoinhibition and capability to acclimate to long-term light variations. The ability to employ photoprotective mechanisms (i.e. non-photochemical quenching, NPQ), was also found to be different among the investigated strains. In spite of their different geographical origin, the five strains showed similar regulation of photosynthesis and respiration to rapid temperature changes. These results indicate that metabolic temperature dependence does not necessarily reflect species distribution under various temperature regimes.

## Ecophysiology and morphology the of snow alga *Chloromonas krienitzii* (Chlorophyceae) from the High Tatra Mountains

Snow algae are well-known extremophiles coloring melting snow during alpine and polar summers. We investigated a population of *Chloromonas krienitzii* causing a reddish monospecific bloom above timberline in the High Tatra Mountains, Poland. The taxon has been recently described from Japan and was formerly part of the collective *Chloromonas* cf. *brevispina*. However, ecology and life cycle were still unknown, and a morphologic description of the spores abundantly present at the snow surface was also not performed yet.

Our sample was identical for the highly variable marker of internal transcribed spacer 2 with *Chloromonas krienitzii* from Japan, except for three nucleotide positions out of 288 bp, and no compensatory base changes were found.

The spores of *Chloromonas krienitzii* differed from all other known snow algae of this genus by the fact that their primary cell walls had secondary spiky surface structures, which were abandoned during maturation, while the long-lasting secondary wall remained smoothly. Only the secondary cell wall showed a blue autofluorescence. This indicated, rarely for green algae sensu stricto, the presence of unknown UV-absorbing protective compounds. The cytoplasm contained a large number of orange lipid bodies, likely deposits of the secondary carotenoid astaxanthin. The chloroplast was sectioned into several discoid parts, which is typical for snow dwelling zygotes of this genus.

The fatty acids and pigment composition of spores was analysed. Fluorometric measurements (using PAM) showed that the photosystem II of the spores was well adapted to high levels of irradiation and showed no sign of photoinhibition up to 2100  $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ .

In course of this study, *Chloromonas krienitzii* was found also in the Sarntal Alps (Italy) and in Rocky Mountains (USA), suggesting a cosmopolitan distribution at alpine sites.

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## Toward a monograph of non-marine Ulvophyceae using an integrative approach

Phylogenetic analyses of SSU rDNA sequences have shown that coccoid and filamentous green algae are distributed among all classes of the Chlorophyta. One of these classes, the Ulvophyceae, mostly contains marine seaweeds and microalgae. However, new studies have shown that there are filamentous and sarcinoid freshwater and terrestrial species (including symbionts in lichens) among the Ulvophyceae, but very little is known about these species. Ultrastructural studies of some of them have confirmed that the flagellar apparatus of zoospores (counterclockwise basal body orientation) is typical for the Ulvophyceae.

In addition to ultrastructural features, the presence of a “Codiolum”-stage is characteristic of some members of this algal class. We studied more than 50 strains of freshwater and terrestrial ulvophycean microalgae obtained from the different public culture collection and our own isolates using an integrative approach. Three independent lineages of the Ulvophyceae containing terrestrial species were revealed by these methods. Unexpectedly each of these lineages contained several isolates that morphologically developed a high degree of phenotypic plasticity, and included hidden phylogenetic diversity that let us to the description of several new genera and species.

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## ***Ochromonas* sp. DR75b (Chrysophyceae) isolated from alpine snow: physiological and biochemical characterization**

Unicellular photoautotrophic Chrysophyceae (golden algae) are predominantly known for their presence in plankton during spring and autumn. They also occur in long lasting, melting snowfields in polar and mountainous environments, where they give the snow a yellowish color. Little is known about their adaptations to cold and oligotrophic habitats or about their biotechnological potential.

We isolated a strain that we preliminarily assigned to the genus *Ochromonas* from a seasonal snowfield in Tyrol, Austrian Alps and optimized its growth in inorganic medium (DY-V, modified). The cells occurred either as typical heterokont flagellates or as loose aggregates of

immotile capsal stage cells. Lyophilised cells were analyzed for fatty acids, pigments and soluble carbohydrates that might act as anti-stress metabolites. For example the macroscopic golden alga *Hydrurus* sp. accumulates the polyunsaturated fatty acid eicosapentaenoic acid (EPA; C20:5), which has become popular as a substitute for fish oil in vegan diets.

*Ochromonas* sp. DR75b appears to secrete a protein that affects the growth of ice. In other ice-associated algae, such proteins mitigate the damage to the cells by ice or help to maintain an aqueous environment. This is the first time an ice-active substance has been observed in golden algae.

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## **Genome editing in the diatom *Phaeo- dactylum tricornu- tum* methods and applications**

Reverse genetics techniques are powerful tools for studying gene functions or for tailoring genetically optimized cell lines for biotechnological applications. In the model diatom *Phaeodactylum tricornutum*, genome-editing approaches like TALEN (Transcription activator-like effector nucleases), and/or CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats), recently became available, which allow targeted mutations of genes of interest. In my presentation, I will present concise guidelines for routine utili-

zation of these tools and for the characterization of the resulting mutant cell lines. By adapting a cost-effective TALEN generation system previously established for mammalian cells for the use in *P. tricornutum*, the assembly of TALENs is now possible in about two weeks. I will present strategies for choosing a TALEN target site in order to avoid potentially ineffective and/or off-target prone TALEN constructs, for the efficient transformation of *P. tricornutum* with both TALEN constructs, for simultaneous utilization of two antibiotics resistance markers and for effective screening of the transformants. First applications of these strategies revealed that most TALEN transformed cell lines were found to be genetically homogeneous without the need for re-plating, which greatly facilitates the screening process.



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## Managing light use efficiency in *Chlamydomonas reinhardtii*

Photosynthetic organisms have to tolerate rapid changes in light intensity, which is facilitated by non-photochemical quenching (NPQ) and involves modification of energy transfer from light-harvesting complexes (LHC) to the photosystem reaction centres. Mechanisms of NPQ include dissipating excess light energy to heat (qE) and the reversible attachment of LHCs to photosystems (state transitions/qT), which are considered separate NPQ mechanisms. The contribution of the xanthophyll cycle to qE in *Chlamydomonas reinhardtii* is less than in other alga species and plants, whereas the LHCSR3 protein has a clear role in qE and photoprotection. Here, it is shown in the npq4 mutant, deficient

in LHCSR3, that energy coupling from the LHC to the photosystems (PSII and PSI) during qT is also disrupted. Stt7-mediated phosphorylation is required for qT, but no major differences in LHC phosphorylation levels or LHC compositions were found in npq4 compared to wild-type cells. Further inclusion in the study of stt7, that is absent in Stt7 kinase, showed that LHCSR3 is involved in the rapid qT transitions (<2 min), whereas Stt7-mediated kinase activity corresponds with the slower qT transitions (up to 10 min). It is concluded that NPQ in *C. reinhardtii* has a much greater mechanistic overlap than previously recognised.

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## The Xanthophyceae – a well-studied algal group on the road to molecular oblivion?

The Xanthophyceae or yellow-green algae (Stramenopiles) are the second largest group of photoautotrophic eukaryotes in soil habitats, second only to green algae, and they are also abundant in freshwaters and a few grow along seashores. Approximately 100 genera and 600 species are described using morphological characters, but only about 20 genera have been investigated using DNA sequence analyses. There are less than 1000 sequences from a variety of genes that are deposited in GenBank, and only one chloroplast genome is sequenced. Thus, the lack of a deep reference database hampers the generation and analysis of Next Generation Sequence (NGS) data. We have already produced group-targeted PCR that provided assess to xan-

thophycean diversity in soil samples from Antarctica and Germany. Long ITS1-5.8S-ITS2 rDNA amplicons were separated by cloning and sequenced. Short fragments are necessary for Illumina MiSeq (2 x 250) paired-ends sequencing, and to achieve this, full ITS2 regions must be re-amplified using unspecific primers. Some primers are not specific and some sequences are too long for NGS. We recognized this problem because longer ITS2 sequences (>380 bp) were recovered from clone libraries. Therefore, the xanthophycean diversity revealed by NGS may be biased. Our goal is to provide a deeper reference base so that xanthophyte sequences are recognized and identified. Failing this effort, xanthophytes are on a molecular road to oblivion.

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## ***Klebsormidium* from biological soil crusts in Chile**

Biological soil crusts (BSCs) are associations of different microorganisms and soil particles in the top millimeters of soil. Formed by algae, cyanobacteria, microfungi, bacteria, bryophytes, lichens and their by-products, BSCs play important role in the primary production, nitrogen fixation, mineralization and stabilization of soils. Representatives of cosmopolitan genus *Klebsormidium* are important constituents of BSCs worldwide, they contribute to physical and functional stability of BSC community. Until

recently G-clade was designated as “desert” clade since it was reported as a component of BSCs in hot and cold deserts. Our study of *Klebsormidium* representatives in almost unstudied BSCs of South America revealed that G-clade strains are indispensable element in Chilean BSCs, in both arid and temperate habitats. Based on morphological and molecular characters (ITS1 and ITS2) we observed phylogenetically diverse representatives within the G-clade. In order to describe a potential link between phylogenetic diversity and habitat preference, we investigated the physiological performance of selected G-clade representatives in a range of controlled temperature and light conditions, as well as their dehydration endurance.

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## ***Chlamydomonas reinhardtii* as a model system to study the interactions of microalgae with other microorganisms**

The biotic interactions of microalgae with other microbes shape aquatic microbial communities and influence the global photosynthetic capacity<sup>[1]</sup>. So far, limited information is available on molecular factors and chemical signals that govern these interactions. Here, we have established the unicellular alga *Chlamydomonas reinhardtii* (Chlorophyta) as a model for these studies<sup>[2]</sup>. We identified a heterotrophic bacterium, *Pseudomonas protegens*, which strongly inhibits the growth and alters the morphology of *C. reinhardtii*. This antagonistic effect is decreased in a bacterial mutant lacking orfamides, demonstrating that these secreted cyclic lipopeptides play an important role in the algal-bacterial interaction.

Using a new aequorin Ca<sup>2+</sup> reporter assay, we show that orfamide A triggers an increase in cytosolic Ca<sup>2+</sup> in *C. reinhardtii* and causes deflagellation of algal cells. These effects of orfamide A, which are specific to the algal class of Chlorophyceae and appear to target a Ca<sup>2+</sup> channel in the plasma membrane, represent a novel biological activity for cyclic lipopeptides.

<sup>[1]</sup>Hom, E.F.Y. et al., A chemical perspective on microalgal-microbial interactions, *Trends Plant Sci.* 20, 689-693 (2015)

<sup>[2]</sup>Aiyar, P. et al. Antagonistic bacteria disrupt calcium homeostasis and immobilize algal cells. *Nat. Commun.* 8, 1756 (2017)

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## High Arctic kelps maintain their photosynthetic functions throughout the polar night

Kelps, seaweeds of the order Laminariales, are important ecosystem engineers in arctic coastal ecosystems. With respect to this, increasing global temperatures, which have been observed to be most severe in the wintertime Arctic, necessitate to assess the capacity of adaptive life strategies of polar kelps during polar night. However, data on seaweed ecosystem functioning under polar night conditions is scarcely available.

We assessed several physiological parameters (photosynthesis, pigment content, respiration, and carbohydrate storage) in two species of arctic kelps, the boreal-temperate *Saccharina latissima* and the arctic-endemic *Laminaria solidungula*, during the period of polar night 2016/17. Plants were sampled from Kongsfjorden, Svalbard, at 78°55'N, shortly before the onset of the dark period in October, as well as towards the end of polar night in early February. Analyses were carried out for differ-

ent sections along the phylloid (Meristem, Centre, Distal Part).

Our data suggest that kelps do largely maintain their photosynthetic functions throughout the entire winter period, as indicated by PI-curves, and matching Chl a and antenna pigment contents. The maintenance of the photosynthetic functions is most likely fuelled by the storage carbohydrate laminaran. Overall laminaran content was reduced by ~96% in *S. latissima*, and by ~90% in *L. solidungula* by the end of the winter. However, strong differences were observed between the different phylloid regions across species, indicating specific adaptive strategies between boreal-temperate and arctic-endemic species.

The data presented here form the baseline for subsequent studies investigating the effects of further increases in temperature on arctic seaweeds during the polar night.

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## Heading northward to Scandinavia – *Undaria pinnatifida* in the northern Wadden Sea

The kelp *Undaria pinnatifida*, native to East Asian shores, was accidentally introduced with Pacific oysters into the Mediterranean in 1971. Intentional introduction from there to the French Atlantic coast 12 years later led to a gradual spread to the British Isles and the North Sea. Here, we report on the northernmost established population in continental Europe, and suggest a further spread into Scandinavian waters to be almost inevitable.

In 2016, several thalli were found washed ashore at the eastern side of the island of Sylt in the northern Wadden Sea (German Bight, Eastern

North Sea). Most specimens bore fertile sporophylls and thallus lengths of > 1 m were common. In June 2017, 91 sporophytes were found attached to a mixed bed of Pacific oysters and native blue mussels, located just below low tide level in a moderately sheltered position. Mean thallus length was 0.2 m and the longest 0.7 m. Most had distinctive sporophylls and released spores in the laboratory. From sporophylls collected in the previous year, we successfully reared a new generation, demonstrating the kelp's potential for further spread by natural means or human vectors.

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## Characterization of two CRY-DASH like proteins in *Chlamydomonas reinhardtii*

Past studies identified the family of cryptochromes as blue light receptors in bacteria, fungi, plants and insects. Interestingly, the unicellular green alga *Chlamydomonas reinhardtii* encodes four different cryptochrome proteins, a typical plant cryptochrome (pCRY), an animal-like cryptochrome (aCRY) and two CRY-DASH (*Drosophila*, *Arabidopsis*, *Synechocystis*, *Homo*)-like proteins (Beel et al., 2012). Characterization of pCRY, a typical blue-light receptor, revealed that it is involved in the circadian clock as well as in the sexual cycle of *Chlamydomonas* (Müller et al., 2017). Intriguingly, aCRY was found to be not only a sensory blue light, but also a sensory red light receptor (Beel et al., 2012). It controls some of the clock-related genes and is also involved in the control of the *Chlamydomonas* life cycle (Beel et al., 2012; Zou et al., 2017). Here, we have started to characterize the two CRY-DASH like proteins of *Chlamydomonas*. We

expressed their genes in *E. coli*, purified the protein and generated anti-CRY-DASH1 and 2 antibodies, respectively. Using these antibodies, we investigated the diurnal protein expression patterns of the CRY-DASH proteins and found that their expression differs from that of the other CRYs. Moreover, we determined the subcellular localizations of both proteins using the soluble and membrane associated cellular fractions as well as subcellular compartments (cytosol, mitochondria and chloroplasts). A screen for potential interaction partners is in progress.

<sup>[1]</sup>Beel et al., 2012, *Plant Cell*,  
doi: 10.1105/tpc.112.098947

<sup>[2]</sup>Müller et al., 2017, *Plant Physiol*,  
doi: 10.1104/pp.17.00349

<sup>[3]</sup>Zou et al., 2017, *Plant Physiol*,  
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## DNA Barcoding for assessing distribution patterns in cryptic seaweed species: the “*Codium*” case study in the Bay of Biscay, Northeast Atlantic Ocean

The infamous seaweed invader *Codium fragile* ssp. *fragile*, distributed on rocky shores worldwide, is officially listed as one of the 100 worst invading species in Europe. As it is prone to crypsis, the application of genetic molecular data is needed to support species identification. *Codium* spp. are recorded in a checklist of the benthic flora in the Cantabrian Sea (North of Spain) but detailed biogeographical distributions, origins, ecological mechanisms explaining their successful establishment and genetic structuring data from existing species are scarce. In this work, the main aim was to assess the *Codium* species distribution along the central southern area of the Bay of Biscay, supporting these data with DNA – based identifications using sequencing of the RuBisCO and plastids elongation factor TU (*tufA*) genes. Initial results showed that three different species of *Codium*: the invasive *Codium fragile* ssp. *fragile* and the

native *Codium vermilara* and *Codium tomentosum* were found cohabiting this area, but they have different seasonal and geographic patterns. More samples from the Portuguese and Spanish Atlantic areas are currently being included in this study to extend the scope of the results and conclusions. This is the first extensive genetic and distribution study on cryptic species from the genus *Codium* in the Bay of Biscay, northeast Atlantic Ocean, and it is significant not only for assessing species distributions, but also morphology-environment-genetics interactions. The primary results indicate differences in a winter and summer sampling of invasive *Codium fragile* ssp. *fragile* and native *Codium* species along Cantabrian coast. This knowledge will help to identify relevant key points for the prevention and management of seaweed biological invasions.

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## Constitutive endocytosis in *Chara* internodal cells revealed by plasma membrane dyes and fluid phase markers

Eukaryotic cells internalize plasma membrane and extracellular material by endocytosis. Endocytosis is required for the recycling of plasma membrane components, for nutrient uptake and for signaling. It can be classified according to the cargo (e.g. fluid-phase endocytosis) or according to the mechanism of endocytosis which is used to form an endocytic vesicle from the plasma membrane (e.g. clathrin-mediated endocytosis). The process of endocytosis can be followed in vivo with the aid of fluorescent dyes. Among them, FM styryl dyes are most frequently used. They incorporate into the plasma membrane and are taken up via endocytic vesicles which fuse with other organelles thereby revealing the endocytic pathway. In the present study we compared the internalization of these membrane markers with the uptake of Alexa 488 hydrazide, a fluid phase marker, in characean internodal cells. Both dyes were

actively taken up into the cytoplasm and stained various classes of endosomes, including brefeldin A- and wortmannin-sensitive organelles (trans Golgi network and multivesicular endosomes). Uptake of FM-dyes as well as of Alexa 488 hydrazide was independent of an intact actin cytoskeleton but could be inhibited by ikarugamycin and methyl  $\beta$ -cyclodextrin indicating the involvement of clathrin and sterols, respectively. In spite of these similarities, membrane endocytosis markers and fluid-phase markers co-localized only partially. Furthermore, Alexa 488 hydrazide distributed not only to rapidly recycling compartments but was also sequestered in long-lived endosomes. The significance of these findings for the mechanism of constitutive endocytosis in *Chara* internodal cells and for the use of Alexa 488 hydrazide as endocytic marker is discussed.

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## Restoration of potash spoil heaps by the establishment of biological soil crust algae

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With their broad range of protection mechanisms biocrust algae withstand multiple environmental stressors and thus are able to survive in extreme environments. By nutrient input as well as increasing water retention and erosion protection these organisms initiate the establishment of higher plants. Thus, soil crust algae could be used to green barren potash soil heaps.

These hypersaline sites result from the production of potash fertilizers. As sodium chloride is also a component of the mined potash salt, it must be divided from the valuable potassium chloride. Consequently, a vast amount of unusable salt is deposited in waste dumps close to the factory which grow to big heaps. With every rainfall event some salt dissolves and will be spread into the

surrounding. This leads to a continuous salinification of the environment. A plant cover could reduce the salt out-put by trapping rainwater. However, generally there is no satisfactory concept for greening the heaps known. According to that, the aim of this project is to use terrestrial algae from biological soil crusts to green the heaps. At first, soil crust organisms have been isolated from potash heaps and their surroundings. Typical taxa are e.g. *Chloroidium ellipsoideum*, *Tetracystis vinatzerii*, *Diplosphaera chodatii* and *Stichococcus bacillaris*.

In the following growth experiments with different salinities algae with the highest salt tolerance will be selected to finally build artificial soil crusts on salt material. Optimal growth of the crust will be provided by mixing heap material with nutrients and a salt buffer.



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# Stress induces mitochondrial fusion in the unicellular model alga *Micrasterias*

The freshwater model alga *Micrasterias* is closely related to higher plants and is an outstanding model system for research in plant cell biology. It inhabits acidic peat bogs and may be exposed to extreme environmental conditions such as high UV-radiation, temperature fluctuation and anthropogenic influences like heavy metal- or salt induction. Main targets of such stressors are cell organelles like mitochondria, which may physiologically react by disturbance of respiration, ROS production and release of cytochrome c into the cytoplasm. Previous investigations have revealed 2-D changes of mitochondrial structure in *Micrasterias* due to ionic stress. However, in order to correlate structural changes to physiological reactions, 3-D visualization is inevitable. Therefore, the present study focuses on 3-D reconstruction of mitochondria of high-pressure frozen *Micrasterias* cells that were exposed to stress. Both focused ion beam scanning

microscopy (FIB-SEM) and transmission electron microscopy (TEM) were employed to acquire tomography series. Our study shows that mitochondria in *Micrasterias* fuse to form elongated networks as consequence of ionic and or osmotic stress induced by exposure to KCl, but also to other stressors. Moreover it is shown that respiration is maintained during ionic stress and structural mitochondrial alterations are reversible. Similar results were obtained in the higher freshwater plant *Lemna* sp. which we investigated for comparison. Therefore we assume that mitochondrial fusion is an ubiquitous process that may help the cells to cope with stress. This may occur by interconnecting the respiratory chains of the individual mitochondria and by enhancing the buffer capacity against stress induced ionic imbalance. Our results are corroborated by reports of similar structural alterations in animal cells during disease.

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# Conspecificity of the model organism *Ulva mutabilis* and the potential nuisance algae *Ulva compressa* (Ulvophyceae, Chlorophyta)

As one of the most abundant and ubiquitous representatives of marine and brackish coastal macrophytobenthos communities the genus *Ulva* is not only an important primary producer but also of ecological and morphogenetic interest to many scientists. *Ulva mutabilis* became an important model organism to study morphogenesis, morphogenetics and mutualistic interactions of macroalgae and microorganisms. Here we report that *Ulva compressa* Linnaeus 1753 from Germany is conspecific with the type strain of the model organism *Ulva mutabilis* Føyn 1958, which was originally collected at Faro in Portugal and is from that time onward preserved as a game-

tophytic and parthenogenetic lab strain. Different approaches were used to examine conspecificity: (1) Comparisons on vegetative and reproductive features of cultured material of *Ulva mutabilis* and German *Ulva compressa* resulted in congruent results proving that a certain morphogenetic mutation pattern is shared. (2) Gametes of *Ulva compressa* and *Ulva mutabilis* were successfully crossed and developed into fertile sporophytic first-generation offspring. (3) Phylogenetic and species delimitation analyses were carried out on tufA sequences of 126 *Ulva compressa* specimen sampled in 2014-2016 at Baltic and North Sea areas of Germany and on respectively one tufA sequence of clones of the strains *Ulva mutabilis* (sl-G[mt+]) and *Ulva mutabilis* (wt-G[mt-]) to identify Molecular Operational Taxonomic Units (MOTUs). The Generalized Mixed Yule-Coalescent (GMYC) method supports their monophyly by comprising one major MOTU containing all included sequences of *Ulva compressa* and *Ulva mutabilis*. Thus *Ulva mutabilis* and *Ulva compressa* can be treated as the same species.

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## Impacts of skyglow on phytoplankton species

Since the invention of artificial light sources in the 19<sup>th</sup> century, there has been a massive increase in anthropogenic light emission on earth. This intense use of artificial light at night can be viewed as light pollution as it disrupts the natural diel photoperiod. Apart from direct illumination by street lights, ecosystems can experience low levels of indirect light pollution caused by emitted light that is backscattered to the earth's surface mainly from clouds (skyglow). Here we focus on direct effects of skyglow on phytoplankton ecology and physiology by testing in a laboratory experiment the effect of very low light levels at night on 4 algal species belonging to 3 main algal groups (cyanobacteria, diatoms, chlorophytes). After one week of acclimation to a 14:10 hour day:night cycle, algae were exposed to low light levels of about 6 lux (simulating skyglow) or kept in

complete darkness at night. At the start of the experiment and after one week we assessed the physiological status of the algae by measuring concentrations of particulate carbon, phosphorus, nitrogen, chlorophyll-a, other algal pigments and photosystem II activity at the end of both day and night. Responses of the algae to skyglow were species-specific. The chlorophyte showed higher chlorophyll-a fluorescence after exposure to skyglow, whereas the two tested cyanobacteria did not. The photosynthetic yield increased under skyglow in the diatom and in one species of cyanobacteria, but not in the other, nor in the chlorophyte. Overall our experiment shows that low levels of light pollution by skyglow have potential to affect phytoplankton directly, but responses of different physiological traits vary among species.

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Zygnematophycean green algae occur predominantly in shallow freshwater habitats where they produce high amounts of biomass and form characteristic, extensive mats. In such habitats, these algae are dominant and ecologically important, but they are also affected by environmental stress like UV radiation, desiccation or frost. Previous studies have shown that *Zygnema* sp. is able to survive various stress conditions in the form of pre-akinetes, resistant vegetative cells. Despite that, field observations confirmed that mats formed by *Zygnema* sp. have only annual character. We tested frost resistance of *Zygnema* sp. strains of different culture age (2 weeks and 8 months) using programmable freezers. We exposed young cultures to freezing temperatures down to -14°C, whereas old cultures were frozen down to

## Effect of frost on viability of conjugating green alga *Zygnema* (Zygnematophyceae)

-70°C. Survival rate of cells after freezing was tested using fluorescence staining with 0.1% Auramine O dye and measurement of photosynthetic activity by the steady-state quantum yield of PSII in the light ( $\Phi$ PSII). LT50 value for young cultures was -5.9°C, determined 24 h after the freezing process, whereas old cultures could survive even freezing down to -70°C. However, their LT50 value was -26.2°C. Cryo-microscopical observations and examination of the ultrastructure allowed us to visualize the cellular effects of freezing temperatures and frost injury. Our results show that frost hardening via formation of pre-akinetes is crucial for the survival of harsh temperature conditions prevailing in High Arctic in winter and plays a key role in seasonal dynamics of this alga.

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The Cymbellaceae (Bacillariophyta) comprise one of the most common families of diatoms in freshwater systems and has been researched since the early 19<sup>th</sup> century. Historical classification and separation of members in this family is based primarily on their life-history data and the morphological characteristics visible in the light microscope, such as frustule form and – if seen alive – on chloroplast form. However, with the advent of molecular analysis, it has become apparent that these characters are not always sufficient in delimitating species. Recent phylogenetic research has suggested that some established genera within the Cymbellaceae are not monophyletic and that a revision of their taxonomic and phylogenetic relationships is due.

Here we aimed to perform an integrative taxonomic study and review of the Cymbellaceae using

## Reconsidering the phylogenetic relationships of selected taxa within the Cymbellaceae (Bacillariophyta) with an integrative approach

molecular (18SV4 SSU DNA and rbcL markers) and morphological data; furthermore, we supplement the methodology used to delimitate diatom species. Emphasis was placed on internal and external microstructures visible only at SEM resolution, such as foramina form, stigma alveoli, and apical pore field structure. Less importance was given to gross structure and general habitus. Our findings suggest that *Cymbella* Agardh is polyphyletic and that new autopomorphies need to be defined for each group. In addition, we propose to re-evaluate the members of *Encyonema* Kützinger sensu stricto, and to establish new groupings for the *Encyonema* species not fitting this description. Finally, we also reexamined the phylogenetic positions of *Encyonopsis* Krammer, *Cymbopleura* Krammer, and *Didymosphenia* M. Schmidt within the Cymbellaceae.

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## Towards establishment of a genetic transformation system in charophyte green algae – Zygnemato- phyceae

The Zygnematophyceae occupy an important phylogenetic position as the sister group of land plants. However, little is known about this class of green algae, especially concerning functional genomics. Until now, no genomic sequence data for Zygnematophyceae are available and no robust genetic experimental platform is established. We use our Microalgae and Zygnematophyceae Collection Hamburg (MZCH) as a source to screen for streptophytic algae strains allowing high-throughput transformation as a prerequisite for potential model organisms. *Cosmarium regenesii* and *Spirogyra pratensis* were chosen as fast growing

candidate species representing the orders Desmidiaceae and Zygnematales. For both, axenic cultures were established. Different zeocin resistance cassettes driven by heterologous promoters have been successfully introduced to *C. regenesii* using microparticle bombardment. However, none of the heterologous promoters seem to work in *S. pratensis*. Two homologous promoters were isolated by Tail-PCR and the transformation showed that the native tubulin promoter can drive GFP expression in *S. pratensis* protoplasts. After the optimization of the transformation protocol, the selection of stable transformants is expected.

## **German Barcode of Life 2 (GBOL2) – eDNA metabarcoding of diatoms in the context of the EU Water Frame- work Directive (EU WFD)**

The GBOL2 project, funded by the German Federal Ministry of Education and Research, focuses on the extension of the thitherto erected DNA barcode reference library to integrate all frequent, common and indicator organisms, those in the Council Directive 92/43/EEC on the Conservation of Natural Habitats and of Wild Fauna/Flora (FFH) and the German Red Lists, as well as health-relevant and invasive organisms.

The project part located at the BGBM Berlin is focussing on compiling a diatom DNA barcode reference library, as diatoms are very important bioindicator organisms implemented within the EU WFD.

Hitherto, no concerted strategy for the molecular registration of diatom species exists in Germany and the quality as well as the taxonomical validation of the deposited diatom sequences in the INSDC is often not adequate. Therefore, it is planned to DNA barcode the 400 most important indicator species of the 1700 species expected to live in German waters.

The DNA barcodes and all correlated information will be publicly available. The DNA stocks will be deposited in the BGBM DNA Bank connected to taxonomically validated voucher specimens deposited at Herbarium Berolinense (B) following specific standardised requirements.

As a best practice use case for documenting and displaying environmental and eDNA data, the diatom DNA barcode reference library will use the EDIT platform for cybertaxonomy and provides the possibility to assign environmental sequences gained from eDNA metabarcoding to the taxonomically validated reference sequences in the database as well as providing a platform for the complete automation of the eDNA metabarcoding data evaluation. Both are crucial prerequisites for developing a sequenced-based time and cost efficient method to analyse the diatom community composition in environmental samples via eDNA metabarcoding for water quality assessments.

**S. 95–100**

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